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RESEARCH**

APPLICATION NUMBER:

214094Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review

Clinical Review

Non-Clinical Review

Statistical Review

Clinical Pharmacology Review

NDA Multidisciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	214094
Priority or Standard	Standard
Submit Date(s)	December 3, 2019
Received Date(s)	December 3, 2019
PDUFA Goal Date	December 3, 2020
Division/Office	DPACC/OII
Review Completion Date	December 3, 2020
Established/Proper Name	Berotralstat
(Proposed) Trade Name	Orladeyo
Pharmacologic Class	Kallikrein inhibitor
Code Name	BCX7353
Applicant	BioCryst Pharmaceuticals
Dosage Form	Capsule
Applicant-Proposed Dosing Regimen	150 mg orally once daily and 110 mg orally once daily
Applicant Proposed Indication(s)/Population(s)	For prophylaxis to prevent attacks (b) (4) of hereditary angioedema in patients 12 years of age and older
Applicant Proposed SNOMED CT Indication Disease Term for Each Proposed Indication	Hereditary angioedema (disorder)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s)	For prophylaxis to prevent attacks (b) (4) of hereditary angioedema in patients 12 years of age and older
Recommended SNOMED CT Indication Disease Term for Each Indication	Hereditary Angioedema (disorder)
Recommended Dosing Regimen	150 mg orally once daily 110 mg orally once daily (dose adjustment for moderate to severe hepatic impairment or persistent GI adverse reactions)

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DPACC, Division of Pulmonology, Allergy, and Critical Care
 DIIP, Division of Inflammation and Immune Pharmacology
 DPT-II, Division of Pharmacology/Toxicology for Immunology and Inflammation
 OB, Office of Biostatistics
 OCP, Office of Clinical Pharmacology
 OII, Office of Immunology and Inflammation
 OND, Office of New Drugs
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OPQ, Office of Pharmaceutical Quality
 OPDP, Office of Prescription Drug Promotion
 DMPP, Division of Medical Policy Programs
 OSI, Office of Scientific Investigations
 OSE, Office of Surveillance and Epidemiology
 DEPI, Division of Epidemiology

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DMEPA, Division of Medication Error Prevention and Analysis
 DRISK, Division of Risk Management

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Glossary

AE	adverse event
AE-QoL	angioedema quality-of-life
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC ₀₋₂₄	area under the curve from time zero to 24 hours
AUC _{tau}	area under the curve over the dosing interval
BCRP	breast cancer resistance protein
BLA	biologics license application
C1-INH	C1-esterase inhibitor
CL _{CR}	creatinine clearance
CL _F	apparent oral clearance
C _{max}	maximum plasma concentration
C _{tau}	maximum plasma concentration over the dosing interval
CV	cardiovascular
CYP	cytochrome P450
DARRTS	Document Archiving, Reporting, and Regulatory Tracking System
DDI	drug–drug interaction
DMID	Division of Microbiology and Infectious Diseases
EC ₅₀	half-maximal effective concentration
ECAC	Executive Carcinogenicity Assessment Committee
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
GCP	good clinical practices
GD	gestational day
GI	gastrointestinal
GLP	good laboratory practices
HAE	hereditary angioedema
HD	high dose
HMWK	high-molecular-weight kininogen
IC ₅₀	half-maximal inhibitory concentration
ICH	International Council on Harmonisation
IND	investigational new drug
IRT	integrated review team
ISS	integrated summary of safety
ITT	intent to treat
K _a	absorption rate
LAD	lactation day
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD	low dose
LFT	liver function test

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LLOQ	lower limit of quantification
LSM	least-squares mean
MD	mid dose
NDA	new drug application
NOAEL	no observable adverse effect level
OCS	Office of Computational Science
OLE	open-label extension
OSE	Office of Surveillance and Epidemiology
PD	pharmacodynamics
P-gp	P-glycoprotein
PK	pharmacokinetics
PPND	peri- and post-natal development
QD	once daily
QTc	corrected QT
QTcF	Fridericia-corrected QT
QT-IRT	QT-Interdisciplinary Review Team
REMS	risk evaluation and mitigation strategy
RSE	relative standard error
SAE	serious adverse event
SAEM	stochastic approximation/expectation maximization
SOC	system organ class
TBM	to be marketed
TE	treatment emergent
TEAE	treatment-emergent adverse event
T _{max}	time to C _{max}
V _{cF}	apparent central volume

1. Executive Summary

1.1. Product Introduction

Berotralstat is an orally administered inhibitor of active plasma kallikrein for prophylaxis to prevent attacks of hereditary angioedema (HAE) in patients 12 years of age and older. Berotralstat is a new molecular entity (NME) and not approved for use in any country. The dosing regimen for approval consists of a recommended starting dose of 150 mg orally once daily and 110 mg orally once daily for patients with moderate-to-severe hepatic impairment or for patients with persistent gastrointestinal (GI) adverse reactions.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The recommended regulatory action for this application is Approval of orally administered berotralstat 150 mg once daily for prophylaxis to prevent attacks of HAE in patients 12 years of age and older. To support this application, the Applicant conducted a single 24-week, adequate, well-controlled pivotal efficacy and safety trial in adolescents and adults with type I or II HAE. The results from this trial demonstrated evidence of efficacy for both the 110 mg and 150 mg doses of berotralstat compared to placebo for the primary endpoint of HAE attack rate over 24 weeks and the predefined exploratory endpoint of a $\geq 50\%$ reduction in attack rate compared to baseline. The study had adequate statistical power to detect a clinically meaningful difference between berotralstat treatment versus placebo. Based on the robustness of the results and the rarity of the disease, a single pivotal trial is adequate to demonstrate substantial evidence of effectiveness. The Division did not deem the results from the first key secondary endpoint, the angioedema quality-of-life (AE-QoL) questionnaire score, fit for purpose for labeling. Nevertheless the results were not statistically significant versus placebo for either treatment group and further secondary endpoints were therefore not tested because of the hierarchal analysis. The numerically greater and more clinically significant treatment effect in the high-dose arm supports a recommended dose of 150 mg once daily. However, the totality of safety and efficacy data also support use of the 110 mg once-daily dose for patients who may not tolerate the higher dose due to GI-related adverse reactions or who have moderate to severe hepatic impairment.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Berotralstat is an orally administered inhibitor of active plasma kallikrein for prophylaxis to prevent attacks of hereditary angioedema (HAE) in patients 12 years of age and older. The recommended regulatory action of this NDA is Approval.

Hereditary angioedema (HAE) is a rare, autosomal dominant, inherited condition characterized by intermittent, unpredictable attacks of angioedema. HAE is caused by mutations in the *SERPING1* gene, resulting in deficient or dysfunctional C1-esterase inhibitor (C1-INH) protein, a serine protease inhibitor. Absence of functional C1-INH leads to dysregulation of the contact system, unchecked kallikrein activity, and ultimately widespread release of bradykinin, which increases vascular permeability causing the characteristic swelling of acute HAE attacks. Although the exact prevalence is unknown, HAE is estimated to affect 1 in 10,000 to 50,000 individuals worldwide (approximately 6,000 to 10,000 individuals in the United States) and is categorized as an orphan disease. Acute attacks of HAE are potentially life-threatening, particularly in cases of laryngeal edema resulting in airway compromise. Attacks at other anatomic sites (e.g., gastrointestinal [GI] tract, genitourinary tract, and skin on the hands, feet, and face) can cause disabling pain and significant morbidity. These attacks are highly variable in frequency and location among individuals and even within a given individual. Patients with particularly severe and/or frequent attacks may be treated with prophylactic therapies to prevent or reduce acute attacks. While the context of available therapies for prophylaxis as well as treatment of acute attacks has expanded greatly in the last decade, most therapies require subcutaneous injection or intravenous infusion. Danazol, an attenuated androgen, is the only oral therapy approved for HAE prophylaxis; however, safety and tolerability issues limit its use.

The efficacy and safety of berotralstat was evaluated in a single, well-controlled, adequately designed pivotal trial in Type I and II HAE patients 12 years of age and older. Patients had a minimum of 2 HAE attacks over a 2-month run-in period. Berotralstat treatment for 24 weeks versus placebo resulted in statistically significant and clinically meaningful reductions in the rate of HAE attacks. The 110 and 150 mg doses of berotralstat reduced the rate of HAE attacks by 30% ($p=0.024$) and 44% ($p<0.001$) relative to placebo, respectively. Efficacy was further supported by additional endpoints such as proportion of patients with at least a 50% reduction in HAE attacks compared to baseline. While the study demonstrated a significant treatment benefit in a patient population with frequent attacks who are likely to require prophylaxis, the treatment response was modest as few subjects achieved complete elimination of attacks. However, an oral therapy provides an additional, convenient option to the treatment armamentarium.

The safety data submitted with the NDA were sufficient to assess the safety of berotralstat in the proposed HAE population. Gastrointestinal-related adverse events and transaminase elevations emerged as the main safety signals. Adverse reactions such as abdominal pain, vomiting, diarrhea, dyspepsia and gastroesophageal reflux occurred more frequently, often in a dose-

dependent manner, with berotralstat compared to placebo. However, GI-related events were generally mild-to-moderate severity and self-limited, and typically did not lead to study drug discontinuation. Adverse events related to transaminase elevations, primarily increased ALT, were reported with berotralstat treatment in the controlled and open label extension studies. Few subjects had marked elevations triggering treatment discontinuation, and no potential Hy's law cases were identified in the program. Transaminase elevations were generally transient, asymptomatic, not dose-dependent, and in some cases confounded by underlying co-morbidities and prior androgen use. In general, safety data from the open label extension study were consistent with the placebo controlled data and support chronic berotralstat use. Although not observed in clinical trials, QT prolongation is an anticipated risk with higher than recommended doses based on the pharmacologic action of the drug. No safety concerns were identified that should preclude approval or require a REMS.

Efficacy and safety results across various demographic and baseline characteristic subgroups were generally consistent with the overall findings. Given the small number of adolescent subjects, efficacy in this age group is primarily based on extrapolation from efficacy data in adults because the pathophysiology of disease is similar irrespective of age. The numerically greater and more clinically significant treatment effect in the high-dose arm supports a recommended dose of 150 mg once daily. However, the totality of safety and efficacy data also support use of the 110 mg once-daily dose for patients who may not tolerate the higher dose due to GI-related adverse reactions or who have moderate to severe hepatic impairment. Overall, the risk/benefit profile for berotralstat in the treatment of a rare, serious disease such as HAE is favorable.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• HAE is a rare genetic condition caused by mutations in the <i>SERPING1</i> gene, resulting in deficiency or dysfunction of C1-INH protein. • Approximately 85% of patients have type I HAE, characterized by low production of normal C1-INH protein; the remaining 15% of patients have type II HAE, characterized by normal production and level of dysfunctional C1-INH. • The absence of functional C1-INH leads to the characteristic swelling of acute HAE attacks. • The exact prevalence is unknown, but HAE is estimated to affect 1 in 10,000 to 50,000 individuals worldwide (approximately 6,000 to 10,000 individuals in the United States). • Acute attacks of HAE are potentially life-threatening, particularly in cases of laryngeal edema resulting in airway compromise. Attacks at other anatomic sites (e.g., GI tract, genitourinary tract, and skin) can cause	HAE is a rare, genetic, potentially life-threatening disease characterized by unpredictable, recurrent swelling attacks. The HAE types included in the proposed indication encompass most patients with HAE in the United States. While the disease can be highly variable, patients with severe and/or frequent attacks benefit from prophylactic therapy.

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ORLADEYO (berotralstat)

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	disabling pain and significant morbidity. These attacks are unpredictable and highly variable in frequency and location among individuals and even within a given individual. • Patients with severe and/or frequent attacks are candidates for prophylactic therapies	
Current Treatment Options	• No cure is available; however, there are several approved therapies for prevention (i.e., prophylaxis) and treatment of acute attacks • Therapies for prophylaxis include a biologic monoclonal antibody targeting active plasma kallikrein, plasma-derived/recombinant C1-INH replacement, and oral attenuated androgens. C1-INH therapies have short half-lives and must be administered every 3 to 4 days intravenously or subcutaneously. Oral androgens are associated with numerous side effects that limit tolerability. The approved monoclonal antibody, while highly effective, requires subcutaneous injection. • Prophylactic therapies do not eliminate all HAE attacks in all patients.	Although there are effective approved prophylactic therapies for patients with HAE, the availability of additional, convenient treatment options is desirable for those who prefer an orally administered therapy and/or are unable to tolerate existing treatments.
Benefit	• The Applicant has demonstrated substantial evidence of efficacy for berotralstat in patients with type I or II HAE based on reductions in the HAE attack rate from baseline over a 24-week treatment period. • The 110 and 150 mg doses of berotralstat reduced the rate of HAE attacks by 30% (p=0.024) and 44% (p<0.001), respectively relative to placebo. • Efficacy was further supported by additional exploratory endpoints such as proportion of patients with at least a 50% reduction in HAE attacks compared to baseline. • A larger treatment effect was observed with the higher dose; therefore, 150 mg once daily is the recommended dose. • Due to the small number of adolescents, efficacy in patients 12-17 years of age is supported by extrapolation from efficacy data in adults; the pathophysiology of disease is similar irrespective of age	Berotralstat demonstrated statistically significant and clinically relevant, though relatively modest, reductions in HAE attack rates relative to placebo. Both doses studied were effective compared to placebo; however, the higher dose of 150 mg once daily was more effective and is the recommended dose.
Risk and Risk Management	• The safety program for berotralstat identified potential risks for gastrointestinal-related side effects and elevated liver enzymes. • Gastrointestinal adverse reactions were dose-related.	No substantial safety findings that outweigh the potential benefit were identified in the clinical trials. Potential risks of adverse gastrointestinal symptoms and elevated liver

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Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• QT prolongation is an anticipated risk with doses higher than 150 mg based on the pharmacologic effect of the drug. • No risk evaluation and mitigation strategy is proposed.	enzymes can be managed by means of the labeling and routine pharmacovigilance. The 110 mg dose is recommended for patients with moderate to severe hepatic impairment and also available as step-down therapy for patients who cannot tolerate the 150mg dose due to persistent gastrointestinal symptoms.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (Check All that Apply)

☐	The patient experience data that were submitted as part of the application include:	Section of review where discussed, if applicable
x	Clinical outcome assessment (COA) data, such as	
	x Patient-reported outcome (PRO)	8.1.1
	☐ Observer-reported outcome (ObsRO)	
	☐ Clinician-reported outcome (ClinRO)	
	☐ Performance outcome (PerfO)	
	☐ Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
	☐ Patient-focused drug development or other stakeholder meeting summary reports	
	☐ Observational survey studies designed to capture patient experience data	
	☐ Natural history studies	
	☐ Patient preference studies (e.g., submitted studies or scientific publications)	
	☐ Other: (Please specify):	
☐	Patient experience data that were not submitted in the application, but were considered in this review:	
	☐ Input informed from participation in meetings with patient stakeholders	
x	Patient-focused drug development or other stakeholder meeting summary reports	2.1
	☐ Observational survey studies designed to capture patient experience data	
	☐ Other: (Please specify):	
☐	Patient experience data was not submitted as part of this application.	

2. Therapeutic Context

2.1. Analysis of Condition

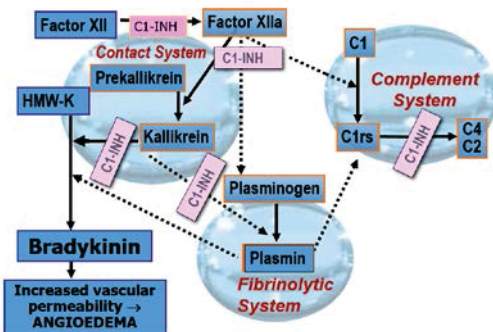
Hereditary angioedema (HAE) is a rare, autosomal dominant, inherited condition characterized by intermittent, unpredictable attacks of angioedema. HAE is caused by mutations in the *SERPING1* gene, resulting in deficiency or dysfunction of C1-esterase inhibitor (C1-INH) protein, a serine protease inhibitor. Approximately 85% of patients have type I HAE, characterized by low production of normal C1-INH protein; the remaining 15% of patients have type II HAE, characterized by normal production and levels of dysfunctional C1-INH. The absence of functional C1-INH leads to dysregulation of the contact system, a plasma protease cascade initiated by factor XII that activates the proinflammatory kallikrein-kinin system (Figure 1) and the procoagulant intrinsic coagulation pathway. Ordinarily, kallikrein activity is regulated by C1-INH, but in patients with HAE, kallikrein activity is unchecked, leading to widespread release of bradykinin. The resulting elevated level of bradykinin increases vascular permeability, leading to the characteristic swelling of acute HAE attacks.

Figure 1 provides a more detailed representation of how C1-INH functions within the contact, complement, and fibrinolytic systems. C1-INH is an endogenous inhibitor of the serine protease enzymes Factor XIIa and plasma kallikrein. Factor XIIa converts prekallikrein to active plasma kallikrein, which in turn cleaves high-molecular-weight kininogen (HMWK) to form bradykinin and cleaved HMWK. Bradykinin is a polypeptide signaling molecule that participates in multiple biological processes, including blood coagulation, activation of inflammation, and regulation of blood pressure.

Patients with HAE have uncontrolled plasma kallikrein production and pathologic levels of bradykinin. The key triggering events of HAE attacks are not completely understood. Activation by bradykinin of bradykinin B2 receptors on the surface of endothelial cells leads to vasodilation and decreased blood pressure. Activation of B2 receptors also results in the vascular leakage and subsequent tissue swelling characteristic of HAE attacks. Bradykinin is converted to des-Arg⁹ bradykinin in the plasma by carboxypeptidase N. Des-Arg⁹ bradykinin activates bradykinin B1 receptors, thus contributing to symptoms of HAE. Berotralstat is designed to block the enzymatic function of plasma kallikrein and so prevent the proteolytic conversion of HMWK to bradykinin¹.

¹ Kenniston, JA, RR Faucette, D Martik, SR Comeau, AP Lindberg, KJ Kopacz, GP Conley, J Chen, M Viswanathan, N Kastropeli, J Cosic, S Mason, M DiLeo, J Abendroth, P Kuzmic, RC Ladner, TE Edwards, C TenHoor, BA Adelman, AE Nixon, and DJ Sexton, 2014, Inhibition of plasma kallikrein by a highly specific active site blocking antibody, *J Biol Chem*, 289(34):23596-23608; and Zuraw, BL, JA Bernstein, DM Lang, T Craig, D Dreyfus, F Hsieh, D Khan, J Sheikh, D Weldon, DI Bernstein, J Blessing-Moore, L Cox, RA Nicklas, J Oppenheimer, JM Portnoy, CR Randolph, DE Schuller, SL Spector, SA Tilles, and D Wallace, 2013, A focused parameter update: hereditary angioedema, acquired C1 inhibitor deficiency, and angiotensin-converting enzyme inhibitor-associated angioedema, *J Allergy Clin Immunol*, 131(6):1491-1493.

Figure 1. The Plasma Kallikrein-Kinin System
C1-INH involved in 3 systems → C1-INH depletion



Source: World Allergy Organization Global Hereditary Angioedema Practice Parameter
Abbreviations: C, complement; C1-INH, C1-esterase inhibitor; HMW-K, high-molecular-weight kininogen

Although the exact prevalence is unknown, HAE is estimated to affect 1 in 10,000 to 50,000 individuals worldwide (approximately 6,000 to 10,000 individuals in the United States) and is categorized as an orphan disease. Despite its autosomal dominant inheritance pattern, roughly one quarter of patients with HAE have spontaneous mutations, and diagnosis is often delayed by several years. The age at which attacks begin is variable, but symptoms during infancy are exceedingly rare. Based on literature reports, the median age of symptom onset is between 6 and 11 years. The attack frequency increases after puberty and continues throughout an individual's life. The acute attacks of HAE are potentially life-threatening, particularly in cases of laryngeal edema resulting in airway compromise. Attacks at other anatomic sites (e.g., gastrointestinal [GI] tract, genitourinary tract, hands, feet, and face) can cause disabling pain and significant morbidity. These attacks are highly variable in frequency and location both among individuals and within a given individual. If untreated, attacks may occur on average every 1 to 2 weeks and typically last 48 to 72 hours. The therapies currently used by patients with HAE to manage their disease are listed in Section [2.2](#).

The Food and Drug Administration (FDA) convened a Patient-Focused Drug Development public meeting for HAE on September 25, 2017, during which FDA staff heard directly from patients, families, and caregivers about their experiences with HAE attacks and the life-changing impact of available therapies. Approximately 110 patients with HAE and their representatives were present at the meeting and a further 52 participated by means of a live webcast. The discussion at the meeting focused on two key topics: 1) the disease symptoms and daily impacts that matter most to patients and caregivers and 2) patients' perspectives on current approaches to treating HAE and on participating in clinical studies. The *Voice of the Patient* report highlighted the following overarching themes from the discussion that took place during the meeting:

- Diagnostic delays and lack of awareness of HAE and recognition of signs/symptoms by healthcare providers.
- Unpredictability of attacks.
- Pain, disability, and disfigurement that result from attacks.
- Emotional stress that accompanies the condition.
- Impact on work, family, and social life.
- Life-changing effect of newer approved treatments.

- Unmet need for treatments that are less invasive, improve predictability of attacks, have fewer side effects, and ultimately lead to cure.
- Areas for future research include hormonal influences on the disease, therapies for children, and therapies for HAE types that are less responsive to currently available therapies.
- Willingness to participate in clinical trials and natural history studies.

2.2. Analysis of Current Treatment Options

Medications used to treat HAE patients are typically categorized as treatments for acute attacks or prophylaxis. Although currently available treatments ([Table 1](#)) for routine prophylaxis of acute HAE attacks are effective in reducing their number and frequency, they do not eliminate all attacks in every individual. While fresh frozen plasma and antifibrinolytics (tranexamic acid, ε-aminocaproic acid) are available for off-label use in HAE, their use has fallen out of favor following the availability of effective FDA-approved therapies.²

Table 1. Summary of the Treatment Armamentarium for HAE

Product Name	Pharmacologic Class	Year of Approval	Dosing/ Administration	Pediatric Indication	Important Safety and Tolerability Issues
<i>FDA-Approved Treatments for Prophylaxis</i>					
Danazol	Androgen	1980	200 mg po BID-TID	No age limit	Thromboembolism, hepatic dysfunction, hepatic adenoma, dyslipidemia, myopathy, weight gain, acne, hirsutism, menstrual disturbance
Cinryze	Plasma-derived C1-INH	2008	1000 units IV q3-4 days	6 years and older	Thromboembolism, hypersensitivity, transmissible infection
Haegarda	Plasma-derived C1-INH	2017	60 IU/kg SC q3-4 days	12 years and older	Same as Cinryze
Tahkzyro	Monoclonal antibody; plasma kallikrein inhibitor	2018	300 mg SC q2-4 weeks	12 years and older	Injection-site reactions, hepatic dysfunction

² Busse PJ, Christiansen SC, Riedl MA, Banerji A, Bernstein JA, Castaldo AJ, Craig T, Davis-Lorton M, Frank MM, Li HH, Lumry WR, Zuraw BL. US HAEA Medical Advisory Board 2020 Guidelines for the Management of Hereditary Angioedema. *J Allergy Clin Immunol Pract.* 2020 Sep 6:S2213-2198(20)30878-3.

Product Name	Pharmacologic Class	Year of Approval	Dosing/ Administration	Pediatric Indication	Important Safety and Tolerability Issues
<i>FDA-Approved Treatments for Acute Attacks</i>					
Berinert	Plasma-derived C1-INH	2009	20 IU/kg IV prn	No age limit	Same as Cinrye
Kalbitor	Plasma kallikrein inhibitor	2009	30 mg SC prn (up to 60 mg/day)	12 years and older	Injection-site reactions, anaphylaxis
Firazyr	Bradykinin B2 receptor antagonist	2011	30 mg SC prn (up to 90 mg/day)	18 years and older	Injection-site reactions, hepatic dysfunction
Ruconest	Recombinant C1-INH	2014	50 units/kg IV (maximum 4200 U/dose)	12 years and older	Thromboembolism, hypersensitivity

Source: Drugs@FDA.gov (<https://www.accessdata.fda.gov/scripts/cder/daf/>)

Abbreviations: BID, twice daily; C1-INH, C1-esterase inhibitor; HAE, hereditary angioedema; IU, international units; IV, intravenous; PO, by mouth; PRN, as needed; q, every; SC, subcutaneous; TID, thrice daily

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Berotralstat is not approved or marketed in the United States or any foreign country for any other indication.

3.2. Summary of Presubmission/Submission Regulatory Activity

Berotralstat (BCX7353) was developed under investigational new drug (IND) application 135058, which was opened on June 22, 2017. Prior to opening the IND, the Applicant had multiple ex-US clinical studies (completed and ongoing) evaluating the use of berotralstat for prevention of HAE attacks. Berotralstat was granted orphan therapy designation on November 1, 2017, and fast-track designation on August 2, 2018. Relevant interactions between the Agency and Applicant to discuss the clinical development program are summarized below.

End-of-Phase 2 meeting: November 2, 2017

- Agreement to submit the final report of 2-year carcinogenicity study of berotralstat in rats prior to submitting NDA
- Discussed that a thorough QTc study may be required
- Disagreement with proposed study design and safety database for a single trial to support an NDA. Six months of blinded, controlled data will be required to support safety of an NME. Provided no major safety issues are identified in the phase 3 trial, the safety database for this program should include approximately 64 to 70 patients in the controlled portion of the study and approximately 100 subjects exposed for at least 1 year

- Approach to dose selection for phase 3 was reasonable; however, the Division could not comment further given that the 150 mg dose had not been previously studied. Advised against dose titration and modifications within the study as this may complicate the safety evaluation and result in insufficient long-term safety data to support the proposed dose
- Exclusion criteria should be limited where possible to ensure generalizability of results
- Primary efficacy endpoint of investigator-confirmed attack rate over 24 weeks acceptable; however, disagreement with proposed definition of HAE attacks. HAE attack definition should require presence of swelling, and premature use of rescue medication to treat prodromal symptoms should not be considered an acute attack. For completeness, all attacks should be captured in the safety database

Pre-NDA meeting: May 29, 2019

- Agreement that the food effect had been adequately studied
- Agreement with proposed format and content of NDA submission
- Preliminary agreement that safety summary data did not suggest need for a risk evaluation and mitigation strategy (REMS) plan in the initial NDA submission
- Size and scope of the safety database appeared adequate to support NDA filing and review

Of note, the Applicant has a related IND 142879 for the treatment of acute HAE attacks with berotralstat. The higher proposed doses for treatment of acute HAE attacks generated concern regarding the potential for significant QT prolongation, particularly if used on top of prophylaxis therapy. The FDA communicated these concerns to the Applicant and further stated that addressing the issue of QT prolongation was an essential component of the development program of berotralstat for the treatment of acute HAE attacks (refer to Meeting Minutes dated August 20, 2019).

4. Significant Issues From Other Review Disciplines Pertinent to the Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations

The pivotal efficacy and safety trial, Study BCX7352-302 Part 1, in the berotralstat development program was a multicenter trial in which each site enrolled a small number of subjects. Therefore, the potential for any individual study center to impact the efficacy and safety findings was limited. Two clinical sites (Dr. Johnston/Site #033 and Dr. Lumry/Site #035) were selected for inspection for Study BCX7352-302 Part 1. Both sites were chosen for having a relatively large enrollment and higher response rates on the primary efficacy endpoint compared to other sites in the United States. The Office of Scientific Investigations reports for these sites found them to be in compliance with Good Clinical Practices and their study data to be reliably supportive of the requested indication.

4.2. Product Quality

CMC information for two strengths (110 and 150 mg) of berotralstat capsules were submitted to the NDA. (b) (4) there were two distinct drug product manufacturing sites. Both strengths have been evaluated from a quality perspective. The proposed drug product specification is appropriate and includes testing and acceptance criteria for description, identification (HPLC and UV), assay, degradation products, content uniformity, dissolution, (b) (4) and microbial limits, as recommended by ICH Q6A. Impurities specification acceptance criteria followed the recommendations of ICH Q3B. The associated analytical methods were found to be suitable for their intended use. There are no plans for specific tests for elemental impurities in the drug product, and this is supported by the risk assessment and data from 12 registration lots. The applicant's request for a categorical exclusion from the requirements to submit an environmental assessment per 21 CFR 25.31(b) is acceptable. All the facilities have been approved based on previous history and with concurrence with ORA recommendations. Based on the drug solubility, drug dissolution rate of the pivotal clinical and registration batches for both strengths of berotralstat capsules, low variability of drug release with the proposed dissolution method, the Applicant's proposed dissolution method and acceptance criterion are considered adequate. The overall recommendation for this product is approval from a product quality perspective.

4.3. Clinical Microbiology

Not applicable.

4.4. Devices and Companion Diagnostic Issues

Not applicable.

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

The nonclinical program of berotralstat consists of studies of its pharmacology, pharmacokinetics (PK), and toxicology. Pharmacology studies showed that berotralstat inhibited kallikrein activity across species. PK studies showed that orally administered berotralstat is bioavailable in animals and humans. There are no major and unique berotralstat metabolites in humans. Toxicology studies evaluated the general toxicity, genetic toxicity, carcinogenicity, and reproductive and developmental toxicity of berotralstat. General toxicity studies included chronic oral toxicity studies in rats and monkeys (treatment durations of up to 26 and 39 weeks, respectively). Genetic toxicity was evaluated by means of a standard battery of assays. Carcinogenicity studies included studies of oral berotralstat with a traditional 2-year bioassay in rats and a 26-week study in transgenic mice. Reproductive and developmental

studies included teratology studies in rats and rabbits, a fertility and early embryonic developmental study in rats, and a peri- and post-natal development (PPND) study in rats.

5.1.1. Carcinogenicity

No evidence of tumorigenicity was found in mice or rats. The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in rats and a 26-week study in B6F1 Tg.RasH2 mice. Wistar rats (60/sex/dose) were dosed by oral gavage with 0, 3, 8 and 20 mg/kg berotralstat once daily for 104 weeks. Mice (25/sex/dose) were dosed by oral gavage with 0, 8, 20, and 50 mg/kg berotralstat once daily for 26 weeks. Neither study revealed treatment-related increases in tumor incidences.

5.1.2. Genetic Toxicity

Berotralstat produced negative results in the bacterial reverse gene mutation assay, an in vitro chromosomal aberration assay in human peripheral blood lymphocytes, and an in vivo micronucleus assay in rats.

5.1.3. Reproductive and Developmental Toxicity

Berotralstat was nonteratogenic in pregnant rats and rabbits dosed with the drug during the organogenesis period. Berotralstat did not affect the fertility or PPND of rats.

5.1.4. Others

Toxicity studies indicated that berotralstat can result in phospholipidosis and affect the cardiovascular (CV) system of animals.

5.1.4.1. Phospholipidosis

Repeat-dose administration of berotralstat can cause phospholipidosis in rats and monkeys. Phospholipidosis-related findings included vacuoles in the epithelium and mucosa of the intestine, kidney, and choroid plexus (rats); foamy macrophages and histiocytes in the spleen, thymus, and intestine; and foamy Kupffer cells in the liver. Phospholipidosis was confirmed by electronic microscopic examination of Kupffer cells (myelinosomes) in the liver and the urinary level of di-22:6-bis(monoacylglycol) phosphate, a biomarker of phospholipidosis. Phospholipidosis is a known drug-storage mechanism. There is no evidence that berotralstat causes phospholipidosis in humans.

5.1.4.2. Cardiovascular Effects

Berotralstat prolonged the QT interval in monkeys and humans. Berotralstat affected the conductance of potassium and sodium channels and the membrane action potential in vitro. Berotralstat also affected blood pressure, heart rate, and electrocardiogram (ECG) (i.e., slight increases in the RR, PR, QT, and QTc intervals, and in the QRS duration) in monkeys. In humans, berotralstat at an oral dose of 450 mg (maximum concentration [C_{max}] 191 ng/mL) prolongs the QT interval by approximately 16 ms. A thorough QT study of the effect of berotralstat on the QT interval in humans has been completed.

5.2. Referenced NDAs, Biologics License Applications, and Drug Master Files

- Biologics license application (BLA) 125277—KALBITOR (ecallantide), a recombinant human plasma kallikrein inhibitor indicated for HAE; approved on December 1, 2009.
- BLA 761090—TAKHZYRO (lanadelumab); approved on August 23, 2018.
- NDA 022150—FIRAZYR (icatibant acetate injectable); approved on August 25, 2011.
- IND 135058—Berotralstat, intended for the prevention of HAE attacks.
- IND 142879—Berotralstat, intended for the treatment of HAE attacks.

5.3. Pharmacology

Berotralstat decreases vascular permeability and edema in patients with HAE by reducing the blood level of bradykinin, a potent vasodilator. Berotralstat decreases the bradykinin level by inhibiting kallikrein, a plasma serine protease responsible for bradykinin production. Kallikrein cleaves and releases bradykinin from HMWK. Kallikrein is inactivated by C1-INH. Patients with HAE exhibit excessive kallikrein activity as a result of C1-INH deficiency or dysfunction.

5.3.1. Primary Pharmacology

Berotralstat inhibited plasma kallikrein from animals and humans. The half-maximal inhibitory concentration (IC_{50}) was 5.2, 5.7, and 5.4nM for rat, monkey, and human kallikrein (healthy subjects), respectively (Study BR-7353-005-REV-01). Kallikrein from patients with HAE was less sensitive to inhibition by berotralstat than was that from healthy subjects. This was indicated by a three-fold difference in IC_{50} (15.9 and 5.4nM for kallikrein from patients with HAE and healthy subjects, respectively; Study BR-7353-006-REV-01). Berotralstat has an inhibition constant of 0.44nM for isolated/purified human plasma kallikrein (Study BR-7353-001), but the IC_{50} of kallikrein from patients with HAE is a better indicator of potency because of the lack of purified enzymes in vivo. Berotralstat inhibited kallikrein-dependent production of bradykinin (half-maximal effective concentration [EC_{50}] 5.56 ± 1.2 nM, Study BR-7353-004). Berotralstat has little activity against other serine proteases—the IC_{50} values for other serine proteases were at least 4500-fold higher than that for kallikrein (Study BR-7353-002).

5.3.2. Secondary Pharmacology

Berotralstat at very high concentrations caused an increase in coagulation time in vitro. The concentration required to produce a doubling of the prothrombin time was about 80 μ M in monkey and pig blood, and greater than 100 μ M in blood from other species. The prothrombin time prolongation at the highest concentration tested (100 μ M) was 30%, 67%, 68%, 80%, 86%, 123%, and 144% in dog, rabbit, rat, human, mouse, pig, and monkey blood, respectively.

A 9-month oral toxicity study in monkeys showed that berotralstat at ≥ 55 mg/kg/day decreased the blood fibrinogen concentration by approximately 25% starting from week 4 (Study 806-162). No such finding was obtained for rats.

5.3.3. Safety Pharmacology

A full battery of in vitro and in vivo safety pharmacology studies of berotralstat was completed. The in vitro studies evaluated the effects of berotralstat on membrane channel conductance and action potential. In ion channel conductance assays, berotralstat inhibited the currents of human ether à-go-go-related gene potassium channels expressed in human embryonic kidney 293 cells, voltage-gated sodium channels (Nav1.5) in rabbit cardiac Purkinje fibers, and human voltage-gated calcium channels (hCav1.2) expressed in Chinese hamster ovary cells (IC₅₀ 0.29, 4.79, and 1.29µM, respectively; [Table 2](#)). Berotralstat also increased the field potential duration and decreased the sodium-spike amplitude in human cell-derived cardiomyocytes (Report 150127.DCS).

Table 2. In Vitro Safety Pharmacology Studies of Berotralstat

Ion Channels			Berotralstat (µM)			Report #
Ion	Name	Cells	Parameter	Tested	IC ₅₀	
Potassium	hERG	HEK293	K ⁺ current	0.03-30	0.29	806-125
Sodium	Nav1.5	Purkinje ¹ fibers	Na ⁺ current	0.9-94	↓APA	141030
Calcium	hCav1.2	CHO cells	Ca ⁺⁺ current	0.3-28	1.30	150123
Calcium	hCav1.5	CHO cells	Ca ⁺⁺ current	0.3-28	4.79	150123
Mixed	NA	Cardiac myocytes ²	Conductance	0.3-28	~1.0	150217

Source: Extracted from Pharmacology Tabulated Summary of the submission, Section 2.6.3, p5 – 6.

¹ Isolated rabbit cardiac Purkinje fibers

² Human stem cell-derived cardiomyocytes

Abbreviations: ↓, decrease; APA, action potential amplitude; CHO, Chinese hamster ovary; HEK, human embryonic kidney; hERG, human ether-à-go-go-related gene; IC₅₀, half-maximal inhibitory concentration; NA, not applicable

In vivo safety pharmacology studies were performed to evaluate the effect of berotralstat on the central nervous, CV, and respiratory systems (Studies 806-123, 806-124, and 806-135, respectively) ([Table 3](#)). No treatment-related effects were observed in the central nervous system and respiratory system. Berotralstat at ≥50 mg/kg caused a slight but nonsignificant increase in the QTc in conscious monkeys (Study 806-135) ([Figure 2](#)). Berotralstat at 100 mg/kg/day caused mild QT prolongation on day 3 in a 4-week toxicity study in monkeys (Study 806-132, discussed below).

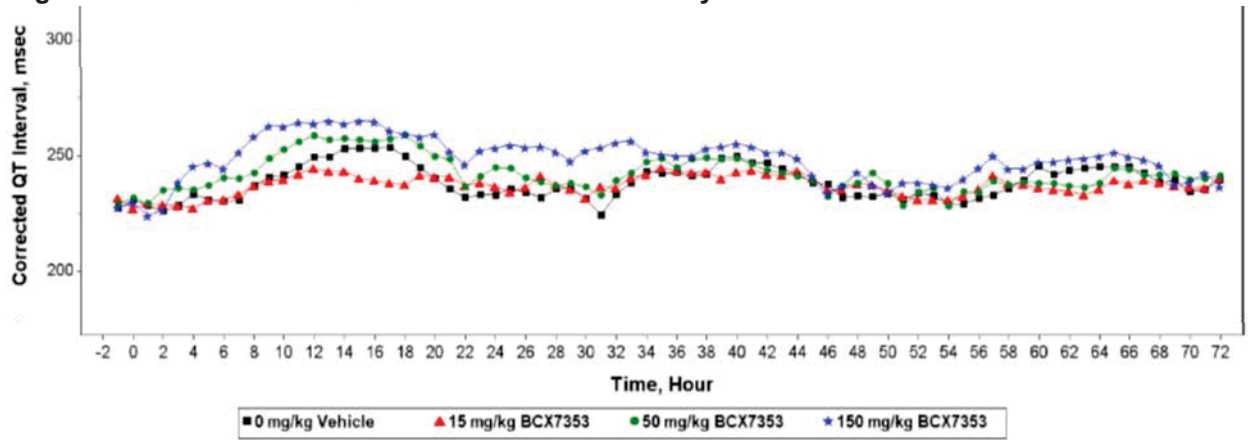
Table 3. In Vivo Safety Pharmacology Studies of Berotralstat

System	Species	ROA	Berotralstat (mg/kg)	N & Sex /Dose	Finding	GLP	Study #
CNS	Rat	PO	0, 25, 100, 450	10 M	None	Yes	806-123
Respiratory	Rat	PO	0, 25, 100, 450	8 M	None	Yes	806-124
CV	Monkey	PO	0, 15, 50, 150	4 F	None	Yes	806-135

Source: Extracted from Pharmacology Tabulated Summary of the submission, Section 2.6.3, p7.

Abbreviations: CNS, central nervous system; CV, cardiovascular; F, female; GLP, good laboratory practices; M, male; PO, oral gavage; ROA, route of administration

Figure 2. Mean Corrected QT Interval in Female Monkeys



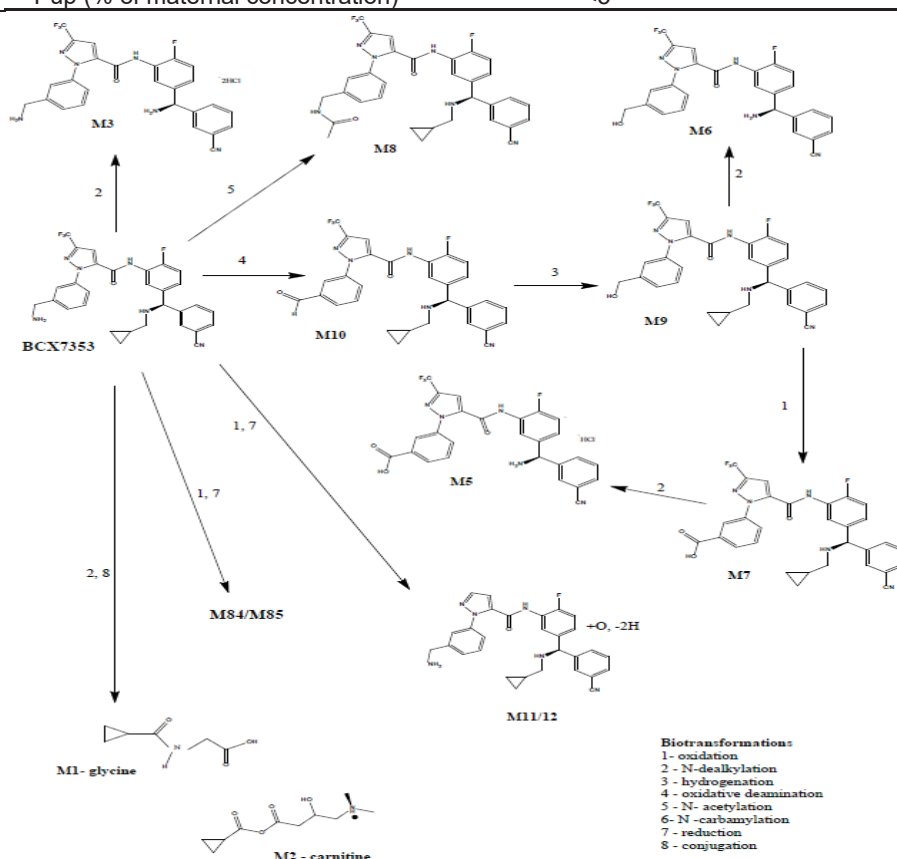
Source: Study Report 806-135, p68.

5.4. Absorption, Distribution, Metabolism, Excretion/Pharmacokinetics

Type of Study	Major Findings																																																																		
Absorption, distribution, and excretion See the table at right for a summary of key berotralstat PK parameters in animals and humans. Table constructed from multiple study reports.	Key Pharmacokinetic Parameters in Animals and Humans																																																																		
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Metabolism

See figure at right for the hypothesized metabolic pathways. Source: eCTD 2.4 Nonclinical Overview (p. 12).



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Metabolism/ excretion See the table for a summary of the excretion parameters of berotralstat metabolites in animals and humans. Table constructed from multiple study reports. Source: eCTD 2.4 Nonclinical Overview (p. 12).	Excretion of Berotralstat Metabolites in Animals and Humans <table border="1"> <thead> <tr> <th rowspan="2">Metabolite</th> <th colspan="3">Rat</th> <th colspan="3">Monkey</th> <th colspan="3">Human</th> </tr> <tr> <th>P</th> <th>U</th> <th>F</th> <th>P</th> <th>U</th> <th>F</th> <th>P</th> <th>U</th> <th>F</th> </tr> </thead> <tbody> <tr> <td>M1</td> <td>18.5</td> <td>34.0</td> <td></td> <td>11.5</td> <td>13.0</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>M2</td> <td>44.9</td> <td></td> <td></td> <td>41.7</td> <td>5.5</td> <td></td> <td colspan="3"><49</td> </tr> <tr> <td>M3</td> <td>13.5</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>M5</td> <td></td> <td></td> <td></td> <td>13.1</td> <td></td> <td>10.5</td> <td></td> <td></td> <td></td> </tr> <tr> <td>M6</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td colspan="3">7.8</td> </tr> <tr> <td>M7</td> <td></td> <td></td> <td>6.6</td> <td>13.6</td> <td></td> <td>11.5</td> <td>5.0</td> <td></td> <td>6.3</td> </tr> <tr> <td>M8</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>5.3</td> <td></td> <td></td> <td>9.4</td> </tr> <tr> <td>M9</td> <td>13.0</td> <td></td> <td></td> <td>7.3</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>M10</td> <td></td> <td></td> <td></td> <td colspan="3">34.6</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Berotralstat</td> <td>60.9</td> <td></td> <td>19.4</td> <td></td> <td></td> <td>16.5</td> <td>34.0</td> <td></td> <td>17.2</td> </tr> <tr> <td>M68</td> <td></td> <td></td> <td></td> <td colspan="3">6.3</td> <td></td> <td></td> <td></td> </tr> <tr> <td>M70</td> <td></td> <td></td> <td></td> <td colspan="3">5.2</td> <td></td> <td></td> <td></td> </tr> <tr> <td>M71</td> <td></td> <td></td> <td></td> <td colspan="3">7.6</td> <td></td> <td></td> <td></td> </tr> <tr> <td>M84</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td colspan="3">7.6</td> </tr> <tr> <td>M85</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td colspan="3">5.6</td> </tr> </tbody> </table>	Metabolite	Rat			Monkey			Human			P	U	F	P	U	F	P	U	F	M1	18.5	34.0		11.5	13.0					M2	44.9			41.7	5.5		<49			M3	13.5									M5				13.1		10.5				M6							7.8			M7			6.6	13.6		11.5	5.0		6.3	M8						5.3			9.4	M9	13.0			7.3						M10				34.6						Berotralstat	60.9		19.4			16.5	34.0		17.2	M68				6.3						M70				5.2						M71				7.6						M84							7.6			M85							5.6		
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39-Week oral (gavage) toxicity studies in monkeys (Studies 8001292 and 806-102)	Monkey T _{1/2} : Not available Accumulation: No Dose proportionality: Yes; see the table below. However, the FDA determined that the toxicokinetic data were unreliable. There were major GLP violations by the study laboratory, (b) (4). See the EIR review completed by Dr. Lynda Lanning on June 18, 2020 (DARRTS ID 4627740).																																																																																																																																																																									
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Toxicokinetic data from reproductive toxicology studies An EFD study (PO) in rats (Study 9000644)	Rat (day 17) AUC ₀₋₂₄ : 3500, 12,400, and 24,600 ng.h/mL at 10, 25, and 75 mg/kg/day levels. The respective plasma drug level in the LD, MD, and HD groups at 4 hours postdosing was 191, 653, and 1440 ng/mL in maternal blood and 7.22, 46.3, and 73.7 ng/mL in fetal blood. The fetal blood drug concentration was 3.8%, 7.1%, and 5.1% of the maternal concentration in the LD, MD, and HD groups, respectively.																																																																																																																																																																									

Type of Study	Major Findings
An EFD study (PO) in rabbits (Study 9000815)	<u>Rabbit (day 19)</u> AUC ₀₋₂₄ : 990, 2350, and 4410 ng.h/mL at the 20, 50, and 100 mg/kg/day levels. The respective plasma drug level in the LD, MD, and HD groups at 3 hours postdosing was 80.3, 266, and 267 ng/mL in maternal blood and 5.81, 30.4, and 40.7 ng/mL in fetal blood. The fetal blood drug concentration was 7%, 11%, and 11% of the maternal concentration in the LD, MD, and HD groups, respectively.
Toxicokinetic data from carcinogenicity studies A 104-week oral carcinogenicity study in rats (Study 806-161)	<u>Rat</u> AUC: The mean AUC between males and females in week 52 was 639, 2720, and 11,500 ng.h/mL in the 3, 8, and 20 mg/kg/day groups, respectively.

Abbreviations: AUC, area under the curve; DARRTS, Document Archiving, Reporting, and Regulatory Tracking System; eCTD, electronic Common Technical Document; EFD, embryo-fetal development; EIR, establishment inspection report; F, feces; GLP, good laboratory practices; HD, high dose; LD, low dose; MD, mid dose; P, plasma; PK, pharmacokinetics; PO, by mouth; U, urine

5.5. Toxicology

5.5.1. General Toxicology

Repeat-dose toxicity studies of berotralstat of durations of up to 6 months in rats and 9 months in monkeys have been completed to evaluate general toxicity. Studies of durations of up to 6 months and 13 weeks in rats and monkeys, respectively, were reviewed previously. See the nonclinical review by Dr. Luqi Pei on July 19, 2017, in IND 135058 (Document Archiving, Reporting, and Regulatory Tracking System [DARRTS] number 4126737).

This review evaluates two 9-month oral toxicity studies of berotralstat in monkeys (Studies 8001292 and 806-162). These studies were completed by two different laboratories at different times and had no overlap in berotralstat doses. Specifically, Studies 8001292 and 806-162 were completed in 2015 and 2016, respectively. The berotralstat doses were 0, 1.0, 2.5, 7.5 and 20 mg/kg/day in Study 8001292 and 0, 30, 55, and 80 mg/kg/day in Study 806-162. The goal of the second study was to identify the target organs of toxicity of berotralstat because the first study showed no treatment-related effects.

Study Title/Number: A 39-Week Study of BCX7353 by Oral Gavage in the Cynomolgus Monkey with a 13-Week Recovery Period (#8001292)

- Monkeys treated orally with up to 20 mg/kg/day berotralstat for 39 weeks did not reveal any significant toxicity.
- Cynomolgus monkeys (4/sex/dose) were dosed by oral gavage with 0, 1, 2.5, 7.5, and 20 mg/kg/day berotralstat for 39 weeks. No treatment-related toxicity was observed in the 1 to 7.5 mg/kg/day groups. Half of the monkeys in the 20 mg/kg/day group showed mild increases (less than three-fold) in the alanine aminotransferase (ALT) level starting from 6 months. One female in the 20 mg/kg/day group showed single-cell necrosis of hepatocytes in the liver, but without corresponding changes in the levels of liver

enzymes. The 20 mg/kg/day group also showed an around eight-fold increase in the urinary level of di-22:6-bis(monoacylglycerol) phosphate, a marker of phospholipidosis.

- The no observable adverse effect level (NOAEL) of berotralstat was 20 mg/kg/day, corresponding to a C_{max} of 196.5 ng/mL and an area under the curve from time zero to 24 hours (AUC_{0-24}) of 2875 hour·ng/mL on day 273.

Conducting laboratory and location: [REDACTED] (b) (4)

Good laboratory practices (GLP) compliance: Yes

Methods

Doses and frequency of dosing: 0, 1, 2.5, 7.5, and 20 mg/kg/day, once daily
Route of administration: Oral gavage
Formulation/vehicle: 0, 0.4, 1.0, 3.0, and 8.0 mg/mL berotralstat in pure water
Species/strain: Cynomolgus monkeys
Number/sex/group: 4/sex/group
Age: 1.5 to 5 years
Satellite groups/unique design: 2/sex/group
Deviation from study protocol affecting interpretation of results: No

Observations and Results: Changes from the Control

Parameter	Major Findings
Mortality	No treatment-related effects
Clinical signs	No treatment-related effects
Body weights	No treatment-related effects
Ophthalmoscopy	No treatment-related effects
ECG	No treatment-related effects
Hematology	No treatment-related effects
Clinical chemistry	Reversible, minimal to mild, increases in alanine aminotransferase activity in a few animals at 20 mg/kg/day (up to three-fold) at weeks 26 and 39
Urinalysis	The HD group showed increases (approximately eight-fold) in urinary BMP level as compared to the controls and to lower doses
Gross pathology	No treatment-related effects
Organ weights	No treatment-related effects
Histopathology	One HD female (#5502) showed mild single-cell necrosis of hepatocytes in the liver, but there were no correlating clinical chemistry findings
Adequate battery: Yes	

Abbreviations: BMP, di-22:6 bis(monoacylglycerol) phosphate; ECG, electrocardiogram; HD, high dose

Toxicokinetics

Dose-related increases in both AUC and C_{max} were observed. However, FDA has determined the data to be unreliable because of significant GLP violations by [REDACTED] (b) (4). For details, see the establishment inspection report completed by Dr. Lynda Lanning on June 18, 2020 (DARRTS 4627740).

Study Title/Number: A 9-Month Study of BCX7353 by Oral Gavage in the Cynomolgus Monkey with a 13-Week Recovery Period (#806-162)

- The target organs of toxicity of berotralstat in monkeys were the kidney, liver, intestine, lung, spleen, lymph nodes, and bone marrow.
- Cynomolgus monkeys were dosed by oral gavage with 0, 30, 55, and 80 mg/kg/day berotralstat for up to 273 days. The mid-dose (MD) and high-dose (HD) groups showed the following microscopic changes: degeneration (kidney, tubular), hyperplasia (kidney, bone marrow, and spleen), hypertrophy (liver, hepatocellular), vacuolation (adrenal, cortical), and foamy macrophages (lung, lymph node, and intestine). The foamy macrophages were caused by phospholipidosis. The low-dose (LD) group showed foamy macrophages in the intestine and lymph nodes. Although the kidney showed the most severe tissue damage (tubular degeneration and hyperplasia), the intestine was the most sensitive organ. The effects of berotralstat were partially reversible.
- The study did not establish a NOAEL for berotralstat.

Conducting laboratory and location: [REDACTED] (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 30, 55, and 80 mg/kg once daily
Route of administration:	Oral gavage
Formulation/vehicle:	0, 12, 22, and 32 mg/mL in purified water
Species/strain:	Cynomolgus monkeys
Number/sex/group:	4/sex/group
Age:	2.2 to 3.6 years
Satellite groups/unique design:	3/sex/group for 13-week interim analysis, 2/sex in control and HD groups for recovery
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: Changes from Control

Parameter	Major Findings
Mortality	No treatment-related effects
Clinical signs	The MD and HD groups showed increases in the incidence of emesis. Four HD monkeys (two of each sex; #242, 245, 262, and 263) had a thin appearance. Six HD females (#222, 245, 246, 248, 263, and 264) showed inappetence.
Bodyweight	Both males and females in the HD group showed dose-dependent decreases in bodyweight. The decrease was 22% and 9% in mean bodyweight in week 39 in HD males and females, respectively (see the graphs below). The HD males showed no increase in mean bodyweight during the study.
Ophthalmoscopy	No treatment-related effects
ECG	No treatment-related effects
Hematology	The MD and HD groups showed a decrease ($\leq 38\%$) in fibrinogen concentration starting from week 4. The HD females showed a decrease ($\leq 13\%$) in hemoglobin concentration starting from week 13.
Clinical chemistry	The MD and HD groups showed increases in AST and ALT levels (up to $4\times$ and $8.7\times$, respectively) starting from week 4. The MD and HD females showed a statistically significant decrease ($\downarrow 16\%$) in albumin level starting from week 13. The HD males showed a statistically significant decrease ($\downarrow 31\%$) in phosphorus level starting from week 13.
Urinalysis	No treatment-related effects
Gross pathology	The MD and HD groups showed discoloration in the kidney, liver, and lymph nodes. The respective total incidence of discoloration at termination sacrifice in the C, LD, MD, and HD groups ($n=8/\text{group}$, M and F combined) was 0, 0, 3, and 5 in the kidney; and 0, 0, 1, and 7 in the liver.
Organ weights	The MD and HD groups showed increases in organ weights of the liver, kidney, and spleen (82%, 67%, and 139%, respectively). These increases in absolute organ weights were replicated in those relative to bodyweight and brain weight.

Parameter	Major Findings
Histopathology Adequate battery: Yes	Treatment-related findings were observed in the adrenal glands, bone marrow, kidney, liver, lung, lymph nodes, intestine, and spleen. As expected, findings were more pronounced in the main study; less-marked effects were apparent in the interim and recovery periods. Histological findings can be grouped into the following: degeneration (kidney, tubular), hyperplasia (kidney, bone marrow and spleen), hypertrophy (liver), vacuolation (adrenal), and foamy macrophages (lung, lymph node, and intestine). Although the kidney showed the most severe tissue damage (tubular degeneration and hyperplasia), the intestine was the most sensitive organ. Only the intestine showed foamy macrophages in the LD group. The findings were not readily reversible. Most findings remained apparent after a recovery period of 13 weeks. The foamy macrophages were caused by phospholipidosis. See Section 16.3 for the incidence table.

Special evaluations
 The study measured BMP levels in urine. BMP is an exploratory biomarker of phospholipidosis. There were dose- and treatment duration-dependent increases in the urinary BMP level (see the table below). The respective increases in the urinary BMP level in the LD, MD, and HD groups at week 39 were 21-, 229-, and 271-fold in males and 29-, 91-, and 192-fold in females.

Urine BMP Level in the 9-Month Monkey Study (806-162)

Berotralstat (mg/kg/day)	Male				Female			
	Control ¹	↑ Over Control (x)			Control ¹	↑ Over Control (x)		
	0	30	55	80	0	30	55	80
Week 13	10.56	36*	107*	207*	17.59	35*	95*	131*
Week 39	8.81	21*	229*	271*	14.62	29*	91*	192*

¹ BMP concentration in ng/mg creatine

* p<0.1

Abbreviations: ↓, decrease; ↑, increase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMP, di-22:6 bis(monoacylglycerol) phosphate; C, control; ECG, electrocardiogram; F, female; HD, high dose; LD, low dose; M, male; MD, mid dose

Toxicokinetics

Dose-related increases in the C_{max} and AUC were observed in both sexes. However, the FDA has determined the data to be unreliable, as described [above](#).

5.5.2. Genetic Toxicology

5.5.2.1. in Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study Title/Number: BCX7353: Bacterial Reverse Mutation Assay (AE02WJ.502ICH.BTL)

Key study findings:

- Berotralstat tested negative in the Ames test.

GLP compliance: Yes

Test system: *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvrA*

Study is valid: Yes. The assay was performed using the plate incorporation method. Berotralstat concentrations of 5.0 to 1500 µg/plate were tested and considered appropriate. Precipitate and toxicity were observed with berotralstat at 1500 µg/plate. The criteria for a positive response

and valid assay were appropriate. The positive controls yielded a significant increase in the number of mutant colonies, as is typical of the assay.

5.5.2.2. In Vitro Assays in Mammalian Cells

Study Title/Number: BCX7353: In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes [AE02WJ.341ICH.BTL]

Key study findings:

- Berotralstat tested negative in the chromosomal aberration assay in vitro.

GLP compliance: Yes

Test system: Human peripheral blood lymphocytes

Study is valid: Yes. The selection of berotralstat concentrations was reasonable. The highest dose analyzed under each treatment condition produced a reduction in mitotic index (50±5%). The criteria for a positive response and valid assay were appropriate. The positive controls yielded significant increases in the rate of chromosomal aberrations, as is typical of the assay.

5.5.2.3. In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title/Number: BCX7353: In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats (AE02WJ.125M0121ICH.BTL)

Key study findings:

- Berotralstat tested negative in the in vivo micronucleus assay in rats.

GLP compliance: Yes

Test system: Sprague–Dawley rats

Study is valid: Yes. The dose selection of BCX7353 was appropriate (0, 125, 250, and 500 mg/kg single dose by oral gavage). The highest dose was half of the lethal dose in the dose-ranging study. The criteria for a positive response and valid assay were appropriate. Bone marrow was extracted and 4000 polychromatic erythrocytes per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of polychromatic erythrocytes and normochromatic erythrocytes in at least the first 500 total erythrocytes for each animal.

5.5.2.4. Other Genetic Toxicity Studies

Three bacterial reverse mutation assays (Studies AE54DM.502ICH.BTL, AE54DL.502ICH.BTL, and AE54DK.502ICH.BTL) were completed to evaluate the mutagenicity of three BCX7353 impurities: (b) (4). Each assay employed the plate incorporation method and used five tester strains: *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 *uvrA*. The results were negative for all assays and tester strains, except for a positive result for (b) (4) in TA98.

Two tests (initial and repeat) were performed to evaluate the effect of (b) (4) on mutant colonies of test strain TA98. The (b) (4) concentrations ranged from 15 to 5000 µg/plate in both tests. Precipitate was observed at 5000 µg/plate. The 5000 µg/plate concentration in both tests led to an at least two-fold increase in revertant count in the presence of S9 activation (Table 4). The increase was also outside of the 95% vehicle control limit. (b) (4) was considered to produce a positive result in the bacterial gene mutation assay under the test conditions. The (b) (4) level in the drug substance and product will be controlled per the International Council for Harmonisation (ICH)-M7 guidance.

Table 4. Effect of (b) (4) on Colony Counts in TA98 in the Ames Test (With S9 Fraction)

(b) (4) (µg/mL)	Revertant Colony Count (Mean ± SD)								(b) (4) (1 µg)
	0	15	50	150	500	1500	3000	5000	
Test 1	20±9	24±5	28±6	30±6	36±5	31±5	-	42±7	234±11
Test 2	27±3	29±7	26±10	29±5	31±5	36±5	39±5	55±2	271±23

Source: Extractaed from Toxicology Tabulated Sumamry of the submission, Section 2.6.7, p81.
 Abbreviations: (b) (4) berotralstat impurity; SD, standard deviation

5.5.3. Carcinogenicity

The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in rats and in a 26-week study in transgenic mice (reports #806-161 and #20088905, respectively). Wistar rats (60/sex/dose) were dosed by oral gavage with 0, 3, 8 and 20 mg/kg berotralstat once daily for 104 weeks. Mice (25/sex/dose) were dosed by oral gavage with 0, 8, 20, and 50 mg/kg/day berotralstat for 26 weeks. No evidence of tumorigenicity was observed in rats or mice.

The Executive Carcinogenicity Assessment Committee (ECAC) of the Agency evaluated reports of these studies on March 10 and July 10, 2020 (final reports in the original submission and addendum to the rat report submitted by the Applicant in SDN 029, respectively). The Committee concluded that 1) both studies were adequate, and 2) no evidence of carcinogenicity was observed in either species. See the ECAC meeting minutes and the addendum thereto completed by Dr. Robeena Ariz on March 16 and July 23, 2020, and the nonclinical review and an addendum thereto completed by Dr. Luqi Pei on March 24 and July 23, 2020, respectively (DARRTS IDs 4576054, 4580256, 4645569, and 4645674, respectively).

The ECAC twice evaluated the carcinogenicity data of the 2-year rat study. The first evaluation was performed on March 10, 2020. The ECAC concluded that: 1) berotralstat at 20 mg/kg/day for 2 years increased the incidence of hemangiosarcoma in the mesenteric lymph nodes in male rats, and 2) the relevance of the tumor finding in rats to humans is unknown.

Th ECAC's conclusion about the carcinogenicity of berotralstat in rats differed from that of the Applicant. The Applicant argued that hemangiosarcoma associated with berotralstat treatment was a species-specific effect. To support their argument, the Applicant assembled a pathology expert working group (EWG) to re-evaluate the microscopic changes in the mesenteric lymph nodes in male and female rats. By consensus, the EWG revised the tumor type and incidence. The Applicant submitted an amendment to the final report on July 9, 2020 (SDN 029), which outlined the EWG's conclusion. The Applicant stated that new data showed that berotralstat

showed no evidence of tumorigenicity in rats. The relevant data from the original and amended reports are presented in [Table 5](#).

Table 5. Tumor Findings in the Mesenteric Lymph Node in Males: Study 806-161

eCTD	Sub Date	Report	Tumor	Incidence (n=60)				P-Value ¹ (C vs HD)
				0 mkd	3 mkd	8 mkd	20 mkd	
0001	12/3/19	Original	Hemangiosarcoma	1	2	2	9	0.005
0029	07/9/20	Revised	Hemangioma	1	0	0	5	0.081

Source: Addendum to Pharmacology/Toxicology Review (DARRTS ID 4580256) completed by Dr. Luqi Pei on July 23, 2020 (DARRTS ID 4645674).

¹ Pairwise comparison

Abbreviations: C, control; eCTD, electronic Common Technical Document; HD, high dose; mkd, mg/kg/day; sub, submission

The ECAC evaluated the amended report on July 10, 2020 (discussion via email). The Committee agreed with the Applicant that there was no evidence of tumorigenicity associated with berotralstat in male rats.

5.5.4. Reproductive and Developmental Toxicology

5.5.4.1. Fertility and Early Embryonic Development

Study Title/Number: BCX7353: A Study of Fertility and Early Embryonic Development to Implantation in Rats (806-156)

Key study findings:

- Berotralstat did not affect fertility in male or female rats under the conditions tested.
- Male and female rats were dosed by oral gavage with 0, 10, 25, and 45 mg/kg/day berotralstat. The berotralstat treatment started before, and continued through and after mating: males—from 28 days before, during, and after mating for a total of 63 days; females—from 14 days before mating to gestational day (GD) 7.
- Males were evaluated for fertility and sperm quality parameters (at the end of treatment). Females were evaluated for fertility and uterine content on GD 13. No treatment-related effects were observed on any of the parameters evaluated.

Conducting laboratory and location: [REDACTED] (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 10, 25, and 45 mg/kg/day
Males: from 28 days prior to mating to euthanasia (treatment day 63)
Females: 14 days prior to mating until GD 7

Route of administration: Oral gavage

Formulation/vehicle: Deionized water

Species/strain: Sprague–Dawley rats

Number/sex/group: 25

Satellite groups: 4

Study design: Males and females were treated from prior to, during, and after mating. Treatment continued after mating until 63 days in males and until GD 7 in females. Males were sacrificed at the end of the treatment period for sperm analysis. Females were sacrificed on GD 13 for examination of uterine content.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameter	Major Findings
Mortality	There were no deaths in the study.
Clinical signs	The HD group showed audible breathing in both sexes (incidence, three and six male and female rats, respectively).
Body weight	The HD males showed decreases of 5% in bodyweight and 12% in bodyweight gain in comparison to the control. This was associated with a decrease in food consumption. The females showed no treatment-related effects.
Necropsy findings	<u>Mating/fertility index</u> : No treatment-related effects were observed in either sex. Mating index was 100% in both sexes in all groups. Both fertility and fecundity indices ranged between 96% to 100% in both sexes. There were no apparent differences in menstrual cycles and duration in females. There were 24 pregnant rats in each dose group. <u>Cesarean section data</u> : No treatment-related effects were observed. The mean number of corpora lutea was 15.7 to 16.8. The mean number of implantation sites was 14.2 to 14.9. The mean number of viable embryos was 13.2 to 14.1. The mean preimplantation loss was 6.2% to 11.2%. The mean number of postimplantation sites was 4.1% to 9.4%.

Abbreviation: HD, high dose

Toxicokinetics

Blood levels of berotralstat were measured at 4 hours postdosing on treatment day 1 in both sexes, day 63 in males, and GD 7 in females in toxicokinetics animals (1.0 mL blood/time point, n=4) (Table 6). The plasma berotralstat level increased in a dose-related fashion. A significant increase (approximately 2.5-fold) in the blood level between the first and last days of treatment was observed in both sexes. There was no sex difference in the plasma berotralstat level.

Table 6. Maternal and Fetal Exposures in a Fertility Study in Rats

Berotralstat (mg/kg/day, PO)	Plasma Berotralstat Concentration (ng/mL) at 4 Hours Postdosing			
	Male		Female	
	Day 1	Day 63	Day 1	GD 7
10	89	182	96	181
25	194	289	284	299
45	302	835	338	795

Source: Report 806-156, Table H, p25.

Abbreviations: GD, gestational day; PO, by mouth

5.5.4.2. Embryo-Fetal Development

Study Title/Number: An EFD Study of BCX7353 by Oral Gavage in Rat (9000644)

Key study findings:

- Berotralstat was nonteratogenic in rats under the conditions tested.
- Pregnant rats were dosed by oral gavage with 0, 10, 25, and 75 mg/kg/day berotralstat during the organogenesis period (GD 6 to 17). They were sacrificed on GD 21 for examination of uterine content.
- The HD group showed marked and statistically significant decreases ($\downarrow$ 34%) in mean bodyweight gain. The decrease was especially apparent in the beginning (91% in GD 6 to 9). No treatment-related effects were observed in any of the embryofetal developmental parameters evaluated.

Conducting laboratory and location:

(b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 10, 25, and 75 mg/kg/day from GD 6 to GD 17
Route of administration:	Oral gavage
Formulation/vehicle:	Ultrapure water
Species/strain:	Sprague–Dawley rats
Number/sex/group:	25 pregnant females
Satellite groups:	8 for TK analysis
Study design:	Pregnant rats were dosed from GD 6 to 17. Cesarean sections were performed on GD 21. The report defines GD as postcoitus.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameter	Major Findings																																							
Mortality	There were no deaths in the study.																																							
Clinical signs	The HD group showed increases in the incidence of salivation, wet fur on the lower jaw, and occasional abnormal breathing sounds. The abnormal breathing sounds were noted before dosing and up to 21 min postdose for four main-study animals (Nos. 4502, 4505, 4519, and 4520) at 75 mg/kg/day during GD 10 to 17.																																							
Body weight	Bodyweight was recorded every 3 days starting from GD 0. The HD group showed significant decreases in bodyweight gains (table below) and bodyweight. The magnitude of the decrease in bodyweight gain was most remarkable at the beginning of treatment and diminished thereafter (e.g., ↓93% and 11% at GD 6 to 9 and 15 to 18, respectively). The overall bodyweight gain for the entire treatment period (GD 6 to 18) was decreased 34%. The MD group showed a transient decrease in bodyweight gain. These findings were associated with decreases in food consumption in MD and HD animals. Body Weight Gain in an EFD Study in Rats <table border="1"> <thead> <tr> <th rowspan="2">Berotralstat (mg/kg/day)</th> <th colspan="4">Body Weight Gain</th> </tr> <tr> <th>Control</th> <th colspan="3">Change from Control (%)</th> </tr> <tr> <th></th> <th>0</th> <th>10</th> <th>25</th> <th>75</th> </tr> </thead> <tbody> <tr> <td>GD 6 to 9</td> <td>15 g</td> <td>-</td> <td>-40</td> <td>-93</td> </tr> <tr> <td>GD 9 to 12</td> <td>20 g</td> <td>-</td> <td>-</td> <td>-40</td> </tr> <tr> <td>GD 12 to 15</td> <td>19 g</td> <td>-</td> <td>-</td> <td>-32</td> </tr> <tr> <td>GD 15 to 18</td> <td>45 g</td> <td>-</td> <td>-</td> <td>-11</td> </tr> <tr> <td>GD 18 to 21</td> <td>55 g</td> <td>-</td> <td>-</td> <td>+11</td> </tr> </tbody> </table> -, not different from the control group	Berotralstat (mg/kg/day)	Body Weight Gain				Control	Change from Control (%)				0	10	25	75	GD 6 to 9	15 g	-	-40	-93	GD 9 to 12	20 g	-	-	-40	GD 12 to 15	19 g	-	-	-32	GD 15 to 18	45 g	-	-	-11	GD 18 to 21	55 g	-	-	+11
Berotralstat (mg/kg/day)	Body Weight Gain																																							
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GD 15 to 18	45 g	-	-	-11																																				
GD 18 to 21	55 g	-	-	+11																																				
Necropsy findings Cesarean section data	The number of litters examined was 21, 21, 22, and 23 in the C, LD, MD, and HD groups, respectively. Dams were examined for the following parameters: numbers of corpora lutea, implantation sites, resorptions, and pre-and post-implantation loss. Fetuses were examined for sex ratios, live and dead fetuses, and variations and malformations of external, visceral, and skeletal examinations. <u>Cesarean section data</u>: No treatment-related effects were observed. The mean number of corpora lutea was 11.5 to 16.3. The mean number of implantation sites was 13.7 to 14.4. There were dose-related, but not statistically significant, increases in mean implantation loss (7.8%, 10.5%, 11.3%, and 15.1% in the C, LD, MD, and HD groups, respectively).																																							
Necropsy findings Offspring	No treatment-related effects were observed. There were no differences in sex ratios, live and dead fetuses, and variations and malformations of external, visceral, and skeletal examinations between the control and treatment groups.																																							

Abbreviations: ↓, decrease; C, control; EFD, embryofetal development GD, gestational day; HD, high dose; LD, low dose; MD, mid dose

Toxicokinetics

Blood levels of berotralstat were measured on the first and last days of treatment (GD 6 and 17) in TK animals. Maternal blood samples were drawn at 0, 1, 2, 4, 8 and 24 hours postdosing on each day (0.5 mL blood/time point, n=4). Fetal blood drug concentrations were determined on GD 17 in TK animals. Fetal blood was drawn when fetuses were still in uterus and dams were under anesthesia at 4 and 24 hours postdosing. The TK results are summarized in [Table 7](#). The plasma berotralstat level increased in a dose-related fashion in maternal and fetal blood.

Significant increases (two- to three-fold) in the berotralstat level were observed in the maternal blood on GD 17 compared to GD 6.

Table 7. Maternal and Fetal Exposures in an EFD Study in Rats

Berotralstat (mg/kg/day)	Maternal AUC ₀₋₂₄				Maternal C _{max}		Fetal Drug Level	
	(ng.h/mL)		(ng.h/mL)/(mg/kg)		(ng/mL)		(ng/mL, GD 17)	
	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17	Hour 4	Hour 24
10	1370	3500	137	351	83.8	191	7.22	7.44
25	3200	12,400	128	496	196	653	46.3	29.1
75	6800	24,600	90.1	328	565	1440	73.7	61.0

Source: Extracted from Toxicology Tabulated Summary, Section 2.6.7, subsection 13.1, Report 900064, p111.

Abbreviations: AUC₀₋₂₄, area under the curve from time zero to 24 hours; C_{max}, maximum concentration; EFD, embryo-fetal development; GD, gestational day

Study Title/Number: An EFD Study of BCX7353 by Oral Gavage in Rabbit (9000815)

Key study findings:

- Berotralstat was nonteratogenic in New Zealand white rabbits under the conditions tested.
- Pregnant rabbits were dosed by oral gavage with 0, 20, 50, and 100 mg/kg/day berotralstat during the organogenesis period (GD 7 to 19). They were sacrificed on GD 29 for examination of uterine content.
- The HD group showed statistically significant decreases in bodyweight gain and food consumption (p<0.05). No treatment-related effects were observed in any of the fetal developmental parameters evaluated. The HD group showed abortions and early terminations (two rabbits each). These findings were not considered significant because they were associated with significant maternal toxicity.
- Berotralstat can cross the placenta. The fetal blood berotralstat concentration may be up to 11% of that in maternal blood.

Conducting laboratory and location: (b) (4)

GLP compliance: yes

Methods

Dose and frequency of dosing: 0, 20, 50, and 100 mg/kg/day
 Route of administration: Oral gavage
 Formulation/vehicle: Ultrapure water
 Species/strain: New Zealand white rabbits
 Number/sex/group: 22 pregnant females
 Satellite groups: 2 to 3 for TK analysis
 Study design: Pregnant rabbits were dosed from gestation days (GD) 7 to 19. Cesarean sections were performed on GD 24. The report uses postcoitus for GD.
 Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameter	Major Findings
Mortality	Four (4) HD rabbits (#4505, #4518, #4519, and #4521) were euthanized during GD 22 to 24 for humane reasons. Rabbits #4505 and #4518 were sacrificed due to significant decreases in bodyweight. Rabbits #4519 and #4521 were sacrificed after they had undergone abortion.
Clinical signs	Prior to sacrifice, the preterminally euthanized rabbits showed decreases in fecal output, dehydration, bodyweight, food consumption, and/or abortion.
Body weight	Bodyweight was recorded every 3 days starting from GD 7. The MD and HD groups showed 50% and 100% decreases in bodyweight gain (vs 0.2 kg gain in the C group) during GD 7 to 20. Only the decrease in the HD group was statistically significant (p<0.01). There was also a decrease in food consumption at all dose levels (9% to 32%).
Necropsy findings Cesarean section data	Rabbits in the main study were sacrificed on GD 29. Dams were examined for the following parameters: numbers of corpora lutea, implantation sites, resorptions, and pre- and post-implantation loss. Fetuses were examined for sex ratios, live and dead fetuses, and variations and malformations of external, visceral, and skeletal examinations. <u>Cesarean section data:</u> No treatment-related effects were observed. There were no apparent differences in the mean of number of corpora lutea (9.5 to 10.3), implantation sites (8.7 to 9.5), and implantation losses (4.39 for the LD group, and 8.48 to 8.72 for the other groups).
Necropsy findings Offspring	No treatment-related effects were observed. There were no differences in sex ratios, live and dead fetuses, and variations and malformations of external, visceral, and skeletal examinations between the control and treatment groups.

Abbreviations: C, control; GD, gestational day; HD, high dose; LD, low dose; MD, mid dose

Toxicokinetics

Blood levels of berotralstat were measured on the first and last days of treatment (GD 7 and 19) in TK animals. Maternal blood samples were drawn at 0, 1, 2, 4, 8 and 24 hours postdosing on each day (0.5 mL blood/time point, n=3). Fetal blood drug concentrations were determined at 3 hours postdosing on GD 19 in TK animals. Fetal blood was drawn when fetuses were in the uterus and dams were under anesthesia. The TK results are summarized in [Table 8](#). The plasma berotralstat level increased in a dose-related fashion in maternal and fetal blood. There was no significant bioaccumulation of berotralstat in pregnant rabbits.

Table 8. Maternal and Fetal Exposures in the EFD Study in Rabbits

Berotralstat (mg/kg/day)	AUC ₀₋₂₄ (ng.h/kg)		Concentration (ng/mL)			Fetal/Dam (%)
	Maternal		Maternal	Fetal	Fetal/Dam (%)	
	GD 7	GD 19	GD 7	GD 19		
20	626	880	64.0	83.3	5.81	7%
50	2040	2350	312	266	30.4	11%
100	3020	4410	532	267	40.7	11%

Source: Extracted from Tables 11, 12, and Section 9.6 in report 9000815, p37-38.

Abbreviations: AUC₀₋₂₄, area under the concentration-time curve from time 0 to 24 hours; EFD, embryo-fetal development; GD, gestational day

Berotralstat can cross the placenta in rabbits. The fetal blood berotralstat level was determined at 3 hours postdosing on day 19. The mean berotralstat level in fetal blood was approximately 11% that in maternal blood.

5.5.4.3. Prenatal and Postnatal Development

Study Title/Number: Toxicity Study to Evaluate the Effect of BCX7353 on Peri- and Post-Natal Development, Including Maternal Function in Rats (806-157)

The study design is outlined in [Table 9](#). The key study findings are as follows:

- The PPND study in rats showed that berotralstat had no effect on PPND parameters under the test conditions.
- Dams (F0) were dosed by oral gavage with 0, 10, 25, and 45 mg/kg/day berotralstat during pregnancy and lactation (i.e., from GD 6 to lactation day [LAD] 20). Dams gave birth to and nurtured pups (F1) naturally. The F1 generation grew up and mated at the age of 10 weeks. They were assessed for growth and behavioral and developmental parameters (e.g., motor activity, learning, and memory) while growing. Pregnant F1 rats were sacrificed on GD 13 for examination of uterine contents.
- Both dams and pups (F0 and F1) in the HD groups showed statistically significant decreases (up to 7.8%, $p < 0.01$) in bodyweight during the second and third weeks of the lactation period. The dams in this period also showed statistically significant decreases in food consumption (up to 10%). No treatment-related effects were observed on any of the other parameters evaluated.
- The NOAEL was 25 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: yes

Methods

Dose and frequency of dosing:	0, 10, 25, and 45 mg/kg/day (GD 6 to LAD 20)
Route of administration:	Oral gavage
Formulation/vehicle:	Deionized water
Species/strain:	Sprague–Dawley rats
Number/sex/group:	25 females in F0, and 8 and 1 for growth/development and reproduction performance, respectively, in F1.
Satellite groups:	4 in F0
Study design:	Effects of berotralstat on PPND and parturition were evaluated by dosing dams during the pregnancy (GD) and lactation (LAD) periods in rats. Dams (F0, 25/dose) were dosed by oral gavage with 0, 10, 25, and 45 mg/kg/day berotralstat from GD 6 to LAD 20. The dams delivered their pups (F1) naturally. The F1 pups grew up naturally and were culled twice: to 4 and 1 to

2/sex/litter on LAD 4 and postnatal day (PND) 28, respectively. The F1 males and females (25/sex/dose) mated at the age of 10 to 11 weeks. Pregnant F1 rats were euthanized on GD 13 for examination of uterine contents (F2).

Deviation from study protocol affecting interpretation of results: No

Table 9. Design of the PPND Toxicity Study of Berotralstat in Rats

Rats	Berotralstat	Key Observations	Times of Measurements
F ₀	0, 10, 25, or 45 mg/kg/day from GD 6 to LAD 20	Maternal general health	GD 6 to LAD 20
		Litter size and fetal parameters	Time of delivery
		Blood drug level (TK rats only)	GD 6, LAD 14
		Termination	LAD 21
F ₁	Exposures via placenta transfer and milk	Survival	PND 0 to 28
		Blood drug level	LAD 14
		General growth and development	PND 0 to 28
		Neurobehavioral assessment	LAD 21, PND 70 to 85
		Sexual maturation	PND 35 to 46
		Fertility and reproductive performance	PND 73 to 84
		Culling	LAD 4 (to 4/sex/litter) LAD 28 (to 1 to 2/sex/litter)
F ₂	None	Early embryofetal development	GD 13

Source: Constructed by the reviewer based on information in the study report.

Abbreviations: GD, gestational day; LAD, lactation day; PND, postnatal day; PPND, pre- and post-natal development; TK, toxicokinetics

The F0 rats were evaluated for parturition and litter parameters. The F1 rats were evaluated for growth, development, and reproductive/fertility performance. The growth and development evaluations included general growth, sexual maturation, and neurobehavioral parameters (n=8/litter/sex/dose).

Observations and Results

Generation	Major Findings																																																																				
F0 Dams	Clinical signs: The MD and HD groups showed audible breathing in both the gestation and lactation periods. The respective incidence of audible breathing in the C, LD, MD and HD groups was 0, 0, 5, and 10 during the gestation period; and 0, 1, 4, 5 during the lactation period. Body weight: The HD group showed statistically significant decreases ($p < 0.05$) in bodyweight and bodyweight gain in the LAD period (~5% and 11 to 20%, respectively). Food consumption: The HD group showed statistically significant decreases ($p < 0.05$) in bodyweight and body weight gain in the LAD period (~10% in each period). Pregnancy and delivering: No treatment-related effects were observed; 24 to 25 dams (out of 25) in each group delivered pups naturally. The mean length of the gestation period was ~21.7 days in all groups. The mean litter size was 11.7 to 12.7 pups. No significant finding in fetal deaths, malformations, or variations was observed.																																																																				
F1 Generation	Body weight: The pups from the HD group showed statistically significant decreases ($p < 0.01$) in bodyweight during the second and third weeks postnatally (up to 7.8% in males and females). Mean Body Weight of the F1 Generation <table border="1"> <thead> <tr> <th rowspan="3">Postnatal Age (Days)</th> <th colspan="4">Male</th> <th colspan="4">Female</th> </tr> <tr> <th rowspan="2">Control (g)</th> <th colspan="3">Change from Control (%)</th> <th rowspan="2">Control (g)</th> <th colspan="3">Change from Control (%)</th> </tr> <tr> <th>LD</th> <th>MD</th> <th>HD</th> <th>LD</th> <th>MD</th> <th>HD</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>7.18</td> <td>-2.2</td> <td>-2.9</td> <td>-1.7</td> <td>6.82</td> <td>-1.5</td> <td>-2.6</td> <td>-2.1</td> </tr> <tr> <td>7</td> <td>19.5</td> <td>0.0</td> <td>-1.0</td> <td>-3.1</td> <td>18.9</td> <td>0.5</td> <td>-1.1</td> <td>-3.7</td> </tr> <tr> <td>14</td> <td>39.1</td> <td>-1.5</td> <td>-3.3</td> <td>-6.9**</td> <td>38.0</td> <td>-1.8</td> <td>-3.4</td> <td>-7.6**</td> </tr> <tr> <td>21</td> <td>64.4</td> <td>-1.9</td> <td>-4.7</td> <td>-7.8**</td> <td>61.3</td> <td>-1.6</td> <td>-3.3</td> <td>-7.0**</td> </tr> <tr> <td>28</td> <td>106.4</td> <td>-0.1</td> <td>2.7</td> <td>-4.7*</td> <td>96.3</td> <td>1.0</td> <td>-0.2</td> <td>-4.2</td> </tr> </tbody> </table> *, $p < 0.05$; **, $p < 0.01$ Postnatal development: No treatment-related effects were observed. The following parameters for postnatal developmental assessments were assessed (time of the assessment in parentheses): pinna detachment (LAD 2), cliff aversion (LAD 11), eye opening (LAD 13), vaginal opening (PND 28), preputial separation (PND 35), motor activity (PND 35), and neurological/behavioral evaluations. The neurological and behavioral evaluations were: static right reflex (LAD 2), Erwin's functional observation battery (LAD 21), auditory response (PND 22), and passive avoidance test (PND 70 to 85). Reproductive and fertility assessment: No treatment-related effects were observed. (PND 70 to 85). Twenty-three to twenty-five rats/sex/dose were confirmed to mate. No differences were noted in estrous cycles in females or the following parameters in either sex: mating index, fertility index, or fecundity index (92% to 100% each). There were five nonpregnant females (incidence: two, zero, two, and one in the C, LD, MD, and HD groups, respectively). Cesarean section: No treatment-related effects were observed. Twenty-one to twenty-two rats/dose had viable embryos. No differences were noted in the following parameters: abortion, resorption, corpora lutea, implantation sites, pre- and post-implantation losses, and litter size.	Postnatal Age (Days)	Male				Female				Control (g)	Change from Control (%)			Control (g)	Change from Control (%)			LD	MD	HD	LD	MD	HD	0	7.18	-2.2	-2.9	-1.7	6.82	-1.5	-2.6	-2.1	7	19.5	0.0	-1.0	-3.1	18.9	0.5	-1.1	-3.7	14	39.1	-1.5	-3.3	-6.9**	38.0	-1.8	-3.4	-7.6**	21	64.4	-1.9	-4.7	-7.8**	61.3	-1.6	-3.3	-7.0**	28	106.4	-0.1	2.7	-4.7*	96.3	1.0	-0.2	-4.2
Postnatal Age (Days)	Male				Female																																																																
	Control (g)		Change from Control (%)			Control (g)	Change from Control (%)																																																														
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F2 Generation	Not evaluated																																																																				

Abbreviations: C, control; GD, gestational day; HD, high dose; LAD, lactation day; LD, low dose; MD, mid dose; PND, postnatal day

Toxicokinetics

The blood of dams and fetuses showed a dose-dependent increase in plasma berotralstat concentration. The fetal blood drug concentration, however, was a fraction (~2.1%) of that in maternal blood ([Table 10](#)).

Table 10. Mean Plasma Berotralstat Concentration in Dams and Pups in Rats

Day	Time (Hours Postdose)	Mean Plasma Berotralstat Concentration (ng/mL)							
		Dam (F0)			Pup (F1)			F1/F0 (%)	
		LD	MD	HD	LD	MD	HD	MD	HD
GD 6	4	46.2	183.3	312.8	-	-	-	-	-
	0 ¹	-	-	-	1.11	5.83	29.5	-	-
LAD 14	2	-	-	-	-	5.04	11.2	-	-
	4	63.2	360.3	688.3	-	6.64	14.6	1.84	2.12
	24	-	-	-	-	3.62	13.7	-	-

Source: Extracted from Section 2.6.7 Toxicology Tabulated Summary, subsection 14.1, Study 806-157, p115-116

¹ Predose

Abbreviations: -, not available; GD, gestational day; HD, high dose; LAD, lactation day; LD, low dose; MD, mid dose

5.5.5. Other Toxicology Studies

Neutral Red Uptake Phototoxicity Assay of BCX7353 in BALB/c 3T3 Mouse Fibroblasts (Study No. 20088288)

Key study findings:

- Berotralstat had no phototoxic potential in vitro.

Conducting Laboratory: (b) (4)

GLP: Yes

This test was performed because berotralstat absorbs light at wavelengths of 290 to 700 nm. Phototoxicity potential was measured by assaying the relative reduction in viability of BALB/c 3T3 mouse fibroblasts treated with berotralstat in the presence and absence of ultraviolet radiation (+UVR and -UVR, respectively). The berotralstat concentration ranged from 1.0 to 56.2 µg/mL. The results showed that berotralstat had no phototoxic potential in vitro.

6. Clinical Pharmacology

6.1. Executive Summary

This is an original (505(b)(1)) NDA submitted by the applicant, BioCryst Pharmaceuticals (BioCryst), on December 3, 2019, seeking marketing approval for ORLADEYO (berotralstat), an orally administered plasma kallikrein inhibitor indicated for prophylaxis to prevent attacks of HAE in patients 12 years of age and older. The proposed dosage form is the active pharmaceutical ingredient (API) (b) (4) in a hard gelatin capsule with a product strength of 150 mg berotralstat

(also referred to as BCX7353) per capsule. The proposed dose is 150 mg³ orally once daily (QD) without regards to food. HAE is a serious, potentially life-threatening, genetically determined disease characterized clinically by recurrent episodes of angioedema of the skin, pharynx, larynx, GI tract, genitals, and extremities. Because there are no oral medications recommended as first-line prophylactic treatment of HAE, berotralstat was granted orphan drug designation on November 1, 2017 and fast track designation on August 2, 2018.

The NDA for berotralstat is supported by studies conducted by BioCryst (IND 135058) and includes 11 phase-1 clinical pharmacology studies in healthy subjects and subjects with renal and hepatic impairment, 2 phase-2 studies, and 1 phase-3 study in subjects with HAE. The program was also supported by the results of several in vitro human biomaterial studies. The major findings for this application are summarized in Section [6.2](#).

The Office of Clinical Pharmacology/Division of Inflammation and Immune Pharmacology and Pharmacometrics have reviewed the clinical pharmacology data submitted in support of NDA 214094 for berotralstat and found them to be acceptable to support approval from a clinical pharmacology perspective.

6.2. Comprehensive Clinical Pharmacology Review

6.2.1. General Pharmacology and Pharmacokinetic Characteristics

Fourteen clinical studies provide data on the clinical pharmacology and pharmacodynamics (PD) of berotralstat in this NDA submission ([Table 11](#)).

Table 11. Clinical Pharmacology Studies Conducted for Berotralstat

Phase	Study #	Description (Dose Studied)
Phase 1	101	SAD and MAD in healthy subjects
	102	DDI cocktail study (300 mg)
	103	BA/food effect study for formulation bridge (300 mg)
	104	Mass-balance (300 mg)
	105	Transporter DDI study (300 mg)
	106	Thorough QTc study (150 and 450 mg)
	107	Renal impairment study (200 mg)
	108	Hepatic impairment study (150 mg)
	112	DDI study (150 mg)
	113	Formulation bridging (110 and 150 mg)
	115	DDI cocktail study (150 mg)
Phase 2	203	Dose-ranging study
	204	Efficacy and safety study (110 and 150 mg)
Phase 3	302	Efficacy and safety study (110 and 150 mg)

Source: Reviewer's summary of information submitted in Module 2.7.2.

Abbreviations: BA, bioavailability; DDI, drug–drug interaction; MAD, multiple ascending doses; QTc, corrected QT; SAD, single ascending dose

The clinical pharmacology findings for berotralstat are summarized in [Table 12](#).

³ Rounded from 153 mg berotralstat and equivalent to 175 mg berotralstat hydrochloride.

Table 12. Clinical Pharmacology Summary

Type of Parameter	Description
General Information	
Berotralstat exposure	GM C _{max} (ng/mL) and %CV
	GM AUC (ng.h/mL) and %CV
	Steady state, 110 mg, Study 101
	97.8 (35%)
	1600 (41%)
	Steady state, 150 mg, Study 106
	158 (24%)
	2770 (24%)
Dose proportionality	The AUC _{tau} , C _{max} , and C _{tau} of berotralstat showed a greater than dose-proportional increase in exposure over the 55 to 300 mg (65 to 350 mg [SN]) daily dose range in subjects with HAE. The exposure increase between 110 and 150 mg doses is around two-fold after multiple doses.
Accumulation	Berotralstat accumulates about five-fold when dosed to steady-state. Berotralstat reaches steady-state within 8 to 12 days.
Absorption	
T _{max} median (range)	The median T _{max} of berotralstat when administered with food is 5 hours (range 1 to 8 hours).
Enterohepatic recirculation	Berotralstat likely undergoes enterohepatic recirculation. Multiple plasma concentration peaks were observed over the dosing interval after single- and multiple-dose administration; the second peak typically occurred after food intake.
Effect of food	No differences in the C _{max} and AUC of berotralstat were observed following administration with a high-fat meal; however, the median T _{max} was delayed 3 hours (median T _{max} 2 hours under fasting conditions compared to 5 hours after a high-fat meal).
Distribution	
Plasma protein binding	Plasma protein binding is approximately 99%.
Elimination	
Half-life	Median (range) elimination t _{1/2} is 93 hours (39 to 152 hours). Effective half-life is 89 hours, consistent with the elimination half-life.
Primary metabolic pathways	In human hepatocytes and S9 fractions, approximately 10% loss of berotralstat was observed over an extended incubation period. In vitro incubations with recombinant CYP3A4 and CYP2D6 resulted in 15% and 22% loss of BCX7353, respectively, with approximately 10% loss in incubations without the cofactor NADPH. Following a single dose of 300 mg [¹⁴ C]-BCX7353, unchanged berotralstat accounted for 34% of plasma radioactivity. Eight metabolites were detected, each accounted for <8% of the radioactivity. In vitro and in vivo metabolic pathways include oxidation, oxidative deamination, hydrogenation, N-dealkylation, N-acetylation, and N-carbamoylation.
Excretion	
Primary excretion pathways	After a single dose of 300 mg [¹⁴ C]-BCX7353, approximately 85.5% of the radioactivity administered was excreted over a 50-day collection period; the predominant component in urine and feces was unchanged berotralstat, but metabolites were also identified in both urine and feces. Overall, 79.4% and 9.2% of the administered dose was excreted in feces and urine, respectively.
Intrinsic Factors	
Renal impairment	The AUC and C _{max} of berotralstat were increased by approximately 13 and 47%, respectively, in subjects with severe renal impairment compared to healthy subjects. This change in exposure does not require dose adjustment of berotralstat.

Type of Parameter	Description
Hepatic impairment	There was no change in berotralstat exposure in subjects with mild hepatic impairment (Child-Pugh A) compared to healthy subjects. The AUC and C _{max} were increased by ~80%, in subjects with moderate hepatic impairment (Child-Pugh B), whereas the C _{max} increase in subjects with severe hepatic impairment (Child-Pugh C) was 27% (there was no AUC increase), in comparison to healthy subjects. Although the exposure increase in subjects with severe hepatic impairment was less than that in those with moderate impairment, there was a 2.6-fold decrease in protein binding in this subpopulation. Additionally, there was a 50% increase in the berotralstat t _{1/2} in subjects with moderate or severe hepatic impairment. In patients with moderate or severe hepatic impairment (Child-Pugh B or C, respectively), the recommended dose of berotralstat is 110 mg QD.
Weight	Slightly higher exposure of berotralstat (~15%) was predicted in adults of 40 to 60 kg bodyweight and adolescents 12 to 18 years of age, compared to adults of 60 to 80 kg bodyweight. This increase is not considered sufficiently clinically meaningful to warrant dose adjustment in this subpopulation. See the pharmacometrics review by Dr. Jing Niu in Section 16.3.2 for further details.
Extrinsic Factors and Drug Interactions	
PK of berotralstat affected	Berotralstat is a substrate of P-gp and BCRP. The berotralstat C _{max} and AUC were increased by 25% and 69%, respectively, with concomitant administration of cyclosporine, a P-gp and BCRP inhibitor. Dose adjustment (110 mg QD) is recommended when berotralstat is administered with P-gp and/or BCRP inhibitors; this will be communicated to the Applicant during labeling interactions. Additionally, P-gp inducers (e.g., rifampin, St. John's wort) may decrease berotralstat plasma concentration, leading to reduced efficacy of berotralstat. Use of berotralstat with P-gp inducers is not recommended.
Effect of berotralstat on the PK of other drugs	Berotralstat 150 mg QD is a moderate inhibitor of CYP2D6 and CYP3A4 and a weak inhibitor of CYP2C9 and CYP2C19. Berotralstat at 300 mg is an inhibitor of P-gp but not of BCRP. Appropriate monitoring and dose titration are recommended for concomitant medications with a narrow therapeutic index that are predominantly metabolized by CYP2D6 (e.g., thioridazine, pimozide) or CYP3A4 (e.g., cyclosporine, fentanyl) or are sensitive P-gp substrates. <u>CYP2C9 substrates:</u> Following administration of berotralstat 150 mg QD, the C_{max} and AUC of tolbutamide were increased by 19% and 73%, respectively. <u>CYP2C19 substrates:</u> Following administration of berotralstat 150 mg QD, the C_{max} and AUC of omeprazole were increased by 21% and 24%, respectively. <u>CYP3A4 substrates:</u> Following administration of berotralstat 150 mg QD, the C_{max} and AUC of midazolam were increased by 45% and 124%, respectively; the C_{max} and AUC of amlodipine were increased by 45% and 77%, respectively; and the C_{max} and AUC of danazol were decreased by approximately 20%. <u>CYP2D6 substrates:</u> Following administration of berotralstat 150 mg QD, the C_{max} and AUC of dextromethorphan were increased by 196% and 178%, respectively, and the C_{max} and AUC of desipramine were increased by 64% and 87%, respectively. <u>P-gp substrates:</u> Following administration of berotralstat 300 mg QD, the C_{max} and AUC of digoxin were increased by 58% and 48%, respectively. <u>BCRP substrates:</u> Following administration of berotralstat 300 mg QD, the C_{max} and AUC of rosuvastatin were decreased by approximately 20%.

Type of Parameter	Description
Pharmacologic Effects	
Kallikrein inhibition	Kallikrein inhibition was evaluated in clinical studies using an ex vivo assay of amidolytic cleavage of a kallikrein-specific substrate in pre- and post-dose samples. E-R analyses demonstrated a high correlation between ex vivo kallikrein inhibition activity and the plasma concentration of berotralstat. The berotralstat concentration and kallikrein inhibition relationship in subjects with HAE was well described by a sigmoidal E _{max} model with a predicted EC ₅₀ of 11 ng/mL. See the pharmacometrics review by Dr. Jing Niu in Section 16.3.2 for further details.

Source: Module 2.7.2, Summary of Clinical Pharmacology Studies, page 16 (modified by the reviewer)
 Abbreviations: AUC, area under the curve; AUC_{tau}, AUC over the dosing interval; BCRP, breast cancer resistance protein; C_{max}, maximum concentration; C_{tau}, concentration over dosing interval; CYP, cytochrome P450; EC₅₀, half-maximal effective concentration; E_{max}, maximum efficacy; E-R, exposure-response; GM, geometric mean; HAE, hereditary angioedema; NAPDH, nicotinamide adenine dinucleotide (reduced); P-gp, P-glycoprotein; QD, four times daily; T_{max}, time to C_{max}

6.3. Summary of the Clinical Pharmacology Assessment

The key issues identified during the clinical pharmacology review are listed in [Table 13](#).

Table 13. Key Issues Identified in the Review Process

Review Issue	Recommendations and Comments
Efficacy and safety	The pivotal randomized, double-blind, placebo-controlled phase 3 study (302) evaluated two doses (110 mg QD ¹ and 150 mg QD) for a 24-week treatment duration. The study met its primary endpoint for both dose levels, with the 110 and 150 mg berotralstat doses reducing HAE attacks by 30% (p=0.024) and 44% (p<0.001), respectively, compared with placebo. Key safety issues noted during the review were GI adverse events of diarrhea, nausea or vomiting, and abdominal pain; transient benign maculopapular rash; and asymptomatic transaminase elevations. In addition, at a dose of 450 mg (i.e., three-fold the 150 mg dose), berotralstat showed a mean QTc increase of 15.9 ms (90% upper CI bound of 23.5 ms); the observed increase in QTcF was concentration-dependent. Refer to the clinical and QT-IRT reviews by Dr. Shaz Siddiqui and Dr. Girish Bende, respectively, for more details.
General dosing instructions	The Applicant recommends a dose of 150 mg QD for the proposed indication. However, based on the clinical assessment of benefit/risk, the review team recommends a starting dose of 150 mg QD, with an option of reducing the dose to 110 mg QD if GI-related adverse events persist. The Applicant proposed to dose berotralstat without regards to food. Although food had no significant impact on the berotralstat PK, the Applicant administered berotralstat with food in the phase 3 study to avoid GI-related adverse events. A recommendation to administer berotralstat with food will be reflected in the product labeling.

Review Issue	Recommendations and Comments
Dosing in patient subgroups (intrinsic factors)	In a dedicated single-dose hepatic impairment study (108), the berotralstat AUC and C_{max} were increased by ~80%, in subjects with moderate hepatic impairment (Child–Pugh B), whereas the C_{max} increase in subjects with severe hepatic impairment (Child–Pugh C) subjects was 27% (there was no AUC increase) in comparison to healthy subjects. While the exposure increase in subjects with severe hepatic impairment was less than that in those with moderate impairment, there was a 2.6-fold decrease in protein binding in this subpopulation. Additionally, there was a 50% increase in the berotralstat terminal half-life in subjects with moderate or severe hepatic impairment. At the time of NDA submission, the Applicant did not propose dose adjustment in subjects with moderate and severe hepatic impairment. However, during the review cycle, the FDA raised concern regarding the concentration-dependent QTc prolongation potential at high berotralstat exposures in patients with moderate or severe hepatic impairment. Thereafter, the Applicant proposed a reduced starting dose of 110 mg QD in patients with moderate or severe hepatic impairment (Child–Pugh Class B or C). See Section 6.3.2 for further details. No dose adjustment is recommended in patients with mild hepatic impairment (Child-Pugh Class A) or renal impairment.
Dosing in patient subgroups (extrinsic factors)	Berotralstat is a substrate of P-gp and BCRP. Cyclosporine, a P-gp and BCRP inhibitor, increased the C_{max} of berotralstat by 25% and the AUC_{inf} by 69%. The Applicant did not propose dose adjustment for this drug interaction. However, a recommendation for dose adjustment (110 mg QD) for berotralstat with concomitant administration of P-gp and/or BCRP inhibitors will be reflected in the product labeling. Additionally, the Applicant did not address the impact of P-gp inducers on the exposure of berotralstat. The use of P-gp inducers with berotralstat is not recommended. See Section 6.3.2 for further details. Berotralstat is a moderate inhibitor of CYP2D6, CYP3A4, and CYP2C9 and a weak inhibitor of CYP2C19. It is also an inhibitor of P-gp. Appropriate monitoring and dose titration are recommended for concomitant medications with sensitive substrates and substrates with a narrow therapeutic index that are predominantly metabolized by CYP2D6 and CYP3A4 and are sensitive P-gp substrates.
Labeling	Recommendations reflected in final labeling.
Bridge between the to-be-marketed and clinical trial formulations	For the initial phase 1 and 2 studies (Studies 101, 102, 103, 105, and 203) API in hydroxypropyl methylcellulose capsules were used. The hydroxypropyl methylcellulose capsules and phase 3 formulation ((b) (4) -capsule) were bridged in Study 103. Subsequently, Study 113 was conducted to demonstrate the bioequivalence of the formulation used in the phase 3 study (two capsules per dose) and the commercial formulation (one capsule per dose).

Source: Reviewer's summary of clinical pharmacology data submitted in NDA 214094.

¹ Rounded from 109 mg berotralstat and equivalent to 125 mg berotralstat hydrochloride.

Abbreviations: API, active pharmaceutical ingredient; AUC_{inf} , area under the curve to infinity; BCRP, breast cancer resistance protein; CI, confidence interval; C_{max} , maximum concentration; CYP, cytochrome P450; GI, gastrointestinal; HAE, hereditary angioedema; NDA, new drug application; P-gp, P-glycoprotein; PK, pharmacokinetics; QD, four times daily; QTc, corrected QT; QTcF, Fridericia-corrected QT; QT-IRT, QT-Interdisciplinary Review Team

6.3.1. Pharmacology and Clinical Pharmacokinetics

6.3.1.1. Pharmacokinetics

- The PK of berotralstat in patients with HAE is similar to that of healthy subjects.
- Berotralstat exposure (C_{max} and AUC) increases greater than proportionally with dose, and steady state is reached within 8 to 12 days. Accumulation is around five-fold at the 110 and 150 mg doses.
- The median time to the C_{max} (T_{max}) of berotralstat when administered with food is 5 hours (range 1 to 8 hours). No differences in the C_{max} and AUC of berotralstat were

observed following administration with a high-fat meal; however, the median T_{max} was delayed by 3 hours, from 2 hours (fasted) to 5 hours (fed).

- Plasma protein binding is approximately 99%. After a single dose of radiolabeled berotralstat 300 mg, the blood-to-plasma ratio was approximately 0.92.
- The median elimination half-life of berotralstat was ~93 hours (range 39 to 152 hours).
- Berotralstat is metabolized by cytochrome P450 (CYP)2D6 and CYP3A4 with low turnover in vitro. After a single oral radiolabeled berotralstat 300 mg dose, unchanged berotralstat represented 34% of the total plasma radioactivity, with each of the eight metabolites accounting for 1.8 to 7.8% of the total radioactivity.
- After a single oral radiolabeled berotralstat 300 mg dose, approximately 9% was excreted in urine (3.4% unchanged; range 1.8 to 4.7%) and 79% was excreted in feces.
- Among the factors evaluated in the population PK analyses (bodyweight, age, gender and race), only bodyweight was identified as a significant predictor of berotralstat clearance and volume of distribution; higher exposure (~15%) was predicted in 40 to 60 kg adults and adolescents 12 to 18 years of age, compared to 60 to 80 kg adults.

6.3.1.2. Pharmacodynamics

Primary and secondary nonclinical PD studies showed that berotralstat is a specific inhibitor of human plasma kallikrein activity. Concentration-dependent inhibition of plasma kallikrein, measured as a reduction from baseline of specific enzyme activity, was demonstrated after oral administration of berotralstat to patients with HAE in the clinical development program. The EC_{50} of kallikrein inhibition measured from a pool of subjects with HAE was 11 ng/mL. A 110 mg dose provided a mean C_{tau} of 4.7-fold the EC_{50} (Study 203).

Cardiac Electrophysiology

The effect of berotralstat was evaluated in thorough QT Study (106), a phase 1, randomized, double-blind, placebo-controlled, parallel-group, multiple-dose study in healthy subjects. At the therapeutic dose of 150 mg QD, berotralstat does not prolong the Fridericia-corrected QT (QTcF) interval to a clinically relevant extent. A positive QT prolongation was observed at a dose of 450 mg (QD for 14 days) ([Table 14](#)). Refer to the QT-IRT review by Dr. Girish Bende for more details.

Table 14. Point Estimates and 90% CIs (FDA Analysis) From TQT Study 106.

ECG Parameter	Treatment	Concentration (ng/mL)	$\Delta\Delta QTcF$ (msec)	90% CI (msec)
QTc	Berotralstat 150 mg*	158.2	3.4	(0.1 to 6.6)
QTc	Berotralstat 450 mg*	591.0	15.9	(8.3 to 23.5)
QTc	Supra-therapeutic Exposure	240.0	5.7	(2.0 to 9.5)

Source: QT-IRT review by Dr. Girish Bende (Reference ID: 4592746).

Abbreviations: CI, confidence interval; ECG, electrocardiogram; FDA, Food and Drug Administration; QTc, corrected QT; QTcF, Fridericia-corrected QT; TQT, thorough QT

6.3.2. General Dosing and Therapeutic Individualization

6.3.2.1. General Dosing

The Applicant's proposed dose of berotralstat is one 150 mg capsule taken orally QD without regard to food. Although the numerically greater treatment effect in the high-dose arm supports the recommended dose of 150 mg QD, the totality of the efficacy and safety data support the option for dose reduction to 110 mg QD if GI adverse events (AEs) are observed with the 150 mg QD dose. Refer to the clinical review by Dr. Shaz Siddiqui for more details (Section 8). The applicant proposed to dose berotralstat without regard to food. Although food had no major impact on the berotralstat PK, berotralstat was administered with food in the phase 3 study to avoid GI-related AEs. A recommendation to administer berotralstat with food will be reflected in the product labeling.

6.3.2.2. Therapeutic Individualization

- In patients with moderate or severe hepatic impairment (Child–Pugh B or C, respectively), the recommended dose of berotralstat is 110 mg QD.
- No adjustment of the starting dose of berotralstat is recommended for patients with mild hepatic impairment (Child–Pugh A).
- No adjustment of the starting dose of berotralstat is recommended for patients with renal impairment.
- Berotralstat is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) and dose adjustment (110 mg QD) is recommended in cases of concomitant administration with P-gp and/or BCRP inhibitors. This recommendation will be reflected in the product labeling. The use of P-gp inducers with berotralstat is not recommended.

6.3.2.3. Outstanding Issues

None.

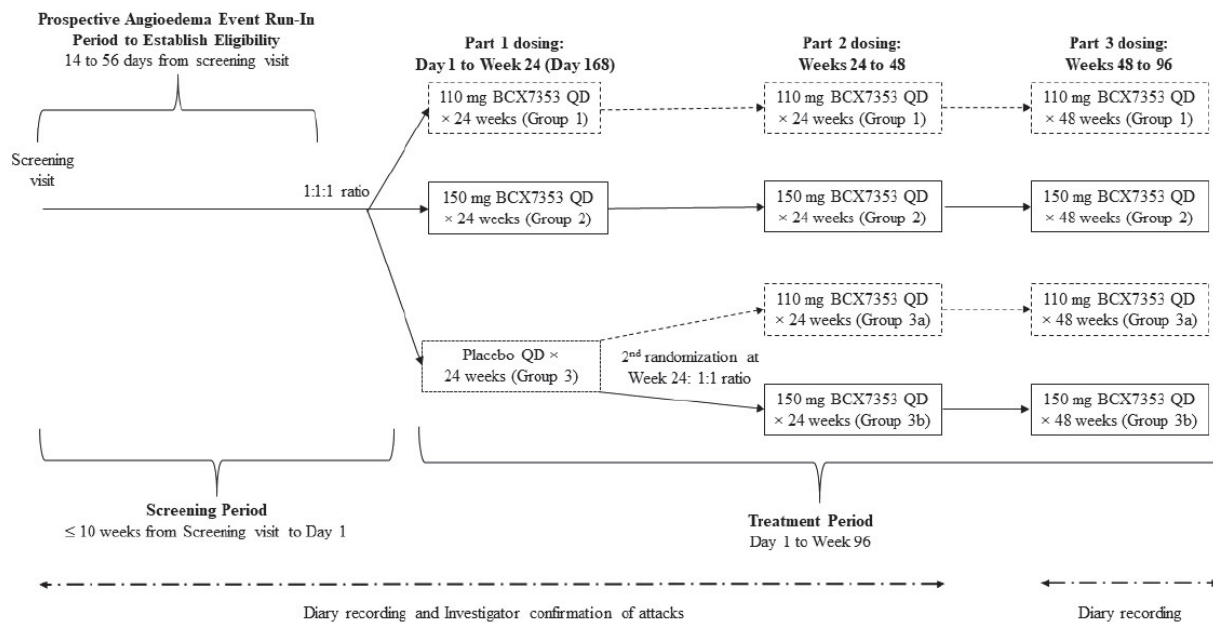
6.3.3. Clinical Pharmacology Questions

Does the Clinical Pharmacology Program Provide Supportive Evidence of Effectiveness?

The efficacy of berotralstat for the prevention of HAE attacks in patients 12 years of age and older with type I or II HAE was demonstrated in Part 1 of the multicenter, randomized, double-blind, placebo-controlled, parallel-group Study 302. Adult and adolescent patients who experienced at least two investigator-confirmed attacks within the first 8 weeks of the run-in period were randomized at a 1:1:1 ratio (berotralstat 110 mg, berotralstat 150 mg, or placebo by oral administration once daily, with food) for the 24-week treatment period (Part 1); 41 subjects were treated with berotralstat 110 mg QD, 40 subjects with berotralstat 150 mg QD, and 40 subjects were treated with placebo QD. Patients discontinued other prophylactic HAE medications prior to entering the study; however, all patients were allowed to use rescue medications for treatment of breakthrough HAE attacks. A history of laryngeal angioedema attacks was reported in 74% of the patients and 75% reported prior use of long-term prophylaxis. The median attack rate during the prospective run-in period (baseline attack rate)

was 2.9/month. Seventy percent of patients enrolled had a baseline attack rate of ≥ 2 attacks/month. Parts 2 and 3 of the study are ongoing. A schematic is shown in [Figure 3](#).

Figure 3. Schematic of Study 302

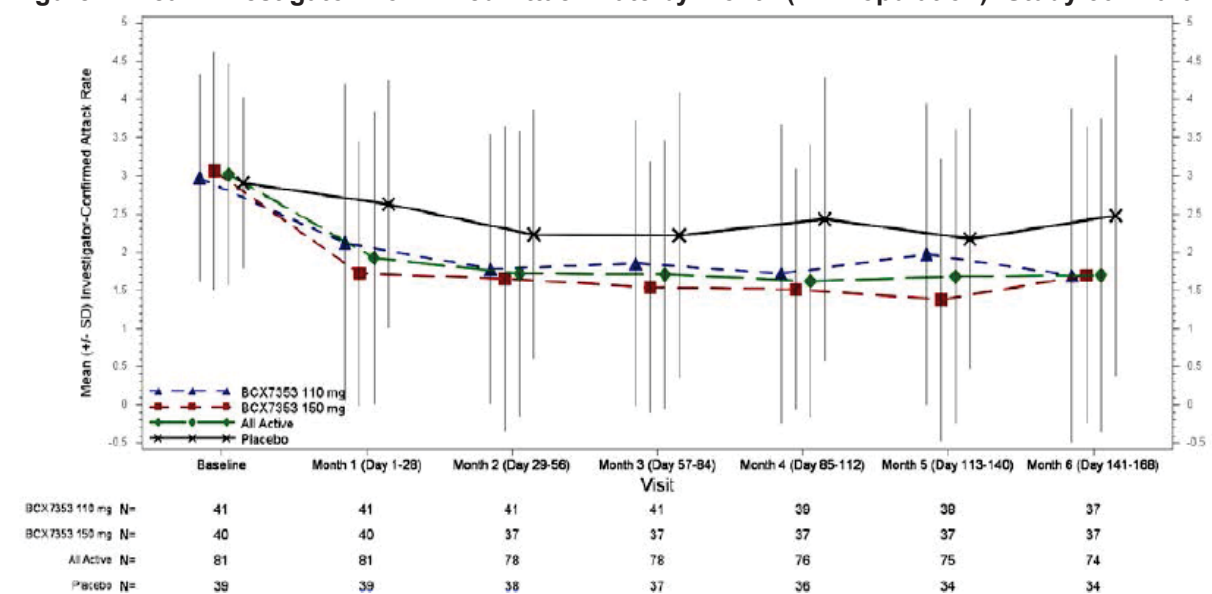


Source: Study 302 protocol, page 48
Abbreviation: QD, once daily

The primary efficacy endpoint was the rate of investigator-confirmed HAE attacks during dosing throughout the 24-week treatment period (Part 1). The study met its primary endpoint for both dose levels, with the 110 and 150 mg berotralstat doses reducing HAE attacks by 30% ($p=0.024$) and 44% ($p<0.001$), respectively, compared with placebo. The negative binomial analysis-estimated attack rates per 28 days were 1.65 for subjects who received berotralstat 110 mg, 1.31 for those administered berotralstat 150 mg, and 2.35 for subjects who received placebo over the 24-week Part 1 dosing period. The reductions in attack rates are shown in [Figure 4](#). Note that a total of six adolescents was enrolled in the study, which was insufficient for adequate interpretation in the subpopulation; however, efficacy may be extrapolated from adult to adolescent patients given that the pathophysiology of the disease is the same in both populations. Refer to Section [8](#) for further details.

In Study 302, in the 174 samples (collected with no particular relationship to the timing of study drug administration), the mean (standard deviation) percentage inhibition of plasma kallikrein averaged across postbaseline visits was 62% ($\pm 15.0\%$), 68% ($\pm 33\%$), and -21% ($\pm 56\%$) for the berotralstat 110 mg QD, berotralstat 150 mg QD, and placebo groups, respectively.

Figure 4. Mean Investigator-Confirmed Attack Rate by Month (ITT Population): Study 302 Part 1



Source: Study 302 clinical study report, page 132

Abbreviations: ITT, intent-to-treat; SD, standard deviation

Is the Proposed Dosing Regimen Appropriate for the General Patient Population for Which the Indication is Being Sought?

Yes, the proposed dosing regimen is reasonable from a clinical pharmacology perspective. The basis for the dose selection and findings from phase 2 Study 203 is discussed below.

Study 203 was a phase 2, randomized, double-blind, placebo-controlled, four-part, parallel-group, dose-response study that evaluated the safety, tolerability, PK, PD, and efficacy of berotralstat in subjects with HAE over a 28-day dosing period; the doses evaluated were 55 mg QD to 300 mg QD. The primary efficacy endpoint was the number of confirmed HAE attacks. A statistical comparison of confirmed attacks per week is shown in [Table 15](#). Treatment with berotralstat at doses ≥ 110 mg for 28 days resulted in a reduction in the rate of angioedema attacks compared with placebo in subjects with type I or type II HAE ($p \leq 0.006$), with a 70% reduction in attack rate at a berotralstat dose of 110 mg representing a clinically meaningful treatment effect. No further increase in efficacy at doses above 110 mg was observed. The Applicant hypothesized that the efficacy of the 218 and 300 mg doses was probably masked by GI AEs that may have been misattributed as early symptoms of abdominal angioedema attacks.

Table 15. Statistical Analysis of the Overall Confirmed Attack Rate: Study BCX7353-203

Treatment	N	LSM	SE	Difference vs. Placebo	Percentage Difference vs. Placebo	95% CI for Treatment Difference	p-Value for Treatment Difference
Berotralstat 300 mg	18	0.525	0.103	-0.387	-42.446	-0.663, -0.112	0.006
Berotralstat 218 mg	14	0.467	0.117	-0.446	-48.865	-0.742, -0.150	0.003
Berotralstat 110 mg	14	0.271	0.117	-0.642	-70.327	-0.938, -0.346	<0.001
Berotralstat 55 mg	7	0.867	0.166	-0.046	-5.001	-0.423, 0.332	0.811
All berotralstat	53	0.488	0.060	-0.425	-46.561	-0.645, -0.205	<0.001
Placebo	22	0.913	0.093				

Source: Study 203 Clinical Study Report, page 95 (dihydrochloride salt converted to free base), with modifications
 Abbreviations: CI, confidence interval; LSM, least-squares mean; SE, standard error

Although there was no apparent correlation between the confirmed attack rate and berotralstat plasma exposure over the dose range studied (55 to 300 mg), the steady-state berotralstat area under the curve over the dosing interval (AUC_{τ}) and maximum plasma concentration over the dosing interval (C_{τ}) were correlated with the confirmed attack rate at the lower dose range of 55 to 110 mg. Refer to the pharmacometrics review by Dr. Jing Niu (Section [16.3.2](#)) for further details. In addition, the results suggested that maintaining a threshold concentration of at least four-fold the EC_{50} for plasma kallikrein inhibition will result in a clinically meaningful reduction in attack rate. The proportions of subjects with an average berotralstat plasma C_{τ} greater than four-fold the EC_{50} were 0/7, 8/14, 14/14, and 16/16 in the 55, 110, 218, and 300 mg dose groups, respectively. A 110 mg QD dose provided a mean C_{τ} of 4.7-fold the EC_{50} , with a corresponding reduction in attack rate of 70% ($p < 0.001$) versus placebo, and a 55 mg QD dose provided a mean C_{τ} of 1.5-fold the EC_{50} , with no change in attack rate versus placebo ($p = 0.811$). Hence, 110 mg QD berotralstat was selected for confirmation of efficacy in the phase 3 study. The higher doses (218 mg and 300 mg QD) were not evaluated in the phase 3 study because of the GI AEs observed in Study 203. The Applicant noted that berotralstat has nonlinear PK, and a two-fold increase in steady-state exposure was observed at 150 mg as compared to 110 mg. Therefore, to evaluate the dose-response between 110 mg and 218 mg, 150 mg QD was selected as the second dose in the phase 3 study. Note that berotralstat has a long half-life of ~93 hours and as such a QD dosing regimen was considered reasonable. Other dosing regimens were not evaluated in the clinical development plan.

Is an Alternative Dosing Regimen or Management Strategy Required for Subpopulations Based on Intrinsic Patient Factors?

Yes, in patients with moderate or severe hepatic impairment (Child–Pugh B or C), a lower 110 mg QD dose of berotralstat is recommended. No dose modification is recommended in patients with mild hepatic impairment (Child–Pugh A) or renal impairment. Additionally, there is no need for dose adjustment based on age or bodyweight. See below for further details and the basis of these recommendations.

Patients with Hepatic Impairment

The impact of hepatic impairment on the berotralstat PK was assessed in a single-dose, open-label study (Study 108) in adult male and female subjects with normal hepatic function and varying degrees of hepatic impairment (Child–Pugh A, B, or C). Six subjects in each group received a single oral dose of 150 mg berotralstat.

The PK results are shown in [Table 16](#). Exposure of berotralstat increased in subjects with hepatic impairment, but the increase in exposure in subjects with severe hepatic impairment was lower than that in those with moderate hepatic impairment. The berotralstat unbound fraction was similar in subjects with mild hepatic impairment compared with matched healthy subjects with normal hepatic function (1.2), and the median unbound fraction was approximately 1.5 and 2.6 in subjects with moderate and severe hepatic impairment, respectively. The Applicant noted that there was no physiological basis for moderate hepatic disease to have a greater impact on berotralstat disposition than severe disease, and so proposed to pool the moderate and severe hepatic impairment groups for comparison with healthy matched controls ([Table 16](#)). However, the reviewer did not agree with this approach because decreased protein binding may impact the pharmacologically active unbound drug in patients with moderate or severe hepatic impairment.

Table 16. Statistical Comparison of Plasma Berotralstat PK Parameters (ANOVA)–PK Population: Study 108

PK Parameter	Group	Geometric Mean	GLSM Ratio (%) (90% CI) ^a
C _{max} (ng/mL)	Healthy/normal hepatic function	45.5	
	Mild hepatic impairment	45.9	101 (54.2, 188)
	Moderate hepatic impairment	80.5	177 (95.0, 329)
	Severe hepatic impairment	57.9	127 (68.4, 237)
	Combined moderate/severe hepatic impairment	68.3	150 (87.9, 256)
AUC _{0-inf} (ng.h/mL)	Healthy/normal hepatic function	907	
	Mild hepatic impairment	945	104 (60.6, 179)
	Moderate hepatic impairment	1620	178 (104, 307)
	Severe hepatic impairment	850	93.8 (51.1, 172)
	Combined moderate/severe hepatic impairment	1250	138 (82.6, 230)
AUC _{0-last} (ng.h/mL)	Healthy/normal hepatic function	737	
	Mild hepatic impairment	769	104 (61.7, 177)
	Moderate hepatic impairment	1250	170 (100, 287)
	Severe hepatic impairment	700	95.0 (56.1, 161)
	Combined moderate/severe hepatic impairment	936	127 (74.8, 206)

Source: Study 108 clinical study report, page 52

Abbreviations: ANOVA, analysis of variance; AUC_{0-inf}, area under the curve from time zero to infinity; AUC_{0-last}, area under the curve to the last quantifiable time point; CI, confidence interval; C_{max}, maximum concentration; GLSM, geometric least-squares mean; PK, pharmacokinetics

Furthermore, based on the pooled PK approach, the Applicant proposed no dose adjustment in subjects with moderate or severe hepatic impairment because higher doses of up to 300 mg QD berotralstat were considered to be safe and generally well-tolerated in subjects with HAE (Study 203). However, the following issues were noted by the reviewer:

- Although a higher dose of 300 mg QD was evaluated in the phase 2 Study 203 and concluded to be generally safe, the study duration was relatively short (28 days) and the number of subjects exposed to 300 mg QD was small (N=18), reducing the level of support for higher exposure of berotralstat.
- The berotralstat half-life was increased by ~50% in subjects with moderate or severe hepatic impairment. Berotralstat accumulates by around five-fold when dosed to steady-state. The accumulation of berotralstat in subjects with moderate or severe hepatic impairment is expected to be elevated because of the longer half-life.
- There was a 1.5- and 2-fold decrease in protein binding in subjects with moderate and severe hepatic impairment, respectively, and so the pharmacologically active unbound drug concentration would be higher in these patients.

- At a 450 mg QD dose of berotralstat ($C_{\max,ss}$ 577 ng/mL), the estimated $\Delta\Delta QTcF$ was 15.9 ms with a two-sided 90% upper bound of 23.5 ms.

Because berotralstat is proposed for chronic use and associated with a potential QTc risk, an information request (IR) was sent to the Applicant (dated June 26, 2020) to consider alternate dosing recommendations in subjects with moderate or severe hepatic impairment. In response to the IR, the applicant simulated the following dosing regimens in subjects with HAE using population PK Model 258 (BCX7353-PPK-01-A2):

- Alternate daily dose of 110 mg QD
- Alternate dosing regimen 150 mg every other day

Based on the simulation results and patient compliance considerations, the Applicant proposed a reduced dose of 110 mg QD berotralstat in subjects with moderate or severe hepatic impairment. This proposed dosing recommendation for subjects with moderate or severe hepatic impairment is acceptable.

Patients with Renal Impairment

The impact of renal impairment on the berotralstat PK was assessed in a single-dose, open-label study (Study 107) in adult male and female subjects with severe renal impairment and matched healthy subjects with normal renal function. Seven subjects in the following two groups received a single oral dose of 200 mg berotralstat.

- Group A: normal renal function (creatinine clearance [CL_{CR}] ≥ 90 mL/min)
- Group B: severe renal impairment (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m²; not yet on dialysis)

Compared to matched healthy subjects, the berotralstat $C_{\max}$ was approximately 39% higher in subjects with severe renal impairment following single-dose administration of 200 mg berotralstat; however, the AUC was similar in the two groups. Note that healthy subjects and those with severe renal impairment were enrolled using different criteria— CL_{CR} and eGFR, respectively. During the NDA review, the Applicant was informed (IR of June 30, 2020) that only one criterion; i.e., CL_{CR} or eGFR, should be used for comparison of PK between the two groups. The Applicant reanalyzed the study data using CL_{CR} as the criterion. For classification of subjects by CL_{CR} , the applicant used the CL_{CR} value, either at screening or baseline, that the respective site had used for classification by MDRD during the study. One subject (b) (6) in the severe renal impairment group had a screening and baseline CL_{CR} of 38 mL/min and was excluded from the severe renal impairment group ($CL_{CR} < 30$ L/min). The updated statistical comparison of the berotralstat PK between subjects with normal and severe renal impairment using the CL_{CR} criterion was provided by the Applicant on July 10, 2020 (Table 17). The $C_{\max}$ and AUC were increased by approximately 47% and 13%, respectively, in subjects with severe renal impairment compared to healthy subjects using the CL_{CR} criterion. The half-life and protein binding were similar in subjects with severe renal impairment and healthy subjects.

Table 17. Statistical Comparison of Plasma Berotralstat PK Parameters in Healthy Subjects and Subjects With Severe Renal Impairment Classified by CL_{CR}: Study 107

PK Parameter	Geometric Mean		Percent GLSM Ratio (90% CI), Severe/Healthy
	Healthy (CL _{CR} > 90 mL/min) N = 7	Severe Renal Impairment (CL _{CR} < 30 mL/min) N = 6	
AUC _{0-inf} (ng.h/mL)	2063	2252	109.1 (67.0, 177.7)
AUC _{0-last} (ng.h/mL)	1608	1832	113.9 (69.9, 185.6)
C _{max} (ng/mL)	89	130	146.9 (89.7, 240.3)

Source: Information request response dated 07/10/2020 (excluding subject (b) (6) from the severe renal impairment group)
Abbreviations: AUC_{0-inf}, area under the curve from time zero to infinity; AUC_{0-last}, area under the curve to the last quantifiable time point; CI, confidence interval; CL_{CR}, creatinine clearance; C_{max}, maximum concentration; GLSM, geometric least-squares mean; PK, pharmacokinetics

The clinical development program of berotralstat allowed enrollment of HAE patients with moderate or mild renal impairment in the clinical trials. Using CL_{CR} to estimate renal function, 25 of 223 subjects (11.2%) enrolled across the two long-term prophylactic studies (204 and 302) who received berotralstat 150 mg QD had mild (60 to <90 mL/min CL_{CR}, n=23) or moderate (30 to <60 mL/min CL_{CR}, n=2) renal impairment at baseline. In these 25 subjects, the safety profile of berotralstat 150 mg QD was generally similar to that of the overall study population or those with normal renal function in both studies.

Overall, considering the above factors, the increased C_{max} in subjects with severe renal impairment is not expected to cause clinically meaningful QTc prolongation, and no dose modification is recommended for patients with severe renal impairment. The PK of berotralstat in patients with ESRD requiring hemodialysis has not been determined.

Covariates (Age, Body Weight)

Based on population PK analyses of pediatric patients 12 to <18 years of age (simulated bodyweight generally 40 to 71 kg), slightly higher exposure (~15%) of berotralstat was predicted compared to adults weighing 60 to 80 kg. The higher exposure in this lower bodyweight group is not expected to cause meaningful QT prolongation, which is a major safety concern correlated with exposure. Therefore, no dose modification is recommended for adolescents and adults weighing 40 to 60 kg. See the pharmacometrics review (Section [16.3.2](#)) by Jing Niu for further details.

Are There Clinically Relevant Food–Drug Or Drug–Drug Interactions, and What is the Appropriate Management Strategy?

Effect of Food

The effect of food on berotralstat exposure was evaluated in two phase 1 studies. Study BCX7353-101 was a pilot study in which six subjects received two single doses of berotralstat 218 mg (250 mg [SN]), in the fasted state and after a high-fat meal. In this study, the API in HPMC capsules (an earlier formulation) was used. Subsequently, a second relative bioavailability and food-effect study (103; three-period, crossover) evaluated berotralstat

(300 mg) administered as the API in the HPMC capsule used in early phase 1 studies and the (b) (4)-capsule used in the phase 3 study. Twenty-four subjects were randomized to the following treatments:

- Treatment A: Reference API in capsule, fasted
- Treatment B: Test phase 3 (b) (4)-capsule, fasted
- Treatment C: Test phase 3 (b) (4)-capsule, fed

The relative effect of a high-fat meal on the PK of berotralstat is shown in [Table 18](#). In the pilot food effect Study 101, berotralstat exposure (AUC) under fed conditions was increased 1.4-fold; however, the 90% CIs around the point estimates were large, likely as a result of the small number of subjects. In Study 103, although the median T_{max} was delayed about 3 hours, food did not impact the rate or extent of berotralstat exposure. As berotralstat progressed into the phase 3 program, the Applicant decided to administer berotralstat with food because there was no effect of food on PK and it was postulated that food may minimize GI AEs of berotralstat. Considering this, it is recommended that berotralstat be administered with food in patients with HAE.

Later in clinical development, a change in the phase 3 formulation was made and a higher strength capsule (e.g., one 110/150 mg capsule instead of two 55/75 mg capsules) was developed as the to-be-marketed (TBM) drug product. The phase 3 formulation and TBM formulation are of identical composition (except for the difference in capsule fill weight) and were bridged in Study 113. The TBM drug product was bioequivalent to the phase 3 formulation for the 110 and 150 mg doses in terms of the PK parameters of interest: AUC_{0-72} , AUC_{0-inf} , and C_{max} . Although a definitive food effect study for the commercial formulation of berotralstat was not conducted, the food effect results from Study 103 are considered applicable to the TBM drug product because the phase 3 clinical study formulation is of composition identical to the TBM formulation and Study 103 evaluated the food effect at a dose of 300 mg.

Table 18. Cross-Study Comparison of Berotralstat Food Effect in Healthy Subjects

Study (n)	Dose (mg)	Median T_{max} (Range) (h)	GLSM ratio (%) (90% CI for C_{max} [Fed vs. Fasted])	GLSM ratio (%) (90% CI for AUC_{inf} [Fed vs. Fasted])
101 (n=6)	250 (SN) fasted	2.0 (2.0, 5.0)	94.3 (66.4, 134)	141 (108, 184)
	250 (SN) fed	5.0 (1.0, 6.0)		
103 (n=22)	300 fasted	2.0 (1.0, 6.0)	112 (103, 122)	102 (93.2, 111)
	300 fed	5.0 (1.0, 8.0)		

Source: Module 2.7.2, Summary of Clinical Pharmacology Studies, page 85

Abbreviations: AUC_{inf} , area under the curve to infinity; CI, confidence interval; C_{max} , maximum concentration; GLSM, geometric least-squares mean; SN, salt notation; T_{max} , time to C_{max}

Drug Interactions

Effects of Coadministered Drugs on the Pharmacokinetics of Berotralstat

1. Cytochrome P450-Mediated Interactions

Using in vitro studies of recombinant cytochrome P450 (CYP) enzymes, the Applicant demonstrated that the greatest decrease in berotralstat concentration (2.7 μ M) occurred with CYP2D6 and CYP3A4 (15.0% and 22.4% decrease, respectively, with ~10% decreases in incubations without cofactors). Given the low level of in vitro metabolism, no single enzyme contributed more than 25% of berotralstat elimination; therefore, no clinical studies were conducted with strong index inhibitors of CYP3A4 and CYP2D6. Similar to the in vitro studies, the mass balance Study 104 showed the presence of multiple metabolites at low levels, none of which exceeded 8% of drug-related radioactivity.

2. Transporter Interaction Studies

The in vitro studies showed that berotralstat was a substrate of P-gp and BCRP, but not of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, or MATE2-K.

Study 105 was conducted to assess the drug interaction potential related to transporters. The probe inhibitor, cyclosporine (inhibits P-gp, OATP, and BCRP) was used to evaluate a possible interaction with berotralstat. As shown in [Table 19](#), cyclosporine increased the exposure of a single dose of berotralstat (C_{max} by 25% and area under the curve from time zero to infinity by 69%). The Applicant proposed no dose adjustment with concomitant administration of P-gp and BCRP inhibitors. However, the following issues were noted by the reviewer:

- Although a higher dose of 300 mg QD was evaluated in phase 2 Study 203 and concluded to be generally safe, the study duration was relatively short (28 days), and the number of subjects exposed to 300 mg QD was small (N=18), reducing the level of support for higher exposure of berotralstat.
- Limited information from long-term studies is available on the safety of berotralstat with P-gp and BCRP inhibitors. According to the information submitted by the Applicant, 22 of 223 subjects (9.9%) enrolled across the two long-term prophylactic studies (204 and 302) took concomitant P-gp inhibitors while receiving berotralstat 150 mg QD. In seven subjects, the P-gp inhibitor was a short-term antibiotic (azithromycin, clarithromycin) or antifungal (fluconazole). Thirteen subjects initiated a concomitant P-gp inhibitor (atorvastatin, simvastatin, telmisartan, carvedilol, or diltiazem) administered chronically with berotralstat 150 mg QD. While no AEs attributable to concomitant dosing of a P-gp inhibitor were reported, the relevance of these data is unclear because the selectivity/sensitivity of these P-gp inhibitors is unknown and may not represent the worst-case scenario. Additionally, this interaction assessment was conducted post hoc. Typically, such analyses should be prespecified to avoid effects of confounding factors on detecting a specific clinically meaningful change in drug exposure.
- Note that cyclosporine inhibits P-gp as well as BCRP. Further studies using more selective inhibitors were not conducted by the Applicant to elucidate the relative contribution of each transporter.

Overall, dose adjustment of berotralstat to 110 mg QD with concomitant administration of P-gp and/or BCRP inhibitors is recommended to be included in the prescribing information.

P-gp inducers (e.g., rifampin, St. John's wort) may decrease berotralstat plasma concentration, leading to reduced efficacy of berotralstat. The Applicant's clinical development program did not address the potential impact of P-gp inducers on the systemic exposure of berotralstat. The use of berotralstat with P-gp inducers is not recommended.

Effect of Berotralstat on the Pharmacokinetics of Coadministered Drugs

In vitro, berotralstat inhibited CYP2C9 and CYP2C19 with IC₅₀ values of 0.24 and 0.36 μM, respectively. The IC₅₀ values were 1 to 10 μM for CYP2D6 (2.3 μM) and CYP3A (2.5 and 2.2 μM). All other CYP isozymes had IC₅₀ values >10 μM, which exceeded the therapeutic C_{max} for berotralstat, approximately 0.28 μM (158 ng/mL) following administration of 150 mg berotralstat QD. In a time-dependent inhibition assay, IC₅₀ shifts of 5 to 12 μM suggested berotralstat to likely be a time-dependent inhibitor of CYP3A.

Berotralstat inhibited P-gp, BCRP, MATE1, and MATE2-K with calculated IC₅₀s of 0.492, 12.0, 3.53, and 4.60 μM, respectively. The IC₅₀s for berotralstat on OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 could not be assessed at concentrations up to 8.9 μM. Berotralstat was an inhibitor of OATP1B1, OCT2, and OATP1A2 with IC₅₀s of 13.2, 13.3, and 11.3 μM, respectively. Berotralstat at concentrations up to 30 μM resulted in <50% inhibition of OATP1B3, OAT1, OAT3, and OATP2B1. Berotralstat at concentrations up to 100 μM resulted in <50% inhibition of MRP2.

Based on these results, in Study 105 berotralstat was tested as a transporter inhibitor using the probe substrates digoxin (P-gp substrate) and rosuvastatin (BCRP substrate).

The clinical drug interaction potential for berotralstat was evaluated in the following studies:

- Study 112: Phase 1 drug–drug interaction (DDI) study to evaluate the effect of berotralstat on the PK of danazol (CYP3A4), amlodipine (CYP3A4), and desipramine (CYP2D6) in healthy subjects.
- Study 115: Phase 1, single sequence, open-label, DDI study to evaluate the effect of berotralstat at steady-state on CYP3A4 (midazolam), 2C9 (tolbutamide), 2C19 (omeprazole), and 2D6 (dextromethorphan) activity using cocktail probe substrates.
- Study 105: Phase 1 study to evaluate the effect of berotralstat on the single-dose PK of a P-gp substrate (digoxin) and BCRP substrate (rosuvastatin).

The results of these studies are presented in [Table 19](#). Note that a second cocktail drug interaction study (102) was conducted using the 300 mg dose of berotralstat. This study was subsequently repeated with the 150 mg dose; only the results for 150 mg berotralstat are listed in [Table 19](#).

NDA/BLA Multidisciplinary Review and Evaluation NDA 214094
ORLADEYO (berotralstat)

Table 19. Summary of Drug Interaction Studies Conducted for Berotralstat

Substrate Drug	Dose and Schedule Perpetrator	Effect on Drug PK	Mean Ratio (90% CI)		Dosing Recommendation
			AUC	C _{max}	
Digoxin (0.25 mg)	BCX 300 mg QD (8 days)	↑ Digoxin	1.48 (1.22, 1.79)	1.58 (1.20, 2.09)	Appropriate monitoring and dose titration
Rosuvastatin (10 mg)	BCX 300 mg QD (8 days)	↓ Rosuvastatin	0.76 (0.67, 0.85)	0.80 (0.73, 0.88)	No dose adjustment
Berotralstat 300 mg QD	Cyclosporine (600 mg)	↑ Berotralstat	1.69 (1.50, 1.91)	1.25 (1.05, 1.48)	110 mg dose
Midazolam (4 mg)	BCX 150 mg QD (9 days)	↑ Midazolam	2.24 (2.05, 2.44)	1.45 (1.29, 1.63)	Appropriate monitoring and dose titration
Danazol (200 mg)	BCX 150 mg QD (9 days)	↓ Danazol	0.78 (0.59, 1.02)	0.77 (0.57, 1.01)	No dose adjustment
Amlodipine (5 mg)	BCX 150 mg QD (12 days)	↑ Amlodipine	1.77 (1.59, 1.98)	1.45 (1.27, 1.64)	Appropriate monitoring and dose titration
Tolbutamide (500 mg)	BCX 150 mg QD (9 days)	↑ Tolbutamide	1.73 (1.63, 1.85)	1.19 (1.11, 1.27)	No dose adjustment
Omeprazole (40 mg)	BCX 150 mg QD (9 days)	↑ Omeprazole	1.24 (1.06, 1.44)	1.21 (1.00, 1.47)	No dose adjustment
Dextromethorphan (30 mg)	BCX 150 mg QD (9 days)	↑ Dextromethorphan	2.78 (2.33, 3.33)	2.96 (2.48, 3.55)	Appropriate monitoring and dose titration
Desipramine (50 mg)	BCX 150 mg QD (11 days)	↑ Desipramine	2.15 (1.87, 2.47)	1.64 (1.48, 1.82)	Appropriate monitoring and dose titration

Source: Reviewer's summary of the data submitted in NDA 214094

Abbreviations: ↑, increase; ↓, decrease; BCX, berotralstat; AUC, area under the curve; CI, confidence interval; C_{max}, maximum concentration; PK, pharmacokinetics; QD, four times daily

Berotralstat increased the exposure of digoxin by ~50% (Table 19). The increase in exposure of digoxin is likely clinically meaningful, given its narrow therapeutic range. Therefore, therapeutic drug monitoring and dose titration of digoxin is recommended if administered concomitantly with berotralstat.

CYP2D6 is a polymorphic enzyme, and variations of the gene give rise to alleles resulting in no, decreased, normal, or increased enzymatic activity. In Studies 102, 112, and 115, subjects with no and increased enzymatic activity, known as poor and ultrarapid metabolizers, respectively, were excluded, but subjects with normal and decreased enzymatic activity; i.e., normal and intermediate metabolizers, respectively, were eligible for enrollment. The increase in exposure of the CYP2D6 substrates, desipramine and dextromethorphan, caused by coadministration with berotralstat is not expected to cause variability in exposure to CYP2D6 substrates greater than the inherent intersubject variability between poor metabolizers, who have no CYP2D6 enzymatic activity, and normal or ultrarapid metabolizers.

Berotralstat is a moderate inhibitor of CYP3A4 and CYP2D6 sensitive substrates; therefore, appropriate monitoring and dose titration is recommended in cases of concomitant administration of sensitive substrates or those with a narrow therapeutic index that are predominantly metabolized by CYP2D6 and CYP3A4.

What is the Bioanalytical Method Utilized to Quantitate Berotralstat?

Berotralstat (BCX7353) plasma levels were determined by a validated liquid chromatography tandem-mass spectrometry (LC-MS/MS) assay. The method employs a least-squares linear regression analysis with a $1/x^2$ weighting factor to generate a 1 to 1000 ng/mL range. Protein precipitation is performed on a 50 μ L aliquot of plasma using acidified acetonitrile. The samples are next centrifuged and the supernatants are injected into a high-performance LC-MS/MS system. Plasma concentrations are back-calculated from the berotralstat peak area ratios compared with those of the calibration standards. The intra-assay precision of the method ranged from 2.9% to 7.3% (8.6% at the lower limit of quantification [LLOQ]), and intra-assay accuracy (bias) ranged from 88.9% to 110.7% (83.5% at LLOQ). Interassay precision ranged from 4.0% to 8.5% (12.1% at LLOQ), and interassay accuracy from 92.0% to 102.7% (96.1% at LLOQ). Berotralstat in human plasma was found to be stable after four freeze/thaw cycles (-20°C and -80°C) and for 363 days when stored frozen at -80°C . This validated plasma assay (vendor, (b) (4)) was used to support analyses of berotralstat in Studies 101, 102, 103, 104, 105, 107, 108, 109, and 203.

The Applicant utilized an additional vendor (b) (4) to quantitate berotralstat levels during the clinical development program. The (b) (4)-validated plasma bioanalytical method (171354 (b) (4)) by LC-MS/MS was used to support analysis of berotralstat in Studies 106, 112, 113, 115, 204, and 302. The method was similar to that used by (b) (4) and a cross-validation (TD-VAL-20-7353-001R) of the two assays was performed. The intra-assay precision of the plasma method ranged from 3.0% to 4.4% (4.5% at LLOQ), and intra-assay accuracy (bias) from 99.9% to 105% (104% at LLOQ). Interassay precision ranged from 3.5% to 5.3% (4.9% at LLOQ) and interassay accuracy from 103.5% to 107.3% (106% at LLOQ). For

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processing purposes, BCX7353 was stable in whole blood for 2 hours at room temperature and in an ice bath. BCX7353 in human plasma was found to be stable on the bench top for at least 26 hours, after five freeze/thaw cycles (-20°C and -80°C), and for 43 days when stored frozen at -20°C or -80°C, the longest period tested.

The performance of the bioanalytical assays used in the clinical pharmacology studies is shown in [Table 20](#). The incurred sample reanalysis met the acceptance criteria. Samples were analyzed within the established freezer stability limit at -80°C. In all bioanalytical reports, the interassay accuracy and precision of quality control samples and calibration standards were within the acceptance criteria: accuracy within ±15% of the nominal concentration (±20% at the LLOQ) and precision of ≤15% (≤20% at the LLOQ).

Table 20. Summary of Bioanalytical Assay Performance in Clinical Pharmacology Studies

Clinical Study Number	Calibration Range (ng/mL)	Range of Values				Site of Analysis	Study Report
		Calibration Standards Accuracy (%RE)	Calibration Standards Precision (%CV)	QC Accuracy (%RE)	QC Precision (%CV)		
Study 101	1 – 1000 (QC: 3, 50, 750)	Plasma -2.0 to 3.0	Plasma ≤ 7.7	Plasma -1.0 to 1.7	Plasma ≤ 8.8	(b) (4)	(b) (4) 276639QB01
	Urine 5 – 5000 (QC: 15, 250, 3750)	Urine -3.3 to 2.2	Urine ≤ 6.8	Urine -13.7 to 6.5	Urine ≤ 11.8		
Study 102	1 – 1000 (QC: 3, 50, 750)	-3.2 to 2.4	≤ 11.4	-3.0 to 5.3	≤ 9.3	(b) (4)	(b) (4) 282786QB01
Study 103	1 – 1000 (QC: 3, 50, 750)	-6.4 to 7.0	≤ 5.8	-7.6 to 6.0	≤ 7.3		(b) (4) 297833QB01
Study 105	1 – 1000 (QC: 3, 50, 750)	Plasma -6.8 to 5.4	Plasma ≤ 7.4	Plasma -0.6 to 9.7	Plasma ≤ 5.6		(b) (4) 294300QB01 (plasma)
	Urine 5 – 5000 (QC: 15, 250, 3750)	Urine -3.4 to 1.8	Urine ≤ 7.3	Urine -9.3 to 2.4	Urine ≤ 7.1	(b) (4) 276623QB01 (urine)	
Study 106	1 – 1000 (QC: 3, 30, 300, 750)	-5.5 to 4.0	≤ 4.0	1.3 to 6.0	≤ 9.7	190232 (b) (4)	

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Clinical Study Number	Calibration Range (ng/mL)	Range of Values				Site of Analysis	Study Report
		Calibration Standards Accuracy (%RE)	Calibration Standards Precision (%CV)	QC Accuracy (%RE)	QC Precision (%CV)		
Study 107	Plasma 1 – 1000 (QC: 3, 50, 750) Urine 5 – 5000 (QC: 15, 250, 3750)	Plasma -2.6 to 1.4 Urine - acid -1.3 to 1.7 Urine - CHAPS -7.4 to 4.4	Plasma ≤ 6.2 Urine - acid ≤ 6.4 Urine - CHAPS ≤ 14.2	Plasma -5.7 to 0.0 Urine - acid 3.2 to 6.0 Urine - CHAPS 0.7 to 8.8	Plasma ≤ 3.4 Urine - acid ≤ 5.3 Urine - CHAPS ≤ 5.8	(b) (4)	(b) (4) 304080QB20 304080QB10 309263QB10
Study 108	Plasma 1-1000 (QC: 3, 50, and 750) RED 0.2 – 200 (QC: 0.6, 10, 150)	Plasma -1.0 to 1.6 RED -2.8 to 2.0	Plasma ≤ 8.2 RED ≤ 8.3	Plasma 1.7 to 4.3 RED 1.3 to 7.5	Plasma ≤ 4.9 RED ≤ 5.2	(b) (4)	315930QB20
Study 112	1 – 1000 (QC: 3, 30, 300, 750)	-4.0 to 5.0	≤ 3.1	0.1 to 4.7	≤ 3.1	(b) (4)	180581 (b) (4)
Study 113	1 – 1000 (QC: 3, 30, 300, 750)	-2.5 to 2.0	≤ 3.2	-3.7 to 3.7	≤ 3.6	(b) (4)	181921 (b) (4)
Study 115	1 – 1000 (QC: 3, 30, 300, 750)	-1.3 to 1.5	≤ 3.8	0.3 to 1.7	≤ 3.0	(b) (4)	182289 (b) (4)
Study 203	1 – 1000 (QC: 3, 50, 750)	-6.0 to 5.3	≤ 7.1	0.3 to 3.6	≤ 10.7	(b) (4)	81599QB01
Clinical Study Number	Calibration Range (ng/mL)	Range of Values				Site of Analysis	Study Report
		Calibration Standards Accuracy (%RE)	Calibration Standards Precision (%CV)	QC Accuracy (%RE)	QC Precision (%CV)		
Study 204	1 – 1000 (QC: 3, 30, 300, 750)	-3.3 to 3.0	≤ 3.4	-8.8 to 5.0	≤ 5.1	(b) (4)	171914 (b) (4) Interim
Study 302	1 – 1000 (QC: 3, 30, 300, 750)	-4.3 to 2.0	≤ 3.7%	1.1 to 5.0	≤ 5.6	(b) (4)	180314 (b) (4) Interim

Abbreviations: CHAPS = 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate; CV = coefficient of variation; (b) (4) (b) (4) QC = quality control; RE = relative error; RED = rapid equilibrium dialysis

Note: Cross validation of BCX7353 between (b) (4) was performed early in 2020 to illustrate analytical results for plasma samples from each site were comparable (Study report: TD-VAL-20-7353-001R).
Source: Information request response dated 07/02/2020

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

The sources of the clinical data used in this review are summarized in [Table 21](#).

Table 21. Listing of Clinical Trials Relevant to this NDA

Trial Identifier	Trial Design	Treatment Regimen [†] / Duration	Primary and Key Secondary Endpoints	No. of Subjects	Trial Population	No. of Centers/Countries
Controlled Phase 3 Study to Support Efficacy and Safety						
BCX7353-302	Part 1: R, DB, PC, PG	110 mg po QD 150 mg po QD	- Rate (per 28 days) of investigator-confirmed HAE attacks during dosing period - Change from baseline in AE-QoL total score - Number and proportion of days with angioedema symptoms - Rate of investigator-confirmed HAE attacks during dosing in the effective treatment period	Planned: 96 Actual: 121 Randomized: 120	Type I or II HAE patients ≥ 12 years	40 sites in 11 countries: US, EU, Canada
NCT# 03485911	Part 2: R, DB, PG	24 weeks		110mg: 41 150mg: 40 Placebo: 39		
	Part 3: OLE	24 weeks 24 weeks 48 weeks				
Uncontrolled Study to Support Safety						
BCX7353-204	Phase 2, uncontrolled, OL	110 mg po QD 150 mg po QD 240 weeks	- The proportion of subjects with TEAEs, SAEs, grade 3 or 4 AE, grade 3 or 4 lab abnormalities, discontinuations due to AE, drug rash - Number and rate of HAE attacks - Durability of response (attack rate trend over time) - Number and proportion of days with angioedema symptoms - Patient-reported outcomes (HAE disease-specific AE-QoL questionnaire and TSQM global satisfaction scores)	Planned: 475 Actual: 227 110mg: 100 150mg: 127	Type I or II HAE patients ≥12 years	49 sites in 22 countries: US, EU, Israel, South Africa, Australia, South Korea, Hong Kong, New Zealand, Serbia, Switzerland, Macedonia
NCT# 03472040						
Phase 2 Dose-Ranging Study						
BCX7353-203	R, DB, PC	55, 110, 218 and 300 mg po QD	- Number of confirmed HAE attacks. - Pharmacokinetic and pharmacodynamic parameters	Randomized: 75 300mg: 18 218mg: 14 110mg: 14 55mg: 7 placebo: 22	Healthy Subjects ≥18 years	75 sites in 11 countries: EU, Switzerland, Macedonia, Australia
NCT# 02870972		28 days				

Source: Reviewer generated and adapted from Applicant provided Synopsis of Individual Studies (pages 13-15, 130-171) and Clinical Study Report (page 31, Table 6)

[†] Doses presented as free base equivalent

Abbreviations: AE, adverse event; DB, double-blind; DR, dose ranging; EU, European Union; HAE, hereditary angioedema; NDA, new drug application; OLE, open label, PC, placebo-controlled; PD, pharmacodynamics; PG, parallel group; PK, pharmacokinetics; PO, orally; QD, once daily; QoL, quality of life; R, randomized; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TSQM, treatment satisfaction score for medication

7.2. Review Strategy

Support for the efficacy and safety of berotralstat in the proposed indication is primarily based on a single, 24-week multicenter, randomized, double-blind, placebo-controlled, parallel-group trial in 120 adult and adolescent patients with type I or II HAE, Study BCX7353-302. The efficacy results from Study BCX7353-302 are presented in Section [8.1.1](#). The FDA biostatistician, Mingyu Xi, Ph.D. confirmed the Applicant's efficacy analyses and generated tables and figures for this review. For the evaluation of safety, FDA medical officer Shaz Siddiqi, M.D. analyzed data from Study BCX7353-302 Parts 1 and 2, as well as from the open-label extension (OLE) of Phase 2 study BCX7353-204 using CoreFit Data Analysis, JMP, JMP Clinical, JReview, MAED, Office of Computational Science (OCS) Analysis Studio, and the Demographic Tool in the OCS Analysis Toolbox. The safety results in Section [8.3](#) are from the analysis by the medical officer reviewer. For brevity, trials/studies may also be referred to in this review by the three-digit study number 302 or 204.

The intent-to-treat (ITT) population included all randomized subjects, irrespective of whether they received the study drug. This was the primary population used for the efficacy analyses as well as for reporting of demographic information and baseline disease characteristics. Data were analyzed according to randomized treatment.

The safety population included all subjects who received at least one dose of study drug. This population was used in the assessment and reporting of safety data.

8. Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study BCX7353-302 Protocol

Trial Design

Study BCX7353-302 (Figure 5) is a phase 3, randomized, double-blind, placebo-controlled, parallel-group, three-part study in adolescent and adult subjects 12 years of age and older with type I or II HAE. The schedule of assessments is shown in Table 22. The trial was multinational; most centers were in the United States with some in Europe and Canada. The primary objective of Part 1 of the study was to determine the safety and efficacy of prophylactic berotralstat 110 or 150 mg administered orally once daily for 24 weeks compared to placebo in subjects with HAE. The primary efficacy analysis (the rate of investigator-confirmed HAE attacks during dosing in the entire 24-week treatment period) was performed after the last subject completed Part 1. The primary objective of parts 2 and 3 of the study was to assess long term safety with chronic use of berotralstat.

To be eligible for randomization for Part 1, subjects had to experience two HAE attacks meeting the requirements outlined in the inclusion criteria. These two attacks needed to occur in the 14

to 56 day-long window starting at the date of the screening visit. The baseline attack rate included all qualifying attacks during the run-in period and up to the date of first dose.⁴ Parts 1, 2, and 3 were conducted in sequence, with Parts 2 and 3 conducted as continuous rollovers from Parts 1 and 2, respectively.

Part 1 (24-Week Blinded Evaluation of the Efficacy and Safety of Berotralstat)

The population of the single pivotal study comprised adolescent (12 to 17 years of age) and adult subjects (≥ 18 years of age). Eligible subjects were equally randomized to one of the following three treatment groups:

- Group 1: Berotralstat 110 mg administered orally once daily for 24 weeks
- Group 2: Berotralstat 150 mg administered orally once daily for 24 weeks
- Group 3: Placebo administered orally once daily for 24 weeks

Randomization into the treatment groups was stratified by the baseline HAE attack rate (≥ 2 versus < 2 attacks/month). Study visits in Part 1 occurred at screening, baseline, and weeks 2, 4, 8, 12, 18, and 24. The primary efficacy analysis occurred after the last subject completed their week-24 visit and included all data through week 24.

Part 2 (24-Week Blinded Evaluation of the Safety of Berotralstat)

Part 2 of the study began with administration of the study drug at the week-24 visit. Subjects in Groups 1 and 2 continued to receive the same berotralstat dose to which they were randomized in Part 1 of the study in a blinded manner. Subjects originally randomized to placebo in Group 3 underwent a second randomization to receive either 110 or 150 mg (1:1) in a blinded manner beginning at the week-24 visit through week 48. Study visits during Part 2 occurred during weeks 26, 28, 32, 36 and 48, with telephone contact at weeks 40 and 44. Efficacy and safety assessments continued throughout Part 2, including investigator confirmation of HAE. Interim safety analyses were conducted while Part 2 was ongoing.

Part 3 (48-Week Evaluation of the Safety of Open-Label Berotralstat)

Part 3 of the study began with the administration of the study drug at the week-48 visit and is ongoing. Subjects continued to receive the same berotralstat dose in Part 3 as in Part 2, albeit in an open-label manner. Study visits during Part 3 were scheduled to occur during weeks 60, 72, 84, and 96, with telephone contact at weeks 52, 56, 64, 68, 76, 80, 88, and 92. A final study follow-up visit was scheduled approximately 3 weeks following the last administration of the study drug (week 99). Investigator confirmation of attacks was not required in Part 3.

⁴ While the run-in period was 14-56 days, the period over which the baseline attack rate was calculated included the entire time between screening and start of study drug (i.e., run-in period + time between run-in period and 1st dose of study drug).

Assessment	Screening Period		Baseline	Part 1 Double-Blind, Placebo-Controlled Study Drug Administration					Week 24 Day 169 ^d
	Screening Visit ^{a,b} (up to Week -10)	Run-in Period ^a		Day 1 ^a	Week 2 ^c Day 15 ±2 days	Week 4 Day 29 ±2 days	Week 8 Day 57 ±2 days	Week 12 Day 85 ±2 days	
AE-QoL, TSQM and WPAI ^f			X		X	X	X	X	X
Concomitant medications	←-----→								
AEs	←-----→								
Randomization ⁱ			X						X
e-Diary instruction/review/ set-up ^u	X	X	X		X	X	X	X	X
e-Diary daily completion ^v	←-----→								
Study drug dosing ^w			←-----→						←-----→
Investigator confirmation of attacks ^x	←-----→								
Study drug accountability/ dispensing			X		X	X	X	X	X
Plasma for PK analysis ^y			X		X	X	X	X	X
Plasma for kallikrein inhibition ^z			X		X	X	X	X	X

Source: Applicant's Clinical Study Report for Study BCX7353-302 (Page 43, Table 3)

Abbreviations: AE = adverse event; AE-QoL = Angioedema Quality of Life; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; C1-INH = complement 1 esterase inh bitor; C3 = complement 3; C4 = complement 4; CK-MB = creatine kinase MB isoenzyme; ECG = electrocardiogram; eCRF = electronic case report form; e-diary = electronic diary; EQ-5D-5L = EuroQoL 5-dimensional, 5-level questionnaire; FSH = follicle stimulating hormone; GGT = gamma-glutamyl transpeptidase; HAE = hereditary angioedema; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HLA = human leukocyte antigen; IXRS = interactive (web or voice) response system; LFT = liver function test; LLN = lower limit of normal; NGAL = neutrophil gelatinase-associated lipocalin; PD = pharmacodynamic; PK = pharmacokinetic; QTcF = QT interval corrected by Fridericia's formula; TSQM = Treatment Satisfaction Questionnaire for Medication; WPAI = Work Productivity and Activity Impairment Questionnaire

Trial Population

The study population comprised adults (18 years or older) and adolescents (12 to 17 years of age) with a confirmed diagnosis of type I or II HAE and a minimum baseline attack rate of two investigator-confirmed attacks during the run-in period of 14 to 56 days from the screening visit.

Key Inclusion Criteria

- Males and nonpregnant, nonlactating females ≥12 years of age.
- Bodyweight of ≥40 kg.
- A confirmed diagnosis of type I or II HAE based on :
 A clinical diagnosis of HAE Type I or Type II, defined as having a C1-INH functional level below 50% and a complement 4 (C4) level below the lower limit of the normal (LLN) reference range, as assessed during the Screening period. In the absence of a low C4 value drawn during the intercritical period (ie, subject is not having an HAE attack), one of the following was acceptable to confirm the diagnosis of HAE: a SERPING-1 gene mutation known or likely to be associated with HAE type I or II assessed during the screening period; a confirmed family history of C1-INH deficiency; a C4 redrawn and retested during an attack in the screening period with the results below the LLN reference range. For subjects with C1-INH function ≥50% but less than the assay LLN, a SERPING-1 gene mutation known or likely to be associated with HAE type I or II, as assessed during the screening period OR a repeat C1-INH functional level <50% will be considered acceptable for enrollment.

4. The subject must have had at least two HAE attacks that met all of the requirements below during the run-in period of a maximum of 56 days from the screening visit:
 - a. The attacks were unique, defined as an attack that began at least 48 hours after the end of a previous attack.
 - b. The attacks must have been treated, required medical attention, or been documented to cause functional impairment based on information in the e-diary.
 - c. The attacks included symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may include symptoms in the oropharyngeal or abdominal region indicative of internal swelling.
 - d. The attacks were confirmed by the investigator to be HAE attacks. Subjects were contacted within 2 business days of the attack to discuss the attack and any queries on the data entered in the e-diary, as applicable.

Key Exclusion Criteria

1. Use of C1-INH for prophylaxis of HAE attacks within the 14 days prior to the screening visit or initiation during the study. Use of a C1-INH for treatment of acute attacks was not excluded at any time, nor was C1-INH for preprocedural prophylaxis for an unplanned/unforeseen procedure.
2. Use of androgens or tranexamic acid for prophylaxis of HAE attacks within the 28 days prior to the screening visit or initiation during the study.
3. Use of an angiotensin-converting enzyme inhibitor within 7 days of the baseline visit or planned initiation during the study.
4. Any significant cardiovascular disease or sudden cardiac death.
5. Laboratory abnormalities: Creatinine clearance ≤ 30 mL/min and aspartate aminotransferase (AST) or ALT level greater than three-fold the ULN.
6. Prior enrollment in a berotralstat study.
7. Pregnant, planning to become pregnant during the study, or nursing.

Key Subject Discontinuation Criteria

A subject was discontinued from the study drug for any of the following reasons, which was recorded in the source documents and the electronic case report form:

1. Emergence of any laboratory abnormality or AE that in the judgment of the investigator compromised the ability of the subject to continue study-specific procedures or altered the subject's benefit-risk profile such that it was considered not to be in the subject's best interest to continue.
2. Confirmed ALT or AST elevation as follows: greater than eight-fold the ULN; greater than five-fold the ULN for >2 weeks; greater than three-fold the ULN and a total bilirubin level greater than two-fold the ULN; and greater than three-fold the ULN if accompanied by fatigue, nausea, vomiting, right upper quadrant pain and tenderness, fever, rash and/or eosinophilia (>5%).
3. Subject noncompliance with the study drug or the study protocol.
4. A QTcF of >500 ms (confirmed on repeat ECG testing).

5. Subjects with a study drug-related grade 3 or 4 rash as described by the Division of Microbiology and Infectious Diseases (DMID) skin-mucocutaneous criteria were discontinued from the study and treated according to best medical practice.

In all cases, subjects who discontinued the study drug during Part 1 were asked to complete all regularly scheduled visits and procedures through week 24.

Reviewer comments: The Applicant's target population and overall study design was consistent with HAE programs for other approved products for prophylaxis. A similar patient population, primary endpoint, and treatment period to assess efficacy (26 weeks) were used in the recent lanadelumab program.

Although HAE is a rare disease, the development program for HAE prophylaxis is established and the primary clinical endpoint of HAE attacks is a meaningful, established endpoint. The randomized, controlled treatment period of 26 weeks is acceptable for this program. Most sites in the pivotal trial were located in the United States; therefore, the data should be reflective of the U.S. population of patients with HAE.

The inclusion, exclusion, and subject discontinuation criteria were also acceptable and consistent with prior studies of approved HAE products. Long-term prophylaxis of HAE is an individualized decision made between the patient and physician taking into account disease activity, frequency of attacks, quality of life, health-care resources, and ability to achieve adequate control with on-demand therapy. However, based on the minimum attack frequency, the eligibility criteria captured a target population likely to require prophylactic therapy.

Study Treatments and Blinding

- Berotralstat 110 mg orally once daily
- Berotralstat 150 mg orally once daily
- Placebo orally once daily

The study drug was blinded to both the subjects and study staff. With the exception of the week-2 and week-26 visits, subjects were instructed to bring all drug kits (including unused and used bottles) and e-diaries with them to each study visit. Compliance and adherence were reviewed at these visits. During study drug administration, subjects were to record in the e-diary the times of day the study drug was taken on a daily basis. Real-time access to e-diary entries for subjects was available via a website, and the investigator (or designee) proactively assessed compliance.

Concomitant Medications

Allowed

Therapies for coexisting conditions, treatment for acute HAE attacks (including C1-INH for acute-attack therapy, but not for long-term prophylaxis), treatments for short-term HAE prophylaxis, and therapies for any AEs.

Prohibited

Long-term prophylaxis for HAE (e.g., C1-INH, androgens, antifibrinolytics), angiotensin-converting enzyme inhibitors, estrogen-containing medications with systemic absorption, androgens, and any other investigational drug or device.

Study Endpoints

Primary Efficacy Endpoint

The primary endpoint for Part 1 is the rate of investigator-confirmed HAE attacks during dosing in the entire 24-week treatment period, defined as day 1 to the time of the first dose of study drug in Part 2 (day 1 to day 168).

Details of acute attacks of angioedema were recorded in an electronic diary (e-diary) beginning at screening and continuing through week 48. Subjects were required to complete the HAE attack e-diary daily, noting whether or not symptoms of an HAE attack were experienced in the previous 24 hours. Subjects were required to complete the e-diary irrespective of the presence of HAE symptoms. If a subject did report an attack, additional details about the attack were required to be entered into the e-diary, including the start and stop times, symptoms, anatomical location of swelling (if applicable), severity, treatment(s) administered and the times of administration, and whether additional medical care was sought for the attack. Attack severity was assessed by the subject as negligible, mild, moderate, or severe. Attacks were treated in accordance with the subject's normal standard of care. Medications for angioedema attacks were not provided by the Applicant.

For all attacks that occurred from screening through the end of Part 1, the investigator (or designee) reviewed the e-diary record of the attack. Subjects were contacted within approximately 2 business days of the end of the attack to discuss its clinical characteristics, any questions the investigator had on the entered data or to gain additional information not included in the e-diary that the investigator deemed important for clinical evaluation of the event. This information, in conjunction with the e-diary record, was used by the investigator to verify or reject the event recorded in the e-diary as an HAE attack. Investigators used their judgment to confirm or reject a reported event as an HAE attack. All investigator-confirmed attacks of HAE must have included symptoms of swelling irrespective of treatment. In addition to visible swelling, symptoms in the oropharyngeal or abdominal regions indicative of internal swelling were also included. Investigator-confirmed attacks were entered into the AE database as mild, moderate, or severe. Safety and tolerability were evaluated by assessments of AEs, laboratory parameters (clinical chemistry, hematology, and urinalysis), vital signs, ECGs, and physical examinations.

Secondary Efficacy Endpoint

Three secondary efficacy endpoints were specified in the Statistical Analysis Plan:

- Change from baseline in the Angioedema Quality of Life Questionnaire (AE-QoL) total score at week 24.
- Number and proportion of days with angioedema symptoms through 24 weeks.

- Rate of investigator-confirmed HAE attacks during dosing in the effective treatment period, defined as beginning on day 8 through to the first dose of study drug in Part 2.

Quality-of-life data were obtained using the Angioedema Quality of Life Questionnaire (AE-QoL) and Treatment Satisfaction Questionnaire for Medications (TSQM)⁵ at baseline and subsequently at weeks 4, 8, 12, 18, and 24 (Table 22).

The AE-QoL is an AE-specific, quality-of-life questionnaire used in the clinical setting and captured in some prior HAE clinical trials. The AE-QoL consists of 17 questions with five answers each and has a recall period of 4 weeks. A total score can be computed over all questions, as in Study 302 Part 1, but it is also possible to apply the AE-QoL as a profile instrument by grouping the questions into four domains (*Functioning, Fatigue/Mood, Fears/Shame, and Nutrition*) and calculating the individual domain scores. These are transformed to a linear 0 to 100 scale, with higher scores indicative of greater QoL impairment (0 best, 100 worst). Therefore, based on the scoring algorithm a decrease in score should correspond to improvement in QoL (Section 16.4). The 14-item TSQM is used in clinical practice to assess patients' satisfaction with medication. It comprises scores on four scales: side effects, effectiveness, convenience, and global satisfaction.

Exploratory Efficacy Endpoints

Three exploratory efficacy endpoints were specified in the Statistical Analysis Plan:

- Number and proportion of subjects with no attacks over 24 weeks.
- Use of HAE attack medications over 24 weeks.
- The proportion of responders to study drug, defined as at least a 50% relative reduction in the rate of investigator-confirmed attacks.

Reviewer comments: A reduction in the frequency of acute HAE attacks is a clinically meaningful outcome for HAE patients and therefore acceptable as a primary endpoint for an HAE clinical trial intended to demonstrate efficacy for a product indicated for prophylactic treatment.

Investigator-confirmed attacks (rather than patient-reported attacks) have been used as the primary efficacy endpoint in other HAE programs to ensure that only swelling attacks and not prodromal symptoms or pre-emptive rescue medication use are counted as a distinct HAE attack. Secondary endpoints primarily assessed outcomes related to HAE attack reduction.

Endpoints derived from the AE-QoL instrument are not considered well-defined and reliable assessments of HAE-related quality of life for use by the clinical division in HAE prophylaxis trials or fit for purpose for labeling. While its use in recurrent angioedema patients has been described in the literature, this includes patients with recurrent histamine or bradykinin-mediated angioedema. Furthermore, the recall period is relatively long at 4 weeks, and the tool contains domains related to nutrition and mood that may not be specific to HAE. Within person

⁵ Bharmal, M, K Payne, MJ Atkinson, MP Desrosiers, DE Morisky, and E Gemmen, 2009, Validation of an abbreviated Treatment Satisfaction Questionnaire for Medication (TSQM-9) among patients on antihypertensive medications, Health Qual Life Outcomes, 7:36.

clinically meaningful change has not been established for the AE-QoL or derived endpoints; therefore, the clinical meaningfulness of any results are uncertain.

Safety Assessments

Safety was evaluated by assessments of AEs, laboratory parameters (clinical chemistry, hematology, urinalysis, coagulation, drug screen, and additional tests), vital signs, ECGs, and physical examinations according to the schedule in [Table 22](#). Furthermore, should a pregnant woman have received blinded study drug, the outcome of the pregnancy was to be followed.

Statistical Analysis Plan

Statistical Methods

The efficacy analyses were conducted on the ITT population, defined as all randomized subjects, regardless of whether study drug was administered. The primary endpoint was the rate of investigator-confirmed HAE attacks during dosing in the entire treatment period of Part 1, expressed as attacks per month (1 month was defined as 28 days). Comparisons of the rate of investigator-confirmed HAE attacks between each berotralstat dose and placebo during the entire dosing period for Part 1 was conducted using a Poisson regression model. The number of investigator-confirmed attacks was included as the dependent variable, the treatment was included as a fixed effect, the baseline attack rate was included as a covariate, and the logarithm of duration on treatment was included as an offset variable. The estimated monthly rate of attacks for each treatment group and the treatment differences expressed as attack rate ratios (berotralstat over placebo rate ratio) and their associated 95% confidence intervals (CIs) were obtained from the Poisson regression model.

Analyses of Secondary Endpoints for Part 1

The AE-QoL total score change from baseline was analyzed using a mixed model for repeated measures with fixed effects for treatment, baseline attack rate, baseline AE-QoL, visit, a visit-by-treatment interaction, and a random effect for subject. The estimated treatment difference for each berotralstat dose versus placebo at each visit, the 95% CI, and the associated p-value were calculated. Primary inferences were drawn from treatment differences for the changes from baseline estimated from the mixed model for repeated measures model at week 24.

The number and proportion of days with angioedema symptoms through week 24 were computed. The proportion of days with angioedema symptoms through week 24 was evaluated using an analysis of covariance model with baseline attack rate as a covariate and treatment included as a fixed effect. The estimated treatment difference comparing each active treatment to placebo, the 95% CI, and the associated p-value were calculated.

For investigator-confirmed HAE attacks during the effective treatment period, between-treatment comparisons were performed using a Poisson regression model similar to that used for the primary endpoint analysis.

The Statistical Analysis Plan specified that for all analyses using the Poisson regression model, goodness of fit will be evaluated by examining the dispersion parameter. In addition, the

Applicant proposed to analyze the data using a negative binomial model to evaluate the dispersion parameter. If the dispersion parameter of the negative binomial model was significantly greater than zero, the negative binomial model was to be considered primary.

Multiplicity Control

A combination of hierarchical testing and the Hochberg step-up procedure was used to control the Type I error rate for testing the primary and secondary endpoints in Part 1. Four endpoints were evaluated, for each of which two potential doses were tested against placebo. The four endpoints were tested in a hierarchical fashion, and the two doses were tested using the Hochberg step-up procedure at each level of the hierarchy to which both doses progress.

The first endpoint to be tested was the primary endpoint, the rate of investigator-confirmed HAE attacks during dosing in the entire 24-week treatment period of Part 1. Using the Hochberg step-up procedure, each of the two doses was tested at the $\alpha=0.05$ level, comparing active treatment to placebo. If the maximum of the two p-values was <0.05 , the null hypotheses of no difference between the rate of attacks for subjects on active and placebo treatment was rejected for both doses and testing proceeded to the next endpoint in the hierarchy with $\alpha=0.05$. If the maximum of the two p-values was >0.05 but the minimum was <0.025 , the null hypothesis for the dose with $p<0.025$ was rejected and testing for that dose only proceeded to the next endpoint in the hierarchy with $\alpha=0.025$. Otherwise, the null hypotheses for both doses were not rejected, testing stopped, and the next endpoint in the hierarchy could not be tested.

The first, second, third, and fourth endpoints in the hierarchy are:

1. The rate of investigator-confirmed HAE attacks during dosing in the entire 24-week treatment period (day 1 to day 168).
2. Change from baseline in the total AE-QoL score at week 24.
3. Number and proportion of days with angioedema symptoms through 24 weeks.
4. Rate of investigator-confirmed HAE attacks during dosing in the effective treatment period (beginning on day 8 through 24 weeks).

The process described above was continued for each endpoint in the hierarchy until either all four endpoints had been tested or testing was stopped due to nonrejection of the null hypotheses for both doses for endpoints earlier in the hierarchy. At each level of the hierarchy, the Hochberg step-up procedure was used to control the Type I error rate if two doses were tested. Otherwise, if only one dose was tested, the single test was conducted with $\alpha=0.025$.

Subgroup Analysis

For the primary endpoint, subgroup analyses were conducted by the following categories:

- Age (12 to 17, 18 to 64, and ≥ 65 years)
- Gender (male and female)
- Race (white and other)
- Region (North America and Europe)

Missing Data Sensitivity Analysis

Missing data sensitivity analyses were conducted for the analysis of the primary endpoint using three methods:

1. A missing data sensitivity analysis was conducted in which observed data post-study drug discontinuation were included in the attack-rate determination.
2. A missing data sensitivity analysis was conducted in which observed data post-study drug discontinuation were used in combination with imputed data for subjects who discontinued study drug prior to the end of Part 1 and did not continue to provide attack information.
3. A tipping-point analysis was conducted in which observed data post-study drug discontinuation were combined with imputed data for subjects who discontinue study drug prior to the end of Part 1 and did not continue to provide attack information by varying assumptions for missingness.

The analyses of safety data for Parts 1, 2, and 3 and of effectiveness data for Parts 2 and 3 were primarily descriptive.

Protocol Amendments

The Applicant amended the protocol once, making the following key changes:

Amendment 1 (October 11, 2018)

- Removed participating study centers in the Asia–Pacific region.
- Added objectives and endpoints for Study 302 Part 3.
- Clarified the diagnosis of HAE, which allowed patients with C1-INH function $\geq 50\%$ but lower than the lower limit of normal (LLN) to be included in study.
- Provide updated information on the berotralstat clinical program.

8.1.2. Study BCX7353-302 Part 1 Results

Data Quality and Integrity

The Applicant conducted clinical studies according to procedures that incorporate the ethical principles of good clinical practices (GCP). BioCryst also undertook a GCP audit program. Audits were performed by auditors from (b) (4) who operated independently of the study monitors. The audits within the clinical program were aimed at study documentation, investigator sites, and clinical study reports. A total of 10 site audits was performed through April 10, 2019 and audit certificates were provided. No issues were identified.

Compliance with GCP

The Applicant states that the study was conducted in accordance with GCP as described in the guidelines of the ICH. The study protocol, amendments, informed consent, and other necessary documents were reviewed and approved by an independent ethics committee or institutional review board before initiation of the study. Written informed consent and assent, where applicable, were obtained from each subject before participating in the study.

Financial Disclosure

The Applicant has adequately disclosed its financial interests and arrangements with clinical investigators as recommended in the guidance for industry entitled Financial Disclosure by Clinical Investigators ([Financial Disclosure](#)).

Subject Disposition

Overall, a total of 160 subjects were screened, 121 subjects were randomized (ITT population), and 120 of these subjects (99%) were treated (safety population). Most screening failures were caused by normal C1-INH and/or C4 results and an insufficient number of baseline HAE attacks during the run-in period. Subject (b) (6) was randomized to placebo but not treated due to withdrawal of consent; this subject was included in the ITT population but not the safety population. A total of 108 subjects (89%) completed study drug dosing in Part 1: 37 subjects who received berotralstat 110 mg (90%), 37 who received berotralstat 150 mg (93%), and 34 who received placebo (85%). No subject was lost to follow up. [Table 23](#) shows the subject disposition.

Table 23. Subject Disposition: Study 302 Part 1

	Berotralstat			Total N=121
	110 mg N=41	150 mg N=40	Placebo N=40	
Subjects randomized (ITT population)	41 (100%)	40 (100%)	40 (100%)	121 (100%)
Subjects randomized, not dosed, and withdrawn from study	0	0	1 (2.5%)	1 (0.8%)
Safety population (received ≥1 dose of study drug)	41 (100%)	40 (100%)	39 (97.5%)	120 (99.2%)
Early study drug discontinuation	4 (9.8%)	3 (7.5%)	5 (12.5%)	12 (9.9%)
Lab abnormality or AE	3 (7.3%)	1 (2.5%)	1 (2.5%)	5 (4.1%)
Perceived lack of efficacy	1 (2.4%)	1 (2.5%)	2 (5.0%)	4 (3.3%)
Subject withdrew consent	0	1 (2.5%)	1 (2.5%)	2 (1.7%)
Other	0	0	1 (2.5%)	1 (0.8%)
Early study drug discontinuation with study completion	3 (7.3%)	1 (2.5%)	2 (5.0%)	6 (5.0%)
Completed treatment	37 (90.2%)	37 (92.5%)	34 (85.0%)	108 (89.3%)
Completed Part 1 study assessments (completers population)	40 (97.6%)	38 (95.0%)	36 (90.0%)	114 (94.2%)

Source: Adapted from the Applicant's Clinical Study Report (Page 96, Table 9); confirmed by the FDA statistical reviewer.
 Abbreviations: AE, adverse event; ITT, intent-to-treat

Protocol Violations/Deviations

A total of four subjects had major protocol deviations that resulted in exclusion from the per protocol population:

- Berotralstat 110 mg group:
 - Subject (b) (6) received a prohibited concomitant medication (desogestrel) that may have affected the HAE attack rate.

- Berotralstat 150 mg group:
 - Subject (b) (6) was informed of the run-in qualifying attack requirement during the screening period. This subject did meet the run-in attack rate requirement, but the protocol specified that the run-in attack rate should not be disclosed to study subjects; therefore, the sponsor listed as a protocol deviation.
- Placebo group:
 - Subject (b) (6) was randomized to placebo and was dispensed study drug prior to completion of all baseline procedures. The subject was never dosed with study drug. The subject withdrew consent and discontinued the study on May 31, 2018.
 - Subject (b) (6) received a prohibited concomitant medication (prophylactic Berinert).

Although these major protocol deviations were unlikely to have affected the overall quality or interpretation of the study data, two of the four patients continued in the trial despite receiving adjunct and prohibited HAE therapies that may have improved their treatment response. In view of the small total sample size, there was concern this could be significant. However, an efficacy analysis of the ITT population yielded robust results whether these two patients were included or excluded from the ITT population. Numerous other minor protocol deviations occurred; these were related to the timing of study visits, timing of procedures, and one ECG assessment that showed a PR interval longer than recommended for entry. The extended PR interval was deemed not to be clinically meaningful by the site principal investigator and cardiologist.

Demographic Characteristics

The demographic characteristics of the subjects are shown in [Table 24](#), and were generally similar across treatment arms. Overall, the study enrolled more females than males. The proportion of females was higher in the berotralstat 110 mg group (73%) than in the berotralstat 150 mg group (57%). Relatively small proportions of the subjects were in the youngest (12 to 17 years) and oldest (≥ 65 years) age groups. The majority of subjects (93% across treatment arms) were white. Most subjects (72%) were from North America with over 60% of that population from the United States. The remainder were from various European countries. Also, there was a total of six adolescent patients enrolled, which is relatively few, even for this orphan population. However, this may be expected because symptoms tend to present for the first time in puberty and patients are typically diagnosed several years after initial presentation.

Reviewer comment: Although HAE affects males and females equally, the literature reports a clear female predominance, most likely because women tend to be more symptomatic than men as a result of hormonal factors such as puberty, contraception, and pregnancy. Therefore, the predominantly female study population does not raise concerns about the generalizability of the study but is merely a reflection of the most symptomatic patients, who are most likely to use and benefit from prophylactic treatment. All ethnicities and races are affected by types I and II HAE; however, most of the extant epidemiologic and genetic data are derived from European

populations. The interaction between race and disease expression is poorly understood. The proportion of nonwhite patients in this study, while relatively small, is adequate for such a rare disease. The proportion of adolescent subjects in this study is small (N=6); however, the pathophysiology of the disease is unaffected by age.

Table 24. Demographic Characteristics (ITT Population): Study 302 Part 1

Characteristic	Berotralstat		Placebo N=40	Total N=121
	110 mg N=41	150 mg N=40		
Age at time of consent (years)				
N	41	40	40	121
Mean (SD)	40.4 (17.5)	40.0 (14.0)	44.5 (14.1)	41.6 (15.3)
Median	39.0	39.0	43.5	40.0
Range	12 to 74	12 to 69	14 to 72	12 to 74
Age group, n (%)				
12-17 years	2 (4.9%)	2 (5.0%)	2 (5.0%)	6 (5.0%)
18-64 years	34 (82.9%)	37 (92.5%)	35 (87.5%)	106 (87.6%)
≥65 years	5 (12.2%)	1 (2.5%)	3 (7.5%)	9 (7.4%)
Sex, n (%)				
Male	11 (26.8%)	17 (42.5%)	13 (32.5%)	41 (33.9%)
Female	30 (73.2%)	23 (57.5%)	27 (67.5%)	80 (66.1%)
Race, n (%)				
Asian	1 (2.4%)	0	0	1 (0.8%)
Black or African American	2 (4.9%)	1 (2.5%)	2 (5.0%)	5 (4.1%)
White	38 (92.7%)	38 (95.0%)	37 (92.5%)	113 (93.4%)
Other	0	1 (2.5%)	1 (2.5%)	2 (1.7%)
Ethnicity, n (%)				
Hispanic or Latino	0	2 (5.0%)	1 (2.5%)	3 (2.5%)
Not Hispanic or Latino	39 (95.1%)	38 (95.0%)	38 (95.0%)	115 (95.0%)
Not reported	2 (4.9%)	0	1 (2.5%)	3 (2.5%)
Region, n (%)				
North America	32 (78.0%)	27 (67.5%)	28 (70.0%)	87 (71.9%)
Europe	9 (22.0%)	13 (32.5%)	12 (30%)	34 (28.1%)
Weight at screening, kg				
N	41	40	39	120
Mean (SD)	78.75 (21.5)	87.62 (20.4)	84.87 (21.4)	83.70 (21.2)
Median	72.50	82.05	77.00	78.96
Range	52.6 to 136.7	57.5 to 149.7	57.3 to 134.7	52.6 to 149.7
BMI at screening, kg/m²				
N	41	40	39	120
Mean (SD)	27.50 (7.3)	30.35 (6.7)	29.29 (6.8)	29.03 (7.0)
Median	25.6	28.6	27.8	27.5
Range	18.5 to 44.9	21.0 to 54.1	19.9 to 51.0	18.5 to 54.1

Source: Applicant's Clinical Study Report (Page 103, Table 13); confirmed by the FDA statistical reviewer.

Abbreviations: BMI, body mass index; ITT, intent to treat; N, number of subjects; n, number of subjects who experienced the event; SD, standard deviation.

Other Baseline Characteristics (e.g., Disease Characteristics, Important Concomitant Drugs)

The baseline disease characteristics were generally similar across treatment groups (Table 25). The vast majority of patients had HAE diagnosis confirmed by a low C4 and <50% functional level of C1-inhibitor (88%). The Applicant did not provide data to categorize subjects as having

type I or II HAE; however, low C4 and low functional C1-INH are pathognomonic for types I and II HAE. Type III HAE is exceedingly rare and characterized by normal complement studies. Based on prevalence data, the vast majority of subjects (~85%) are expected to have type I disease. Further, phenotypically types I and II HAE are indistinguishable and respond similarly to HAE treatments. SERPING-1 gene mutations were only evaluated if a patient had a normal C4 level and despite a clinical history suggestive of type I or II HAE. The time between screening and first dose of study drug was similar between treatment groups. The mean baseline attack rate per month was also similar among the treatment groups, with an average of approximately three attacks per month. Subjects reported the primary location of previous HAE attacks as a combination of abdominal and peripheral attacks. All of the treatment groups had on average about two laryngeal attacks per year. The study population reflects a population with predominantly moderate-to-severe HAE and a fairly equal disease burden among treatment groups in terms of the frequency of attacks and attack location; including laryngeal involvement. Patients were most likely to be on prior prophylactic therapy with androgens, followed by C1 inhibitor replacement. This was consistent in all three treatment groups. Overall, the study population was representative of a population of patients with HAE for which long-term prophylaxis with an oral kallikrein inhibitor would be appropriate.

Reviewer comment: The baseline attack rate was calculated from the start of the run-in period to the date of first dose of study drug. Once subjects experienced 2 or more attacks during the 14 to 56-day run-in period, they were randomized into the study, but may not have received the first dose of study drug right away. Some subjects had a longer time interval between the end of run-in and first dose of study drug (up to 16 days); given the variability of disease, these subjects may not have experienced any further attacks during this time period. Because the baseline attack rate was calculated from the run-in period to the first day of study drug, some subjects had a baseline attack rate < 1 attack per month; this scenario was most apparent in subjects with a long run-in period and lengthier delay between run-in and start of study drug.

Table 25. Baseline Disease Characteristics (ITT Population): Study 302 Part 1

Summary	Berotralstat			Total N=121
	110 mg N=41	150 mg N=40	Placebo N=40	
Diagnosis Confirmed				
C4 < LLN + C1-INH function < 50%	34 (82.9%)	35 (87.5%)	38 (95.0%)	107 (88.4%)
C4 < LLN + C1-INH function 50% to 74% + SERPING-1 mutation	0	1 (2.5%) ¹	0	1 (0.8%)
C4 ≥ LLN + C1-INH function < 50% + family history				
C4 ≥ LLN + C1-INH function < 50% + SERPING-1 mutation	4 (9.8%)	3 (7.5%)	1 (2.5%)	8 (6.6%)
C4 ≥ LLN + C1-INH function 50% to 74% + SERPING-1 mutation	3 (7.3%)	0	1 (2.5%)	4 (3.3%)
C4 ≥ LLN + C1-INH function 50% to 74% + SERPING-1 mutation	0	1 (2.5%) ¹	0	1 (0.8%)
Baseline investigator-confirmed attack rate (attacks/month)²				
N	41	40	39 ³	120 ³
Mean (SD)	2.97 (1.4)	3.06 (1.6)	2.91 (1.1)	2.98 (1.4)
Median	2.90	2.70	3.00	2.90
Range	0.9 to 6.1	0.9 to 6.7	1.3 to 6.2	0.9 to 6.7
Estimated number of laryngeal attacks in the past year				
N	10	9	17	36
Mean (SD)	2.0 (1.5)	2.2 (2.4)	2.4 (1.7)	2.2 (1.8)
Median	1	1	2	1
Range	1-5	1-8	1-7	1-8
Categorized baseline attack rate: n, (%)				
≥ 2 attacks/month	28 (68.3%)	30 (75.0%)	27 (67.5%)	85 (70.2%)
< 2 attacks/month	13 (31.7%)	10 (25.0%)	12 (30.0%)	35 (28.9%)
Number of days in the period between screening and first dose of study drug				
N	41	40	39 ³	120 ³
Mean (SD)	41.3 (14.2)	41.8 (15.2)	39.5 (12.5)	40.9 (13.9)
Median	36.0	36.5	36.0	36.0
Range	17 to 71	15 to 72	20 to 66	15 to 72

Source: Applicant's Clinical Study Report (Pages 105-106,112 and Tables 14,15,18); confirmed by the FDA statistical reviewer.

¹ Two subjects had levels C1-INH function levels > 50% at screening but C1-INH Ag levels were < 10% at baseline (Subjects (b) (6))

² Baseline investigator-confirmed attack rate was defined as (total number of investigator-confirmed HAE attacks experienced in the period between screening and first date/time of study drug) × 28/(date of first dose - date of screening + 1).

³ Subject (b) (6) was randomized but did not receive study drug. As this subject did not receive drug, the subject had no baseline calculations.

Abbreviations: Ag, antigen; C4, complement 4; C1-INH, complement 1 esterase inhibitor; HAE, hereditary angioedema; ITT, intent to treat; LLN, lower limit of normal; N, number of subjects; HAE, hereditary angioedema; ITT, intent to treat; IXRS, interactive (web or voice) response system; N, number; n, number of subjects who experienced the event; SD, standard deviation

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The rate of treatment compliance was high and fairly equal across all treatment groups, with an average total mean compliance rate of 98% (Table 26). Subjects were not allowed to take concomitant prophylactic HAE medications; however, they were allowed rescue medications for an HAE attack that occurred during the study. Rescue medications were not supplied by the Applicant.

Table 26. Summary of Treatment Compliance (Safety Population): Study 302 Part 1

Compliance Summary	Berotralstat		Placebo N=39	Total N=120
	110 mg N=41	150 mg N=40		
Overall, Part 1				
N	41	40	39	120
Mean (SD)	97.8% (4.6)	98.5% (3.9)	97.1% (7.5)	97.8% (5.5)
Median	98.8%	99.4%	99.4%	99.4%
Range	74.0% to 103.0%	85.0% to 108.8%	67.6% to 107.1%	67.6% to 108.8%
Compliance category, n (%)				
<80%	1 (2.4%)	0	2 (5.1%)	3 (2.5%)
80% to <90%	1 (2.4%)	2 (5.0%)	3 (7.7%)	6 (5.0%)
90% to 110%	39 (95.1%)	38 (95.0%)	34 (87.2%)	111 (92.5%)
>110%	0	0	0	0

Source: Applicant's table from the clinical study report (page 124, Table 25), verified by the Clinical Reviewer.
Abbreviation: SD, standard deviation

Efficacy Results—Primary Endpoint

[Table 27](#) lists the investigator-confirmed HAE attacks that occurred during Part 1 in the ITT population.

Table 27. Summary of HAE Attacks (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo N=40
	110 mg N=41	150 mg N=40	
Investigator-confirmed attacks			
Number of subjects with ≥1 investigator-confirmed attack, n (%)	40 (97.6%)	37 (92.5%)	38 (95.0%)
Total number of investigator-confirmed attacks	443	357	508
Number of subjects with ≥1 TIC attack, n (%)	38 (92.7%)	32 (80.0%)	37 (92.5%)
Total number of TIC attacks (% of TIC attacks)	385 (86.9%)	311 (87.1%)	449 (88.4%)
Number of subjects with ≥1 untreated investigator-confirmed attack, n (%)	15 (36.6%)	15 (37.5%)	19 (47.5%)

Source: Excerpted from the Applicant's clinical study report (page 128, Table 28)
Abbreviations: HAE, hereditary angioedema; ITT, intent-to-treat; TIC, treated investigator-confirmed

The total number of investigator-confirmed attacks was lower in the 150 mg group than the 110mg dose, and berotralstat-treated subjects had fewer total attacks than subjects treated with placebo. Numerically, the proportion of subjects with one or more HAE attacks requiring treatment with rescue medication was lowest in the 150 mg group. Notably, however, very few subjects in the study achieved complete elimination of attacks during the treatment period: one (2%), three (8%), and two (5%) subjects in the 110 mg, 150 mg, and placebo groups, respectively.

Analysis of the HAE attacks during the 24-week treatment period confirmed that the primary endpoint was met for both dose levels, with the 110 and 150 mg doses reducing the HAE attack rate by 30% (p=0.024) and 44% (p<0.001), respectively, relative to placebo. The negative binomial regression analysis-estimated attack rate per 28 days was 1.65 for berotralstat 110 mg

subjects, 1.31 for berotralstat 150 mg subjects, and 2.35 for placebo subjects over the 24-week Part 1 dosing period. [Table 28](#) presents the analysis results of the primary endpoint.

Table 28. Primary Endpoint of Investigator-Confirmed Attack Rate During the Entire Dosing Period (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo (N=40)
	110 mg N=41	150 mg N=40	
Estimated rate per 28 days	1.65	1.31	2.35
Attack rate ratio (relative to placebo) (95% CI)	0.70 (0.51, 0.95)	0.56 (0.41, 0.77)	Reference
p-Value	0.024	<0.001	Reference
Rate reduction (relative to placebo): % (95% CI)	30.0 (4.6, 48.7)	44.2 (23.0, 59.5)	Reference

Source: FDA Statistical Reviewer

Abbreviations: CI, confidence interval; ITT, intent-to-treat

Note: Statistical analysis was based on a negative binomial regression model. The number of investigator-confirmed attacks was included as the dependent variable, the treatment was included as a fixed effect, baseline investigator-confirmed attack rate was included as a covariate, and the logarithm of duration on treatment was included as an offset variable.

The Applicant reported that 10 to 15% of subject-reported HAE attacks were not confirmed by the investigator either due to absence of swelling symptoms or investigator judgment. However, rejection rates were similar across treatment groups, and the Applicant's supplementary analysis of subject-reported attacks showed that the rate ratios and p-values were similar to those from the primary endpoint analysis.

In an ad hoc analysis of the rate of investigator-confirmed HAE attacks rated as moderate or severe by the subject during Part 1, the 110 and 150 mg berotralstat doses reduced the rate of moderate and severe HAE attacks by 10% (p=0.608) and 40% (p=0.018), respectively, compared with placebo.

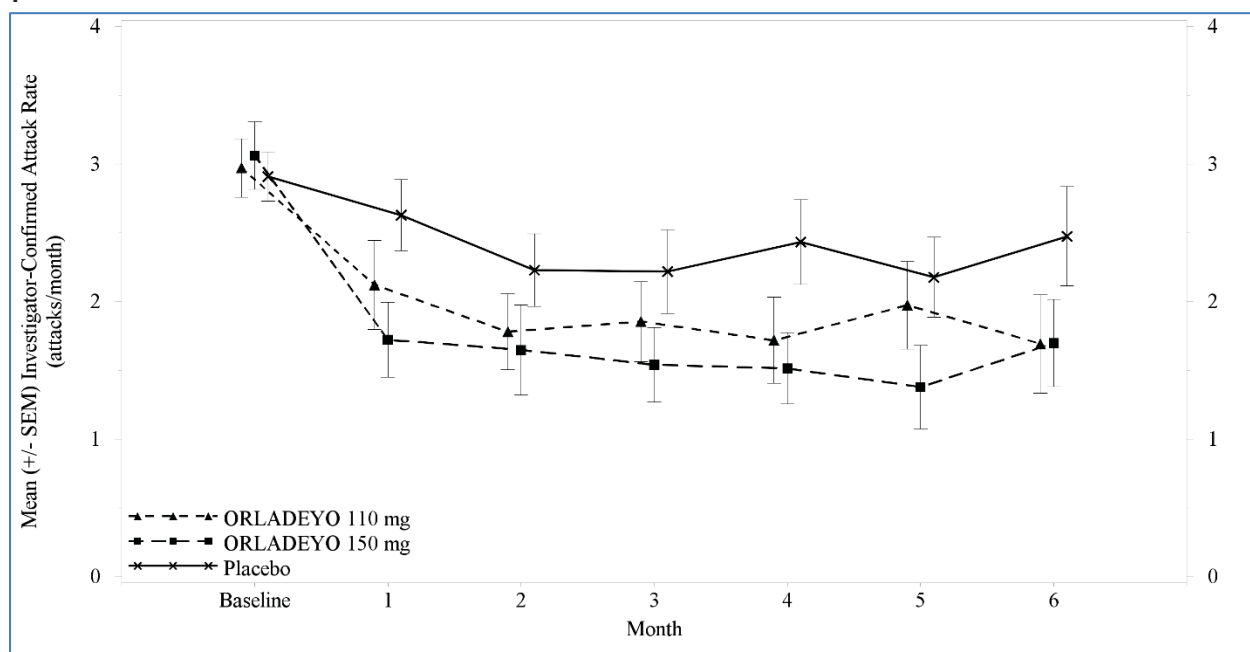
Dose/Dose Response

Subjects receiving the 150 mg dose had a moderately higher reduction in the HAE attack rate compared to those receiving the 110 mg dose. Attack rate ratio between 150 mg and 110 mg doses is 0.80 with 95% CI (0.58, 1.10). The dose response is preserved in the evaluation of secondary and exploratory endpoints, which also demonstrate numerically better outcomes with the 150 mg dose compared to the 110 mg dose. Within the development program, there were more treatment discontinuations due to lack of perceived efficacy in the 110 mg group compared to the 150 mg group, albeit this was mainly seen in the open label studies. Overall, the evidence supports the Applicant's recommended dose of 150 mg once daily.

Durability of Response

Figure 6 presents the mean investigator-confirmed attack rate by month. Berotralstat reduced the attack rate in month 1 and the effect was stable over the 6-month duration of Part 1.

Figure 6. Mean ($\pm$ SEM) HAE Attack Rate/Month Through 24 Weeks (ITT Population): Study 302 Part 1



Source: Excerpted from the Applicant's labeling (Figure 2, page 11).
Abbreviations: ITT, intent-to-treat; SEM, standard error of the mean

Persistence of Effect

Patients in this study continued the study drug in an open-label fashion in Part 3. Because the effect of berotralstat was not evaluated after cessation of study drug, no data are available to analyze the persistence of its effect.

Efficacy Results—Secondary or Exploratory Clinical Outcome Assessment (Patient-Reported Outcome) Endpoints

Change from Baseline in AE-QoL Total Score at Week 24

[Table 29](#) shows the analysis of the secondary endpoint of AE-QoL score at week 24. The least-squares mean (LSM) differences from placebo in AE-QoL total score were -2.8 (95% CI $-10.1, 4.5$; $p=0.453$) and -4.9 (95% CI $-12.2, 2.4$; $p=0.188$) for the berotralstat 110 and 150 mg groups, respectively. The treatment effect of berotralstat compared to placebo was not statistically significant for either dose group. Given that the AE-QoL total score did not significantly improve with berotralstat treatment, the individual components and domains were not analyzed. Because this endpoint failed to reach statistical significance and the Applicant did not seek a labeling claim for AE-QoL, the Division of Clinical Outcome Assessments was not consulted to evaluate this tool during the NDA review.

Table 29. Secondary Endpoint AE-QoL Total Score at Week 24 (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo N=40
	110 mg N=41	150 mg N=40	
N	40	38	36
LSM AE-QoL total score (SE)	-12.5 (2.5)	-14.6 (2.6)	-9.7 (2.6)
LSM difference from placebo (95% CI)	-2.8 (-10.1, 4.5)	-4.9 (-12.2, 2.4)	Reference
p-Value	0.453	0.188	Reference

Source: FDA Statistical Reviewer

Abbreviations: AE-QoL, angioedema quality of life; CI, confidence interval; ITT, intent-to-treat; LSM, least-squares mean; SE, standard error

Number and Proportion of Days with Angioedema Symptoms to 24 Weeks

[Table 30](#) lists the proportion of days with angioedema symptoms up to 24 weeks.

Table 30. Secondary Endpoint of Number and Proportion of Days with Angioedema Symptoms From Investigator-Confirmed Attacks (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo N=40
	110 mg N=41	150 mg N=40	
N	41	40	39
LSM Proportion of days with angioedema symptoms from investigator-confirmed attacks (SE)	0.13 (0.02)	0.12 (0.02)	0.20 (0.02)
LSM Difference from placebo (95% CI)	-0.06 (-0.12, -0.01)	-0.08 (-0.13, -0.02)	Reference
p-Value	0.025	0.006	Reference

Source: FDA Statistical Reviewer

Abbreviations: CI, confidence interval; ITT, intent to treat; LSM, least-squares mean; SE, standard error

The proportion of days on which subjects had angioedema symptoms from investigator-confirmed attacks were 0.13, 0.12, and 0.20 over the 24-week treatment period for the berotralstat 110 mg, berotralstat 150 mg, and placebo groups, respectively. The differences from the placebo in the proportion of the 169 days of treatment with angioedema symptoms were -0.06 (95% CI -0.12, -0.01; nominal p=0.025; approximately 10 more symptom-free days over the 6 months of Part 1) and -0.08 (95% CI -0.13, -0.02; nominal p=0.006; about 13 more symptom-free days over the 6 months of Part 1) for the berotralstat 110 and 150 mg groups, respectively.

Rate of Investigator-Confirmed HAE Attacks During Dosing in the Effective Treatment Period

[Table 31](#) shows the analysis of the rate of investigator-confirmed HAE attacks during dosing in the effective treatment period. The rate of investigator-confirmed attacks for the effective treatment period is an indication of the efficacy of active treatment after berotralstat had reached steady-state concentration. The reduction in attack rate relative to the placebo group was 30% (95% CI 4, 49; nominal p=0.026) and 47% (95% CI 26, 62; nominal p<0.001) for the berotralstat 110 and 150 mg groups, respectively.

Table 31. Secondary Endpoint of Investigator-Confirmed Attack Rate During the Effective Treatment Period (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo N=40
	110 mg N=41	150 mg N=40	
Estimated rate per 28 days	1.65	1.27	2.38
Attack rate ratio (relative to placebo) (95% CI)	0.70 (0.51, 0.95)	0.54 (0.39, 0.74)	Reference
p-Value	0.026	<0.001	Reference
Rate reduction (relative to placebo): % (95% CI)	30 (4, 49)	47 (26, 62)	Reference

Source: FDA Statistical Reviewer
 Abbreviations: CI, confidence interval; ITT, intent-to-treat

The first secondary endpoint of AE-QoL score did not reach statistical significance, halting the hierarchical testing of the other two secondary endpoints.

Additional Analyses Conducted on the Individual Trials

Exploratory Efficacy Analysis

One of the exploratory efficacy endpoints was the proportion of responders to study drug, defined as at least a 50% relative reduction in the rate of investigator-confirmed HAE attacks during treatment compared with the baseline attack rate. The analysis of this endpoint is presented in [Table 32](#). For this exploratory endpoint, 58% of patients receiving 150 mg berotralstat and 51% of those receiving 110 mg berotralstat had a ≥50% reduction in their HAE attack rate compared to baseline versus 25% of patients receiving placebo.

The post hoc analyses of the proportion of responders to study drug, defined as at least 70% and 90% relative reductions in the rate of investigator-confirmed HAE attacks during treatment compared with the baseline attack rate, were also considered. The analyses of these endpoints are also listed in [Table 32](#).

In post hoc analyses, 50% and 23% of patients receiving 150 mg berotralstat, and 27% and 10% of those receiving 110 mg berotralstat, had a ≥70% or ≥90% reduction in HAE attack rate compared to baseline versus 15% and 8% of patients receiving placebo, respectively. While berotralstat demonstrated significant improvements in investigator-confirmed attack rates compared to placebo, these responses suggest that very few subjects experienced complete/near complete elimination of attacks. These responder analysis results should be interpreted with caution because the reduction of attack rate relative to the baseline rate could be potentially highly variable due to the limited baseline observation period.

Table 32. Relative Reduction From Baseline in the Adjusted Investigator-Confirmed Attack Rate (ITT Population): Study 302 Part 1

Parameter	Berotralstat		Placebo N=40
	110 mg N=41	150 mg N=40	
N	41	40	39
Subjects with ≥50% relative reduction			
Yes	21 (51.2%)	23 (57.5%)	10 (25.0%)
Odds ratio (95% CI)	3.0 (1.2, 7.8)	3.9 (1.5, 10.2)	Reference
p-Value	0.025	0.006	Reference
Subjects with ≥70% relative reduction			
Yes	11 (26.8%)	20 (50.0%)	6 (15.0%)
Odds ratio (95% CI)	2.0 (0.7, 6.2)	5.6 (1.9, 16.5)	Reference
p-Value	0.212	0.002	Reference
Subjects with ≥90% relative reduction			
Yes	4 (9.8%)	9 (22.5%)	3 (7.5%)
Odds ratio (95% CI)	1.3 (0.3, 6.3)	3.6 (0.9, 14.7)	Reference
p-Value	0.748	0.073	Reference

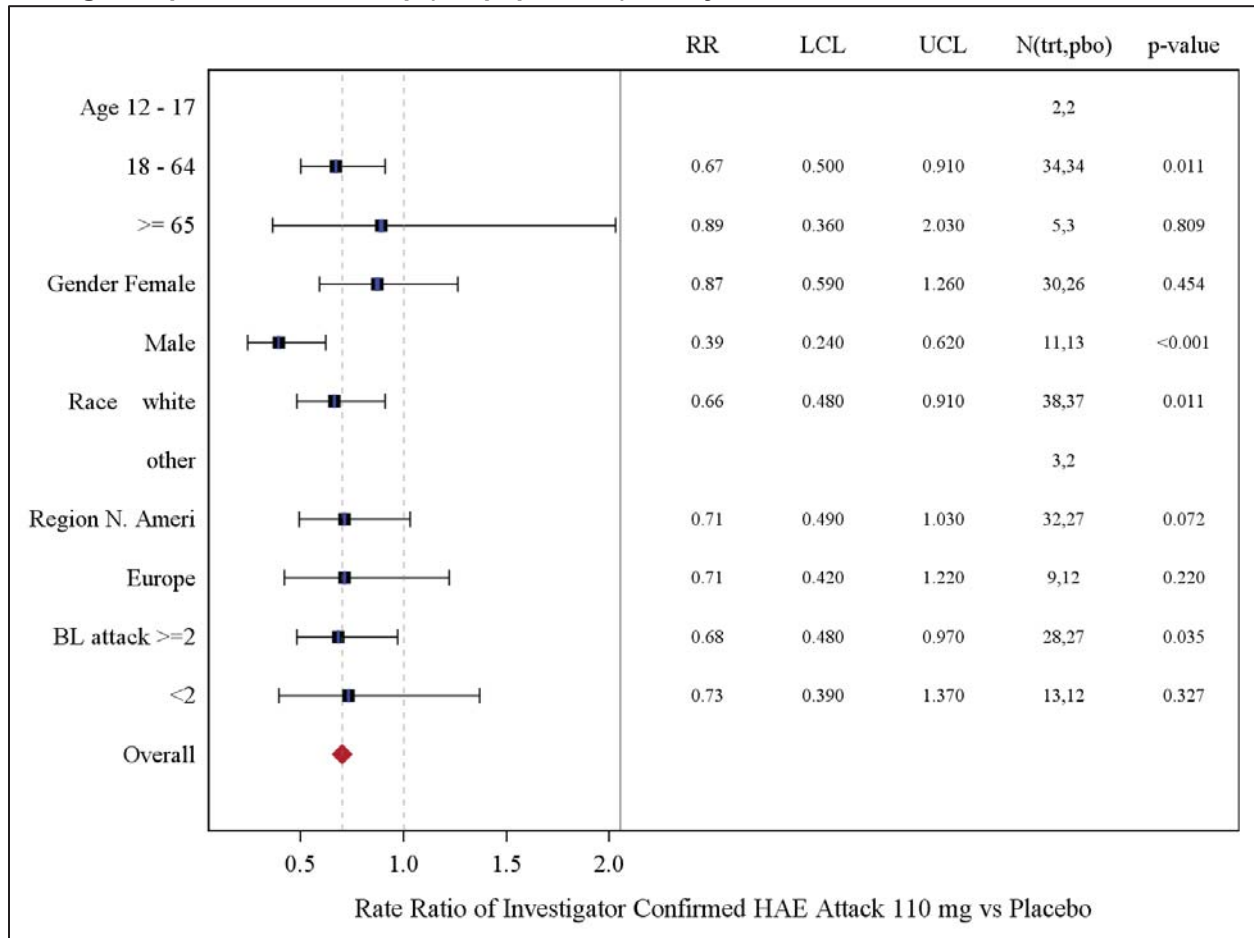
Source: Excerpted from the Applicant's clinical study report (Table 39, page 157).
 Abbreviations: CI, confidence interval; ITT, intent-to-treat; SD, standard deviation

Subgroup Analysis

To examine whether the treatment effects vary by baseline demographic factors, such as age, gender, race, or region, we conducted subgroup analyses on the primary endpoint for these four factors. These subgroup analyses used the same analysis model used in the overall analyses.

Figure 7 presents the subgroup analysis results of primary endpoint investigator-confirmed HAE attack rate comparing the 110 mg group to the placebo group. Figure 8 presents the subgroup analysis results comparing the 150 mg group to the placebo group. Subgroup analyses showed that the treatment effect was consistent with the overall effect across all categories, with the exception of 3 subgroups whose sample sizes were too small to allow adequate interpretation (adolescents [n=6], elderly [n=9], and other race [n=8]). Due to the small number of subjects in those subgroups, model based analysis could not be completed or the results were not reliable. We left fields of these subgroups blank on the graphs and only reported the number of subjects in each group. Because HAE is caused by genetic mutations leading to low levels of functional C1-INH, the pathophysiology of the disease is the same irrespective of age. Even though the number of adolescents evaluated in the pivotal trial was small, efficacy in adolescents is expected to be similar to that in adults and may be extrapolated from the adult data.

Figure 7. Subgroup Analysis of the Investigator-Confirmed HAE Attack Rate Comparing the 110 mg Group vs Placebo Group (ITT population): Study 302 Part 1

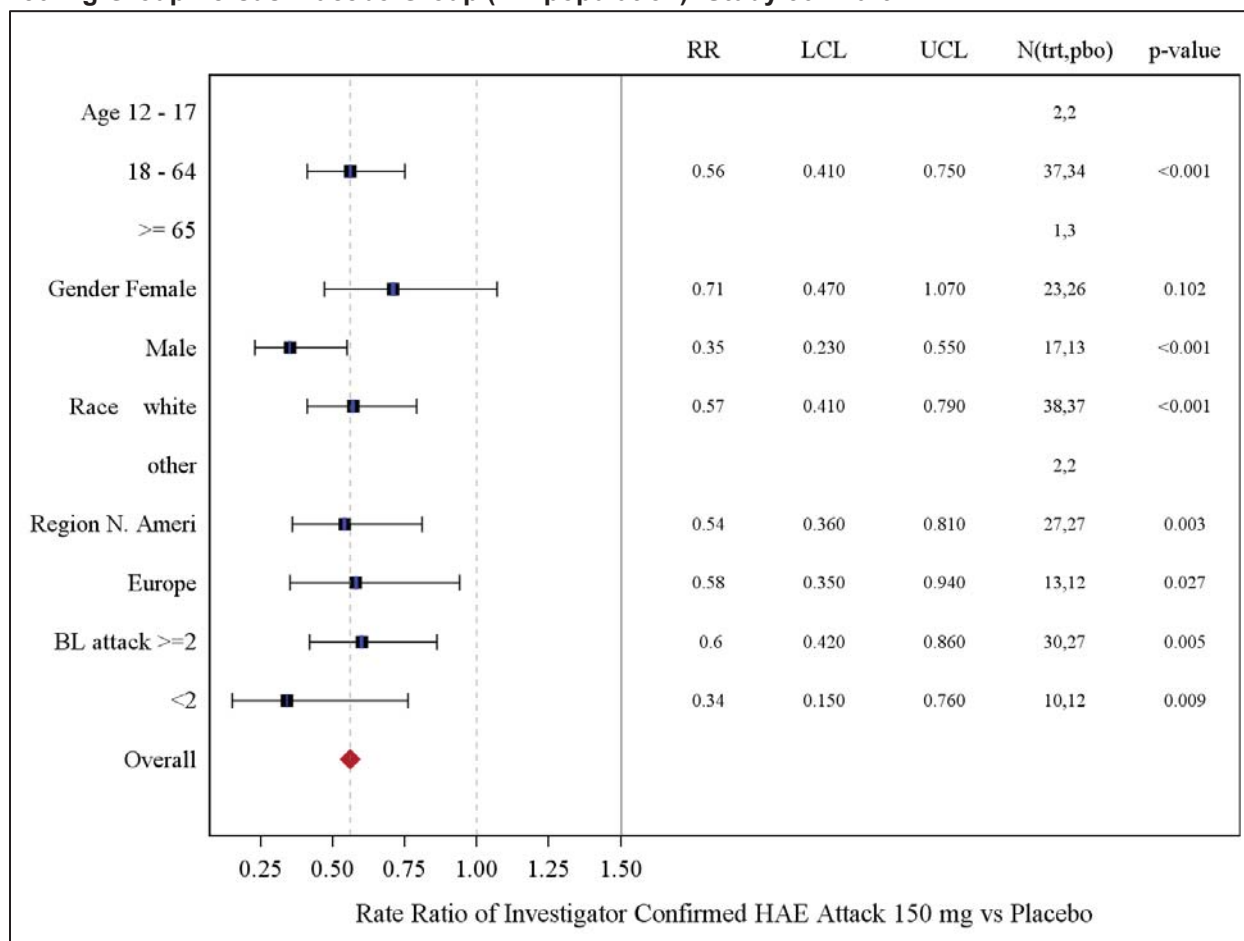


Source: FDA Statistical Reviewer

Note: Due to extremely small number of subjects in subgroups of adolescents and other race, model-based estimate of rate ratio and CIs are considered unreliable and misleading. Therefore, they are not presented in the plot.

Abbreviations: BL attack, baseline number of attacks per month ; LCL, lower confidence limit; N. Ameri, North America; pbo, placebo group; RR, rate ratio; trt, treatment group; UCL, upper confidence limit

Figure 8. Subgroup Analysis of the Investigator-Confirmed HAE Attack Rate Comparing the 150 mg Group Versus Placebo Group (ITT population): Study 302 Part 1



Source: FDA Statistical Reviewer

Note: Due to extremely small number of subjects in subgroups of adolescents, elderly and other race, model-based estimate of rate ratio and CIs are considered unreliable and misleading. Therefore they are not presented in the plot.

Abbreviations: BL attack, baseline number of attacks per month; LCL, lower confidence limit; N. Ameri, North America; pbo, placebo group; RR, rate ratio; trt, treatment group; UCL, upper confidence limit

Missing Data Sensitivity Analysis

The Applicant conducted three missing data sensitivity analyses on the primary endpoint. The first missing data sensitivity analysis included observed data post-treatment discontinuation. The analysis was supportive of the primary endpoint for both dose levels, with the 110 and 150 mg doses reducing the HAE attack rate by 31% (95% CI 6, 49; p=0.020) and 42% (95% CI 20, 58; p<0.001), respectively, versus placebo. The negative binomial analysis-estimated attack rate per 28 days was 1.60 for berotralstat 110 mg subjects, 1.34 for berotralstat 150 mg subjects, and 2.31 for placebo subjects over the 24-week Part 1 dosing period.

The second missing data sensitivity analysis included observed data post-treatment discontinuation and imputing missing data post-treatment discontinuation. The analysis was supportive of the primary endpoint for both dose levels, with the 110 and 150 mg doses reducing the HAE attack rate by 31% (95% CI 5, 50; p=0.023) and 35% (95% CI: 9, 53; p=0.011), respectively, versus placebo. The negative binomial analysis-estimated attack rate per 28 days

was 1.64 for berotralstat 110 mg subjects, 1.55 for berotralstat 150 mg subjects, and 2.37 for placebo subjects over the 24-week Part 1 dosing period.

The third missing data sensitivity analysis was the tipping point analysis on the primary endpoint. The analysis showed that three and two-fold increase in expected attack rates for the 110 and 150 mg doses (attack rates of 5.0 and 2.6, respectively) would be needed for the missing data to alter the result of the analysis. The expected attack rate at week 24 for the 100 and 150 mg groups was 1.65 and 1.31, respectively. Therefore, it is highly unlikely that the conclusion would be altered by the missing data.

Overall, the missing data sensitivity analyses of both doses of berotralstat were supportive of the robustness of the outcomes of the primary analysis.

8.2. Statistical Issues

8.2.1. Substantial Evidence of Effectiveness

The effectiveness of berotralstat was studied in a single ongoing Study 302 based on its Part 1 results. The study met its primary endpoint; the 110 and 150 mg doses of berotralstat reducing the rate of HAE attacks by 30% ($p=0.024$) and 44% ($p<0.001$) relative to placebo, respectively.

The effectiveness of berotralstat was also evaluated in terms of the secondary endpoints of change from baseline in the AE-QoL, number and proportion of days with angioedema symptoms, and rate of investigator-confirmed HAE attacks during the effective treatment period. Testing of the first secondary endpoint, a patient-reported outcome in the hierarchy, failed for both doses, halting formal efficacy evaluation on the remaining secondary endpoints. Of note, in an exploratory analysis, the trend favoring berotralstat in the proportion of patients with at least a 50% reduction in HAE attacks compared to baseline lends support to the primary findings.

The study achieved clinical and statistical significance on the primary endpoint. Endpoints derived from similar data (e.g., investigator confirmed attack rate during the effective treatment period, proportion of days with angioedema symptoms in Part 1) also achieved nominal clinical and statistical significance. There were no statistically significant secondary endpoints after adjusting for multiple testing using the Hochberg procedure. Considering the rarity of the disease, the current unmet need and the robustness of the primary endpoint analysis, the statistical reviewer concludes that substantial evidence of effectiveness was met.

8.2.2. Multiplicity Control

The study hypothesis testing combined hierarchical testing and the Hochberg step-up procedure. Testing of primary and secondary endpoints proceeded in hierarchical fashion, and two doses at each level were tested using the Hochberg step-up procedure. Table 33 summarizes the results of the hierarchical testing. Note that there were no statistically significant secondary endpoints after adjusting for multiple testing.

Table 33. Summary of Hierarchical Testing for Efficacy Endpoints (ITT Population): Study 302 Part 1

Parameter	Berotralstat 110 mg			Berotralstat 150 mg		
	Unadjusted P-value	Hochberg Adjusted α Level	Statistically Significant	Unadjusted P-value	Hochberg Adjusted α Level	Statistically Significant
Investigator-confirmed attack rate during entire dosing period in Part 1	0.024	0.05	Yes	<0.001	0.05	Yes
AE-QoL total score–CFB to week 24	0.453	0.05	No	0.188	0.025	No
Proportion of days with angioedema symptoms in Part 1	0.025	N/A	No	0.006	N/A	No
Investigator-confirmed attack rate during effective treatment period in Part 1	0.026	N/A	No	<0.001	N/A	No

Source: FDA Statistical Reviewer

Abbreviations: AE-QoL, angioedema quality of life; CFB, change from baseline; ITT, intent-to-treat; N/A, not applicable

8.2.3. Estimand

The primary analyses were conducted on the ITT population but included only observed on-treatment data. This is an on-treatment estimand according to the ICH E9 R1 guidance. The Applicant also conducted an analysis of all collected data, including the data collected after treatment discontinuation. The results based on this treatment-policy estimand were consistent with those based on the on-treatment estimand.

8.2.4. Missing Data and Sensitivity Analysis

Table 34 presents the number of subjects who completed study treatment in Part 1. One subject in the 110 mg group, two subjects in the 150 mg group, and four subjects in the placebo group discontinued the study treatment early. Of them, one in the 110 mg group, one in the 150 mg group, and two in the placebo group were due to lack of efficacy.

Table 34: Overall Subject Disposition: Study 302 Part 1

Summary	Berotralstat 110 mg n=41	Berotralstat 150 mg n=40	Placebo n=40	All Subjects n=121
Subjects randomized (ITT population)	41 (100%)	40 (100%)	40 (100%)	121 (100%)
Subjects withdrawn from study in Part 1	1 (2.4%)	2 (5.0%)	4 (10.0%)	7 (5.8%)
Subject withdrew consent	0	2 (5.0%)	2 (5.0%)	4 (3.3%)
Other	1 (2.4%)	0	2 (5.0%)	3 (2.5%)
Subjects with early study drug discontinuation	4 (9.8%)	3 (7.5%)	5 (12.5%)	12 (9.9%)
Laboratory abnormality or AE	3 (7.3%)	1 (2.5%)	1 (2.5%)	5 (4.1%)
Perceived lack of efficacy	1 (2.4%)	1 (2.5%)	1 (2.5%)	4 (3.3%)
Subject withdrew consent	0	1 (2.5%)	1 (2.5%)	2 (1.7%)
Other	0	0	1 (2.5%)	1 (0.8%)

Source: FDA statistical Reviewer
 Abbreviations: AE, adverse event; ITT, intent to treat

The Applicant conducted a missing data sensitivity analysis using three approaches. The first approach used all collected data including the data after treatment discontinuation but without imputation, the second approach used the same data as in the first approach plus imputation for the remaining missing data, and the third approach used all collected data plus imputed data based on the tipping point analysis method. All three analyses supported the conclusion from the primary analysis.

The sensitivity analyses of both doses of berotralstat were supportive of the robustness of the primary analysis outcomes.

Overall, the statistical reviewer of this NDA concludes that the efficacy of both doses of berotralstat was demonstrated. The statistical reviewer’s recommended regulatory action for this application is approval.

8.3. Review of Safety

8.3.1. Safety Review Approach

All clinical studies conducted as part of the berotralstat development program were evaluated for safety; however, given the short exposure periods and absence of additional safety findings in the phase 1 and shorter 2 studies, this safety review focuses on Part 1 of the 24-week placebo-controlled phase 3 trial, Study 302. Supportive data for safety were derived from the analysis of interim data from Part 2 of Studies 302 and 204, discussed in Section 8.3.4, Additional Safety Assessments and Section 8.3.7, Special Safety Studies, respectively. The review tools used to conduct independent reviewer analyses were MAED, JMP Clinical, JMP, JReview, OCS Analysis Studio, and the Demographic Tool in the OCS Analysis Toolbox.

8.3.2. Review of the Safety Database

Overall Exposure

The population of subjects exposed to berotralstat in Study 302 and Study 204 for type I or II HAE is outlined in Table 35. The table includes exposure from Part 2 and Part 3 of Study 302

(data cutoff 12/1/19). Overall, 218 subjects have been exposed to berotralstat 110 and/or 150 mg for ≥48 weeks (337 days), and 63 subjects have been exposed for ≥72 weeks. At the time of NDA submission, 103 subjects had received 150 mg berotralstat for ≥48 weeks—73 subjects from Study 204 and 30 subjects from Study 302. With the 120-day safety update, 123 subjects had been exposed to the 150 mg dose for at least 1 year.

Table 35: Overall Exposure in Phase 2 and 3 Long-Term HAE Prophylaxis Studies (Safety Population): Study 204 and 302

Parameter	Berotralstat		Total	Placebo
	110 mg to 150 mg	150 mg		
Duration of study drug dosing (days)				
N	158	223	381	39
Mean (SD)	350.9 (164.7)	316.4 (202)	330.7 (188)	154.8 (36.5)
Median	410.0	374.0	398.0	168.0
Minimum, maximum	14, 628	6, 643	6, 643	23, 168
1 day	0	0	0	0
2-7 days	0	1 (0.4%)	1 (0.3%)	0
>7-28 days	6 (3.8%)	29 (13.0%)	35 (9.2%)	1 (2.6%)
>28-168 days	23 (14.6%)	40 (17.9%)	63 (16.5%)	38 (97.4%)
>168-336 days	34 (21.5%)	30 (13.5%)	64 (16.8%)	0
>336-504 days	73 (46.2%)	82 (36.8%)	155 (40.7%)	0
>504-672 days	22 (13.9%)	41 (18.4%)	63 (16.5%)	0
>672 days	0	0	0	0

Source: Applicant's 120-Day Safety Update Report (page 19, Table 2)
Abbreviations: HAE, hereditary angioedema; SD, standard deviation

Adequacy of the Safety Database

A total of 15 clinical studies have been completed in the berotralstat HAE clinical development program and 6 studies are ongoing. As of April 22, 2020, a cumulative total of 1104 subjects have been enrolled in berotralstat studies. Among them, 1030 subjects received berotralstat and 165 subjects received placebo, with 91 placebo subjects also receiving berotralstat in crossover studies.

Study 302 included 120 adult and adolescent patients with HAE, 81 of whom received at least one dose of berotralstat during the controlled treatment period. A total of 227 subjects received open-label berotralstat in the OLE Study 204. A total of 16 adolescent patients in the two trials were exposed to the study drug.

Overall, the safety database is of sufficient size and duration for a rare disease such as HAE to assess the safety of the proposed dose of berotralstat when taken chronically. Of note, the safety database exceeds the Division's recommendation for 1 year exposure in at least 100 subjects. Study 302 Part 3 and the OLE Study 204 are ongoing to provide further long-term safety data.

8.3.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

Overall, no data quality issues were identified in the review of this NDA. OSI audits of sites 33 and 35 from Study 302 did not reveal any issues related to data integrity. As noted earlier in this review, our initial review of the ADaM datasets for pivotal Study 302 Part 1, showed that the record sequence numbers for the ADVS and ADLB datasets were missing. An IR was sent on February 12, 2020 to resubmit the ADVS and ADLB datasets for Study 302 Part 1, Study 204, the NDA analysis, and the ISS with the appropriate sequence number identifiers included. A data analysis was performed on these new datasets, which were received on February 20, 2020. Since the record sequence numbers did not change the numerical and non-numerical data, the analysis was identical to the safety reports generated previously, and did not impact the integrity of the data.

Categorization of Adverse Events

The definitions of AEs and SAEs in the protocols were consistent with 21 CFR 312.32. Reports of all AEs and SAEs, regardless of investigator attribution, were collected from the time of signing of informed consent to the last study visit. Treatment-emergent adverse events (TEAEs) were defined as any AE that increased in severity or that was newly developed at or after the first dose of study drug through the final follow-up visit. AEs were coded using the Medical Dictionary for Regulatory Activities version 19.1.

All AEs were assessed (graded) for severity and classified using the DMID criteria for grading AEs (published November 2007). Any AEs not covered by the DMID criteria were assessed and classified into one of four clearly defined categories as follows: mild (1), moderate (2), severe (3), and life-threatening (4). The Applicant's coding of verbatim terms to preferred terms (PTs) was appropriate. The investigator or medically qualified designee also reviewed each AE to make a determination of its relationship to the study drug using the following categories: not related, unlikely, possibly related, probably related, and definitely related.

Routine Clinical Tests

Laboratory tests were performed at time points specified in the schedule of assessments for Studies 302. Only clinically significant abnormalities (as determined by the investigator) in laboratory values, physical examination findings, or vital signs were reported as AEs.

8.3.4. Safety

Controlled study drug exposure is shown in [Table 36](#). Drug exposure was fairly uniform across all three treatment groups, with an average mean exposure of 159 days for all subjects. The total exposure period for Part 1 of Study 302 was 168 days (24 weeks). There was adequate drug exposure during the study to determine the treatment effect and perform a safety analysis.

Table 36. Summary of Treatment Exposure (Safety Population): Study 302 Part 1

Exposure Outcome	Berotralstat			Placebo N=39
	110 mg N=41	150 mg N=40	All Active N=81	
Duration of exposure (days)				
Mean (SD)	161 (22.5)	157 (40.3)	159 (32.4)	155 (36.5)
Median	168	168	168	168
Range	81-171	14-170	14-171	23-168
Duration of exposure, n (%)				
≤84 days (≤12 weeks)	2 (4.9%)	3 (7.5%)	5 (6.2%)	3 (7.7%)
85 to 168 days (>12 to ≤24 weeks)	36 (87.8%)	34 (85.0%)	70 (86.4%)	36 (92.3%)
169 to 252 days (>24 to ≤36 weeks)	3 (7.3%)	3 (7.5%)	6 (7.4%)	0

Source: Applicant table from the clinical study report (page 189, Table 52), verified by the reviewer.
 Abbreviations: N, number of subjects; n, number of subjects who experienced the event; SD, standard deviation

The safety results for Study BCX7353-302 Part 1 are listed in Table 37. There were more overall TEAEs in the berotralstat groups than the placebo group (84% versus 77%). Grade 3 or 4 TEAEs and TEAEs leading to discontinuation of study drug were more frequent in the berotralstat 110 mg group. GI TEAEs were more common in the berotralstat 150 mg group compared to the berotralstat 110 mg group or the placebo group.

Table 37. Overall Summary of TEAEs (Safety Population): Study 302 Part 1

TEAE Summary, n (%)	Berotralstat			Placebo N=39
	110 mg N=41	150 mg N=40	All Active N=81	
Any TEAE	34 (83%)	34 (85%)	68 (84%)	30 (77%)
Any SAE	1 (2%)	0	1 (1%)	3 (8%)
Any grade 3 or 4 TEAE	5 (12%)	1 (3%)	6 (7%)	4 (10%)
Any TEAE leading to discontinuation of study drug	3 (7%)	1 (3%)	4 (5%)	1 (3%)
Any GI abdominal TEAE	17 (42%)	20 (50%)	37 (46%)	14 (36%)
Any GI abdominal TEAE leading to discontinuation of study drug	1 (2%)	0	1 (1%)	0

Source: Generated by the reviewer in JReview using ADSL and ADAE datasets where SAFFL=Y, TRTEMFL=Y, ATOXGRN=3 or 4, AESER=Y

Abbreviations: GI, gastrointestinal; SAE, serious adverse event; TEAE, treatment-emergent adverse event

Deaths

No deaths were reported in Study 302 Part 1.

Serious Adverse Events

In Study 302, there were four patients with SAEs ([Table 38](#)). Three of these occurred in the placebo group (pneumonia, leiomyoma, and transient ischemic attack/diverticular intestinal hemorrhage in the same patient). One patient in the berotralstat 110 mg group developed myeloma. A retrospective review revealed an elevated serum protein level in this subject at initial intake prior to receiving berotralstat; therefore, myeloma was suspected to have been present prior to study drug administration. This SAE led to treatment discontinuation. There were no SAEs in the 150 mg berotralstat group, which is the Applicant's recommended dose for patients without hepatic impairment. The total number of SAEs was small, even for this

relatively small safety population (N=120). In addition, most SAEs occurred in the placebo group, and no SAE was attributed to berotralstat.

Table 38. Nonfatal Treatment-Emergent SAEs by SOC and PT (Safety Population): Study 302 Part 1

Parameter, n (%)	Berotralstat		Placebo N=39	Total N=120
	110 mg N=41	150 mg N=40		
Subjects with at least one SAE	1 (2.4%)	0	3 (7.7%)	4 (3.3%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (2.4%)	0	1 (2.6%)	2 (1.7%)
Plasma cell myeloma	1 (2.4%)	0	0	1 (0.8%)
Uterine leiomyoma	0	0	1 (2.6%)	1 (0.8%)
Infections and infestations	0	0	1 (2.6%)	1 (0.8%)
Pneumonia	0	0	1 (2.6%)	1 (0.8%)
Nervous system disorders	0	0	1 (2.6%)	1 (0.8%)
Transient ischemic attack	0	0	1 (2.6%)	1 (0.8%)
Gastrointestinal disorders	0	0	1 (2.6%)	1 (0.8%)
Diverticulum intestinal hemorrhagic	0	0	1 (2.6%)	1 (0.8%)

Source: Reviewer generated the table in JReview using the ADAE datasets where SAFFL=Y, TRTEMFL=Y, and AESER=Y
 Abbreviations: SAE, serious adverse event; SOC, system organ class; PT, preferred term

Dropouts and/or Discontinuations Due to AEs

Five subjects (<1%) in Study 302 prematurely discontinued treatment due to an AE, as shown in [Table 39](#) and summarized in more detail below.

Table 39. Discontinuations Due to TEAEs (Safety Population): Study 302 Part 1

USUBJID	Treatment Arm	Age (Years)	Sex	Ethnicity	System Organ Class	Preferred Term	SAE
BCX7353-302- (b) (6)	110 mg	74	F	White	Skin and subcutaneous tissue disorders	Purpura	No
BCX7353-302- (b) (6)	110 mg	45	F	Black	Neoplasms benign, malignant and unspecified (including cysts and polyps)	Plasma cell myeloma	Yes
BCX7353-302- (b) (6)	110 mg	56	M	White	Gastrointestinal disorders	Dyspepsia	No
BCX7353-302- (b) (6)	150 mg	39	M	White	Investigations	Liver function test abnormal	No
BCX7353-302- (b) (6)	Placebo	49	M	White	Psychiatric disorders	Depression	No

Source: Reviewer generated the table in JMP using the ADSL and ADAE datasets where AEDISC=Y, AESER=Y/N and variables AGE, SEX, RACE

Abbreviations: F, female; M, male; SAE, serious adverse event, TEAE, treatment-emergent adverse event; USUBJID, unique subject identification

Berotralstat 150 mg

Subject (b) (6) developed a grade 3 ALT elevation (238 U/L) on day 14 which progressed to grade 4 elevation (430 U/L) on day 22. The subject stopped study drug on day 17 but continued in the study. He remained asymptomatic and without other laboratory abnormalities. His ALT level normalized by day 57 (30 U/L). However, on Study Day 127, he developed a grade 2 ALT elevation which persisted as a Grade 1 elevation at the end of the study on day 169 (grade 1). He had a history of androgen use from 2013 to 2017, but a year since the most recent exposure. He also had normal liver enzyme levels at baseline.

Reviewer comment: In view of the of the subject's liver enzyme re-elevation despite earlier cessation of study drug and normalization of his ALT level, he likely had another hepatic disorder contributing to his abnormal liver enzymes. This may or may not have been accentuated by berotralstat. He did not have any reported history of alcohol or illicit drug use. He was taking cetirizine, azelastine, and fluticasone for nasal allergies.

Berotralstat 110 mg

Subject (b) (6) discontinued study drug after 3 months of therapy due to dyspepsia, nausea and vomiting of grade 2 severity. Symptoms began on day 5 of treatment, but resolved 3 days after stopping study drug. He was also taking hydrocodone, esomeprazole and calcium as the time.

Reviewer comment: Although patient was on medication which may cause gastrointestinal distress (hydrocodone) and taking anti-reflux medication, the timing of symptom onset soon after drug initiation and prompt resolution after drug cessation, make it likely symptoms were due to berotralstat.

Subject (b) (6) discontinued study after developing a purpuric rash of grade 3 severity which was suspected by the investigator to be related to study drug. The rash began on day 78 of treatment originally affecting both arms, resolved without treatment in 14 days, then recurred 3 more times with involvement to both legs. Berotralstat was stopped on day 138; however, the rash was still present as of day 188. A skin biopsy did not show any evidence of leukocytoclastic vasculitis. She had no evidence of coagulopathy on labwork and was not taking any medications which would promote easy bruisability.

Reviewer comment: Upon further review of detailed analysis of photographs provided by the Applicant, the clinical reviewer assessment is that the purpuric rash is unrelated to treatment. This was based on the morphology and distribution of the rash, its waxing and waning without steroid treatment, and persistence of the rash despite stopping the study drug 3 months prior.

Subject (b) (6) was diagnosed with grade 4 multiple myeloma on day 80. This event was categorized as an SAE and led to treatment discontinuation. It was noted that laboratory results included persistently elevated serum protein levels prior to study drug exposure and preceding the onset of the event: 10.0 g/dL at study screening, 9.7 g/dL at study baseline, 10.1 g/dL at study week 4), and 10.1 at study week 8 (normal range: 6.0 to 8.0 g/dL). Consequently, a serum

protein electrophoresis test revealed gammopathy, and the diagnosis of plasma cell myeloma was confirmed by the hematology-oncology consultant.

Reviewer comment: In view of the elevated serum protein at baseline, the clinical reviewer agrees with the Applicant's assessment that myeloma was likely already present in early stages at start of study entry and therefore un-related to berotralstat exposure.

Significant Adverse Events

Ten subjects reported 11 TEAEs of grade 3 or 4 severity (Table 40), several of which were discussed under SAEs or TEAEs leading to treatment discontinuation. Nearly half of these events occurred in the placebo group, and only one event (back pain) occurred in the 150 mg group. Other than abdominal pain, all severe AEs were single occurrences. No new safety signals were identified based on the analysis of severe TEAEs.

Table 40. Grade 3 or 4 TEAEs (Safety Population): Study 302 Part 1

SOC and PT, n (%)	Berotralstat		Placebo N=39	Total N=120
	110 mg N=41	150 mg N=40		
Subjects with any grade 3 or 4 TEAE	5 (12.2%)	1 (2.5%)	4 (10.3%)	10 (8.3%)
Gastrointestinal disorders	3 (7.3%)	0	1 (2.6%)	4 (3.3%)
Abdominal pain	2 (4.9%)	0	0	2 (1.7%)
Toothache	1 (2.4%)	0	0	1 (0.8%)
Diverticulum intestinal hemorrhagic	0	0	1 (2.6%)	1 (0.8%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (2.4%)	0	1 (2.6%)	2 (1.7%)
Plasma cell myeloma	1 (2.4%)	0	0	1 (0.8%)
Uterine leiomyoma	0	0	1 (2.6%)	1 (0.8%)
Infections and infestations	0	0	1 (2.6%)	1 (0.8%)
Pneumonia	0	0	1 (2.6%)	1 (0.8%)
Investigations	0	0	1 (2.6%)	1 (0.8%)
Gamma-glutamyltransferase increased	0	0	1 (2.6%)	1 (0.8%)
Musculoskeletal and connective tissue disorders	0	1 (2.5%)	0	1 (0.8%)
Back pain	0	1 (2.5%)	0	1 (0.8%)
Nervous system disorders	0	0	1 (2.6%)	1 (0.8%)
Transient ischemic attack	0	0	1 (2.6%)	1 (0.8%)
Skin and subcutaneous tissue disorders	1 (2.4%)	0	0	1 (0.8%)
Purpura	1 (2.4%)	0	0	1 (0.8%)

Reviewer generated the table in JReview using ADSL and ADAE datasets where SAFFL=Y, TRTEMFL=Y, ATOXGRN=3/4
Abbreviation: PT, preferred term; SOC, system organ class; TEAE, treatment-emergent adverse event

Common Treatment-Emergent Adverse Events and Adverse Reactions

The most common TEAEs occurring in $\geq 10\%$ subjects and greater than placebo are shown in Table 41. Gastrointestinal adverse events were the most commonly reported TEAE in all treatment groups. The most common GI-related preferred terms (PTs) observed were nausea, vomiting, abdominal pain and diarrhea. Similar/related PTs were pooled according to the FDA MedDRA Query for abdominal pain (abdominal pain, abdominal discomfort, abdominal pain upper, abdominal tenderness). Abdominal distention was not included because this may signify

a different disease process. GI adverse events appeared to be dose-related. Subjects in the 150 mg treatment group had more GI-related adverse events (1.5- to 2-fold higher) than the 110 mg treatment group or placebo. There was high rate of nausea across all three treatment arms with the highest frequency placebo (14.8% versus 17.9%). Other adverse reactions with an incidence of 5% to <10% and a higher incidence in berotralstat-treated patients compared to those on placebo were headache (9% versus 5%), fatigue (6% versus 3%), and flatulence (6% versus 3%).

Table 41. Common TEAEs Occurring in ≥10% of Subjects and Greater than Placebo (Safety Population): Study 302 Part 1

SOC and PT, n (%)	Berotralstat		Placebo N=39	Total N=120
	110 mg N=41	150 mg N=40		
Gastrointestinal disorders	20 (48.8%)	20 (50.0%)	16 (41.0%)	56 (46.7%)
Abdominal pain ¹	4 (10%)	9 (23%)	4 (10%)	17 (14.2%)
Vomiting	4 (10%)	6 (15%)	1 (3%)	11 (9.2%)
Diarrhea	4 (10%)	6 (15%)	0	9 (7.5%)
Gastroesophageal reflux disease	4 (10%)	2 (5%)	0	6 (5.0%)
Musculoskeletal and connective tissue disorders	3 (7.3%)	6 (15.0%)	7 (17.9%)	16 (13.3%)
Back pain	1 (2.4%)	4 (10.0%)	1 (2.6%)	6 (5.0%)

Source: Reviewer generated in JReview using ADSL and ADAE datasets where SAFFL=Y, TRTEMFL=Y

¹ Abdominal pain includes the following PTs: abdominal pain, abdominal discomfort, abdominal pain upper, abdominal tenderness. Abbreviation: PT, preferred term; SOC, system organ class; TEAE, treatment-emergent adverse event

Table 42 presents all reported PTs in the gastrointestinal SOC. GI-related TEAEs occurred at a high rate across all treatment groups, but in a dose-dependent manner relative to placebo. Despite the frequency of events, most TEAEs were of mild-to-moderate severity and did not lead to treatment discontinuation. Further, the analysis of all GI SOC PTs confirmed that the table of common TEAEs has adequately captured the safety signal related to GI events.

Table 42. All GI TEAEs (Safety Population): Study 302 Part 1

SOC/PT, n (%)	Berotralstat		Placebo N=39	Total N=120
	110 mg N=41	150 mg N=40		
Gastrointestinal disorders (SOC)	20 (48.8%)	20 (50.0%)	16 (41.0%)	56 (46.7%)
PTs				
Abdominal pain ¹	4 (9.8%)	9 (22.5%)	4 (10.3%)	17 (14.2%)
Nausea	6 (14.6%)	6 (15.0%)	7 (17.9%)	19 (15.8%)
Vomiting	4 (9.8%)	6 (15.0%)	1 (2.6%)	11 (9.2%)
Diarrhea	4 (9.8%)	5 (12.5%)	0	9 (7.5%)
Flatulence	2 (4.9%)	3 (7.5%)	1 (2.6%)	6 (5.0%)
Dyspepsia	4 (9.8%)	3 (7.5%)	3 (7.7%)	10 (8.3%)
Gastroesophageal reflux disease	4 (9.8%)	2 (5.0%)	0	6 (5.0%)
Frequent bowel movements	0	1 (2.5%)	0	1 (0.8%)
Food poisoning	1 (2.4%)	1 (2.5%)	0	2 (1.7%)
Dental caries	0	1 (2.5%)	0	1 (0.8%)
Abdominal distension	1 (2.4%)	1 (2.5%)	2 (5.1%)	4 (3.3%)
Toothache	2 (4.9%)	0	2 (5.1%)	4 (3.3%)
Change of bowel habit	1 (2.4%)	0	0 (0.0%)	1 (0.8%)
Esophagitis	1 (2.4%)	0	0 (0.0%)	1 (0.8%)
Constipation	1 (2.4%)	0	1 (2.6%)	2 (1.7%)
Eructation	1 (2.4%)	0	0	1 (0.8%)
Diverticulum intestinal hemorrhagic	0	0	1 (2.6%)	1 (0.8%)
Odynophagia	0	0	1 (2.6%)	1 (0.8%)

Source: Reviewer generated in JReview using the ADAE datasets where SAFFL=Y, TRTEMFL=Y, AEBODSYS=gastrointestinal disorders

¹ Abdominal pain includes the following PTs: abdominal pain, abdominal discomfort, abdominal pain upper, abdominal tenderness. Abbreviations: GI, gastrointestinal; TEAE, treatment-emergent adverse event, SOC, system organ class; PT, preferred term

Laboratory Findings

Routine clinical testing included hematology, serum chemistry with LFTs, and urinalysis. The results of LFTs are discussed in detail below. Otherwise, there was no significant clinical difference between the berotralstat and placebo groups in laboratory parameters, including coagulation tests, during the study.

Liver Function Tests

Table 43, Table 44, and Table 45 list the maximum ALT, AST and bilirubin elevations, respectively, experienced by subjects within each treatment arm. Of note, patients with AST/ALT >3× ULN were excluded from the study; therefore, most subjects had normal transaminase and bilirubin values at baseline. Few subjects experienced transaminase or bilirubin elevation; however, one subject in the 150 mg berotralstat group had marked increase in ALT that prompted discontinuation of study drug as described earlier in this review. A single subject had a maximum AST elevation >3× ULN, and no subjects had bilirubin levels >2× ULN. There were no potential Hy's law cases in this study or the development program as a whole.

Table 43. Maximum ALT Elevations by Treatment Group (Safety Population): Study 302 Part 1

Treatment	Baseline ALT (×ULN)	Maximum ALT Elevation during Study (×ULN)				
		≤1.5	1.5 to ≤3	>3 to ≤5	>5 to ≤10	>10
Berotralstat 110 mg N=41	≤1.5	39 (95.1%)	1 (2.4%)	0	0	0
	>1.5 to ≤3	0	1 (2.4%)	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0
Berotralstat 150 mg N=39	≤1.5	35 (89.7%)	2 (5.1%)	0	1 (2.6%)	0
	>1.5 to ≤3	0	1 (2.6%)	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0
Placebo N=39	≤1.5	35 (89.7%)	4 (10.3%)	0	0	0
	>1.5 to ≤3	0	0	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0

Source: Reviewer generated in OCS Analysis Studio, Shift Table Builder
Population filter: SAFFL (safety population flag)='Y'; X-axis filter: LBBLFL (baseline flag)='Y'; Y-axis filter: EPOCH (epoch)='PART 1'
(N =), number of subjects from each group in the shift table
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

Table 44. Maximum AST Elevations by Treatment Group (Safety Population): Study 302 Part 1

Treatment	Baseline AST (×ULN)	Maximum AST Elevation during Study (×ULN)				
		≤1.5	1.5 to ≤3	>3 to ≤5	>5 to ≤10	>10
Berotralstat 110 mg N=41	≤1.5	41 (100%)	0	0	0	0
	1.5 to ≤3	0	0	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0
Berotralstat 150 mg N=39	≤1.5	37 (94.9%)	1 (2.6%)	1 (2.6%)	0	0
	>1.5 to ≤3	0	0	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0
Placebo N=39	≤1.5	39 (100%)	0	0	0	0
	>1.5 to ≤3	0	0	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0

Source: Reviewer generated in OCS Analysis Studio, Shift Table Builder
Population filter: SAFFL (safety population flag)='Y'; X-axis filter: LBBLFL (baseline flag)='Y'; Y-axis filter: EPOCH (epoch)='PART 1'
(N=) indicates the number of subjects in each group in the shift table.
Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal

Table 45. Maximum Bilirubin Elevations by Treatment Group (Safety Population): Study 302 Part 1

Treatment	Baseline Bilirubin (xULN)	Maximum Bilirubin Elevation (xULN)			
		≤1	>1 to ≤1.5	>1.5 to ≤2	>2
Berotralstat 110 mg N=41	≤1	40 (97.6%)	0	0	0
	>1 to ≤1.5	0	0	0	0
	>1.5 to ≤2	0	1 (2.4%)	0	0
	>2	0	0	0	0
Berotralstat 150 mg N=39	≤1	36 (92.3%)	1 (2.6%)	0	0
	>1 to ≤1.5	1 (2.6%)	1 (2.6%)	0	0
	>1.5 to ≤2	0	0	0	0
	>2	0	0	0	0
Placebo N=39	≤1	36 (92.3%)	1 (2.6%)	0	0
	>1 to ≤1.5	0	0	2 (5.1%)	0
	>1.5 to ≤2	0	0	0	0
	>2	0	0	0	0

Source: Reviewer generated in OCS Analysis Studio, Shift Table Builder
Population filter: SAFFL (safety population flag)="Y"; X-axis filter: LBBLFL (baseline flag)="Y"; Y-axis filter: EPOCH (epoch)="PART 1"
(N=) indicates number of subjects in each group in the shift table.
Abbreviations: BCX, berotralstat; BILI, bilirubin; ULN, upper limit of normal

The Applicant asserted that a history of androgen use was a risk factor for elevated liver enzyme levels in the phase 2 and 3 studies. Table 46 shows maximum ALT elevation by prior androgen use (yes/no). Although in the 110 mg berotralstat group, the rate of ALT elevation in patients who had and had not used androgens was 2.4% and 4.9%, respectively, no such effect was seen in the 150 mg group. Patients in the placebo group with prior androgen use also tended to have a higher incidence of an elevated ALT level compared to those who had never taken androgens. Given the small sample size, it is unclear if the combination of berotralstat and prior androgen use had an additive effect or if the risk of increased ALT was present with prior androgen use alone. This potential relationship was evaluated further in safety assessments from Study 204 (Section 8.3.7.1, Table 57).

Table 46. Maximum ALT Elevation by Prior Androgen Use (Safety Population): Study 302 Part 1

Parameter	Berotralstat					
	110 mg N=41		150 mg N=40		Placebo N=39	
	Never Used	Used	Never Used	Used	Never Used	Used
Postbaseline maximum ALT, n (%)						
≤ULN	21 (51.2%)	15 (36.6%)	13 (32.5%)	17 (42.5%)	12 (30.8%)	20 (51.3%)
>ULN to 1.5x ULN	0	3 (7.3%)	3 (7.5%)	3 (7.5%)	1 (2.6%)	2 (5.1%)
>1.5x ULN to 3x ULN	1 (2.4%)	1 (2.4%)	2 (5.0%)	1 (2.5%)	1 (2.6%)	3 (7.7%)
<3 to ≤10x ULN	0	0	0	0	0	0
>10x ULN to 20x ULN	0	0	0	1 (2.5%)	0	0
Duration of prior androgen use, n(%)						
Never used	22 (53.7%)	0	18 (45.0%)	0	14 (35.9%)	0
<6 months	0	0	0	2 (5.0%)	0	2 (5.1%)
6 months to <1 year	0	1 (2.4%)	0	0	0	2 (5.1%)
1 year to <5 years	0	7 (17.1%)	0	5 (12.5%)	0	10 (25.6%)
5 years to <10 years	0	3 (7.3%)	0	2 (5.0%)	0	4 (10.3%)
≥10 years	0	8 (19.5%)	0	13 (32.5%)	0	7 (17.9%)

Source: Reviewer generated table in JReview using ADSL and ADLB datasets where SAFFL=Y
Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

Additional Safety Assessments from Study 302, Part 2

In addition to the 6 month placebo-controlled safety data from Part 1, the NDA submission included interim safety data from 4 months of Study 302, Part 2. No new safety signals were identified during the review of Part 2 interim data. Similar to Part 1, GI adverse reactions such as abdominal pain, vomiting and diarrhea were common and occurred more frequently with the higher 150 mg dose than the lower 110 mg dose. No significant elevations in transaminases or bilirubin were reported. There were 31 study drug discontinuations, mostly occurring due to subject withdrawal for reasons unrelated to study drug or perceived lack of efficacy. Four subjects in the 150 mg group discontinued due to an adverse event (palpitations/tachycardia, abdominal pain, abdominal pain/diarrhea, and anal incontinence). One subject in the 110 mg group discontinued due to nausea. There were two SAEs: obesity in the 110 mg group and a medical observation in the 150 mg group. The medical observation SAE was due to hospitalization for a routine thyroid cancer screening procedure using radiolabeled iodine in a subject with a history of treated thyroid papillary carcinoma and hypothyroidism. Neither event appeared related to the study drug. No AESIs occurred in Part 2 of Study 302.

Vital Signs

Overall, no clinically meaningful changes within or between treatment groups were observed for pulse rate, respiratory rate, or mean blood pressure.

Electrocardiograms

Among all treatment groups, one subject in the placebo group had a clinically significant abnormal ECG finding. This subject developed sinus bradycardia with first degree atrioventricular block, which was reported as an AE. The Applicant conducted a thorough QT study, which is summarized below.

QT

A thorough QT study (Study BCX7353-106) was conducted in healthy volunteers evaluating berotralstat doses of 150 mg and 450 mg QD for 14 days and reviewed by the Interdisciplinary Review Team (IRT) for Cardiac Safety Studies. Berotralstat caused a dose and concentration-dependent increase in the QT interval over the range of doses evaluated; however, at the peak concentrations with the recommended dose of 150 mg QD, the QT interval was not prolonged to any clinically relevant extent at Day 14. At a dose of 450 mg QD (Day 14), exposures were ~3-fold higher than achieved at the recommended 150 mg dose, and the mean QTc increased significantly (refer to the IRT review by Dr. Girish Bende and Clinical Pharmacology review in this document for details).

Reviewer comment: Because the QT prolongation observed with berotralstat is dose and concentration dependent and berotralstat exhibits nonlinear PK, administering even twice the recommended dose carries a potential risk of clinically significant QTc prolongation. Of note, this safety issue has been discussed with the Applicant during previous interactions for the acute

HAE attack development program which proposes to administer higher doses of berotralstat. In light of an ongoing development program for acute attacks, Sections 1 (Limitation of Use statement), 2 (Dosage and Administration), 5 (Warnings and Precautions) and 12 (Pharmacodynamics and Pharmacokinetics) of the PI and the PPI will be revised to ensure that prescribers are aware of this potential risk and patients do not take higher or additional doses.

Immunogenicity

Immunogenicity studies were not required or performed as part of this study.

8.3.5. Analysis of Submission-Specific Safety Issues

Rash was designated *a priori* as an adverse event of special interest. Reports of skin disorders were rare and occurred as single events: pruritis, acne, dry skin, alopecia, petechiae, purpura and echymosis. One subject treated with 150 mg berotralstat experienced mild petechiae, which was assessed to be caused by mechanical irritation. The purpuric rash was discussed under treatment discontinuations due to TEAEs, but was not felt to be related to study drug.

Although gastrointestinal adverse events were common and a few cases of transaminase elevation occurred, no hepatobiliary adverse events were reported with berotralstat treatment in Part 1 of Study 302.

8.3.6. Safety Analyses by Demographic Subgroups

The OCS Analysis Toolbox DM Tool and OCS Analysis studio risk assessment tools were used to analyze safety in Study 302 by the following demographic subgroups: sex, age, race, ethnicity, and region. This review did not identify a meaningful difference in overall TEAEs between berotralstat and placebo within any subgroup. However, the relatively low frequency of other SAEs and adverse dropouts in addition to the small number of subjects within certain subgroups made it difficult to draw any further conclusions regarding an imbalance in safety based on demographic or disease characteristics. Study 302 only enrolled six adolescent subjects. Because the Applicant is seeking an indication for patients 12 years of age and older, safety signals in the 12 to 17-year age group were analyzed in more detail (Table 47).

Table 47. TEAEs in Adolescent Subjects (Safety Population): Study 302 Part 1

USUBJID	Age (Years)	Sex	Race	Treatment Arm	SAE	Preferred Term
BCX7353-302- (b) (6)	12	F	White	150 mg	N	Nasopharyngitis Proteinuria
BCX7353-302- (b) (6)	15	F	White	110 mg	N	Sweat gland disorder
BCX7353-302- (b) (6)	17	F	White	Placebo	N	Sinusitis
BCX7353-302- (b) (6)	14	F	White	Placebo	N	Nausea
BCX7353-302- (b) (6)	12	M	White	110 mg	N	Contusion
BCX7353-302- (b) (6)	12	M	Other	150 mg	N	Dyspepsia Pharyngitis streptococcal Tooth fracture

Source: Reviewer generated in JMP using the ADAE and ADSL datasets where SAFFL=Y, TRETEMFL=Y and variables age, aex, race

Abbreviations: TEAE, treatment-emergent adverse event; USUBJID, Unique Subject ID; SAE, serious adverse event; F, female; M, male; N, no

The majority of adolescent subjects were white similar to the general safety population. Although small in number, there was adequate representation across the age spectrum with half of the subjects being 12 years of age. There were no SAEs or TEAEs leading to treatment discontinuation. Infections and GI disorders were the most common TEAEs, all of which were grade 1 or 2 severity. The types of infection were typical for this age group. Despite the small numbers, there was no apparent difference in the safety profile of berotralstat in adolescent subjects compared to adults that would raise concern or alter the benefit-risk assessment in this age group.

8.3.7. Specific Safety Studies: BCX7353-204

Trial Design

This is an ongoing two-arm open-label phase 2 study designed to evaluate the long-term safety of prophylactic treatment with daily oral berotralstat at two dose levels in subjects with HAE. The study is also evaluating the long-term effectiveness and impact on QoL or general well-being of subjects who receive berotralstat prophylactic treatment, and the subject's satisfaction with the medication. Target enrollment is 475 subjects with approximately 250 subjects from the United States. The study population includes adults (18 years and older) and adolescents (12 to 17 years of age) with a confirmed diagnosis of type I or II HAE who are either naïve or have received berotralstat in another study. Both 110 mg QD and 150 mg QD doses of berotralstat were initially being evaluated; however, following the completion of Study 302 Part 1, all subjects are receiving berotralstat 150 mg QD.

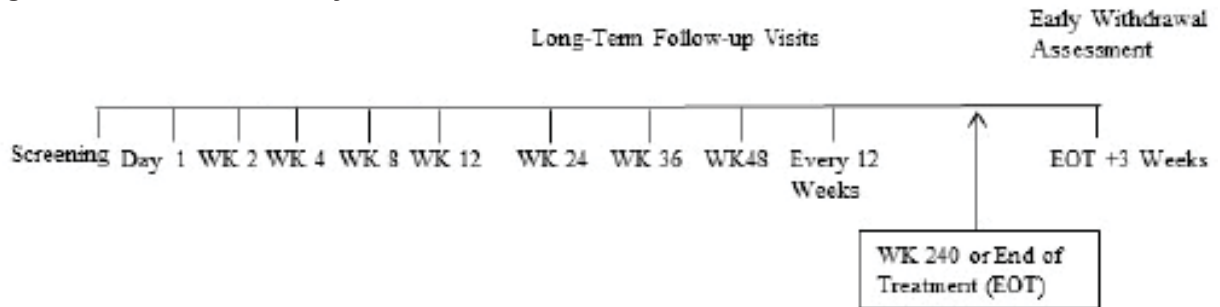
Screening visits are not required for previous berotralstat study participants if the last dose of study drug was <84 days prior. Subjects rolling over from another berotralstat study without treatment interruption or with <14 days of study drug interruption could have final study visit assessments for the parent study and baseline assessments for Study 204 performed at the same visit. The same values may be used for both assessments when possible.

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On-treatment study visits occur at weeks 2, 4, 8, 12 and every 12 weeks thereafter, for a study duration of up to 240 weeks (approximately 5 years), until another mechanism becomes available to provide the drug to the subject (e.g., market access), or until the Applicant discontinues development of the product for the prevention of angioedema attacks, whichever occurs first. Telephone contact will occur at weeks 16, 20, 28, 32, 40, 44, 52, 56, 64, 68, 76, 80, 88, and 92.

A schematic of the study is provided in Figure 9 and a schedule of assessments in Table 48.

Figure 9. Schematic of Study BCX7353-204



Abbreviations: EOT = end of treatment; WK = week.

Note: Subjects who have not interrupted BCX7353 treatment for < 84 days do not require a screening visit.

Note: Day 1 may be the EOT visit from the previous BCX7353 efficacy study.

Source: Sponsor's Study BCX7353-204 Protocol (Page 43, Figure 3)

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Table 48. Schedule of Assessments: Study BCX7353-204

Assessment	Screening Period	Baseline Visit ^e	Treatment Period (Visit schedule)					End of Study Assessment
	Screening Visit (Up to Day -28)	(Day 1)	Week 2 (Day 15 ± 2 days) ^b	Week 4 (Day 29 ± 2 days)	Week 8 (Day 57 ± 2 days)	Week 12 (Day 85 ± 2 days)	Visits every 12 weeks until 240 weeks ^g (± 6 days)	3 weeks (± 3 days) after last dose of study drug
Informed consent/assent (as applicable) ^f	X	X						
Clinic staff phone call/ communication							X ^d	
Inclusion-exclusion criteria	X	X						
Medical and medication history, HAE medical and medication history ^e	X	X						
Weight/height/BMI ^f	X	X		X	X	X	X	X
Drugs of abuse screen ^g	X							
Physical examination ^h	X	X		X	X	X	X	X
Pregnancy test ⁱ	X	X		X	X	X	X	X
Vital signs ^j	X	X		X	X	X	X	X
FSH ^k	X	X						
HIV, HCV, HBV serology	X							
Diagnosis of HAE established ^l		X						
Clinical chemistry/hematology/coagulation ^g	X	X	X ^b	X	X	X	X	X
Troponin I and Troponin T, CK-MB		X		X	X	X	X	X
NGAL and ACR		X				X	X	X
Urinalysis ^g	X	X	X ^b	X	X	X	X	X
Sample for HLA analysis and C3 ^m		X						
Optional sample for exploratory PG ⁿ		X						
ECG ^o	X	X		X	X	X	X	X
AE-QoL, days lost from work and/or school, experience on study and TSQM ^p		X		X	X	X	X	X
Concomitant medications ^q	X	X	X	X	X	X	X	X
Plasma PK sample ^r		X		X	X	X	X ⁱ	X ⁱ
AEs	X	X	X	X	X	X	X	X
Diary instruction/ review/set-up ^s	X	X		X	X	X	X	X
Allocation to study drug ^t		X						
Diary completion ^u	←-----→							
Study drug dosing	←-----→							
Study drug accountability/ dispensing		X		X	X	X	X	X

Source: Applicant's clinical study report of Study BCX7353-204 (page 47, Table 3)

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Abbreviations: ACR = spot urine microalbumin to creatinine ratio; AE = adverse event; AE-QoL = Angioedema Quality of Life questionnaire; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; C1-INH = C1 esterase inhibitor; BMI = body mass index; C3 = complement 3; C4 = complement 4; CK-MB = creatine kinase MB isoenzyme; CRF = case report form; ECG = electrocardiogram; EOS = end of study; FSH = follicle stimulating hormone; GGT = gamma glutamyl transferase; HAE = hereditary angioedema; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HLA = human leukocyte antigen; IXRS = interactive (voice/web) response system; LLN = lower limit of normal; NGAL = neutrophil gelatinase-associated lipocalin; PG = pharmacogenomic; PK = pharmacokinetic; QoL = quality of life; QTcF = QT interval corrected using Fridericia's method; TSQM = Treatment Satisfaction Questionnaire for Medication.

- ^a The baseline visit should be within 28 days of the screening visit. Subjects who have interrupted study drug from a previous BCX7353 study for < 84 days are not required to undergo a screening visit. For subjects directly rolling over from another BCX7353 study without treatment interruption or subjects who interrupt study drug for < 14 days, the final on-treatment visit in the prior BCX7353 study will be considered the baseline visit for this study.
- ^b The Week 2 visit will consist of monitoring liver function tests only (ALT, AST, GGT, total and direct bilirubin, ALP); urine and additional tubes of blood may be required to accommodate reflex testing for abnormal GGT (see Table 4). If preferred by the subject and clinical site, laboratory values may be drawn and resulted locally, with results entered into the CRF.
- ^c Informed consent will be signed at the screening visit for subjects requiring a screening visit and will be signed for all other subjects at the baseline visit or final on treatment visit in the parent study (which serves as the subject's baseline visit in this study).
- ^d After the Week 12 visit, clinic staff must contact the subjects at least once every 4 weeks between clinic visits to assess the subject's overall well-being and any issues with regards to study drug tolerability. During all calls, sites will also discuss study drug compliance and any issues with the diary or study drug.
- ^e Medical history and medication history will be completed at screening (for subjects requiring a screening visit) and at baseline. At the Screening visit (or baseline for those with no screening visit), the HAE Medical and Medication history form will be updated from the previous HAE study, as applicable, with additional questions to be answered if provided.
- ^f BMI calculation and height at screening; weight is to be recorded at each in-clinic visit. Adolescents should also have height recorded at Weeks 48, 96, 144, 192, and 240.
- ^g Please see Table 4 for analytes to be assessed. The lipid panel as part of clinical chemistry will only be measured at baseline, Week 12 and then approximately every 12 weeks.
- ^h Full physical examinations will be performed at screening and baseline; symptom-directed physical examinations will be performed at all post-baseline visits.
- ⁱ For women of childbearing potential (including adolescents), regardless of contraception or lifestyle, a serum pregnancy test will be administered at screening, urine pregnancy tests will be assessed at all subsequent visits as indicated in the table. Demonstration of a negative urine pregnancy test will be required prior to dispensing study drug.
- ^j To include blood pressure and pulse rate. Temperature and respiratory rate will be captured at screening and baseline only. Prior to obtaining vital signs, subjects should rest in a supine position for at least 5 minutes.
- ^k For a woman who declares that she has been post-menopausal ≤ 2 years or was considered a woman of childbearing potential in the previous study. The sample may be drawn at baseline for those who do not undergo a screening visit; however, the subject must have a negative urine pregnancy test at baseline prior to receiving study drug.
- ^l For subjects that have not participated in a previous BCX7353 study, a clinical diagnosis of HAE Type I or II, defined as having a C1-INH functional level below 50% and a C4 level below the lower LLN reference range, as assessed during the screening period. In the absence of a low C4 value drawn during the inter-critical period, 1 of the following is acceptable to confirm the diagnosis of HAE assessed during the Screening period: 1) a SERPING-1 gene mutation known or likely to be associated with HAE Type I or II; 2) a confirmed family history of C1-INH deficiency; 3) a C4 redrawn and retested during an attack with the results below the LLN reference range. For subjects with C1-INH function $\geq 50\%$ but less than the assay LLN, a SERPING-1 gene mutation known or likely to be associated with HAE Type I or II assessed during the screening period or a repeat C1-INH functional level $< 50\%$ will be considered acceptable for enrollment.
- ^m A sample for HLA analysis will be drawn on subjects who have not donated one in a previous study. The sample for HLA and C3 may be drawn at baseline or at any subsequent visit.
- ⁿ A blood sample for possible exploratory pharmacogenomic testing will be drawn at the baseline/Day 1 visit for subjects who have not had a sample collected in a previous study; however, the sample will be analyzed only if consent/assent is obtained for this optional analysis. If a blood sample is not obtained at baseline, the sample may be drawn at any time during the study following consent obtained from the subject.
- ^o All ECGs during the study will be single assessments with the exception of baseline which will be obtained in triplicate. An ECG should be repeated for a change from baseline in QTcF > 60 msec or a QTcF interval > 500 msec. Prior to obtaining an ECG, subjects should rest quietly in a supine position for at least 10 minutes. ECGs should be obtained prior to any blood sampling.
- ^p AE-QoL will be administered once at baseline and at Weeks 4, 8, 12, and every 12 weeks thereafter. The TSQM will be administered once at baseline and at Weeks 4, 8, 12, and every 12 weeks through Week 96. After Week 96, the TSQM will be administered every 24 weeks (Weeks 120, 144, 168, 192, 216, and 240). Additional questions regarding loss of time from work/school will be instituted at each visit to more fully characterize QoL. Long-term experience on study will be assessed every 24 weeks beginning at Week 96.
- ^q Concomitant medications will be reviewed at each visit and on treatment, new concomitant medications will be reviewed to determine whether there are any contraindications or dosage adjustments required.
- ^r The actual date and time of PK blood draw, and time of last study drug dose (relative to PK sample draw) will be captured in the CRF. Samples for PK will only be collected through the Week 48 visit.
- ^s Site staff will review the diary instructions with the subjects at the first clinic visit. Subjects will be instructed to bring the completed diary with them to their visits. Any issues with the entered data in the diary will be discussed with the subject and corrections will be made by the subject as required.
- ^t Sites will request a treatment allocation in the IXRS for eligible subjects at the Day 1 visit.
- ^u At any time the diary is in a subject's possession (diary is dispensed at screening visit), they will enter HAE attacks and relevant details (screening through follow-up) and dosing information (as applicable, prior to Week 48 only) once per day. No dosing information will be collected in the diary after Week 48.
- ^v Up to 240 weeks or until another mechanism is available to provide drug to the subject, or until the Sponsor discontinues development of the product for the prevention of angioedema attacks, whichever comes first.

Key Inclusion Criteria

1. Males and females ≥ 12 years of age with a clinical diagnosis of type I or II HAE who have participated in a berotralstat study OR reside in countries open to recruitment of treatment-naïve subjects, and who have a clinical diagnosis of HAE type I or II, defined as:
 - a. Having a C1-INH functional level below 50% and a C4 level below the LLN reference range, as assessed during the screening period OR laboratory documentation of a

historical C1-INH functional level <50% and a C4 level below the LLN reference range.

- b. In the absence of a low C4 level, the following was acceptable to confirm the diagnosis of HAE:
 - 1) SERPING-1 gene mutation known or likely to be associated with HAE type I or II assessed during the screening period; 2) a confirmed family history of C1-INH deficiency; 3) a C4 redrawn and retested during an attack with the results below the LLN reference range measured during the screening period.

Key Exclusion Criteria

1. Clinically significant abnormal ECG including but not limited to a QTcF >470 ms for women, a QTcF >450 ms for men, a PR interval >220 ms (both sexes), or ventricular and/or atrial premature contractions that are more frequent than occasional, and/or as couplets or higher in grouping.
2. Known family history of sudden cardiac death.
3. Calculated CL_{CR} of ≤30 mL/min or AST or ALT value at least three-fold the ULN reference range at screening or at the last available visit prior to enrollment.
4. Pregnancy or breast feeding or planned pregnancy during the study period.
5. Use of any of the following prohibited medications:
 - a. Angiotensin-converting enzyme inhibitors within 7 days of the baseline visit or planned initiation during the study (potential for exacerbation of HAE).
 - b. Investigational drug exposure, other than berotralstat, within 30 days prior to the screening visit (or baseline if no screening visit) or initiation during the study.
 - c. Use of a medication that is transported by P-gp and has a narrow therapeutic range within 7 days of the baseline visit or planned initiation during the study. For the purposes of this protocol, these are: aliskiren, digoxin, posaconazole, and talinolol.
 - d. Use of a concomitant medication that is metabolized by CYP2D6, CYP2C9, CYP2C19, or CYP3A4 and has a narrow therapeutic range, within 7 days of the baseline visit or planned initiation during the study, including those known to prolong the QT interval.

Reviewer comment: The protocol initially included only subjects from prior berotralstat studies (version 6), however, was later amended (version 7) to allow enrollment of naïve patients. There was no minimum attack rate requirement for entry into the study; thus, this study population could potentially have less severe disease than the subjects in Study 302.

Subject Removal Criteria

Subjects were permanently withdrawn from the study drug if any of the following circumstances occurred:

1. Emergence of any laboratory abnormality or AE that in the judgment of the investigator compromised the ability of the subject to continue study-specific procedures or it was considered not to be in the subject's best interest as a result of an altered benefit-risk profile.

2. Recurrence of any significant treatment-emergent AST or ALT elevations or abnormalities indicative of significant hepatic toxicity.
3. Subsequent determination that inclusion/exclusion criteria were not met.
4. Intercurrent illness or emergence of a new illness/medical condition that, in the judgment of the investigator, affected assessment of clinical status to a significant degree.
5. Subject noncompliance with study drug or the protocol.
6. The subject had a QTcF >500 ms (confirmed on repeat testing) or other significant QTcF changes.
7. Subjects with a study drug-related grade 4 rash according to the DMID criteria.

Study Treatments and Blinding

Study treatment was not blinded. All subjects received open-label berotralstat capsules for oral administration at a dose of 110 or 150 mg QD. Subjects initially allocated to the 110 mg QD group received a daily dose as 110 mg capsules (one capsule per day). Subjects initially allocated to the 150 mg QD treatment group received a daily dose supplied as 50 mg capsules (three capsules per day) or 150 mg capsules (one capsule per day). Based on the results of Part 1 of Study 302, all subjects will be transitioned to berotralstat 150 mg QD.

Primary Endpoints

The primary objective of the study is to assess the long-term safety of berotralstat. The safety endpoints are as follows:

- The proportion of subjects who discontinue berotralstat as a result of a TEAE.
- The proportion of subjects with treatment-emergent serious adverse events (SAEs).
- The proportion of subjects with TEAEs.
- The proportion of subjects with treatment-emergent grade 3 or 4 AEs.
- The proportion of subjects with treatment-emergent, treatment-related AE consistent with a drug rash.
- The proportion of subjects with treatment-emergent grade 3 or 4 laboratory abnormalities.

Secondary and Other Endpoints

Efficacy endpoints include:

- Number and rate of HAE attacks.
- Durability of response (trend in attack rate over time).
- Number and proportion of days with angioedema symptoms.
- Patient-reported outcomes (HAE disease-specific AE-QoL questionnaire scores and TSQM global satisfaction scores).
- Number of attacks requiring attack medication.
- Discontinuations because of lack of efficacy (through week 48 only).
- Severity of attacks.

Reviewer comment: The results of this open label, uncontrolled study primarily serve as supportive evidence of safety. Although the study captured efficacy outcomes, we did not rely on the results to provide evidence of effectiveness for approval, and thus the efficacy results will not be covered in this review. Safety results are discussed below.

8.3.7.1. Study BCX7353-204 Results

This section will focus on the safety results from the 18-month interim analysis period from the date of first subject enrollment (February 27, 2018) to the data cutoff date (August 20, 2019). As mentioned above, subjects were transitioned from the 110 mg to the higher 150 mg dose following data analysis from Part 1 of Study 302. Between June 11, 2019 and August 20, 2019 (data cutoff), 22 of the 100 subjects initially allocated to the 110 mg group were transitioned to berotralstat 150 mg. However, all safety analyses were conducted based on the subject's initial treatment dose assignment. As very few TEAEs occurred in subjects after crossing over to the higher dose given the short time period between dose escalation and data cutoff, this did not have a major impact on the conduct of the safety analyses. TEAEs which occurred in subjects after the transition to the 150 mg dose are discussed under Adverse Events. beginning on 11 June 2019.

Disposition

Overall, a total of 254 subjects were screened and 227 were allocated to receive study drug—100 subjects to berotralstat 110 mg and 127 to berotralstat 150 mg (Table 49).

The most common reason for screening failure was failure to meet the clinical diagnosis of HAE type I or II. Subjects were enrolled at 49 sites in 22 countries, mostly in Europe. There was a fairly high rate of early discontinuation of study drug (59 subjects [26%]). The most common reasons for discontinuation of study drug overall and prior to week 48 were perceived lack of efficacy (n=28/227 [12.3%]) and laboratory abnormalities or AEs (n=19/227 [8.4%]), respectively. Patients who stopped the study drug as a result of a perceived lack of efficacy were more likely to be in the 110 mg berotralstat group than the 150 mg berotralstat group (17 versus 11 subjects, respectively). In contrast, patients who stopped the study drug because of an AE or laboratory abnormality were more likely to be in the 150 mg berotralstat group than the 110 mg berotralstat group (13 versus 6 subjects, respectively).

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Table 49. Subject Disposition (Safety Population): Study 204

Parameter	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Early study withdrawal			
Lost to follow up	0	1	0
Other	17 (17%)	24 (19%)	41 (18%)
Subject withdrew consent	9 (9%)	8 (6%)	17 (8%)
Early study drug discontinuation	26 (26%)	33 (26%)	59 (26%)
Intercurrent illness or emergence of new illness/medical condition/pregnancy	0	1 (0.8%)	1 (0.4%)
Investigator judgement	0	1 (0.8%)	1
Laboratory abnormality or adverse event	6 (6%)	13 (10%)	19 (8%)
Other	1 (1%)	2 (2%)	3 (1%)

Source: Reviewer generated in OCS Analysis Studio, Custom Table Builder.

Columns—dataset, demographics; filter, TRT01P='BCX7353 150 mg' or 'BCX7353 110 mg.'

Subjects withdrawn from study—dataset, demographics; filter, DISCSTN='1' or '4' or '2.'

Subjects with early study drug discontinuation—dataset, demographics; filter, DISCSDN='10' or '1' or '9' or '11' or '7' or '3.'

Patient Demographics

Table 50 displays the demographic data of the subjects in Study 204. Similar to Study 302, there was a predominance of white, middle-aged females. Unlike Study 302, the vast majority of study sites were outside the United States. There were more adolescent subjects (n=10) than in Study 302 (n=6), providing further safety data for this subpopulation. There was a small number of elderly patients ≥65 years of age (n=5). Across the two treatment groups, there was a higher proportion of patients in the 150 mg group (56%) than the 110 mg group (44%).

Table 50. Subject Demographics (Safety Population): Study 204

Parameter	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Age			
Mean [standard deviation]	37.6 [14.0]	42.5 [13.4]	40.3 [13.9]
Median	37	44	41
Range	14-72	12-72	12-72
Age group, n (%)			
18-64 years	93 (93.0%)	119 (93.7%)	212 (93.4%)
12-17 years	5 (5.0%)	5 (3.9%)	10 (4.4%)
65 years and older	2 (2.0%)	3 (2.4%)	5 (2.2%)
Sex, n (%)			
Female	62 (62.0%)	77 (60.6%)	139 (61.2%)
Male	38 (38.0%)	50 (39.4%)	88 (38.8%)
Race, n (%)			
White	82 (82.0%)	110 (86.6%)	192 (84.6%)
Asian	12 (12.0%)	10 (7.9%)	22 (9.7%)
Other	5 (5.0%)	5 (3.9%)	10 (4.4%)
Black or African American	0	2 (1.6%)	2 (0.9%)
Native Hawaiian or other Pacific Islander	1 (1.0%)	0	1 (0.4%)

Parameter	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Ethnicity, n (%)			
Not Hispanic or Latino	96 (96.0%)	115 (90.6%)	211 (93.0%)
Not reported	4 (4.0%)	9 (7.1%)	13 (5.7%)
Hispanic or Latino	0	2 (1.6%)	2 (0.9%)
Unknown	0	1 (0.8%)	1 (0.4%)
Region, n (%)			
Europe	24 (24.0%)	68 (53.5%)	92 (40.5%)
Other region	76 (76.0%)	57 (44.9%)	133 (58.6%)
United States	0	2 (1.6%)	2 (0.9%)

Source: Reviewer generated table in JReview using the ADSL datasets where SAFFL=Y and variables AGE, SEX, RACE, ETHNICITY, COUNTRY

Baseline Disease Characteristics

Table 51 lists the baseline characteristics of subjects in Study 204. The majority of subjects (66.5%) were enrolled directly into this study without having participated in a prior berotralstat study, and the proportion of subjects who had participated in a previous berotralstat study was higher in the 150 mg group (48%) than the 110 mg group (15%). The mean age of symptom onset and age at diagnosis is consistent with the epidemiology of the disease where symptoms commonly manifest with increasing frequency and severity around puberty and patients are diagnosed within the next few years after. A combination of abdominal and peripheral attacks being the most common locations is also consistent with the usual disease presentation. Patients in the 150mg treatment group had more laryngeal attacks within the past year and therefore this group may have had a greater disease burden at the start of the study.

Table 51. Baseline Disease Characteristics: Study 204

Parameter	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Age at HAE diagnosis (years)			
Mean [SD]	19 [13]	21 [13]	20 [13]
Median (Range)	18 (0.5-52)	20	19
Range	0.5-52	0-54	0-54
Age at symptoms onset (years)			
Subject count with data	100	127	227
Mean [SD]	11 [8]	11 [8]	11 [8]
Median	10	10	10
Range	1-50	0.5-49	0.5-50
Number of laryngeal attacks in past year			
Subject count with data	29	35	64
Mean [SD]	2.5 [3]	4.1 [6]	3.4 [5]
Median	1	2	2
Range	1-12	1-30	1-30

Parameter	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Primary HAE attack location			
Abdominal/peripheral	46 (46.0%)	60 (47.2%)	106 (46.7%)
Abdominal/laryngeal/peripheral	34 (34.0%)	47 (37.0%)	81 (35.7%)
Peripheral	8 (8.0%)	10 (7.9%)	18 (7.9%)
Abdominal	3 (3.0%)	5 (3.9%)	8 (3.5%)
Laryngeal/peripheral	5 (5.0%)	3 (2.4%)	8 (3.5%)

Source: Reviewer generated in JReview using the ADSL datasets where SAFFL=Y
Abbreviations: HAE, hereditary angioedema; INH, inhibitor; LLN, lower limit of normal; SD, standard deviation

Berotralstat Exposure

Table 52 shows exposure to berotralstat in Study 204. The mean total exposure to study drug was 283 days. For subjects in the berotralstat 150 mg group, the mean time exposed to study drug (307 days) was greater than that in the berotralstat 110 mg group (253 days). This discrepancy is noted by the Applicant to be due to early preferential dosing allocation to 150 mg. A substantial number (49%) of subjects in the 150 mg group received 337 to 504 days of exposure, equivalent to 1 to almost 2 years of therapy.

Table 52. Berotralstat Exposure: Study 204

Summary	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Duration of exposure (days)			
Mean (standard deviation)	253 (129)	307 (143)	283 (139)
Median	307	342	328
Range	14 - 429	11-540	11-540
Duration of exposure (weeks)			
≤ 12 weeks	22 (22.0%)	18 (14.2%)	40 (17.6%)
<12 to ≥ 24 weeks	1 (1.0%)	8 (6.3%)	9 (4.0%)
< 24 to ≥ 36 weeks	16 (16.0%)	6 (4.7%)	22 (9.7%)
< 36 to ≥ 48 weeks	33 (33.0%)	26 (20.5%)	59 (26.0%)
< 48 to ≥ 72 weeks	28 (28.0%)	62 (48.8%)	90 (39.6%)
< 72 to ≥ 96 weeks	0	7 (5.5%)	7 (3.1%)
< 96 weeks	0	0	0
Person-years of exposure	69.1	106.6	175.7

Source: Applicant's Study BCX7353-204 clinical study report (Page 182, Table 54)

Adverse Events

Rollover Subjects

A total of three subjects reported 5 TEAEs after switching from berotralstat 110 to 150 mg. However, in the tables below, these TEAEs are attributed to the 110 mg dose group because subjects were analyzed according to the initial treatment group allocation. One subject developed tendinitis, another developed a leg fracture and the third subject had three episodes of HAE attacks. The HAE attacks and the leg fracture were identified as SAEs; however, none of these five TEAEs appear related to study drug. None of the remaining 19 patients who transitioned from the 110 mg to the 150 mg dose reported TEAEs following the dose escalation. Therefore, analysis of the data by this reviewer did not reveal any difference in the safety

profile of berotralstat after accounting for patients transitioned from the 110 mg to the 150 mg dose.

Deaths

No deaths were reported in the OLE Study 204.

Nonfatal SAEs

Serious nonfatal AEs that occurred during the OLE are shown in [Table 53](#). Any SAE which occurred in 2 or more subjects is listed, as well as any single event within a SOC which was previously identified as having a potential safety signal in the pivotal study. For example, hepatobiliary disorder was still included despite only one event, due to the known GI safety profile of the drug. Most treatment-emergent SAEs were attacks of HAE (12 subjects [5.3%]), all of which required hospitalization. The 110 mg group had 10 subjects with HAE attacks with the remaining 2 in the 150 mg group, which was consistent with efficacy results favoring the 150 mg dose. GI SAEs were few—abdominal pain, elevated liver enzymes, and an abnormal liver function test (each in one subject) (Table 53). Other significant SAEs that may be relevant but occurred infrequently were two episodes of pneumonia and one of biliary colic, all in the 150 mg group. There was no clustering of SAEs in any other system organ class.

Table 53. Serious Adverse Events (Safety Population): Study 204

SOC and PT, n (%)	Berotralstat		Total N=227
	110mg N=100	150mg N=127	
Subjects with at least one treatment-emergent SAE, n (%)	18 (18.0%)	12 (9.4%)	30 (13.2%)
Congenital, familial, and genetic disorders	10 (10.0%)	3 (2.4%)	13 (5.7%)
Hereditary angioedema	10 (10.0%)	2 (1.6%)	12 (5.3%)
Splenic hamartoma	0	1 (0.8%)	1 (0.4%)
Investigations	3 (3.0%)	2 (1.6%)	5 (2.2%)
Liver function test abnormal	0	1 (0.8%)	1 (0.4%)
Medical observation	2 (2.0%)	1 (0.8%)	3 (1.3%)
Hepatic enzyme increased	1 (1.0%)	0	1 (0.4%)
Infections and infestations	3 (3.0%)	2 (1.6%)	5 (2.2%)
Pneumonia	0	1 (0.8%)	1 (0.4%)
Pyelonephritis	0	1 (0.8%)	1 (0.4%)
Gastroenteritis	1 (1.0%)	0	1 (0.4%)
Anal abscess	1 (1.0%)	0	1 (0.4%)
Gastroenteritis viral	1 (1.0%)	0	1 (0.4%)
Gastrointestinal disorders	2 (2.0%)	1 (0.8%)	3 (1.3%)
Vomiting	0	1 (0.8%)	1 (0.4%)
Diarrhea	0	1 (0.8%)	1 (0.4%)
Abdominal pain	1 (1.0%)	0	1 (0.4%)
Enteritis	1 (1.0%)	0	1 (0.4%)
Hepatobiliary disorders	0	1 (0.8%)	1 (0.4%)
Biliary colic	0	1 (0.8%)	1 (0.4%)

Source: Reviewer generated in JReview using ADSL and ADAE datasets where SAFFL=Y, TRTEMFL=Y, AESER=Y
 Abbreviation: SAE, serious adverse event; SOC, system organ class; PT, preferred term

AEs Leading to Study Drug Discontinuation

TEAEs leading to premature study drug discontinuation and which occurred in 2 or more subjects or single events related to GI events or LFT labs are outlined in Table 54.

Overall, 19 of 227 subjects (8.4%) discontinued study drug due to a TEAE; 6 subjects (6.0%) in the berotralstat 110 mg group and 13 subjects (10.2%) in the 150 mg group. TEAEs leading to discontinuation in at least one subject were related to GI symptoms (upper abdominal pain, vomiting) or elevated liver enzymes and were more common in the 150 mg group. All patients who discontinued study drug due to nausea, vomiting, or diarrhea were in the 150 mg group.

Table 54. Common TEAEs Leading to Study Drug Discontinuation (Safety Population): Study 204

SOC/PT	Berotralstat		Total N=227 n (%)
	110mg N=100 n (%)	150mg N=127 n (%)	
	[events]	[events]	[events]
Any event leading to discontinuation of study drug	6 (6.0%) [9]	13 (10.2%) [20]	19 (8.4%) [29]
Investigations	3 (3.0%) [4]	5 (3.9%) [6]	8 (3.5%) [10]
Alanine aminotransferase increased	1 (1.0%) [1]	3 (2.4%) [3]	4 (1.8%) [4]
Aspartate aminotransferase increased	1 (1.0%) [1]	1 (0.8%) [1]	2 (0.9%) [2]
Hepatic enzyme increased	1 (1.0%) [1]	1 (0.8%) [1]	2 (0.9%) [2]
Liver function test abnormal	1 (1.0%) [1]	1 (0.8%) [1]	2 (0.9%) [2]
Gastrointestinal disorders	2 (2.0%) [2]	4 (3.1%) [5]	6 (2.6%) [7]
Abdominal pain upper	2 (2.0%) [2]	1 (0.8%) [1]	3 (1.3%) [3]
Vomiting	0	2 (1.6%) [2]	2 (0.9%) [2]
Diarrhea	0	1 (0.8%) [1]	1 (0.4%) [1]
Nausea	0	1 (0.8%) [1]	1 (0.4%) [1]

Source: Applicant's Clinical Study Report for Study 204 (Page 204, Table 66), verified by reviewer

Abbreviations: AE, adverse event; TEAE, treatment-emergent adverse event; SOC, system organ class; PT, preferred term

Treatment-Emergent Adverse Events

The TEAEs with a frequency of $\geq 10\%$ in either of the berotralstat groups or overall are listed in Table 55. The safety profile was similar to that of Study 302, with most AEs in the infections and GI SOCs. Most infections were mild and transient episodes of upper respiratory tract infections and nasopharyngitis. Although diarrhea was a more common AE in the 150 mg group, abdominal pain was more common in the 110 mg group. Therefore, a clear dose-response relationship for overall GI side effects could not be established. Similar to Study 302 Part 1, high rates of GI-related AEs did not dissuade most subjects from continuing the study drug. Most of these AEs were of mild-to-moderate severity; only four subjects had a grade 3 or 4 GI TEAE. Consistent with the efficacy results from Study 302 Part 1, more HAE attacks occurred in the 110 mg versus the 150 mg dose group.

Table 55. Common TEAEs Occurring in ≥10% of Subjects (Safety Population): Study 204

SOC/PT, n (%)	Berotralstat		Total N=227
	110 mg N=100	150 mg N=127	
Subjects with at least one TEAE, n (%)	91 (91.0%)	115 (90.6%)	206 (90.7%)
Infections and infestations	51 (51.0%)	87 (68.5%)	138 (60.8%)
Nasopharyngitis	27 (27.0%)	43 (33.9%)	70 (30.8%)
Upper respiratory tract infection	6 (6.0%)	13 (10.2%)	19 (8.4%)
Gastrointestinal disorders	53 (53.0%)	57 (44.9%)	110 (48.5%)
Diarrhea	12 (12.0%)	18 (14.2%)	30 (13.2%)
Abdominal pain ¹	29 (29.0%)	26 (20.5%)	54 (23.8%)
Nervous system disorders	26 (26.0%)	29 (22.8%)	55 (24.2%)
Headache	21 (21.0%)	19 (15.0%)	40 (17.6%)
Congenital, familial, and genetic disorders	11 (11.0%)	3 (2.4%)	14 (6.2%)
Hereditary angioedema	10 (10.0%)	2 (1.6%)	12 (5.3%)

Source: Reviewer generated in JReview using ADSL and ADAE datasets. where SAFFL=Y, TRTEMFL=Y

¹ Pooled term (abdominal pain, abdominal discomfort, abdominal pain upper, abdominal pain lower)

Abbreviations: PT, preferred term; SOC, system organ class; TEAE, treatment-emergent adverse event

Laboratory Findings

LFTs

As in Study 302, LFT elevations were observed in the OLE Study 204. Table 56 shows the maximum ALT elevation by group. ALT elevations greater than three-fold the ULN occurred more frequently in the 110 mg group (9%) versus the 150 mg group (4.7%). While there was no clear dose-response, the greatest ALT elevation (>10× ULN) occurred in the 150 mg group, as was the case in Study 302 Part 1.

Table 56. Maximum ALT Elevations by Treatment Group (Safety Population): Study 204

Treatment	Baseline ALT (×ULN)	Maximum ALT Elevation During Study (×ULN)				
		≤1.5	1.5 to ≤3	>3 to ≤5	>5 to ≤10	>10
Berotralstat 110 mg N=100	≤1.5	79 (79%)	7 (7%)	3 (3%)	2 (2%)	0
	>1.5 to ≤3	1 (1%)	3 (3%)	2 (2%)	0	0
	>3 to ≤5	1 (1%)	0	1 (1%)	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	1 (1%)	0
Berotralstat 150 mg N=127	≤1.5	111 (87.4%)	8 (6.3%)	2 (1.6%)	3 (2.4%)	1 (0.8%)
	>1.5 to ≤3	1 (0.8%)	1 (0.8%)	0	0	0
	>3 to ≤5	0	0	0	0	0
	<5 to ≤10	0	0	0	0	0
	>10	0	0	0	0	0

Source: Reviewer generated in OCS Analysis Studio, Shift Table Builder

X Cutoffs: ≤1.5× ULN ≤1.5; 1.5 <1.5 <ULN ≤3 ≤3; 3 <3 <ULN ≤5 ≤5; 5 <5 <ULN ≤10 ≤10; 10 <>10× ULN

Y Cutoffs: ≤1.5× ULN ≤1.5; 1.5 <1.5 <ULN ≤3 ≤3; 3 <3 <ULN ≤5 ≤5; 5 <5 <ULN ≤10 ≤10; 10 <>10× ULN

Population filter: SAFFL (safety population flag)= 'Y'; X-axis filter: LBBLFL (baseline flag)= 'Y'; Y-axis filter.

Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

Maximum AST elevations greater than three-fold the ULN were infrequent in both treatment groups; a single subject in the 150 mg group had an AST elevation more than five-fold the ULN. No subjects had total bilirubin levels >2× ULN, and there were no cases meeting Hy's law.

Table 57. Prior Androgen Use and ALT Elevations (Safety Population): Study 204

Parameter	Berotralstat			
	110 mg N=100		150 mg N=127	
	Never Used	Used	Never Used	Used
Post-baseline maximum ALT, n (%)				
≤ULN	29 (29.0%)	46 (46.0%)	39 (30.7%)	61 (48.0%)
>ULN to 1.5× ULN	1 (1.0%)	5 (5.0%)	2 (1.6%)	10 (7.9%)
>1.5× ULN to 3× ULN	1 (1.0%)	9 (9.0%)	2 (1.6%)	6 (4.7%)
>3× ULN to 5× ULN	0	6 (6.0%)	0	2 (1.6%)
>5× ULN to 10× ULN	0	3 (3.0%)	0	3 (2.4%)
>10× ULN to 20× ULN	0	0	0	1 (0.8%)
Missing	0	0	0	1 (0.8%)
Duration of prior androgen use, n (%)				
Never used	31 (31.0%)	0	43 (33.9%)	0
<6 months	0	3 (3.0%)	0	8 (6.3%)
6 months to <1 year	0	3 (3.0%)	0	2 (1.6%)
1 year to <5 years	0	16 (16.0%)	0	21 (16.5%)
5 years to <10 years	0	16 (16.0%)	0	18 (14.2%)
≥10 years	0	31 (31.0%)	0	35 (27.6%)

Source: Reviewer generated table in JReview using ADSL and ADLB datasets
 Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

The Applicant asserted that a history of prior androgen use was a risk factor for the development of elevated liver enzymes in their phase 2 and 3 studies. Although not evident in Study 302 Part 1, the relationship between ALT elevation with berotralstat treatment and history of androgen use for HAE prophylaxis is more apparent in Study 204. Elevations >3× ULN only occurred in subjects who reported prior androgen therapy; however, there were insufficient data provided to determine the impact of other factors such as dose and duration of prior androgen use, time since cessation of androgen therapy, and transaminase elevation with androgen therapy to fully characterize this potential risk. Therefore, in the absence of additional data and a placebo control comparator, it is difficult to interpret this data into meaningful information that could be included in the product label to guide prescribers.

Reviewer comment: Although androgen therapy is clearly associated with a higher risk of hepatocyte injury, there is no known mechanism by which prior androgen-exposed patients would be more susceptible to the hepatotoxic effects of berotralstat. A literature search was performed, and no similar relationship was found in the lanadelumab program or with other currently approved HAE therapeutic agents.

120-Day Safety Update

The Applicant also submitted a 120-day safety update report with a data cutoff at December 1, 2019. This provided integrated safety data from all three parts of Study 302 (first patient enrolled March 14, 2018) and OL Study 204 (first patient enrolled February 27, 2018). Most common TEAEs observed in the integrated Phase 2/3 studies remained consistent with what was reported in the pivotal trial, particularly mild to moderate gastrointestinal adverse events such as abdominal pain and diarrhea. No new safety concerns were identified in laboratory safety monitoring, with the exception of a single grade 3 AE of transient leukocytosis. No TEAEs

of arrhythmia or QT prolongation were reported. Overall, there is no evidence of any increasing or new toxicity with chronic use of berotralstat.

Human Carcinogenicity or Tumor Development

In Study 302, one subject in the 110 mg group developed multiple myeloma, which was deemed unlikely to be related to study treatment, and one subject in the placebo group developed a leiomyoma. In Study 204, one subject in the 150 mg group developed acute myelocytic leukemia. Although rare events such as malignancy are unlikely to appear in a clinical development program of this size, berotralstat is not anticipated to have immunomodulatory effects that increase the risk of cancer in humans. Potential tumor and cancer risks to humans based on non-clinical studies were discussed in discussed in Section [5.1.1](#).

Human Reproduction and Pregnancy

All pregnancy tests administered during Part 1 of Study 302 were negative. Three pregnancies were reported for the OLE Study 204: Subjects (b) (6), and a partner pregnancy for Subject (b) (6). In both female subjects, study drug was stopped, and the estimated maximum drug exposure was 28 days. One female subject had an uncomplicated delivery of a healthy infant and the other had a delivery date of May 21, 2020. The male subject whose partner had become pregnant continued study drug and his partner delivered a healthy infant.

Pediatrics and Assessment of Effects on Growth

The safety profile in adolescent patients 12 to 17 years of age enrolled in Study 302 and OLE Study 204 was similar to that in adults. Berotralstat is not expected to impact growth. Although the consequences of long-term suppression of plasma kallikrein are not fully known, the fact that congenital deficiency of prekallikrein is largely asymptomatic is reassuring.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Based on the mechanism of action, berotralstat is not expected to have any potential for drug abuse or dependence.

8.3.8. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarketing Experience

There is no postmarketing experience with berotralstat.

Expectations on Safety in the Postmarketing Setting

Because Studies 204 and 302 excluded patients with certain levels of pre-existing LFT abnormalities, it is possible that patients with HAE with underlying hepatic dysfunction may experience more pronounced side effects not observed in clinical trials. However, the dosage

recommendation for patients with moderate to severe hepatic impairment will be 110 mg (Section 6.3.3). Overall, the patient population evaluated in Study 302 as well as Study 204 are reasonably representative of the HAE patients who are expected to take berotralstat in the postmarket setting. Although the clinical trial safety database is small due to the rarity of this disease, the safety profile observed in the clinical development program may be generalized to the target population.

8.3.9. Integrated Assessment of Safety

The safety data submitted with the NDA were sufficient to assess the safety of berotralstat in the proposed HAE population. The safety information for berotralstat is primarily derived from the pivotal, placebo-controlled phase 3 trial BCX7353-302, Part 1. Additional support for long-term safety comes from the OLE Study BCX7353-204. Study 302 included 121 adult and adolescent HAE subjects, 81 of whom received at least one dose of berotralstat and of these 40 received the proposed recommended starting dose of 150 mg once daily. A total of 227 subjects (100 in the 110 mg group and 127 in the 150 mg group) received open-label berotralstat in the OLE Study 204. No deaths occurred in the berotralstat development program. SAEs were single events, only one of which occurred with berotralstat (in Study 302) and appeared unrelated to treatment. Gastrointestinal-related adverse events and transaminase elevations emerged as the main safety signals. In Part 1 of Study 302, TEAEs such as abdominal pain, vomiting, and diarrhea occurred more frequently in a dose-dependent manner with berotralstat compared to placebo. Dyspepsia and gastroesophageal reflux events occurred with greater frequency in the 110 mg dose group and led to one treatment discontinuation. That said, GI-related TEAEs were generally mild-to-moderate severity and self-limited, and typically did not lead to study drug discontinuation. TEAEs related to transaminase elevations, primarily increased ALT, were reported with berotralstat treatment in the controlled and OLE studies. No potential Hy's law cases were identified in the program. In Study 302, only one subject had AST/ALT $>3\times$ ULN; this subject was in the 150 mg group and had ALT $>10\times$ ULN, which led to treatment discontinuation. In OLE Study 204, there was no clear dose-response with regard to AST/ALT elevations $>3\times$ ULN; however, again, one subject in the 150 mg group experienced ALT $>10\times$ ULN. Overall, though, transaminase elevations were generally transient, asymptomatic, not dose-dependent, and in some cases confounded by underlying comorbidities and prior androgen use. In general, safety data from OLE study 204 was consistent with the placebo controlled data and supports chronic berotralstat use. Although not observed in clinical trials with the 150 mg dose, berotralstat prolongs the QT interval in a dose and concentration dependent manner based on data from the thorough QT study. Therefore, based on the pharmacologic action, QT prolongation is an anticipated risk with higher than recommended doses of 150 mg once daily. Overall, the berotralstat safety profile for the treatment of a rare, serious disease such as HAE is favorable.

8.4. Conclusions and Recommendations

The recommended regulatory action from both the clinical and statistical perspective is Approval. The berotralstat development program for HAE prophylaxis consisted of a single pivotal efficacy and safety trial, BCX7353-302, Part 1 that was adequately conducted and well-

controlled. Results from this trial demonstrated substantial evidence of efficacy with both berotralstat doses compared to placebo in patients with type I or II HAE for the primary endpoint of HAE attack rate over the 24 week treatment period and pre-defined exploratory endpoint of $\geq 50\%$ reduction in attack rate from baseline. Given the small number of adolescent subjects, efficacy in this age group is primarily based on extrapolation from efficacy data in adults because the pathophysiology of disease is similar irrespective of age. The numerically greater and more clinically significant treatment effect in the higher dose arm supports a recommended starting dose of 150 mg once daily, with dose adjustments to 110 mg once daily in patients with moderate-to-severe hepatic impairment and for patients with persistent GI-related adverse reactions to allow flexibility and individualization in dosing while maintaining similar efficacy.

The safety data submitted with the NDA were sufficient to assess the safety of berotralstat in the proposed HAE population. Gastrointestinal-related adverse events and transaminase elevations emerged as the main safety signals. TEAEs such as abdominal pain, vomiting, diarrhea, dyspepsia and gastroesophageal reflux occurred more frequently, often in a dose-dependent manner, with berotralstat compared to placebo. However, GI-related TEAEs were generally mild-to-moderate severity and self-limited, and typically did not lead to study drug discontinuation. TEAEs related to transaminase elevations, primarily increased ALT, were reported with berotralstat treatment in the controlled and OLE studies. Few subjects had marked elevations triggering treatment discontinuation, and no potential Hy's law cases were identified in the program. Transaminase elevations were generally transient, asymptomatic, not dose-dependent, and in some cases confounded by underlying co-morbidities and prior androgen use. In general, safety data from OLE study 204 was consistent with the placebo controlled data and supports chronic berotralstat use. Although not observed in clinical trials, QT prolongation is an anticipated risk with higher than recommended doses based on the pharmacologic action of the drug. Efficacy and safety results across various demographic and baseline characteristic subgroups, including adolescents, were generally consistent with the overall findings. Overall, the risk/benefit profile for berotralstat in the treatment of a rare, serious disease such as HAE is favorable.

9. Advisory Committee Meeting and Other External Consultations

The Pulmonary and Allergy Drug Advisory Committee (PADAC) was not convened to discuss this NDA. Neither a PADAC meeting nor an external consultation was deemed necessary because the review did not raise any significant or unexpected safety or efficacy issues for this drug class or indication.

10. Pediatrics

Berotralstat has been granted orphan drug designation and is exempt from the requirements of the Pediatric Research Equity Act. Adolescents were included in phase 2 and 3 trials, and therefore, the indication will be for patients with type I or II HAE who are 12 years of age and older.

11. Labeling Recommendations

11.1. Prescription Drug Labeling

11.1.1. Prescribing Information

The Applicant submitted proposed prescribing information, patient package insert, and carton and container labeling for berotralstat that included the tradename ORLADEYO. The label was reviewed by the appropriate disciplines within the Division and labeling consultants who recommended various changes to correct formatting errors and to better describe the drug product and indicated population to healthcare providers and fully inform patients. A high-level summary of significant labeling changes is provided in [Table 58](#).

Table 58. Summary of Major Labeling Changes

Section	Proposed Labeling	Approved Labeling	Rationale
Section 1: Indication and Usage	No Limitation of Use statement	Limitations of Use: The safety and effectiveness of ORLADEYO for the treatment of acute HAE attacks have not been established. ORLADEYO should not be used for treatment of acute HAE attacks. Additional doses or doses of ORLADEYO higher than 150 mg once daily are not recommended due to the potential for QT prolongation [see Warnings and Precautions [W&P] (5.1)].	Originally proposed W&P statement relocated as a limitation of use statement and modified to include safety risk of higher than recommended doses.
Section 2: Dosage and Administration	150 mg capsule taken orally once daily	150 mg capsule taken orally once daily with food 110 mg once daily with food for patients with moderate to severe hepatic impairment, concomitant use with P-gP or BCRP inhibitors, or persistent GI reactions	- Although no food effect observed, clinical trials administered with food to mitigate GI adverse reactions - Reflect recommendations for 110 mg dose
Section 3: Dosage Form and Strengths	Capsules: 150 mg	Capsules: 150 mg, 110 mg	Reflect availability of 110 mg strength
Section 5: Warnings and Precautions	(b) (4)	ORLADEYO should not be used for treatment of acute attacks of HAE. Additional doses or doses of ORLADEYO higher than 150 mg	(b) (4)

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 ORLADEYO (berotralstat)

Section	Proposed Labeling	Approved Labeling	Rationale
	(b) (4)	once daily are not recommended. An increase in QT was observed at dosages higher than the recommended 150 mg once daily dosage and was concentration dependent [see Clinical Pharmacology (12.2)].	(b) (4) W&P statement revised to characterize the risk of taking higher than recommended doses
Section 6: Adverse Reactions	(b) (4)	(b) (4) <u>Added/Revised:</u> - Reference to W&P for QT prolongation - Less common AR between 5 and <10% included - New section for Transaminase Elevations: In Part 1 of Trial 1, a single 150 mg ORLADEYO-treated patient discontinued treatment due to asymptomatic elevated transaminases (ALT >8x the upper limit of normal [ULN] and AST >3x ULN). Total bilirubin was normal. No subject receiving 110 mg or placebo developed transaminase levels >3x ULN. In addition to this patient, 2 ORLADEYO-treated patients developed laboratory-related hepatic adverse events compared to 1 placebo-treated patient. No patient reported serious adverse reactions of elevated transaminases.	- Revisions for clarity and removal of language that minimizes safety risks. - Separate subheading for transaminase elevation to better characterize the risk observed in trials. (b) (4)
Section 7: Drug Interactions	(b) (4)	(b) (4)	Reflect recommendations from clinical pharmacology review
Section 8: Use in Specific Populations	(b) (4)	(b) (4)	- No data available in ESRD, so use not recommended - Reflect dose adjustment for mod-severe hepatic impairment based on clin pharm review
Section 11: Description	Description of 150 mg capsule	Added description of 110 mg capsule	To reflect availability of 110 mg dose
Section 12.2: Pharmacodynamics (Cardio Electrophysiology)	(b) (4)	At the recommended dose of 150 mg once daily, ORLADEYO does not prolong the QT interval to any clinically relevant extent. At 3-fold the recommended dose, the mean (upper 90% confidence interval) increase in QTcF was 15.9 ms (23.5 ms). The observed	Reflect values from QT-IRT review analysis from TQT Study 106.

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 ORLADEYO (berotralstat)

Section	Proposed Labeling	Approved Labeling	Rationale
	(b) (4)	increase in QTcF was concentration-dependent.	
Section 12.3: Pharmacokinetics	(b) (4) • Berotralstat 150 mg once daily is a moderate inhibitor of CYP2D6 and CYP3A4, and a weak inhibitor of CYP2C9. (b) (4)	• Based on the population pharmacokinetic analyses that included elderly patients (≥65 to 74 years, N=25), age does not have a clinically meaningful impact on the systemic exposure of berotralstat. • In subjects with moderate hepatic impairment; C_{max} was increased by 77%, while AUC_{0-inf} was increased by 78%. In subjects with severe hepatic impairment, C_{max} was increased by 27%, while AUC_{0-last} was decreased by 5%. The median half-life of berotralstat was increased by 37% and 22% in patients with moderate and severe hepatic impairment, respectively, in comparison to healthy subjects. The percent of unbound berotralstat increased 2-fold from a mean of 1.2% in healthy subjects to a mean of 2.4% in subjects with severe hepatic impairment. • Berotralstat 150 mg once daily is a moderate inhibitor of CYP2D6 and CYP3A4, and a weak inhibitor of CYP2C9 and CYP2C19.	• Reflect changes based on pharmacometrics and clinical pharmacology review. (b) (4)
Section 14: Clinical Studies	(b) (4)		

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Abbreviations: ALT, alanine aminotransferase; AR, adverse reaction; AST, aspartate aminotransferase; AUC, area under the curve; BCRP, breast cancer resistance protein; CI, confidence interval; C_{max} , maximum concentration; CYP, cytochrome P450; ESRD, end-stage renal disease; GI, gastrointestinal; HAE, hereditary angioedema; IRT, integrated review team; P-gp, p-glycoprotein; PI, prescribing information; QTcF, Fredericia-corrected QT; TQT, thorough QT; ULN, upper limit of normal

11.1.2. Other Prescription Drug Labeling

The patient package insert was reviewed by Patient Labeling (DMPP) and revised to ensure consistency with the PI and to improve readability and comprehension by using patient-friendly terminology.

12. Risk Evaluation and Mitigation Strategies

No additional risk management strategies beyond labeling and routine postmarketing pharmacovigilance are required.

13. Postmarketing Requirements and Commitment

A Postmarketing Commitment will be issued for the following:

PMC #1—Submit the final clinical study report from Parts 2 and 3 of ongoing Study BCX7353-302. Agreed-upon submission date: December 2023

Submit the final clinical study report from Parts 2 and 3 of ongoing Study BCX7353-302 to (b) (4) long-term efficacy and safety assessments. The study will be completed in June 2023 and the final report submitted in December 2023.

14. Division Director (DPACC) Comments

This NDA provides for berotralstat as a prophylaxis to prevent attacks of HAE in patients 12 years of age and older. Berotralstat is an NME that inhibits plasma kallikrein. The tradename is Orladeyo. HAE is a rare disease with variable attacks of swelling/edema that can potentially be life threatening. In patients with HAE, deficient or dysfunctional C1-INH leads to increased kallikrein activity and release of bradykinin which leads to the characteristic swelling of acute HAE attacks. Berotralstat decreases plasma kallikrein activity to control excess bradykinin generation in patients with HAE.

The efficacy and safety of berotralstat was evaluated in a single 24-week, randomized, double-blind, placebo controlled, clinical trial in 120 patients with HAE 12 years of age and older (Study 302). While Study 302 had several parts, the focus of the review was on Part 1, the 24-week

placebo-controlled period. Two dose levels were evaluated: 110 mg once daily and 150 mg once daily.

The primary endpoint was the rate of investigator-confirmed HAE attacks during the 24-week treatment period. HAE attacks are an established and meaningful efficacy endpoint for HAE programs. Results from this pivotal trial showed a significant reduction in HAE attacks over the 24 week treatment period for both doses of berotralstat compared to placebo. The HAE attack rate per 28 days was 1.7, 1.3, and 2.4 for berotralstat 110 mg, berotralstat 150 mg, and placebo respectively with a rate ratio of 0.70 (95% CI 0.51, 0.95) [berotralstat 110 mg] and 0.56 (95% CI 0.41, 0.77) [berotralstat 150 mg] relative to placebo. Secondary endpoints were generally supportive of efficacy. While efficacy was demonstrated for both berotralstat doses, there was a numerically greater treatment effect with the 150 mg dose. In addition, there were numerically greater proportions of patients with 50%, 75%, and 90% reductions from baseline of investigator confirmed attack rates with the 150 mg dose compared to the 110 mg dose of berotralstat. Based upon these results, the sponsor has proposed the 150 mg dose of berotralstat. Overall, this single adequate and well-controlled trial is sufficient to provide substantial evidence of efficacy. Efficacy was demonstrated for each dose group of berotralstat and the results for the 150 mg group were statistically persuasive.

The safety data were derived primarily from the single pivotal trial, Study 302, with some supportive data from the open-label Study 204. While the size of the safety database is small, this is not unusual for a rare disease program and overall the safety database is acceptable for this indication. Review of the safety data showed that GI adverse reactions (e.g. abdominal pain, nausea, vomiting, diarrhea) were the most commonly reported adverse reactions and there was a dose response demonstrated. There were no deaths. SAEs were limited and didn't identify a particular safety signal. Elevated transaminases were also noted, but no cases of Hy's law were identified. QT prolongation with doses higher than 150 mg were identified in the thorough QT study. Given the nonlinear PK for berotralstat, information regarding potential for QT prolongation with higher than approved doses will be included in the product labeling.

The sponsor has proposed an indication in patients down to 12 years of age. There were only six adolescents in Study 302. Review of the efficacy and safety data for these six adolescents is consistent with the findings in adult patients. An additional 10 adolescents were evaluated in Study 204. No unique safety signals were identified. The pathophysiology of HAE is the same irrespective of age and efficacy in adolescents is expected to be similar to that in adults and may be extrapolated from the adult data.

Overall, the submitted data support a favorable benefit risk profile for berotralstat for the prevention of HAE attacks in patients 12 years of age and older. All disciplines recommend approval and I agree with approval of this application. Berotralstat provides a new oral treatment option for patients with HAE. Labeling has been agreed to by the FDA and the Applicant. The recommended dosing regimen is 150 mg once daily. A 110 mg daily dose is recommended for patients with moderate-to-severe hepatic impairment or for patients with persistent GI adverse reactions.

15. Office Director (OI) Comments

I concur with the recommendation from the Division of Pulmonology, Allergy and Critical Care to approve berotralstat, an orally administered inhibitor of active plasma kallikrein, for prophylaxis to prevent attacks of hereditary angioedema (HAE) in patients 12 years of age and older. This new molecular entity represents a new prophylaxis option for patients with HAE.

The efficacy of berotralstat for this rare disorder was demonstrated in a single double-blind, placebo-controlled trial. Both doses evaluated, 110 mg and 150 mg administered daily, significantly reduced investigator-confirmed HAE attack rates compared to placebo through 24 weeks of treatment. The numerically greater treatment effect supports a recommended starting dose of 150 mg, with use of 110 mg recommended for patients with moderate-to-severe hepatic impairment and for patients with persistent gastrointestinal-related adverse reactions.

Berotralstat was generally well tolerated with gastrointestinal-related adverse reactions and transaminase elevations as the main safety findings. Prolongation of the QT interval is an expected finding at doses above 150 mg based on the pharmacology of the drug. Product labeling adequately conveys the safety risks associated with use of berotralstat; a REMS will not be required.

The pathophysiology of HAE is similar in adolescents and adults. Although the number of adolescent subjects enrolled in clinical trials was small, efficacy and safety results were similar to those in adults, supporting inclusion of pediatric patients aged 12 years of age and older in the indicated population.

16. Appendices

16.1. Financial Disclosure

Covered Clinical Study (Name and/or Number): BCX7353-302

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 221 (41 primary investigators, 180 sub-investigators)		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
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: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in Sponsor of covered study: _____		
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) <u>Not Applicable</u>		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

16.2. Nonclinical Pharmacology/Toxicology

16.2.1. Nonclinical Pharmacology/Toxicology

16.2.1.1. Integrated Summary and Safety Evaluation

The nonclinical program of berotralstat consists of studies of pharmacology, pharmacokinetics, and toxicology. The toxicology program consists of studies of general toxicity (chronic oral toxicity studies in rats and monkeys), genetic toxicity (standard battery of testing assays), carcinogenicity (oral traditional 2-year bioassay in rats and a 26-week study in a transgenic mouse model), and reproductive and developmental toxicity (teratology studies in rats and rabbits, a fertility and early embryofetal developmental study in rats, and a peri- and post-natal development [PPND] study in rats).

16.2.1.2. Pharmacology

Berotralstat decreases vascular permeability and edema in patients with hereditary angioedema (HAE) by reducing the blood level of bradykinin, a potent vasodilatory agent. Berotralstat decreases the bradykinin level by inhibiting kallikrein (inhibition constant 0.44nM), which cleaves and releases bradykinin from high-molecular-weight kininogen. The half-maximal inhibitory concentration for kallikrein activity in activated human, monkey, and rat plasma is 5.4, 5.2, and 7.2nM, respectively.

Berotralstat inhibited currents of K⁺, Na⁺, and Ca⁺⁺ channels in vitro and prolonged QT intervals in monkeys and humans. It also affected duration and amplitude of action potential in vitro. The effect of berotralstat on QT has been thoroughly investigated in clinical studies. See the section on effects on the [Cardiovascular System](#).

16.2.1.3. Pharmacokinetics and Toxicokinetics

Berotralstat was rapidly absorbed after oral administration to animals. The plasma drug level generally increased in proportion to the administered dose. There were no significant difference in plasma drug level between sexes. Oral bioavailability was 33% and 45% in rats and

monkeys, respectively. The half-life was 18.5, 22.7, and 93 hours in rats, monkeys, and humans, respectively. The protein-binding capacity was high in humans, rats, and mice (98.7 to 99.8%) but low in monkeys and rabbits (74.1% to 81.9%). The tissues showing the highest maximum concentrations of radioactivity were the liver, kidney medulla, spleen, adrenal glands, kidneys, lungs, and pituitary gland.

Metabolism of berotralstat was low across species. About 61% of orally administered berotralstat was excreted unchanged in rats. Less than 10% of berotralstat was metabolized when incubated with human cryopreserved hepatocytes for 360 minutes. No unique or major human metabolites were observed.

Bioaccumulation of berotralstat occurred in rats, but not in monkeys. In rats, the berotralstat area under the curve from time zero to 24 hours (AUC₀₋₂₄) in week 26 was approximately three-fold that in week 1. The monkeys showed no apparent difference in AUC₀₋₂₄ between weeks 1 and 39. For example, the respective mean AUC₀₋₂₄ at 20 mg/kg/day in weeks 1 and 26 was 3190 and 9710 ng.h/mL in rats and 3310 and 3110 ng.h/mL in monkeys.

16.2.1.4. Toxicology

A comprehensive toxicology program was conducted to evaluate the general toxicity, genetic toxicity, carcinogenicity, and reproductive and developmental toxicity of berotralstat. General toxicity studies included chronic oral toxicity studies for up to 6 and 9 months in rats and monkeys, respectively. Evaluation of the genetic and reproductive toxicity potential involved standard batteries of tests. The carcinogenicity studies comprised a standard 2-year bioassay and a study in a transgenic mouse model. A comprehensive evaluation of the effect of berotralstat on growth and development in juvenile animals has not been completed.

Evaluation of the general toxicity of berotralstat included chronic toxicity studies for up to 6 months in rats and 9 months in monkeys. These studies showed that the toxicity of berotralstat was dependent on the dose and treatment duration. The berotralstat doses used in the chronic toxicity studies were up to 20 and 80 mg/kg/day in rats and monkeys, respectively. Higher doses of berotralstat were used in the shorter-term studies (up to 75 and 100 mg/kg/day in rats and monkeys, respectively). The target organs of toxicity were the kidney, liver, intestine, and others ().

Table 59. Repeat-Dose Toxicity Studies of Berotralstat in Animals

Species	Duration (Weeks)	Berotralstat ¹ (mg/kg/day)	Key Findings/Target Organs	Study #
Rat	4	0, 5, 10, 25, 75	Brain, heart, kidney, hepatobiliary	806-131
	13	0, 2.5, 7.5, 20	Liver	806-144
	26	0, 1.0, 2.5, 7.5, 20	Hepatobiliary, intestine, kidney	8001291
Monkey	4	0, 10, 30, 100	Kidney, liver, intestine, spleen	806-132
	13	0, 2.5, 7.5, 20	None	806-145
	39	0, 30, 55, 80	Kidney, liver, intestine	806-162
	39	0, 1.0, 2.5, 7.5, 20	Liver	8001292

Source: Constructed by the reviewer based on information provided in the submission.

¹ Oral gavage dose. The table lists only key good laboratory practices-compliant toxicity studies.

Mortality

Dose- and treatment duration-dependent deaths occurred in rats, but not in monkeys. Deaths in rats occurred at ≥ 750 mg/kg after single doses of 150 mg/kg/day for 7 days (AUC_{0-24} 58,000 ng.h/mL), and 75 mg/kg/day for 4 weeks (AUC_{0-24} 40,500 ng.h/mL). No deaths occurred at a single dose of 450 mg/kg (AUC_{0-24} 11,900 ng.h/mL), 25 mg/kg/day for 4 weeks (AUC_{0-24} 11,200 ng.h/mL), or 20 mg/kg/day for 26 weeks (AUC_{0-24} 16,000 ng.h/mL). No deaths were observed in monkeys at doses up to 100 mg/kg/day for 28 days (AUC_{0-24} 16,000 ng.h/mL).

Studies in Rats

The repeat-dose studies in Wistar Han rats involved oral (gavage) administration of berotralstat for 4, 13, and 26 weeks. The berotralstat dose was up to 75, 20, and 20 mg/kg/day in the 4-, 13-, and 26-week studies, respectively. The results identified the following as the target organs of berotralstat toxicity: liver, kidney, and intestine.

In a 4-week study (#806-131), rats (10 and 5/sex/dose in the main study and recovery period, respectively) were dosed with 0 (water), 5, 10, 25, and 75 mg/kg/day once daily for 28 days. The recovery period was 2 weeks. Additional rats (three and nine of each sex in the vehicle and treatment groups, respectively) were included in the toxicokinetics (TK) analysis. The mean $AUC_{0-24, \text{ day } 28, \text{ male/female}}$ was 1310, 3830, 20,200, and 130,000 ng.h/mL in the low-dose (LD), mid-low-dose, mid-high-dose, and high-dose (HD) groups, respectively. The HD rats showed audible breathing and piloerection, decreased bodyweight (9% to 16%), clinical chemistry changes (decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and an increased number of neutrophils, aspartate aminotransferase [AST] level, and alanine aminotransferase [ALT] level), and increased liver weight. The microscopic changes included vacuolation/foamy macrophages in multiple organs (the adrenal glands [cortical], brain [choroid plexus], liver, lung, lymph nodes [mesenteric], ovaries, pituitary glands, salivary glands, and intestine); tubular regeneration in the kidney; and minimal degeneration/necrosis and hypertrophy of the bile ducts. Most of the microscopic changes were confined to the HD group, but liver and bile duct changes were observed in all of the groups. The findings in the LD groups were of low incidence and minimal severity.

In a 13-week study (#806-144), rats (15 and 5/sex/dose in the main study and recovery period, respectively) were dosed by oral gavage with 0, 2.5, 7.5, and 20 mg/kg/day berotralstat for 26 weeks. The recovery period was 6 weeks. Additional rats (12/sex/dose) were included in the TK analysis. The respective mean $AUC_{0-24, \text{ day } 90}$ in the LD, MD, and HD groups was 318, 1890, and 6330 ng.h/mL in males; and 476, 3480, and 16,000 ng.h/mL in females. The MD and HD groups showed minimal to mild bile duct hypertrophy in males and females (incidence 10 MD males, 14 HD males, 15 MD females, and 15 HD females). The HD group showed bile duct hyperplasia (6 females only) and vacuolated macrophages (13 and 15 males and females, respectively). The no observable adverse effects level [NOAEL] of the study was 2.5 mg/kg/day, corresponding to a mean AUC_{0-24} of 397 ng.h/mL.

In a 26-week study (#8001291), rats (15 and 5/sex/dose in the main study and recovery section, respectively) were dosed by oral gavage with 0, 1.0, 2.5, 7.5, and 20 mg/kg/day berotralstat for

26 weeks. Additional rats (nine/sex/dose) were included in the TK analysis. The recovery period was 13 weeks in duration. The mean $AUC_{0-24, \text{ day } 182, \text{ male/female}}$ was 56.9, 297, 2030, and 9710 ng.h/mL in the LD, mid-low-dose, mid-high-dose, and HD groups, respectively. Microscopic changes were observed in the hepatobiliary system, lymph nodes (mesenteric), spleen, intestine (jejunum and duodenum), and nasal cavity in the ≥ 7.5 mg/kg/day groups. Most of the changes were vacuolation in the mucosa and foamy histiocytes. The bile duct showed minimal-to-moderate hyperplasia. Changes in the nasal cavity were believed to be related to gastric reflux and irritation. The NOAEL of the study was 2.5 mg/kg/day, corresponding to a mean AUC_{0-24} of 297 ng.h/mL.

Studies in Monkeys

Studies completed in nonhuman primates involved oral (gavage) administration of berotralstat for 4, 13, and 39 weeks in cynomolgus monkeys. In the 4-week study (Study 806-132), monkeys (five and two/sex/dose in the control, MD, and HD groups in the main study and recovery period, respectively; and three/sex in the MD main study) were dosed with 0, 10, 30, and 100 mg/kg/day berotralstat for 28 days. The mean $AUC_{0-24, \text{ day } 28, \text{ male/female}}$ was 1350, 5320, and 146,000 ng.h/mL in the LD, MD, and HD groups, respectively. The HD group showed emesis and watery feces; changes in electrocardiogram parameters; increases in lipase (>7-fold), AST (~3-fold), and ALT (6- to 10-fold) levels, increases in the organ weight of the kidney and liver; and microscopic changes. Microscopic changes were observed in the kidney (basophilic tubules and tubular degeneration/regeneration), liver (diffused fatty changes and increased cytoplasmic collagen and hepatocellular hypertrophy), intestine (histiocytic infiltration), and spleen (follicular lymphoid hyperplasia). No significant findings were observed in the recovery animals after the 3-week recovery period.

In a 13-week study (Study 806-145), monkeys (four and two/sex/dose in main study and recovery sections, respectively) were dosed with 0, 2.5, 7.5, and 20 mg/kg/day berotralstat for 91 days. The recovery period was 6 weeks. The mean $AUC_{0-24, \text{ day } 91, \text{ male/female}}$ was 278, 930, and 3350 ng.h/mL in the LD, MD, and HD groups, respectively. No treatment-related findings were observed in any berotralstat treatment groups. The NOAEL was 20 mg/kg/day.

In a 39-week study (Study 8001292), monkeys (four and two/sex/dose in the main study and recovery section, respectively) were dosed with 0, 2.5, 7.5, and 20 mg/kg/day berotralstat for 273 days. The recovery period was 13 weeks. The mean $AUC_{0-24, \text{ day } 273, \text{ male/female}}$ was 240, 559, and 2880 ng.h/mL in the LD, MD, and HD groups, respectively. No treatment-related findings were observed in any of the berotralstat treatment groups. The NOAEL was 20 mg/kg/day.

In another 39-week study (#806-162), monkeys (6, 4, 4, and 6/sex in the C, LD, MD, and HD groups, respectively) were dosed by oral gavage with 0, 30, 55, and 80 mg/kg/day berotralstat for 273 days. Additional monkeys were included for a 13-week interim analysis (three/sex/dose) and recovery (two/sex/dose in the control and HD groups only). The mean $AUC_{0-24, \text{ day } 273, \text{ male/female}}$ was 3950, 7780, and 13,500 ng.h/mL in the LD, MD, and HD groups, respectively. The MD and HD groups showed decreases (up to 38%) in fibrinogen concentration, increases in AST and ALT levels (up to 4- and 8.7-fold, respectively) and a decrease in albumin

level (16%). The histological findings in the MD and HD groups were as follows (Table 60): degeneration (kidney, tubular), hyperplasia (kidney, bone marrow, and spleen), hypertrophy (liver, hepatocellular), vacuolation (adrenal, cortical), and foamy macrophages (lung, lymph node, and intestine). The foamy macrophages were a result of phospholipidosis. The LD group showed foamy macrophages in the intestine and lymph nodes. The kidney showed the most severe tissue damage (tubular degeneration and hyperplasia), but the intestine was the most sensitive organ. The findings were most pronounced in the main study and were of lesser magnitude in the interim and recovery sections. The results indicate that the effects of berotralstat in monkeys were partially reversible. This study did not establish a NOAEL.

Table 60. Microscopic Findings in a 9-Month Study in Monkeys (806-162)

Organ	Berotralstat (mg/kg/day)	Incidence							
		Male				Female			
		0	30	55	80	0	30	55	80
<i>Main study (n=4/sex/dose)</i>									
Adrenal gland	Vacuolation, increased; ≤mild	0	3	2	4	0	3	3	4
Bone marrow	Hyperplasia, follicular lymphoid, ≤mod	0	0	0	0	0	1	2	4
Kidney	Hyperplasia, tubular, ≤mod	0	0	4	4	0	0	4	4
	Degeneration, tubular, ≤mild	0	0	1	4	0	0	1	4
Liver	Hypertrophy, hepatocellular, ≤mod	0	0	3	4	0	0	4	4
	Pigment, Kupffer cell, ≤mild	0	0	1	3	0	0	4	3
Lung	Macrophage, foamy/pigmented, min	0	0	0	2	0	0	0	0
Lymph node	Foamy macrophages, mesenteric, ≤mod	0	2	4	4	0	2	4	4
Small intestine	Foamy macrophages, duodenum, min	0	1	2	2	0	0	2	4
	Foamy macrophages, ileum, ≤mod	0	2	4	4	0	3	3	4
	Foamy macrophages, jejunum, ≤mod	0	2	2	1	0	2	3	4
Spleen	Hyperplasia, focal lymphoid, min	0	0	0	0	0	0	0	2
<i>Interim (n=3/sex/dose)</i>									
Kidney	Hyperplasia, tubular, ≤mild	0	0	3	2	0	1	1	3
	Degeneration, tubular, ≤mild	0	0	2	0	0	0	1	2
	Vacuolation, tubular, mild	0	0	0	1	0	0	0	1
Liver	Hypertrophy, hepatocellular, ≤mod	0	0	2	2	0	3	2	3
Lymph node	Foamy macrophages, mesenteric, ≤moderate	0	0	2	2	0	0	1	2
Small intestine	Foamy macrophages, duodenum, minimal	0	0	0	0	0	0	0	2
	Foamy macrophages, ileum, ≤mod	0	0	3	3	0	2	2	3
Thymus	Lymphoid depletion, ≤mild	0	0	2	3	0	0	1	3
<i>Recovery (n=2/sex/dose)</i>									
Bone marrow	Hyperplasia, follicular lymphoid, minimal	0	-	-	1	0	-	-	1
Kidney	Basophilic tubules, ≤mild	0	-	-	1	0	-	-	2
Liver	Pigment, Kupffer cell, ≤mild	0	-	-	2	0	-	-	2
Lymph node	Foamy macrophages, mesenteric, minimal	0	-	-	0	0	-	-	1
Small intestine	Foamy macrophages, ileum, ≤moderate	0	-	-	1	0	-	-	2

Source: Extracted from Section 2.6.7 Toxicology Tabulated Summary, pages 70-77.
 Abbreviations: min, minimal; mod, moderate; -, not applicable

Targets of Berotralstat Toxicity

Kidney

The kidneys are target organs of berotralstat toxicity in both rats and monkeys. Dose-dependent increases in the incidence of tubular degeneration and regeneration were observed at 75 mg/kg/day berotralstat in the 4-week rat study and at ≥ 55 mg/kg/day berotralstat in the 39-week study in monkeys. The finding was absent at 20 mg/kg/day berotralstat in the 26-week study in rats and at 30 mg/kg/day berotralstat in the 39-week study in monkeys.

Phospholipidosis

Phospholipidosis was a consistent finding in rats and monkeys (Section [5.1](#)). Phospholipidosis, however, was not observed in humans in clinical trials (refer to the end-of-phase 2 internal meeting held on October 25, 2017).

Cardiovascular System

Berotralstat caused dose- and plasma-concentration-dependent increases in the QT interval in monkeys and humans. See Section [5.1](#) and the interdisciplinary review team review completed by Dr. Girish Bende et al. on April 15, 2020 (DARRTS ID 4592746).

Safety Margins

Animal toxicity studies generally provided safety margins of greater than one for relevant findings in general toxicity studies on a plasma drug AUC basis (Table 61). Some safety margins were smaller than 1 (i.e., 0.1 to 0.8), but these were generally irrelevant, being attributable to phospholipidosis, which was absent in humans. The safety margins for reproductive and developmental toxicity and carcinogenicity are discussed in the labeling review.

Table 61. Safety Margins of General Toxicity Studies of Berotralstat

Organ	Toxicity	Species	Duration (Weeks)	Study #	NOAEL		Safety Margin ²
					mg/kg	AUC ¹	
Adrenal gland	Vacuolation	Monkey	39	8001292	20	2880	1.1
Bone	Hyperplasia, follicular lymphoid	Monkey	39	8001292	20 (F)	2880	1.1
Heart	QT prolongation	Monkey	39	806-102	30	3950	1.6
Kidney	Tubular degeneration	Monkey	39	806-102	30	3950	1.6
Liver	Hepatocellular hypertrophy	Monkey	39	806-102	30	3950	1.6
Bile duct	Vacuolation	Rat	26	8001291	2.5	297	0.1
	Histiocytosis	Rat	26	8001291	2.5	297	0.1
	Hyperplasia	Rat	26	8001291	7.5	2030	0.8
Lung	Foamy macrophages	Monkey	39	806-102	80 (M)	14,500	5.7
Lymph node	Foamy macrophages	Monkey	39	806-102	30	3950	1.6
		Rat	26	8001291	7.5	2030	0.8
Intestine	Foamy macrophages	Monkey	39	806-102	30	3950	1.6
		Rat	26	8001291	7.5	2030	0.8
Spleen	Foamy macrophages	Rat	26	8001291	7.5	2030	0.8
Thymus	Lymphoid depletion	Monkey	39	806-102	30	3950	1.6

Source: Constructed by the reviewer based on the information provided in the submission.

¹ In ng.h/mL.

² The safety margin was calculated on an AUC basis. Calculation based on an AUC of 2540 ng.hr/mL at 150 mg/day in human. Abbreviations: AUC, area under the curve; F, female; M, male; NOAEL, no observable adverse effect level

Genetic Toxicity and Carcinogenicity

See Section [5.1](#).

Reproductive Toxicity

A standard battery of tests for reproductive and developmental toxicity of berotralstat did not reveal any significant treatment effect (Table 62). The battery included a fertility and early embryonic developmental study in rats, embryofetal development (EFD) studies in rats and rabbits (one each), and a PPND toxicity study in rats. None of the studies showed findings of significant concern. Berotralstat was nonteratogenic under the test conditions and did not affect fertility or PPND in rats.

Table 62. Reproductive and Developmental Toxicity Studies of Berotralstat

Category	Species	Treatment	n/Dose	Berotralstat (mg/kg/day)	Study #
Fertility	Rat	Premating ^a to GD 7	25	0, 10, 25, 45	806-156
EFD	Rat	6 to 17	21-23	0, 10, 25, 75	9000644
	Rabbit	7 to 19	20-21	0, 20, 50, 100	9000815
PPND	Rat	GD 6 to LAD 20	25 ^b	0, 10, 25, 45	806-157

Source: Extracted from Section 2.6.7 Toxicology Tabulated Summary, pages 107-109.

^a Dosing was initiated 28 and 14 days prior to mating in males and females, respectively.

^b Dosing ended on GD 6 in females and the pups were culled to 4 and 1 on postnatal days 4 and 28, respectively.

Abbreviations: EFD, embryofetal development; GD, gestation day; LAD, lactation day; PPND, peri- and post-natal development

In a fertility study (Study #806-156), male and female Sprague–Dawley rats (25/sex/dose) were dosed by oral gavage with 0, 10, 25, and 45 mg/kg/day berotralstat. The treatment started 4 weeks prior to mating and continued through and after mating for a total of 9 weeks. The treatment in females started 2 weeks prior to mating and continued until gestational day

(GD) 7. Males were evaluated for fertility and sperm quality (day 63). Females were evaluated for fertility and uterine contents on GD 13. The HD group showed increases in the incidence of audible breathing in both sexes (incidence three and six male and female rats, respectively). The HD males showed a statistically significant decrease in bodyweight at the end of treatment (5%, $p < 0.05$) and a decrease in food consumption (up to 14%, $p < 0.01$). The mean plasma drug level was 835 and 795 ng/mL in males and females at the last day of treatment (on treatment day 63 and GD 7, respectively). No treatment-related effects were observed in any of the fertility parameters in either sex and in the sperm analysis in males. Berotralstat did not affect fertility in male or female rats under the test conditions.

In an EFD study in rats (Study #9000644), pregnant dams (21 to 23/dose) were dosed by oral gavage with 0, 10, 25, and 75 mg/kg/day berotralstat during the organogenesis period (GD 6 to 17). They were euthanized on GD 21 for examination of uterine contents. Four (of twenty-three) HD dams showed audible breathing. The HD group showed a statistically significant decrease in mean bodyweight gain (34%, $p < 0.01$). No treatment-related effects were observed in any of the fetal structural parameters evaluated. The mean AUC on GD 17 was 3510, 12,400, and 24,600 ng.h/mL in the LD, MD, and HD groups, respectively. Berotralstat was nonteratogenic in Sprague–Dawley rats under the test conditions.

In an EFD study (Study #9000815), pregnant New Zealand white rabbit does were dosed by oral gavage with 0, 20, 50, and 100 mg/kg/day berotralstat during the organogenesis period (GD 7 to 19). They were euthanized on GD 29 for the examination of uterine contents. The mean plasma AUC on day 19 was 880, 2350, and 4410 ng.h/mL in the LD, MD, and HD groups, respectively. The HD group showed statistically significant decreases in bodyweight gain and food consumption (100% and 32%, respectively; $p < 0.05$). The HD group had abortions and early terminations (two rabbits each). These findings were not considered of concern because they were associated with significant maternal toxicity (i.e., significant decreases in bodyweight). No treatment-related effects were observed in any other fetal development parameters. Berotralstat can cross the placenta; the drug was present in fetal blood at up to 11% of the concentration in maternal blood. Berotralstat was nonteratogenic in New Zealand white rabbits under the test conditions.

In a PPND study in rats (Study #806-157), dams (F_0) were dosed by oral gavage with 0, 10, 25, and 45 mg/kg/day berotralstat during pregnancy and lactation (i.e., from GD 6 to lactation day [LAD] 20). The respective mean plasma berotralstat concentration in the LD, MD, and HD dams was 46.2, 183.3, and 312.8 on GD 6 and 63.8, 360.3, and 699 ng/mL on LAD 14. Dams gave births to and nurtured pups (F_1) naturally. The berotralstat concentration in pup plasma on LAD 14 was $< 5\%$ of the maternal maximum plasma concentration (C_{max}). Pups grew and matured naturally and were assessed for growth and behavioral development parameters (e.g., motor activity, learning, and memory) while growing. The F_1 mated at the age of 10 weeks and pregnant females were euthanized on GD 13 for the examination of uterine contents. Both dams and pups (F_0 and F_1 , respectively) in the HD group showed lower bodyweight gain during the lactation period (up to 7.8%, $p < 0.01$). The dams also showed a statistically significant decrease in food consumption (up to 10%). Therefore, berotralstat did not affect PPND parameters in rats under the test conditions. The NOAEL was 25 mg/kg/day.

Juvenile Animal Study

No comprehensive toxicity study in juvenile animals has been completed to evaluate the effect of berotralstat on growth and development. The Applicant states that they evaluated the effect of berotralstat on growth and development in a PPNP study in rats (#806-157). This review disagrees with the Applicant based on the limitation of the design of the PPNP study. Specifically, pups received limited exposure through milk during the lactation period and no drug exposure postweaning. During the lactation period, the plasma berotralstat level in pups was less than 5% that in maternal blood. Such limited exposure to berotralstat cannot fully evaluate its effect on the growth and development of rat pups.

The lack of a juvenile animal study does not preclude approval for the current indication. The Applicant stated that the animals used in their toxicity program were comparable to adolescent humans. Specifically, dosing in the 26-week study in rats was initiated at 10 weeks of age. The 39-week toxicity studies in monkeys commenced dosing at a median age of 2 years 10 months to 3 years 3 months. The human ages equivalent to those of the rats and monkeys are approximately 14 and 12 years, respectively. The Applicant should consider a well-designed study to fully evaluate the effect of berotralstat on growth and development in juvenile animals if they intend to develop berotralstat for younger pediatric patients in the future.

Drug Product Impurities

There are no safety concerns regarding impurities in the drug product. Six impurities were identified and discussed in the new drug application— (b) (4)
The specifications of these impurities were $\leq$ (b) (4) $\mu\text{g}/\text{day}$ (b) (4)

These specifications are considered adequate based on the discussion below.

Safety evaluation of the impurities included structural activity analysis for genetic toxicity and evaluation of impurity levels in the general toxicity studies. Bacterial gene reverse mutation assays (Ames test) were performed for impurities with structural alerts. For impurities with no structural alerts, group features present in the parent compound, and specifications of $>$ (b) (4) %, safety evaluations were conducted by comparing their levels to those in 13-week repeat-dose toxicity studies.

Bacterial gene reverse mutation (Ames) assays were performed on (b) (4)
Only (b) (4) tested positive (in only the TA98 test strain). The level of (b) (4) will be kept below the lower limit of quantitation of (b) (4) ppm. The total daily intake of (b) (4) in patients receiving 150 mg/day berotralstat will be below the safety concern threshold of (b) (4) μg (Source: electronic Common technical Document 3.2.S.3.2, subsection 2.3.4, Table 73, p. 154). (b) (4) considered qualified based on their lack of mutagenicity and the specification (not more than (b) (4) %).

Additional safety evaluations were conducted for the following three impurities: (b) (4)
(b) (4) whose specifications were greater than (b) (4) %. Table 63 lists the daily intake of these impurities and the corresponding safety margins. The Applicant argued that

these impurities are qualified because they were present at similar levels in the 13-week toxicity studies in rats and monkeys (Studies 806-144 and 806-145, respectively). Both of these studies revealed no significant toxicity at the highest dose of 20 mg/kg/day. The monkey study provided a safety margin of at least 2 based on exposure calculated as mg/m².

Table 63. Safety Margins for Impurities

Impurity	Concentration (%)		Exposure (mg/m ²)			Safety Margin	
	Toxicity Study ¹	Proposed ²	Rat ³	Monkey ³	Human ⁴	Rat	Monkey
(b) (4)		(b) (4)	0.29	0.58	(b) (4)	1.25	2.5
			0.30	0.60		1.30	2.6
			3.42	6.84		7.39	14.8

Source: Constructed by the reviewer based on the information provided in the submission.

¹ Lot G2-B1-10 used in the 13-week rat and monkey toxicity studies; data are % areas

² m3.2.S.4.5.1.4

³ The NOAEL in rat and monkey 13-week toxicity studies was 20 mg/kg

⁴ Based on a 150 mg dose in a 60 kg patient

Abbreviations: BCX, berotralstat; NOAEL, no observable adverse effect level

The nonclinical review team consulted the chemistry review team regarding the structural alerts of the impurities and the Applicant's safety assessment (via email dated May 8, 2020). Dr. Lawrence Perez found no structural alerts of potential genotoxicity for any of the three impurities (source, email on May 8, 2020). Further, these compounds have groups present in berotralstat and are considered nonmutagenic per the International Council for Harmonisation-M7 guidance. Because these compounds were present at the proposed levels in repeat-dose toxicity studies in rats and monkeys, these impurities are considered qualified.

Conclusion

The Applicant has conducted adequate nonclinical characterization and safety evaluation of berotralstat. Findings in the general toxicity studies of berotralstat in animals have been adequately monitored and addressed in clinical trials. Genetic toxicity, carcinogenicity, and reproductive and developmental toxicity studies identified no safety signal of concern. The reviewer recommends approval of the drug from the nonclinical perspective.

16.3. OCP Appendices (Technical Documents Supporting OCP Recommendations)

16.3.1. Population Pharmacokinetics Analysis

16.3.1.1. Review Summary

In general, the Applicant's population pharmacokinetics (PK) analysis is considered acceptable to support the claims (Table 64). The Applicant's analyses were verified by the reviewer, with no significant discordance identified.

Table 64. Specific Comments on the Applicant’s Final Population PK Model

Utility of the Final Model		Reviewer’s Comments
Support labeling statements about intrinsic factors	“Body weight, age, gender, and race did not have a clinically meaningful influence on the systemic exposure of berotralstat.” “Based on the population pharmacokinetic analyses that included elderly patients (≥ 65 to 74 years, N=25), age does not have a clinically meaningful impact on the systemic exposure of berotralstat.”	The statement is acceptable. Covariate analysis by the Applicant demonstrates no evident difference based on age, gender, and ethnicity. The higher exposure in lighter patients is not expected to cause meaningful QT prolongation, so no dose adjustment is recommended.
Derive exposure metrics in pediatric population for the labeling	“Based on population pharmacokinetic analyses that included pediatric patients 12 to <18 years of age, exposure at steady state following oral administration of berotralstat 150 mg once daily was approximately 20% higher (b) (4) compared to adults.”	The Applicant’s final model is acceptable for generating exposure metrics in pediatric patients aged 12 to 18 years (Table 68).

Source: Pharmacometrics Reviewer

Abbreviations: AUC_{tau}, area under the curve over the dosing interval; C_{max}, maximum concentration; CV, coefficient of variation

Introduction

The primary objectives of the Applicant’s analysis were to:

- Characterize the structural PK model and quantify the population variability in the PK parameters of berotralstat.
- Evaluate the effects of intrinsic factors on berotralstat exposure.

Model Development

Data

The analysis was based on PK data from 14 studies. Brief descriptions of those studies are presented in [Table 65](#). The reasons for exclusion of PK samples were predose observation, missing date/time, PK without dosing, and outliers. The final Nonlinear Mixed-Effects Modeling data file for analysis contained 10,437 PK observations from 771 subjects. [Table 66](#) provides summary statistics of the baseline demographic covariates in the analysis dataset.

Table 65. Summary of Studies With PK Sampling Included in the Population PK Analysis

Study	Study ID (b) (6)	Number of Subjects		Number of PK Observations		
		Included/ Total	Excluded	Included/ Total	BLQ	Other Exclusions
BCX7353-102		21/21	0	255/276	21	0
BCX7353-103		24/24	0	979/1003	24	0
BCX7353-105		54/54	0	723/777	54	0
BCX7353-106		28/40	12	471/687	216	0
BCX7353-107		14/14	0	223/237	14	0
BCX7353-108		24/24	0	551/575	24	0
BCX7353-112		50/50	0	503/503	0	0
BCX7353-113		60/60	0	2240/2300	60	0
BCX7353-203		52/75	23	509/602	92	1
BCX7353-204		227/253	26	1141/1403	240	22
BCX7353-301		13/19	6	64/114	49	1
BCX7353-302		108/120	12	598/907	309	0
BCX7353-101 (SAD)		46/62	16	733/797	64	0
BCX7353-101 (MAD)		50/60	10	1447/1517	70	0
Total count		771/876	105	10,437/11,698	1237	24

Source: Applicant's population PK report (BCX7353-PPK-01-A2 Addendum), Appendix Table 1.

Abbreviations: BCX, berotralstat; BLQ, below the lower limit of quantification; ID, identification; MAD, multiple ascending doses; PK, pharmacokinetics; SAD, single ascending dose

Table 66. Summary of Baseline Covariates for Analysis

Covariate	Statistic	Total
Baseline body weight (kg)	N	771
	Mean (SD)	77.7 (17.2)
	Median (minimum, maximum)	75.1 (40.1, 150)
Age (years)	N	771
	Mean (SD)	38.1 (13.9)
	Median (minimum, maximum)	37.0 (12.0, 74.0)
Baseline eGFR (mL/min/1.73 m ²)	N	771
	Mean (SD)	94.2 (21.9)
	Median (minimum, maximum)	92.4 (7.16, 266)
Sex		
Male		414 (53.7%)
Female	N (%)	357 (46.3%)
Ethnicity		
White		642 (83.3%)
Black		35 (4.54%)
Asian	N (%)	67 (8.69%)
Other		27 (3.50%)
Patient status		
Healthy volunteer		346 (44.9%)
Severe renal impairment		7 (0.908%)
Child–Pugh A		6 (0.778%)
Child–Pugh B	N (%)	6 (0.778%)
Child–Pugh C		6 (0.778%)
Hereditary angioedema		400 (51.9%)

Covariate	Statistic	Total
Formulation		
API in capsule	N (%)	231 (30.0%)
(b) (4) capsule		540 (70.0%)
Food status		
Fasted	N (%)	172 (22.3%)
High-fat diet		8 (1.04%)
Regular meal ¹		591 (76.7%)

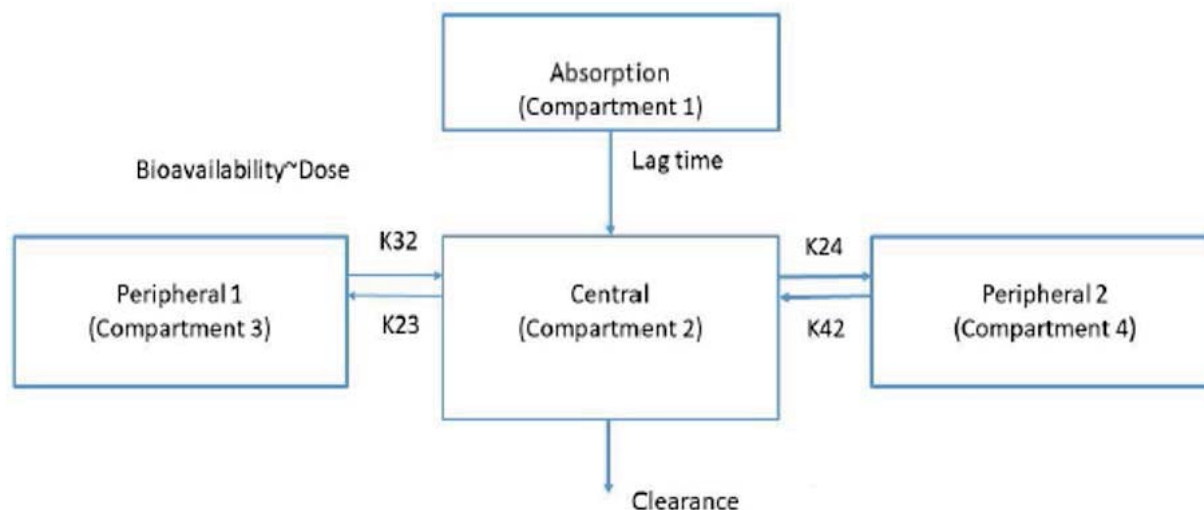
Source: Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum), Appendix Table 6.

¹ The fed/fasted state was not recoded in Studies 203, 204, and 302. The food status for those studies was imputed as *regular diet*. Abbreviations: eGFR, estimated glomerular filtration rate; N, number of subjects, SD, standard deviation

Base Model

The base model for berotralstat was a linear three-compartment model with first-order absorption, an absorption lag time, and linear elimination (Figure 10). An effect of dose on relative bioavailability was included in the base model to address the nonlinear PK of berotralstat. Interindividual variability was modelled assuming a log-normal distribution for patient-level random effects. Residual variability was tested as additive, proportional, or both on the dependent variable. The stochastic approximation/expectation maximization (SAEM) method was used for parameter estimation, and the relative standard error (RSE) was used for hypothesis testing, with a forward-addition RSE of 0.510 (p=0.05) and a backward-elimination RSE of 0.388 (p=0.005). The SAEM was used because it can handle complex models with many parameters.

Figure 10. Diagram of the Base Model



Source: Revised from Figure 9 of the Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).

Covariate Analysis

Clinical judgment, physiologic relevance, and mechanistic plausibility were used to determine which covariates should be tested with the various PK parameters. Additionally, the collinearity of covariates was assessed to ensure that no collinear covariates were added to the model. The effects of the covariate parameters body weight, age, gender, and ethnicity on apparent oral clearance (CL/F) and apparent central volume (V_c/F); bilirubin on CL/F; and food effect on the absorption rate (K_a) were tested in the covariate analysis. Continuous covariates were

evaluated using a power function and categorical covariates were parameterized as a fractional change.

Final Model

The final model (Model 258) was a three-compartment model with first-order absorption with an absorption lag time and linear elimination. The effect of between-subject variability on clearance, volume of distribution, K_a , and absorption lag time was evaluated. A between-study random effect on clearance was included. Bodyweight was the only covariate for clearance and volume. An effect of dose on relative bioavailability was included to address the nonlinear PK of berotralstat (higher dose predicting higher bioavailability). Residual variability was modeled by a combined additive and proportional component.

The parameter estimates for the final model are listed in [Table 67](#), and the goodness-of-fit plots in [Figure 11](#). Selected visual predictive check plots for the final model are shown in [Figure 12](#). [Figure 13](#) shows that inclusion of bodyweight in the final model accounted for the trend between the random effects of berotralstat PK parameters and bodyweight in the base model.

Table 67. Parameter Estimates (RSE) for the Final Model (Model 258)

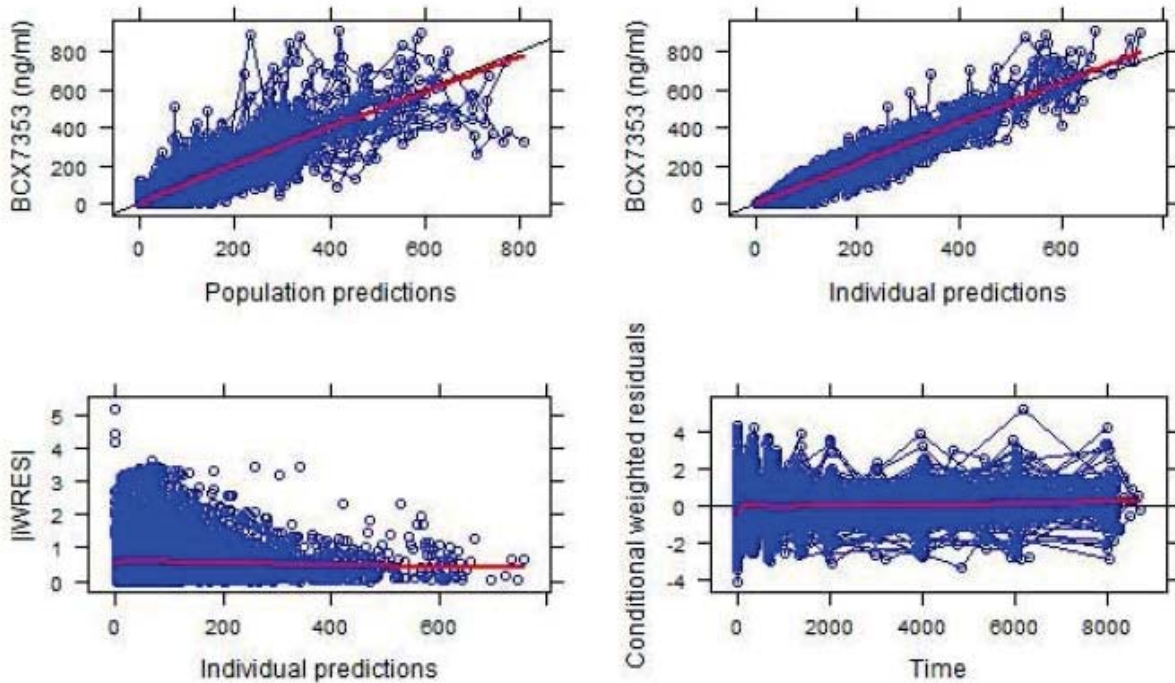
Parameter	Parameter Estimate (RSE%)
THETA(1) CL (L/hr for 75.2 kg subject*)	47.3 (2.4)
THETA(2) V (L for 75.2 kg subject)	1650 (2.49)
THETA(3) Additive error (ng/mL)	0.483 (3.75)
THETA(4) Proportional error	0.286 (0.404)
THETA(5) K_a (1/hr)	1.12 (0.0891)
THETA(6) K_{23} (1/hr)	0.0812 (2.32)
THETA(7) K_{32} (1/hr)	0.0309 (2.52)
THETA(8) ALAG1 (hr)	0.468 (2.15)
THETA(9) K_{24} (1/hr)	0.00281 (17.7)
THETA(10) K_{42} (1/hr)	0.00136 (25.4)
THETA(11) Dose on relative bioavailability	0.497 (5.51)
THETA(13) V~Weight	1.00 (8.28)
THETA(15) CL~Weight	0.480 (14.3)

* 75.2 kg was the median weight across all studies

Source: Table 8 of Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).

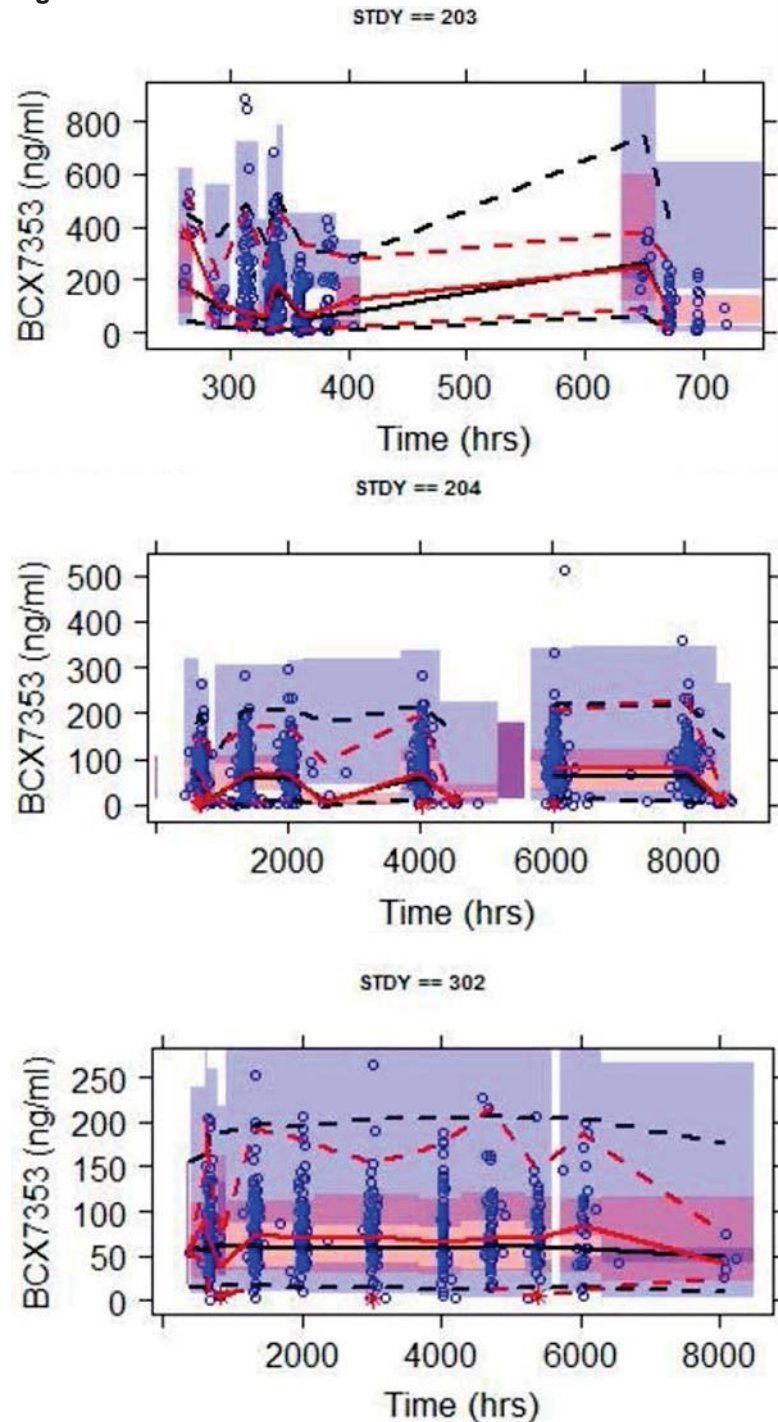
Abbreviations: ~, is a function of; CI, confidence interval; CL, clearance; F1, bioavailability for dose in compartment 1; K_a , absorption rate; RSE, relative standard error; V, volume

Figure 11. Goodness-of-Fit Plots for the Final Model



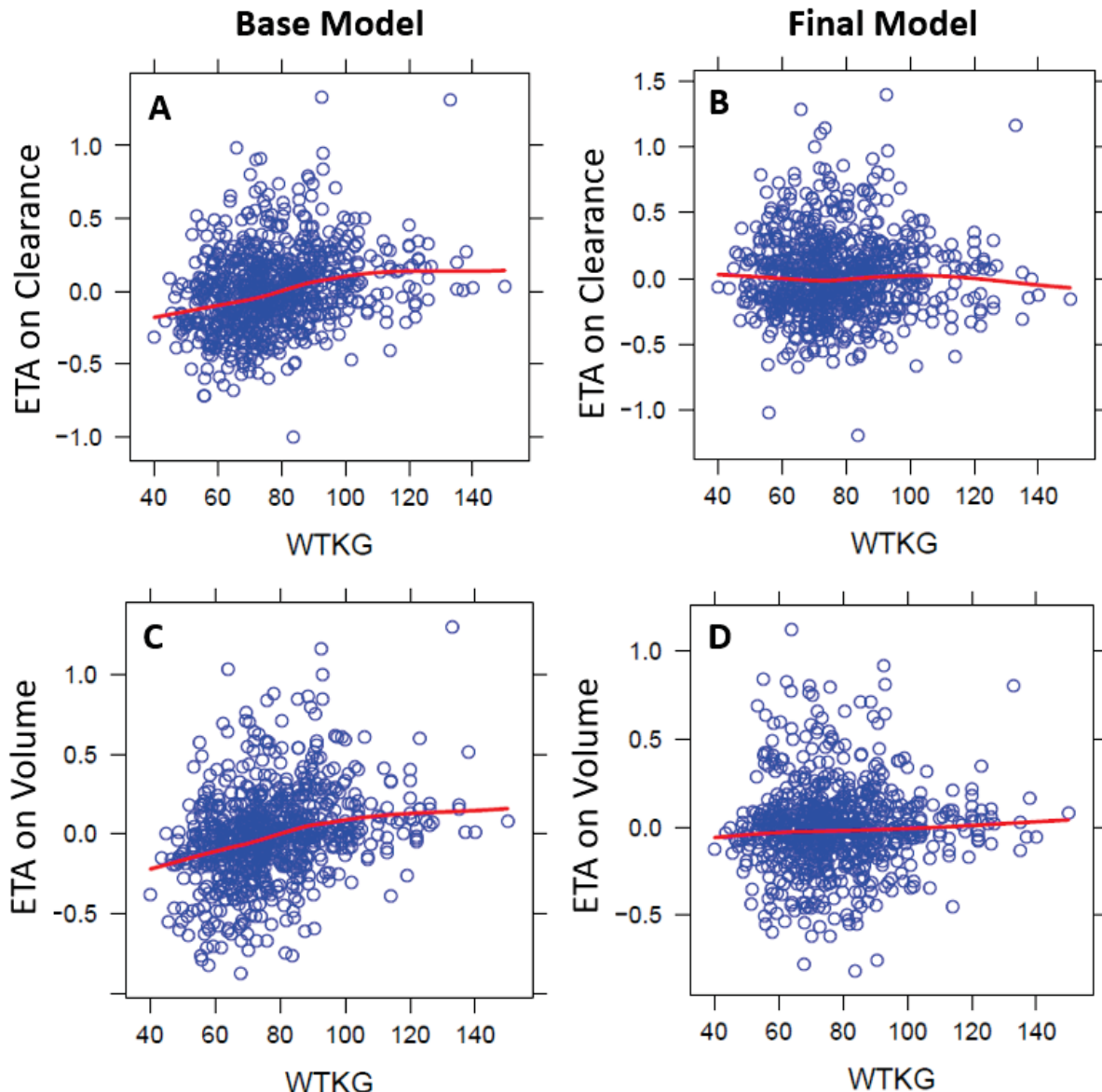
Source: Figure 10 of the Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).
Black line in DV versus predictions/individual predictions plots represents the line of unity ($y=x$). Black line in IWRES versus individual predictions and CWRES versus time plots represents the horizontal line ($y=0$). The red line is a smooth regression line.
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable; IWRES, individual weighted residuals

Figure 12. Visual Predictive Check Plots for the Final Model: Studies BCX7353-203, -204, and -302



Source: Figure 12 of Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).
Blue circles represent the observed plasma concentrations. The solid red line represents the median observed plasma concentration. Dashed red lines represent the 2.5th and 97.5th observed percentiles. The black solid line represents the simulated median. Black dashed lines represent the 2.5th and 97.5th simulated percentiles. The semitransparent red area represents a simulation-based 95% confidence interval for the simulated median. Semitransparent blue areas represent simulation-based 95% confidence intervals for the 2.5th and 97.5th percentiles.
Abbreviations: BCX, berotralstat; STDY, study

Figure 13. Random Effects vs Covariate for the Base and Final Models



Source: Reviewer's plots while replicating Applicant's analysis.

A, B: Random effects on CL versus weight for the base and final models, respectively. C, D: Random effects on V versus weight for the base and final models, respectively.

Abbreviations: ETA, individual random effects; WTKG, bodyweight in kilograms

Simulations

Simulations based on the final model were conducted by the Applicant to predict the PK profiles and parameters of the following populations at a dose of 150 mg once daily, using the

(b) (4)-capsule formulation:

- Adolescents (12 to 18 years of age)
- Underweight adults (40 to 60 kg)
- Low-weight adults (60 to 80 kg)
- Normal-weight adults (80 to 100 kg)

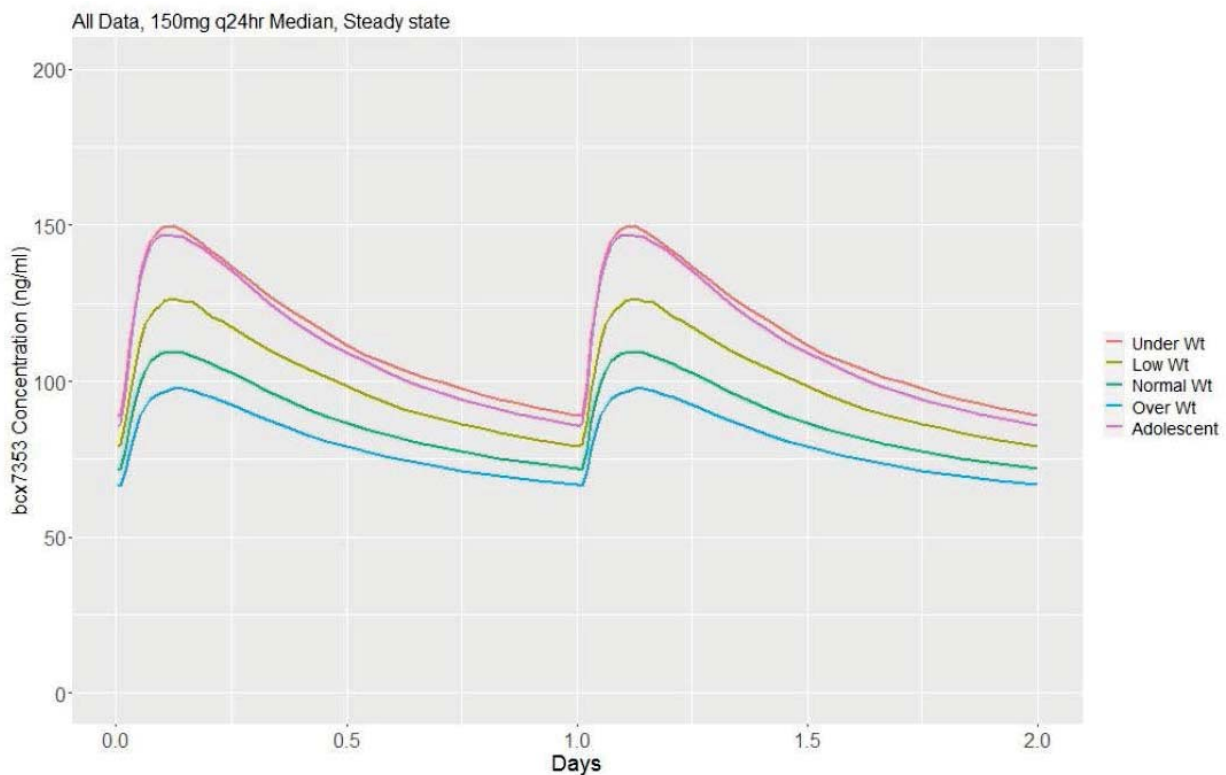
- Overweight adults (100 to 120 kg)

For adult subjects, the mean and variance for weight were derived from the data available in the current dataset. Simulated weight values were generated on the log-transformed values for weight normal distribution.

For the adolescent population, bodyweight and the variance thereof were derived from the Weight-for-Age Tables of the Centers for Disease Control and Prevention and log-transformed. Simulated weight values for adolescents were obtained from a normal distribution.

There were 1000 simulated subjects in each population generated. The predicted median PK profiles of the specified weight groups after reaching steady state following berotralstat 150 mg daily dosing are shown in [Figure 14](#). Steady-state AUC₀₋₂₄ and C_{max} values are listed in [Table 68](#).

Figure 14. Predicted Median PK Profiles After 150 mg QD at Steady State, by Weight Group



Source: Figure 28 of the Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).
Abbreviations: QD, once daily; Wt, weight

Table 68. Predicted Geometric Mean PK Parameters (CV%) After 150 mg QD at Steady State by Subpopulation

Patient Subpopulation	AUC, Steady State (ng·hr/mL)	C_{max}, Steady State (ng/mL)
Underweight adults (40-60 kg)	2574 (36.7)	155 (33.9)
Low-weight adults (60-80 kg)	2211 (38.3)	129 (35.6)
Normal-weight adults (80-100 kg)	1951 (37.0)	111 (34.3)
Overweight adults (100-120 kg)	1818 (36.9)	101 (34.7)
Adolescents 12 to 18 years (40-71 kg) ¹	2515 (38.6)	153 (36.8)

¹ The 5th and 95th percentiles for simulated adolescents aged 12 to 18 years.

Source: Table 15 of the Applicant's Population PK report (BCX7353-PPK-01-A2 Addendum).

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; CV, coefficient of variation; QD, once daily; PK, pharmacokinetics

Reviewer's comments: In general, the Applicant's population PK analysis is considered acceptable to support the labeling claims in Section 12.3 of the prescribing information. The Applicant's analyses were verified by the reviewer, with no significant discordance identified.

The SAEM method was used by the Applicant for parameter estimation. Other estimation methods were evaluated by the reviewer, but the model crashed using the first-order conditional estimation and first-order conditional estimation I methods. This might explain why the SAEM method was selected by the Applicant.

The reviewer noted that bilirubin and food effect were identified as significant covariates on CL and absorption parameters (K_a and bioavailability for dose in compartment 1) by statistical testing; however, both were removed before model finalization as not clinically significant. Assessment of clinical significance should be conducted after model development, and a covariate's effect on berotralstat exposure should be interpreted in the context of the known exposure–response relationships for efficacy and safety. Although the reviewer does not fully agree with the Applicant's covariate analysis, this does not change the conclusion of no clinically relevant covariates and no recommended dose adjustment for covariates.

Bodyweight is a major factor that influences the exposure of berotralstat. In Table 68, the median bodyweight in the pooled dataset is 75.1 kg, within the 60 to 80 kg bodyweight group (low-weight adults) in Table 66. Higher exposure (~15%) was predicted in adults of 40 to 60 kg and adolescents 12 to 18 years of age compared to adults of 60 to 80 kg. The higher exposure in lighter patients is not expected to cause meaningful QT prolongation, which is a major safety concern; therefore, no dose adjustment is recommended. Furthermore, berotralstat was effective in subjects of high bodyweight and showed no substantial safety signal in subjects with low bodyweight (Section [16.3.2](#)), supporting the recommendation of no dose adjustment.

16.3.2. Exposure–Response Analysis

16.3.2.1. Exposure–Efficacy Relationship

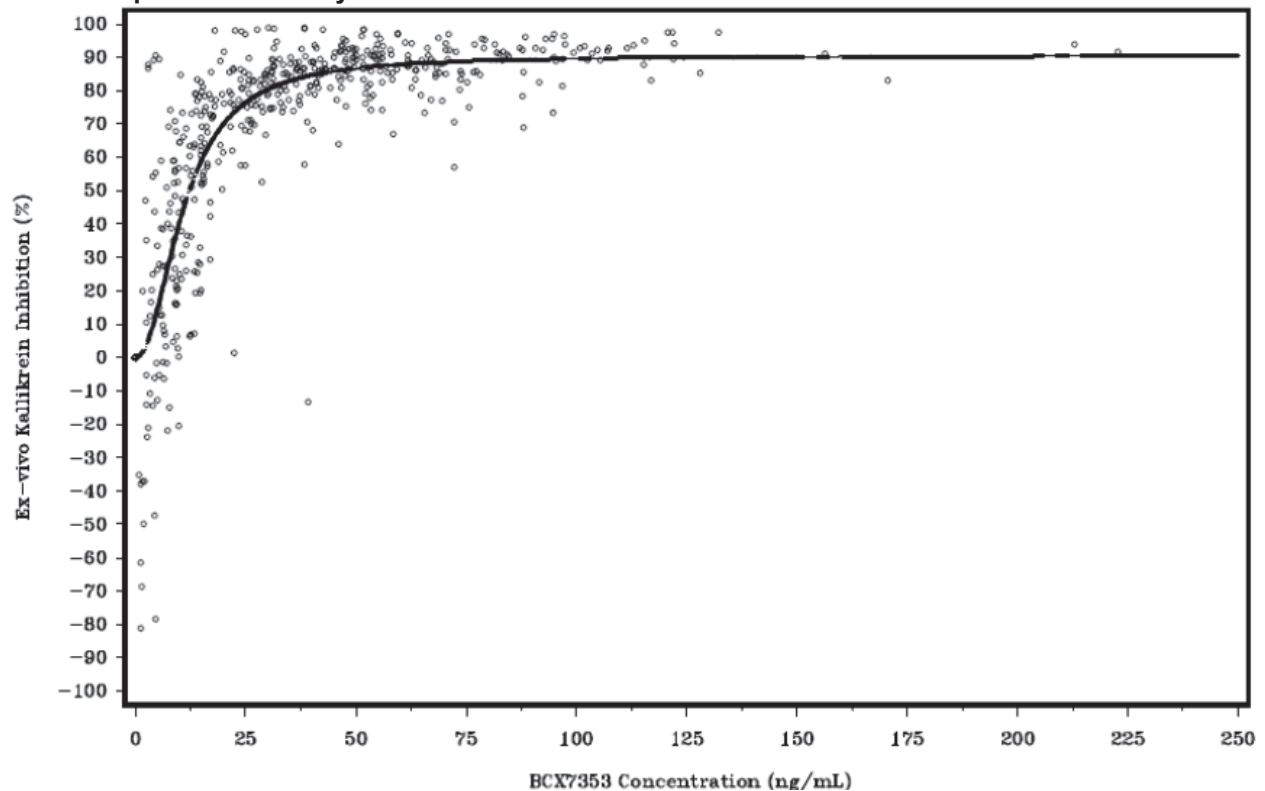
Applicant's Analysis

PK–Pharmacodynamics Relationship

Exposure-response analyses of the relationship between kallikrein inhibition and berotralstat plasma concentrations were conducted using data from subjects with HAE in Study

BCX7353-203. The effect of berotralstat on the target enzyme, plasma kallikrein, was evaluated by comparing specific amidolytic activity in plasma samples taken after drug administration to that in baseline samples. Exposure–response analyses demonstrated a high correlation between ex vivo kallikrein inhibition activity and the plasma berotralstat concentration, which was well-described by a sigmoidal maximum-efficacy model (coefficient of determination 0.75) with a calculated half-maximal effective concentration of 11 ng/mL (Figure 15). The trough concentrations of the 110 and 150 mg doses were 45 and 70 ng/mL; therefore, both reached the plateau for kallikrein inhibition.

Figure 15. Correlation of Plasma Berotralstat Concentrations and PD Effect on Plasma Kallikrein: PK/PD Population in Study BCX7353-203



Source: Study BCX7353-203 clinical study report, Figure 14.2.4.2

Exposure-response relationship was determined with a pharmacologically sigmoid E_{max} model in which: Kallikrein inhibition (E) = $(E_{max} \times [BCX7353]^{\gamma}) \div (EC_{50}^{\gamma} + [BCX7353]^{\gamma})$

Circles represent observed individual kallikrein inhibition values at a given concentration; line represents model-predicted kallikrein inhibition across the concentration range.

Because of dilution during sample preparation for the ex vivo assay, berotralstat concentration in the ex vivo assay represents one quarter of the corresponding in vivo plasma concentration.

Model estimation: E_{max} 90.4% (CV 2.07%); EC_{50} 11.0 ng/mL (CV 4.36%); γ 2.08 (CV 9.49%); R 0.867; EC_{80} 44.0 ng/mL (calculated based on $EC_{80} = 4 \times EC_{50}$).

The model did not account for multiple data pairs within each subject; thus, the results need to be interpreted with caution.

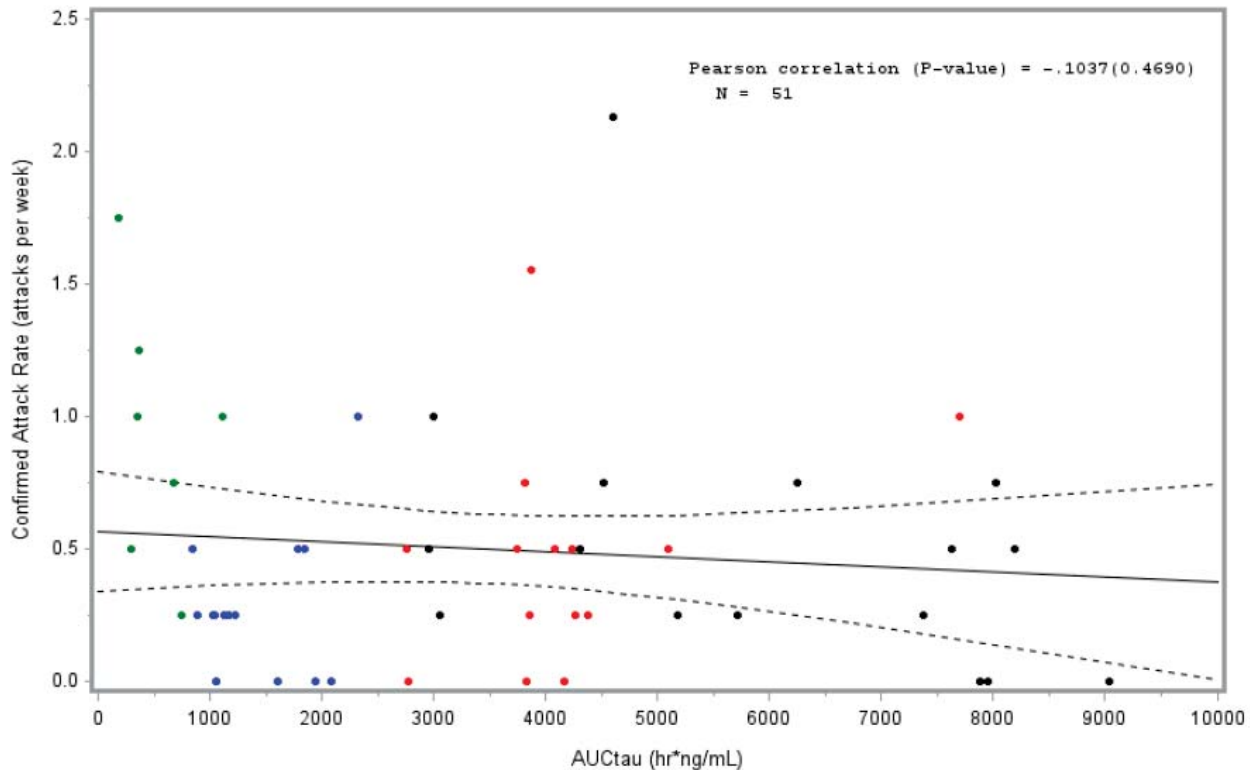
Abbreviations: BCX7353, berotralstat; CV, coefficient of variation; EC_{50} , half-maximal effective concentration; EC_{80} , 80% effective concentration; E_{max} , maximum efficacy; PD, pharmacodynamics; PK, pharmacokinetics

PK–Efficacy Relationship

The efficacy endpoint was the rate of investigator-confirmed HAE attacks. In the dose-ranging Study BCX7353-203, berotralstat at doses of 55, 110, 218, and 300 mg once daily orally for 28 days were tested. The correlation between the investigator-confirmed weekly attack rate and berotralstat plasma exposure was evaluated graphically. None of the berotralstat steady-

state PK parameters (C_{max} , area under the curve over the dosing interval [AUC_{tau}], and maximum plasma concentration over the dosing interval [C_{tau}]) were correlated with the confirmed attack rate, as shown in [Figure 16](#) for AUC_{tau} . The plots for C_{max} and C_{tau} were similar to that for AUC_{tau} and showed no correlation with the attack rate.

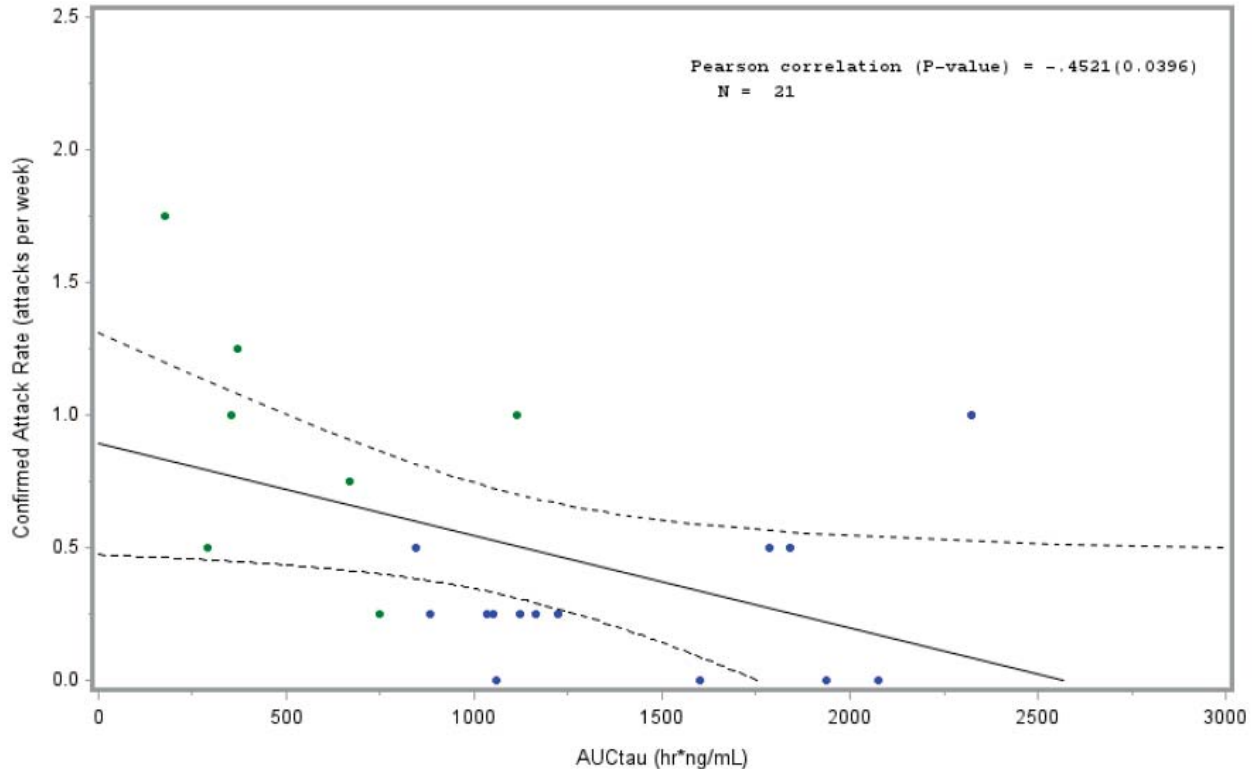
Figure 16. Investigator-Confirmed Attack Rate vs Berotralstat Plasma AUC_{tau} Over the Dose Range of 55 to 300 mg With 95% CI for Mean Predicted Values: Study BCX7353-203



Source: Study BCX7353-203 clinical study report, Figure 7
Treatments: 300, 218, 110, 55 mg
Regression equation: Confirmed attack rate (attacks per week) $0.57 + -0.00002 \times AUC_{tau}$
Abbreviations: AUC_{tau} , area under the curve over the dosing interval; CI, confidence interval

The absence of a correlation might be driven by doses of ≥ 110 mg because the 110, 218 and 300 mg doses showed efficacy; berotralstat significantly reduced the confirmed HAE attack rate in the 28-day treatment period versus placebo. Further exposure-response analyses were conducted with the two lower doses (i.e., 55 and 110 mg). The steady-state berotralstat AUC_{tau} was correlated with the confirmed attack rate in the dose range 55 to 110 mg ([Figure 17](#)). Therefore, 110 and 150 mg free-base doses were selected for the registration trial (Study BCX7353-302).

Figure 17. Investigator-Confirmed Attack Rate vs Berotralstat Plasma AUC_{tau} Over the Dose Range of 55 to 110 mg With 95% CI for Mean Predicted Values in Study BCX7353-203



Source: Study BCX7353-203 clinical study report, Figure 8
Treatments, 110 and 55 mg
Regression equation: Confirmed attack rate (attacks per week) = $0.89 + -0.00035 \times \text{AUC}_{\tau}$
Abbreviations: AUC_{tau}, area under the curve over the dosing interval; CI, confidence interval

Because bodyweight is a significant covariate that influences the exposure of berotralstat, the potential impact of bodyweight on efficacy was examined by summarizing the investigator-confirmed attack rates by bodyweight category using the data collected in Part 1 of Study 302. The mean attack rate varied among the three bodyweight categories: 1.793, 1.810, and 2.219 for berotralstat 110 mg; 1.799, 2.022, and 1.251 for berotralstat 150 mg; and 2.484, 2.015, and 2.901 for placebo low-, middle-, and high-weight subjects, respectively ([Table 69](#)). The 110 and 150 mg doses demonstrated effectiveness in high-weight subjects. The variability in efficacy among bodyweight groups may be attributable to the small sample size of each bodyweight tertile, and/or the nonstratification of baseline disease severity by bodyweight tertile.

Table 69. Summary of Investigator-Confirmed Attack Rates (Attacks/Month) by Baseline Bodyweight Category (Entire Dosing Period; ITT Population in Study 302 Part 1)

Parameter	Berotralstat			Placebo (N=40)
	110 mg (N=41)	150 mg (N=40)	All Active (N=81)	
All subjects, n	41	40	81	39
Mean (SD)	1.902 (1.7084)	1.631 (1.6649)	1.768 (1.6820)	2.465 (1.5984)
Median	1.325	1.160	1.325	2.320
Minimum, maximum	0.00, 7.62	0.00, 7.46	0.00, 7.62	0.00, 7.46
Subjects ≤70 kg, n	19	8	27	11
Mean (SD)	1.793 (1.2241)	1.799 (1.8000)	1.795 (1.3820)	2.484 (1.3936)
Median	1.325	1.243	1.325	2.320
Minimum, maximum	0.00, 3.98	0.00, 4.80	0.00, 4.80	0.17, 4.90
Subjects >70 to 87.5 kg, n	12	14	26	14
Mean (SD)	1.810 (2.0653)	2.022 (1.3940)	1.924 (1.7026)	2.015 (1.5749)
Median	1.243	1.440	1.325	1.657
Minimum, maximum	0.17, 7.62	0.17, 5.33	0.17, 7.62	0.00, 5.63
Subjects >87.5 kg, n	10	18	28	14
Mean (SD)	2.219 (2.1479)	1.251 (1.8019)	1.597 (1.9506)	2.901 (1.7523)
Median	1.160	0.497	0.746	2.899
Minimum, maximum	0.33, 6.46	0.00, 7.46	0.00, 7.46	0.66, 7.46

Source: Response to FDA information request of April 28, 2020 (part 1), Table 4

Abbreviations: BL, baseline; HAE, hereditary angioedema; ITT, intent-to-treat; SD, standard deviation

16.3.2.2. Exposure–Safety Relationship

Exposure–Electrocardiogram Interval Relationship (QT-Integrated Review Team Analysis)

Based on the analyses by the QT-IRT, berotralstat caused a dose- and concentration-dependent increase in the QT interval at the doses (150 and 450 mg) evaluated. However, the QT interval was not prolonged to any clinically relevant extent at the peak concentrations for the recommended dose of 150 mg once daily, and ~1.5-fold higher exposure than the recommended 150 mg dose. This covers the exposure increase (~15%) predicted for subjects of lower bodyweight (underweight adults or adolescents in [Table 68](#)). As a result, the higher exposure in lighter patients is not expected to cause meaningful QT prolongation, and no dose adjustment is recommended. Refer to the QT-Integrated Review Team review (Reference ID, 4592746) for details.

Applicant’s Analysis

An analysis of treatment-emergent adverse events (TEAEs) by dose level was conducted using the data collected in Part 1 of Study BCX7353-302. No substantial safety findings were identified in the 110 and 150 mg groups. The TEAE analysis identified only a slightly higher frequency of gastrointestinal (GI)-related TEAEs for the higher doses: 150 mg (50.0%) >110 mg (41.5%) >placebo (35.9%). No clear dose-dependent trend was identified for other TEAEs.

The potential effect of bodyweight on safety was evaluated by summarizing TEAEs by bodyweight category using the data collected in the integrated phase 2/3 prophylactic long-term studies (Studies 204 and 302). Overall, there were no differences in the percentage of subjects who experienced TEAEs based on baseline bodyweight tertile: no bodyweight-based

trends in the frequency of drug-related TEAEs, grade 3 or 4 TEAEs, TEAEs leading to discontinuation, serious adverse events, or GI-abdominal TEAEs (Table 70). Consistent with the findings of the overall dose-response analysis for safety, lower bodyweight tended to be associated with a slightly higher frequency of GI-related TEAEs. Overall, no substantial safety signal was identified in subjects of low bodyweight.

Table 70. Summary of TEAEs by BL Bodyweight Category: Studies 204 and 302 (Safety Population–Safety Update)

Parameter	Weight ≤70 kg		Weight >70 to 87.5 kg		Weight >87.5 kg	
	Total Berotralstat (N=131)	Placebo (N=11)	Total Berotralstat (N=124)	Placebo (N=14)	Total Berotralstat (N=126)	Placebo (N=14)
Subjects with, n (%)						
TEAEs	117 (89.3%)	8 (72.7%)	108 (87.1%)	11 (78.6%)	102 (81.0%)	11 (78.6%)
Drug-related TEAEs	68 (51.9%)	3 (27.3%)	54 (43.5%)	6 (42.9%)	55 (43.7%)	4 (28.6%)
Grade 3 or 4 TEAEs	15 (11.5%)	0	20 (16.1%)	1 (7.1%)	17 (13.5%)	2 (14.3%)
SAEs	8 (6.1%)	0	19 (15.3%)	1 (7.1%)	13 (10.3%)	1 (7.1%)
Drug-related SAEs	1 (0.8%)	0	1 (0.8%)	0	1 (0.8%)	0
Fatal SAEs (deaths)	0	0	0	0	0	0
TEAEs leading to interruption of study drug	11 (8.4%)	0	16 (12.9%)	1 (7.1%)	11 (8.7%)	1 (7.1%)
TEAEs leading to discontinuation of study drug	10 (7.6%)	0	13 (10.5%)	1 (7.1%)	9 (7.1%)	0
Hepatic disorder (potentially drug related) TEAEs	8 (6.1%)	0	11 (8.9%)	0	6 (4.8%)	1 (7.1%)
GI abdominal-related TEAE	69 (52.7%)	4 (36.4%)	54 (43.5%)	6 (42.9%)	54 (42.9%)	4 (28.6%)

Source: Response to FDA information request of April 28, 2020 (part 1), Table 6

Abbreviations: AE, adverse event; BL, baseline; CRF, case report form; GI, gastrointestinal; HLG, high-level group term; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term; SAE, serious adverse event; SMQ, standardized MedDRA query; TEAE, treatment-emergent adverse event

Reviewer’s comment: The overall exposure–response analyses support the safety and effectiveness of the 150 and 110 mg doses. The latter has numerically lower efficacy and might be a useful option for patients who cannot tolerate the 150 mg dose.

16.3.3. Listing of Analyses Codes and Output Files

File Name	Description	Link to EDR
run258pooled_mod.txt	NONMEM control stream	\\cdsesub1\evsprod\nda214094\0023\m5\datasets\bcx7353-ppk-01\misc\run258pooled_mod.txt
run258pooled_lst.txt	NONMEM output file	\\cdsesub1\evsprod\nda214094\0023\m5\datasets\bcx7353-ppk-01\misc\run258pooled_lst.txt
data2.xpt	Population PK dataset	\\cdsesub1\evsprod\nda214094\0023\m5\datasets\bcx7353-ppk-01\misc\data2.xpt
makesimspooled.txt	R code for simulation	\\cdsesub1\evsprod\nda214094\0023\m5\datasets\bcx7353-ppk-01\misc\makesimspooled.txt
response-to-rfi-poppk-bioa.pdf	Response to FDA information request of January 21, 2020	\\cdsesub1\evsprod\nda214094\0007\m1\us\111-information-amendment\response-to-rfi-poppk-bioa.pdf

NDA/BLA Multidisciplinary Review and Evaluation NDA 214094
ORLADEYO (berotralstat)

File Name	Description	Link to EDR
request-for-clarification-on-01-may-clinical-poppk-request.pdf	Applicant's Clarification Request Pertaining to the FDA Information Request of April 28, 2020	\\cdsesub1\evsprod\nda214094\0018\m1\us\111-information-amendment\request-for-clarification-on-01-may-clinical-poppk-request.pdf
response-to-rfi-dated-28apr2020-pop-pk-qs-b-and-c.pdf	Response to FDA Information Request of April 28, 2020 (part 1)	\\cdsesub1\evsprod\nda214094\0022\m1\us\111-information-amendment\response-to-rfi-dated-28apr2020-pop-pk-qs-b-and-c.pdf
response-to-rfi-dated-28apr2020-pop-pk.pdf	Response to FDA Information Request of April 28, 2020 (part 2)	\\cdsesub1\evsprod\nda214094\0023\m1\us\111-information-amendment\response-to-rfi-dated-28apr2020-pop-pk.pdf
bcx7353-ppk-01-study-report.pdf	Population PK report in the original submission	\\cdsesub1\evsprod\nda214094\0000\m5\53-clin-stud-rep\533-rep-human-pk-stud\5335-popul-pk-stud-rep\bcx7353-ppk-01\bcx7353-ppk-01-study-report.pdf
bcx7353-ppk-01-a1.pdf	Population PK report addendum (to address FDA Information Request of January 21, 2020)	\\cdsesub1\evsprod\nda214094\0007\m5\53-clin-stud-rep\533-rep-human-pk-stud\5335-popul-pk-stud-rep\bcx7353-ppk-01\bcx7353-ppk-01-a1.pdf
bcx7353-ppk-01-study-report.pdf	Population PK report addendum 2 (to address FDA Information Request of April 28, 2020)	\\cdsesub1\evsprod\nda214094\0023\m5\53-clin-stud-rep\533-rep-human-pk-stud\5335-popul-pk-stud-rep\bcx7353-ppk-01\bcx7353-ppk-01-study-report.pdf

16.4. Angioedema Quality of Life Questionnaire (AE-QoL)

Figure 18. Angioedema Quality-of-Life Questionnaire (Provided by Moxie GmbH, Berlin, Germany)

AE-QoL					
Quality of Life Questionnaire for Patients with Recurrent Swelling Episodes					
Patient name: _____					
Date questionnaire completed (dd mmm yyyy): ____ _					
Instructions: This questionnaire asks a number of questions. Please read each question carefully and choose from the five answers the one that fits best for you. Please do not think too long about the questions; be sure to answer all of the questions and to give only one answer to each question, i.e., to check only one box for each question.					
Indicate how often within the last 4 weeks you have been restricted in the areas of your daily life listed below because of swelling episodes (angioedema). (regardless of whether or not you have actually experienced swelling episodes during that time period)	Never	Rarely	Occasionally	Often	Very often
1. Work	☐	☐	☐	☐	☐
2. Physical activity	☐	☐	☐	☐	☐
3. Leisure time	☐	☐	☐	☐	☐
4. Social relations	☐	☐	☐	☐	☐
5. Eating and drinking	☐	☐	☐	☐	☐
In the following questions we would like to get more details about the difficulties and problems that may be associated with your recurrent swelling episodes (angioedema) (during the last 4 weeks)	Never	Rarely	Occasionally	Often	Very often
6. Do you have difficulty falling asleep?	☐	☐	☐	☐	☐
7. Do you wake up during the night?	☐	☐	☐	☐	☐
8. Are you tired during the day because you are not sleeping well at night?	☐	☐	☐	☐	☐

9. Do you have trouble concentrating?	☐	☐	☐	☐	☐
	Never	Rarely	Occasionally	Often	Very often
10. Do you feel depressed?	☐	☐	☐	☐	☐
11. Do you have to limit your choices of food or beverages?	☐	☐	☐	☐	☐
12. Do the swelling episodes place a burden on you?	☐	☐	☐	☐	☐
13. Are you afraid that a swelling episode could occur suddenly?	☐	☐	☐	☐	☐
14. Are you afraid that the frequency of the swelling episodes might increase?	☐	☐	☐	☐	☐
15. Are you ashamed to go out in public because of the swelling episodes?	☐	☐	☐	☐	☐
16. Do the swelling episodes make you embarrassed or self-conscious?	☐	☐	☐	☐	☐
17. Are you afraid that the treatment of the swelling episodes could have negative long-term effects for you?	☐	☐	☐	☐	☐

Source: Weller, K, M Magerl, A Peveling-Oberhag, P Martus, P Staubach, and M Maurer, 2016, The Angioedema Quality of Life Questionnaire (AE-QoL) - assessment of sensitivity to change and minimal clinically important difference, *Allergy*, 71(8):1203-1209.

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CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF NEW DRUGS
OFFICE OF IMMUNOLOGY AND INFLAMMATION
DIVISION OF PHARMACOLOGY/TOXICOLOGY FOR IMMUNOLOGY
AND INFLAMMATION

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: **NDA 214094**

Supporting document/s: **Sequences 0001 and 0029**

Applicant's letter date: **December 3, 2019; July 9, 2020**

CDER stamp date: **December 3, 2019; July 9, 2020**

Product: **ORLADEYO (Berotralstat) oral capsules**

Indication: **Prevention of Hereditary angioedema (HAE)**

Applicant: **BIOCRYST Therapeutics**

Review Division: **Pulmonology, Allergy and Critical Care**

Reviewer: **Luqi Pei, Ph.D.**

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Template Version: September 1, 2010

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LABELING RECOMMENDATION

This review recommends the following text for nonclinical sections of the ORLADEYO (berotralstat) capsule label. The nonclinical sections include the following: USE in SPECIFIC POPULATION (8.1 and 8.2), CLINICAL PHARMACOLOGY (12.1), and NONCLINICAL TOXICOLOGY (13.1).

8.1 Pregnancy

Risk Summary

There are insufficient data (b) (4) in pregnant women to inform drug-related risks with ORLADEYO use in pregnancy. Based on animal reproduction studies, no evidence of structural alterations was observed when berotralstat was administered orally to pregnant rats and rabbits during organogenesis at doses up to approximately 10 and 2 times, respectively, the maximum recommended human daily dose (MRHDD) in adults on an AUC basis [see *Data*].

The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

In animal reproduction studies, oral administration of berotralstat to pregnant rats and rabbits during the period of organogenesis did not cause fetal structural alterations. The berotralstat dose in rats and rabbits was up to approximately 10 and 2 times, respectively, the MRHDD in adults (on an AUC basis at maternal doses of 75 and 100 mg/kg/day, respectively). In a pre- and postnatal development study in rats, oral administration of berotralstat to pregnant rats during the period of organogenesis until delivery at doses up to 45 mg/kg/day (approximately 2 times of the MRHD on a mg/m² basis) did not cause fetal structural alterations. Berotralstat concentrations in the fetal blood was approximately 5 – 11% of the maternal blood.

8.2 Lactation

Risk Summary

There are no data on the presence of berotralstat in human milk, its effects on the breastfed infant, or its effects on milk production. However, when a drug is present in animal milk, it is likely that the drug will be present in human milk. Low levels of berotralstat were detected in the plasma of rat pups when dams were dosed with the drug orally during the lactation period. The berotralstat concentration in the pup plasma was approximately 2% of the maternal plasma (see *Data*).

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ORLADEYO and any potential adverse

effects on the breastfed infant from ORLADEYO or from the underlying maternal condition.

Data

Animal Data

In a pre- and postnatal development study in rats, berotralstat was administered to dams during the pregnancy and lactation periods at doses up to 45 mg/kg/day (approximately 2 times of the MRHD on a mg/m² basis). Berotralstat was detected in the plasma of pups during the lactation period. The berotralstat concentration in the pup plasma was approximately 2% of the maternal plasma. Both dams and pups at 45 mg/kg/day showed statistically significant decreases in body weight gain ($p < 0.05$). No treatment-related effects were observed at 25 mg/kg/day (approximately equal to the MRHD on a mg/m² basis).

12.1 Mechanism of Action

Berotralstat is a plasma kallikrein inhibitor that binds plasma kallikrein and inhibits its proteolytic activity. Plasma kallikrein is a protease that cleaves high-molecular-weight-kininogen (HMWK) to generate cleaved HMWK (cHMWK) and bradykinin, a potent vasodilator that increases vascular permeability resulting in swelling and pain associated with HAE. In patients with HAE due to C1-inhibitor (C1-INH) deficiency or dysfunction, normal regulation of plasma kallikrein activity is not present, which leads to uncontrolled increases in plasma kallikrein activity and results in angioedema attacks. Berotralstat decreases plasma kallikrein activity to control excess bradykinin generation in patients with HAE.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4) in a 2-year study in Wistar rats (b) (4) 26-week study in Tg.rasH2 transgenic mice. The berotralstat doses (oral gavage) were up to 20 and 50 mg/kg/day in rats and mice, (b) (4)

Mutagenesis Berotralstat tested negative in the *in vitro* bacterial reverse mutation assay (Ames test), the *in vitro* (b) (4)

Impairment of Fertility In a fertility study in rats, berotralstat at oral doses up to 45 mg/kg/day (approximately 2 times the MRHD on a mg/m² basis) showed no effect on fertility in males or females.

LABELING REVIEW

Edits to the nonclinical information in the ORLADEYO (berotralstat) label proposed by the Applicant are recommended. The nonclinical information is described in the following sections: 8.1 Pregnancy, 8.2 Lactation, 12.1 Mechanism of Action, and 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility. Edits are made to ensure that the to-be-approved label reflects the Agency's most current thinking.

1. INTRODUCTION

This review evaluates the nonclinical information of berotralstat in the ORLADEYO label proposed by BioCryst on December 3, 2019 and July 9, 2020 (SDNs 0001 and 0029, respectively). Specifically, the review evaluates the nonclinical information in the following subsections of the label: 8.1, 8.2, 12.1, and 13.1. The review recommends major edits, including both the text and dose ratios between animals and humans.

Berotralstat is indicated for the prevention of hereditary angioedema (HAE) in patients 12 years of age and older. The proposed clinical dosing regimen is 150-mg berotralstat once daily. The mean AUC_{0-24} at 150-mg/day berotralstat in patients is 2540 ng.h/mL.

This review consists of 8 sections. Section 1 provides background information relevant to the current review. Section 2 discusses dose ratios between animals and humans in the nonclinical sections. Section 3 discusses methods and rationale used for this review. Sections 4 - 7 evaluate subsections of the proposed labeling, rationale for the recommended editing, and line editing. Section 8 provides a summary of overall recommendations. A clean copy of the recommended text of the nonclinical sections of the ORLADEYO label is presented in the LABELING RECOMMENDATION section which is located at the beginning of the document.

2. EXPOSURE MULTIPLES

This review derives exposure multiples between animals and humans on an AUC and $mg/m^2/day$ basis. Exposure multiples based on AUCs are preferred. When AUC data is unavailable, exposure multiples are calculated on a $mg/m^2/day$ basis [human equivalent dose ($Human_{HED}$)]. The following exposures in humans are used to calculate the exposure multiples: $AUC_{0-24} = 2540$ ng.h/mL and $Human_{HED} = 92.5$ $mg/m^2/day$. Both parameters are based on a maximum recommended human daily dose (MRHDD) of 150 mg. A body weight of 60-kg was used to calculate the $Human_{HED}$ [150 mg/day/ 60 kg $\times$ 37 (km) = 92.5 $mg/m^2/day$]. Briefly, all but one exposure multiple (i.e., 45 $mg/kg/day$ dose in rats) were derived on an AUC basis (Table 1).

Table 1: Berotralstat Exposure Multiples between Animals and Humans

Section	Species	ROA	Berotralstat		Exposure multiple		Study #
			mg/kg /day	AUC (ng.h/mL)	Calculated ^a	Rounded to	
Carcinogenicity							
	Rat	PO	20	11500	4.5	5	806-161
		PO	8	2750	1.1	1	806-161
	Mouse	PO	50	27450	10.8	10	20088905
Reproduction and fertility							
Fertility	Rat	PO	45	-	2.4 ^b	2	806-156
EFD	Rat	PO	75	24600	9.7	10	9000644
	Rabbit	PO	100	4410	1.7	2	9000815
PPND	Rat	PO	45	-	2.4 ^b	2	806-157

a. Based on a mean AUC of 2540 ng.h/mL at a daily oral dose of 150 mg in adult patients.

b. On a mg/m² basis. No AUC data was available in this study. Plasma berotralstat levels were measured at 4 hours post-dosing only. Rat_{HED} = 45 mg/kg x 6 = 270 mg/m²; Human_{HED} = 150 mg/60 kg x 37 = 92.5 mg/m²; and exposure ratio (rat_{HED} / human_{HED}) = 270/92.5 = 2.4.

3. METHODS OF EVALUATION

The review uses product labels of drugs approved for the same indication and similar mechanism of actions as references to maintain consistency across approved labels. Specifically, the review references to the label for KALBITOR, FIRAZYR, and TAKHZYRO (BLA 125277, NDA 022150, and BLA 761090, respectively) for consistency. These products were approved for the treatment of acute attacks or prophylaxis of HAE. The current application proposed to prevent HAE attacks from occurring. See Section 6 (Table 2).

Contents of the proposed labeling are generally acceptable, but the review finds it necessary to edit the proposed text. The edits are made to ensure that the to-be-approved label reflects the Agency's current thinking. Highlights indicate recommended edits. Underlines indicates addition. Strikeouts indicate deletion.

4. PREGNANCY (Section 8.1)

The review finds necessary to edit the proposed text for the pregnancy section. Edits are made to the Risk Summary section. The Animal Data section is rewritten as indicated below.

Risk Summary

There are insufficient data (b) (4) in pregnant women to inform drug-related risks with ORLADEYO use in pregnancy. Based on animal reproduction studies, no evidence of structural alterations was observed when berotralstat was administered orally to pregnant rats and rabbits during organogenesis at doses up to approximately 10 and 2 times, respectively, the maximum recommended human daily dose (MRHDD) in adults on an AUC basis [see Data]. (b) (4)

(b) (4)

The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

In animal reproduction studies, oral administration of berotralstat to pregnant rats and rabbits during the period of organogenesis did not cause fetal structural alterations. The berotralstat dose in rats and rabbits was up to approximately 10 and 2 times, respectively, the MRHDD in adults (on an AUC basis at maternal doses of 75 and 100 mg/kg/day, respectively). In a pre- and postnatal development study in rats, oral administration of berotralstat to pregnant rats during the period of organogenesis until delivery at doses up to 45 mg/kg/day (approximately 2 times of the MRHD on a mg/m² basis) did not cause fetal structural alterations. Berotralstat concentrations in the fetal blood (b) (4) approximately 5 – 11% of the maternal blood.

(b) (4)

5. LACTATION (Section 8.2)

The review finds necessary to edit the proposed text for the pregnancy section. Edits are made to the Risk Summary section. The Animal Data section is rewritten. Below are the recommended edits:

Risk Summary

There are no data on the presence of berotralstat in human milk, its effects on the breastfed infant, or its effects on milk production. **However, when a drug is present in animal milk, it is likely that the drug will be present in human milk. Low levels of berotralstat were detected in the plasma of rat pups when dams were dosed with the drug orally during the lactation period. The berotralstat concentration in the pup plasma was approximately 2% of the maternal plasma (see Data).** (b) (4)

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ORLADEYO and any potential adverse effects on the breastfed infant from ORLADEYO or from the underlying maternal condition.

Data

Animal Data

In a pre- and postnatal development study in rats, berotralstat was administered to dams during the pregnancy and lactation periods at doses up to 45 mg/kg/day (approximately 2 times of the MRHD on a mg/m² basis). Berotralstat was detected in the plasma of pups during the lactation period. The berotralstat concentration in the pup plasma was approximately 2% of the maternal plasma. Both dams and pups at 45 mg/kg/day showed statistically significant decreases in body weight gain ($p < 0.05$). No treatment-related effects were observed at 25 mg/kg/day (approximately equal to the MRHD on a mg/m² basis).

(b) (4)

Reviewer's comment: This review revised the plasma concentration of berotralstat in pups (b) (4) 2%). (b) (4)

According to data presented in Section 5.5.4 of the Unireview of the NDA, the berotralstat concentration in pup plasma (b) (4)

6. MECHANISM OF ACTION (Section 12.1)

Edits to the proposed text of Section 12.1 Mechanism of Action of the berotralstat label are recommended. Edits were made to ensure consistency in language between similar products. There are currently three approved and currently marketed products indicated HAE (Table 2). Berotralstat application is included for reference.

Table 2: Drug Products Indicated for HAE

Application #	BLA 125277	NDA 022150	BLA 761090	NDA 214094
Drug name	KALBITOR	FIRAZYR	TAKHZYRO	ORLADEYO
Chemical name	Ecallantide	Icatibant	Lanadelumab-flyo	Berotralstat
Chemistry	Human TFPI ^a	Peptide (10 AAs)	Monoclonal antibody	Small molecule
Indication	HAE attacks	HAE attacks	Prophylaxis	Prophylaxis
ROA	SC injection	SC Injection	SC injection	Oral capsules
MOA	Kallikrein inhibitor	Bradykinin (B2) receptor antagonist	Anti-Kallikrein antibody	Kallikrein inhibitor
Approval date	12/01/2009	08/25/2011	08/23/2018	Pending

a. TFPI = tissue factor pathway inhibitor (recombinant).

The review uses the TAKHZYRO label as a template for 2 reasons: 1) TAKHZYRO and berotralstat have the same indication – prophylaxis of HAE, and 2) the TAKHZYRO label approved recently reflects the Agency’s most current thinking. Table 3 (next page) presents the text of Section 12.1 in the TAKHZYRO label and Berotralstat label (proposed).

Table 3: Section 12.1 Mechanism of Action of Approved and Proposed Label

TAKHZYRO (BLA 761090, approved)	ORLADEYO (NDA214094, proposed)
Lanadelumab-flyo is a fully human monoclonal antibody (IgG1/k-light chain) that binds plasma kallikrein and inhibits its proteolytic activity. Plasma kallikrein is a protease that cleaves high-molecular-weight-kininogen (HMWK) to generate cleaved HMWK (cHMWK) and bradykinin, a potent vasodilator that increases vascular permeability resulting in swelling and pain associated with HAE. In patients with HAE due to C1-inhibitor (C1-INH) deficiency or dysfunction, normal regulation of plasma kallikrein activity is not present, which leads to uncontrolled increases in plasma kallikrein activity and results in angioedema attacks. Lanadelumab-flyo decreases plasma kallikrein activity to control excess bradykinin generation in patients with HAE.	(b) (4) Plasma kallikrein is a protease that cleaves high-molecular-weight-kininogen (HMWK), (b) (4) bradykinin, a potent vasodilator that increases vascular permeability. In patients with HAE due to C1-inhibitor (C1-INH) deficiency or dysfunction, normal regulation of plasma kallikrein activity is (b) (4) which leads to uncontrolled increases in plasma kallikrein activity (b) (4) Bertralstat decreases plasma kallikrein activity to control excess bradykinin generation in patients with HAE.

The proposed text for bertralstat label mostly reflects the content of the TAKHZYRO label, but modifications exist. These modifications except for drug names are unwarranted. Based on the above discussions, the review recommends the following text for Section 12.1 of the bertralstat label:

12.1 Mechanism of Action

Bertralstat is a plasma kallikrein inhibitor that binds plasma kallikrein and inhibits its proteolytic activity. Plasma kallikrein is a protease that cleaves high-molecular-weight-kininogen (HMWK) to generate cleaved HMWK (cHMWK) and bradykinin, a potent vasodilator that increases vascular permeability resulting in swelling and pain associated with HAE. In patients with HAE due to C1-inhibitor (C1-INH) deficiency or dysfunction, normal regulation of plasma kallikrein activity is not present, which leads to uncontrolled increases in plasma kallikrein activity and results in angioedema attacks. Bertralstat decreases plasma kallikrein activity to control excess bradykinin generation in patients with HAE.

7. CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY (Section 13.1)

Carcinogenicity of bertralstat was evaluated in a 2-year study in Wistar rats and a 26-week study in Tg.rasH2 transgenic mice (b) (4)

(b) (4) No evidence of tumorigenicity was observed in (b) (4) See the Agency's Executive Carcinogenesis Assessment Committee minutes completed on March 16, 2020, and an addendum to the minutes completed on July 23, 2020 (DARRTS IDs 4576054 and 4645569). Also see the nonclinical review completed by Dr. Luqi Pei on March 24, 2020 and an addendum to the review completed by Dr. Pei on July 23, 2020 (DARRTS ID 4580256 and 4645674, respectively).

The review recommends a rewrite of the carcinogenesis section (13.1) of berotralstat label proposed on July 9, 2020 (SDN 029). The rewrite is to reflect the Agency conclusions and thinking.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

[REDACTED] (b) (4)
The berotralstat doses (oral gavage) were up to 20 and 50 mg/kg/day in rats and mice, [REDACTED] (b) (4)
[REDACTED]

[REDACTED] (b) (4)

Mutagenesis Berotralstat tested negative in the *in vitro* bacterial reverse mutation assay (Ames test), the *in vitro* human peripheral blood lymphocyte [REDACTED] (b) (4)
[REDACTED]

Impairment of Fertility In a fertility study in rats, berotralstat at oral doses up to 45 mg/kg/day (approximately 2 times the MRHD on a mg/m² basis) showed no effect on fertility in males or females.

8. RECOMMENDATIONS

1. Revised the proposed text so that the labeling language is consistent with the Agency's current thinking and labeling practice.
2. See LABELING RECOMMENDATION for a clean copy of the recommended text for Sections 8, 10, 12, and 13.

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/s/

LUQI PEI
07/24/2020 02:23:16 PM

CAROL M GALVIS
07/24/2020 02:25:14 PM
I concur.

Addendum to Pharmacology/Toxicology Review
Completed by Dr. Luqi Pei in NDA 214094 on March 24, 2020 (DARRTS 4580256)

Date: July 23, 2020
NDA No.: 214094
Submission No.: SDNs 0001 and 29
Submission date: December 3, 2019 and July 9, 2020
Drug Name: Berotralstat
Reviewer: Luqi Pei, PhD
Subject: Re-evaluation of tumors in the mesenteric lymph nodes in male rats in 2-year oral carcinogenicity study of berotralstat (Study 801-161)

This document is an addendum to a nonclinical review completed by Dr. Luqi Pei on March 24, 2020 in NDA 214094 (DARRTS ID 4580256). The addendum documents the Agency's re-evaluation of tumor findings in the mesenteric lymph nodes in males in a 2-year oral carcinogenicity study of berotralstat in rats (Study 806-161). The re-evaluation was prompted by the Applicant's recent submission of an amendment to the final report of Study 806-161 (SDN 029). The final report of the study was submitted to the original NDA on December 3, 2019 (SDN 0001).

Dr. Luqi Pei of the Division of Pharmacology and Toxicology for Immunology and Inflammation (DPT-II) completed a review of the final report of Study 806-161 on March 24, 2020 (DARRTS ID 4580256). The Executive Carcinogenicity Assessment Committee (Exec CAC) evaluated the findings of the study (as reported in the original study report) on March 10, 2020. The Committee concluded that berotralstat tested positive for carcinogenicity. The conclusion was based on the dose-dependent increase in the incidence of hemangiosarcoma in the lymph nodes in male rats. See Exec CAC Meeting minutes dated March 16, 2020 (DARRTS ID 4576064).

BioCryst submitted an amendment to the final report of Study 806-161 on July 9, 2020 (NDA 214094, eCTD 029). The amendment revised neoplastic findings in the vasculature, particularly the mesenteric lymph node in male rats. Table 1 presents the tumor incidence in the mesenteric lymph nodes in male rats.

Table 1: Tumor Findings in the Mesenteric Lymph Node in Males (Study 806-161)

eCTD	Sub Date	Report	Tumor	Incidence (n = 60)				P value ^a (C v HD)
				0 mkd	3 mkd	8 mkd	20 mkd	
0001	12/3/19	Original	Hemangiosarcoma	1	2	2	9	0.005
0029	7/9/20	Revised	Hemangioma	1	0	0	5	0.081 ^b

a. Pairwise comparison.

b. Provided by Dr. Miao Zhuang via email on July 17, 2020.

Briefly, the amendment revised both tumor type and incidence in this tissue. The tumor type changed from malignant hemangiosarcoma to benign hemangioma. The tumor incidence was decreased by approximately half. These differences resulted in a change in carcinogenic potential of berotralstat: from positive to negative, according to the current evaluation criteria. That is, the original report shows that berotralstat tested positive for carcinogenicity while the amended report showed that berotralstat tested negative.

The revision of the tumor type and incidence was the result of a pathology expert working group (EWG) assembled by BioCryst to re-evaluate neoplastic findings in the mesenteric lymph nodes. The re-evaluation was prompted by a difference in interpretation between the Agency and BioCryst regarding the relevance of the tumor findings to humans, based on data presented in the original study report.

As discussed previously, the Exec CAC concluded that berotralstat tested positive because berotralstat at 20 mg/kg/day caused a statistically significant increase in the incidence of hemangiosarcoma (Ref. DARRTS ID 4576064). The Committee also concluded that the relevance of the finding to humans is unknown. However, BioCryst has maintained their position that the findings of hemangiosarcoma in rats is a species-specific effect. The Agency's position was conveyed to BioCryst in the Filing Communication dated February 14, 2020 and was briefly discussed at the mid-cycle meeting with the Applicant.

BioCryst assembled the EWG to re-evaluate the slides with tumor findings. The EWG conveyed on April 28, and May 12, 2020 via videoconference. The group reviewed "H&E stained digitized slides of selected tissue sections of proliferative vascular findings in mesenteric lymph nodes and all diagnosed hemangiomas and hemangiosarcomas from other tissues as reported". Members of the EWG included Drs. Paul W. Snyder (Chair), Jerry Hardisty (reviewing pathologist), Joshua Decker (Study Pathologist), Kenneth Schaffer, Andrew Suttie, and Samuel Cohen. The EWG issued its report on June 11, 2020 (p. 2401 – 2427 of the amended study report). Table 2 summarizes the diagnosis of pathological findings in the original report and the EWG.

The Exec CAC evaluated the findings of the EWG via email communications on July 10, 2020. Based on the new re-adjudication provided in the amended study report, the Committee concluded that berotralstat is negative for tumorigenicity in male rats in Study 806-161. See the Addendum to the March 16, 2020 Exec CAC minutes completed on July 23, 2020 (DARRTS ID 4645569).

Table 2: Summary of Changes in Findings in Mesenteric Lymph Nodes

Mesenteric Lymph Node

Group	Animal No.	Study Pathologist's Diagnosis	Pathology Working Group Diagnosis	Comments/Other
1M	1015	Hemangioma; Benign	Hemangioma; Benign	
1M	1023	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 2	
2M	2032	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 2	
2M	2043	Fibrohistiocytic Proliferation; Minimal	Hyperplasia; Angiomatous, Grade 1	
3M	3043	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 1	
3M	3060	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 2	
4M	4007	Hemangiosarcoma; Malignant	Hemangioma; Benign	
4M	4023	Hemangiosarcoma; Malignant	Hemangioma; Benign	Delineated nodule
4M	4024	Hemangiosarcoma; Malignant	Hemangioma; Benign	Delineated nodule
4M	4037	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 3	
4M	4042	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 3	Multiple areas with different features, cavernous and capillary
4M	4048	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 2	
4M	4052	Hemangiosarcoma; Malignant	Hyperplasia; Angiomatous, Grade 2	
4M	4055	Hemangiosarcoma; Malignant	Hemangioma; Benign	
4M	4059	Hemangiosarcoma; Malignant	Hemangioma; Benign	

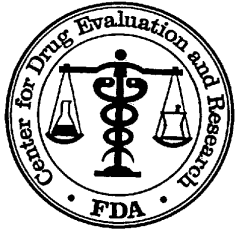
Grade 1 = Minimal; Grade 2 = Slight; Grade 3 = Moderate; Grade 4 = Marked; Grade 5 = Severe

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/s/

LUQI PEI
07/23/2020 01:52:22 PM

CAROL M GALVIS
07/23/2020 02:22:16 PM
I concur.



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research

Office of Translational Science

Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number: NDA 214094

Drug Name: ORLADEYO (BCX7353)

Indication: To prevent attacks of hereditary angioedema (HAE) in patients 12 years and older

Applicant: BioCryst Pharmaceuticals, Inc.
4505 Emperor Boulevard
Durham, NC 27703

Test Facility for Rats Study: [REDACTED] (b) (4)
[REDACTED]
[REDACTED]

Test Facility for Mice Study: [REDACTED] (b) (4)
[REDACTED]
[REDACTED]

Documents Reviewed: Study reports (Study No. 806-161; Study No. 20088905) and electronic data submitted on December 3, 2019 via NDA214094/0000

Biometrics Division: Division of Biometrics -6

Statistical Reviewer: Zhuang Miao, Ph.D.

Secondary Reviewer: Feng Zhou, MS

Concurring Reviewer: Karl Lin, Ph.D.

Medical Division: Division of Pulmonary, Allergy and Rheumatology Products

Reviewing Pharmacologist: Pei, Luqi, Ph.D.

Keywords: Carcinogenicity, Dose response

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Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in Wistar Han [CrI:WI(Han)] rats and one in CByB6F1/Tg rasH2 hemizygous (transgenic) mice. These studies were intended to assess the carcinogenic potential of ORLADEYO (BCX7353), when administered orally by gavage at appropriate drug levels for 104 weeks in rats and 26 weeks in mice.

Rat Study: . Two hundred and forty Wistar Han [CrI:WI(Han)] rats of each sex were randomly assigned to the treated and vehicle control groups in equal size of 60 rats per group. The dose levels for treated groups were 3, 8 and 20 mg/kg/day. The rats in the vehicle control group received the vehicle(deionized water).

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female rats. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female rats.

Tumor analysis:

1. For male rats, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the Hemangiosarcoma, Multicentric (P-value=0.0018<0.005), Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0001<0.005) and combined tumors of Hemangiosarcoma and Hemangioma in whole body (P-value=0.0044<0.005). The pairwise comparison of the Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0049<0.01) between the vehicle control group and the 20 mg/kg/day group for male rats is significant at 0.01 level of significance. The pairwise comparison of the Hemangiosarcoma, Multicentric (p-value=0.0166) and) between the vehicle control group and the 20 mg/kg/day group for male rats is marginally significant at 0.01 level of significance. However, this tumor type for male rats is definitely significant at 0.05 level of significance in the joint test used by the ECAC but with the more appropriate levels of significance recommended in Lin and Rahman (2018, 2019).
2. For female rats, the tumor data analysis showed a statistically significant positive dose response relationship in incidence of the Sarcoma, endometrial stromal, Uterus with cervix (P-value=0.0226<0.025).

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
Male Rats					
MULTICENTRIC NEOPL	HEMANGIOSARCOMA, MALIGNANT	2/60 (52) 0.0018	2/60 (52) 0.6912	4/60 (51) 0.3298	9/60 (47) 0.0166
Lymph Node, Mesenteric	HEMANGIOSARCOMA, MALIGNANT	1/60 (52) 0.0001	1/60 (52) 0.7524	2/60 (51) 0.4851	9/60 (47) 0.0049
Whold Body	C_Hemangiosarcoma+Hemangioma	3/60 (52) 0.0044	2/60 (52) 0.8187	4/60 (51) 0.4891	9/60 (47) 0.0410
Female Rats					
UTERUS WITH CERVIX	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.0226	0/60 (51) NC	2/60 (51) 0.2576	3/60 (49) 0.1211

Mouse Study: One hundred and twenty five hemizygous CByB6F1/Tg rasH2 hemizygous (transgenic) mice of each sex were randomly assigned to the treated, vehicle and positive control group in equal size of 25 mice per group. The dose levels for treated groups were 8, 20, and 50 mg/kg/day for males and females. The mice in the vehicle control group received the vehicle (Reverse Osmosis Deionized (RODI) Water). The study was designed to continue for up to 26 weeks for both sexes, however in accordance with study termination criteria, all surviving mice were sacrificed during Week 27. The mice in the positive control group received 75 mg/kg N-Nitrosomethylurea (NMU) in citrate-buffered saline (pH 4.5) once on Day 1 via intraperitoneal injection.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in either male or female mice. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in either male or female mice.

Tumor analysis:

1. For male mice, the pairwise comparisons between the vehicle control and the positive control showed a statistically significant increase in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Squamous cell carcinoma, stomach (P-

value=0.0073<0.05)

2. For female mice, the pairwise comparisons between the vehicle control and the positive control showed statistically significant increases in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Combined tumor of Papilloma and Carcinoma, Skin (P-value<0.001)

1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in Wistar Han [CrI:WI(Han)] rats and one in CByB6F1/Tg rasH2 hemizygous (transgenic) mice. These studies were intended to assess the carcinogenic potential of ORLADEYO (BCX7353), when administered orally by gavage at appropriate drug levels for 104 weeks in rats and 26 weeks in mice. Results of this review have been discussed with the reviewing pharmacologist Dr. Luqi Pei. This review analyzed the SAS data sets of these studies received from the sponsor on December 3, 2019 via NDA214094/0000.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

2. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were four treated groups and one vehicle control group. Three hundred Wistar Han [CrI:WI(Han)] rats of each sex were randomly assigned to the treated and vehicle control groups in equal size of 60 rats per group. The dose levels for treated groups were 3, 8, 20 and 60/40 mg/kg/day. The rats in the vehicle control group received the vehicle(deionized water). The study for the rats was designed to continue for up to 104 weeks. In accordance with study termination criteria, all surviving male rats were sacrificed during Week 105.

Animals in 60/40 mg/kg/day group were dosed at 60 mg/kg/day from Week 1-17; from Week 18-28 animals were dosed at 40 mg/kg/day. Pharm/Tox reviewer Dr. Pei requested to drop the 60/40 mg/kg/day group due to high death rate. We do not include the 60/40 mg/kg/day group in our analysis.

Table 1: Study Design in Rat Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	60	60
2	3	BCX7353	60	60

3	8	BCX7353	60	60
4	20	BCX7353	60	60
5	60/40	BCX7353	60	60

2.1. Sponsor's analyses

2.1.1. Survival analysis

Intercurrent mortality data was analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test. If this overall test was significant ($p < 0.05$) and there were more than two groups, then a follow up analysis was done where each treatment group was compared to the control group using a log-rank test. Results of all pair-wise comparisons were reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

Sponsor's findings: Sponsor's analysis showed the numbers (percents) of survival were 43 (71.67%), 39 (65%), 42 (70%), and 33 (55%) in vehicle control, 3 mg/kg/day, 8 mg/kg/day and 20 mg/kg/day dose groups, respectively in males and 35 (58.33%), 36 (60%), 37 (61.67%), and 28 (46.67%) in vehicle control, 3 mg/kg/day, 8 mg/kg/day and 20 mg/kg/day dose groups, respectively in females.

Morbidity and mortalities occurred in the male and females at 60 mg/kg/day, causing the dose level to be lowered to 40 mg/kg/day on Day 120. However, even though the dose was lowered, the morbidity and mortality continued in this group. Consequently, the animals in this group were euthanized on Day 191 (Week 28).

The sponsor concluded that no significant differences in the survival rate were noted in males or females at 3 or 8 mg/kg/day when compared to the control group. The survival rate for males and females at 20 mg/kg/day was notably less than the survival rate in the control groups, although the difference was not statistically significant. Decreased survival in the males and females at 20 mg/kg/day was considered BCX7353-related.

2.1.2. Tumor data analysis

Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated and Fisher's exact test¹⁴ was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al. Evaluation criteria (pvalues of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%)

Sponsor's findings: The sponsor concluded that the hemangiosarcoma, malignant, Multicentric neoplasm was significant in both the trend test and the pairwise comparison between the vehicle control group and the 20 mg/kg/day group for male rats and the Sarcoma, endometrial stromal, malignant in Uterus with cervix was significant in both the trend test and the pairwise comparison between the vehicle control group and the 20 mg/kg/day group for female rats. The Sarcoma, undifferentiated, malignant in Skin/skin, subcutis was significant in the pairwise comparison between the vehicle control group and the 20 mg/kg/day group for male rats.

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on December 3, 2019, via NDA214094/0000.

2.2.1. Survival analysis

The survival distributions of animals in all five groups were estimated by the Kaplan-Meier product limit method. The dose response relationship and homogeneity of survival distributions were tested for the vehicle controls, low, medium and high dose groups using the Likelihood Ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 7 and 8 in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1 and 2 in the appendix for males and females, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 9, and 10 in the appendix for males and females, respectively.

Reviewer's findings: This reviewer's analysis showed the numbers (percents) of survival were 43 (71.67%), 39 (65%), 42 (70%), and 33 (55%) in vehicle control, 3 mg/kg/day, 8 mg/kg/day and 20 mg/kg/day dose groups, respectively in males and 35 (58.33%), 36 (60%), 37 (61.67%), and 28 (46.67%) in vehicle control, 3 mg/kg/day, 8 mg/kg/day and 20 mg/kg/day dose groups, respectively in females.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female rats. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female rats.

2.2.2. Tumor data analysis

The tumor data were analyzed for the positive dose response relationships and the positive pairwise comparison increases between each of the treated groups with control group. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-K method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of $s_h = 1$. An animal that dies at week w_h without a tumor before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}} \right)^k < 1$. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes were then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons are listed in Tables 11 and 12 in the appendix for male and female rats, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study and one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels $\alpha = 0.005$ for common tumors and $\alpha = 0.025$ for rare tumors for the chronic rat study. For pairwise comparisons for the chronic rat study in the above type of submission with one chronic rat study and one transgenic mouse study, the same guidance document suggests the use of test levels $\alpha = 0.01$ for common tumors and $\alpha = 0.05$ for rare tumors for the chronic rat study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: The tumor types in Tables 2 and 3 below showed p-values less than or equal to 0.05 in the trend tests and the tests for pairwise comparisons between the vehicle and the treated groups for male and female rats, respectively. Based on the above criterion for multiple testing adjustment, we make the following statistical conclusions:

1. For male rats, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the Hemangiosarcoma, Multicentric (P-value=0.0018<0.005), Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0001<0.005) and combined tumors of Hemangiosarcoma and Hemangioma in whole body (P-value=0.0044<0.005). The pairwise comparison of the Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0049<0.01) between the vehicle control group and the 20 mg/kg/day group for male rats is significant at 0.01 level of significance. The pairwise comparison of the Hemangiosarcoma, Multicentric (p-value=0.0166) and) between the vehicle control group and the 20 mg/kg/day group for male rats is marginally significant at 0.01 level of significance. However, this tumor type for male rats is definitely significant at 0.05 level of significance in the joint test used by the ECAC but with the more appropriate levels of significance recommended in Lin and Rahman (2018, 2019).
2. For female rats, the tumor data analysis showed a statistically significant positive

dose response relationship in incidence of the Sarcoma, endometrial stromal, Uterus with cervix (P-value=0.0226<0.025).

Table 2: Tumor Types with P-Values $\leq$ 0.05 for Comparisons between Vehicle Control and Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
MULTICENTRIC NEOPL	HEMANGIOSARCOMA, MALIGNANT	2/60 (52) 0.0018	2/60 (52) 0.6912	4/60 (51) 0.3298	9/60 (47) 0.0166
Lymph Node, Mesenteric	HEMANGIOSARCOMA, MALIGNANT	1/60 (52) 0.0001	1/60 (52) 0.7524	2/60 (51) 0.4851	9/60 (47) 0.0049
SKIN	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.0365	1/60 (52) 0.5000	1/60 (51) 0.4951	3/60 (48) 0.1070
Whold Body	C_Hemangiosarcoma+Hemangioma	3/60 (52) 0.0044	2/60 (52) 0.8187	4/60 (51) 0.4891	9/60 (47) 0.0410
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.					

Table 3: Tumor Types with P-Values $\leq$ 0.05 for Comparisons between Vehicle Control and Treated Groups-Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
MULTICENTRIC NEOPL	HEMANGIOSARCOMA, MALIGNANT	4/60 (49) 0.0113	2/60 (51) 0.9067	1/60 (50) 0.9733	9/60 (50) 0.1245
UTERUS WITH CERVIX	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.0226	0/60 (51) NC	2/60 (51) 0.2576	3/60 (49) 0.1211
Whold Body	C_Hemangiosarcoma+Hemangioma	4/60 (49) 0.0113	2/60 (51) 0.9067	1/60 (50) 0.9733	9/60 (50) 0.1245
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.					

3. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups, one vehicle control group, and one positive control group. One hundred and twenty five hemizygous CByB6F1/Tg rasH2 hemizygous (transgenic) mice of each sex were randomly assigned to the treated, vehicle and positive control group in equal size of 25 mice per group. The dose levels for treated groups were 8, 20, and 50 mg/kg/day for males and females. The mice in the vehicle control group received the vehicle (Reverse Osmosis Deionized (RODI) Water). The study was designed to continue for up to 26 weeks for both sexes, however in accordance with study termination criteria, all surviving mice were sacrificed during Week 27. The mice in the positive control group received 75 mg/kg N-Nitrosomethylurea (NMU) in citrate-buffered saline (pH 4.5) once on Day 1 via intraperitoneal injection.

Table 4: Study Design in Mouse Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	25	25
2	8	Low	25	25
3	20	Middle	25	25
4	50	High	25	25
5	75	Positive	25	25
6		Sentinel	10	10

3.1. Sponsor's analyses

3.1.1. Survival analysis

The sponsor used the same survival analysis methods used for the rats study in this mouse study.

Sponsor's findings: The sponsor's analysis showed that the numbers (percents) of death were 1 (4%), 1 (4%), 1 (4%), 3 (12%), and 17 (68%) in male mice, and 0 (0%), 1 (4%), 3 (12%), 2 (8%), and 25 (100%) in female mice in vehicol control, low, medium, high dose groups and positive control group, respectively.

The sponsor concluded that there were no statistically significant differences in survival for any BCX7353-treated groups in either sex relative to control groups.

3.1.2. Tumor data analysis

The sponsor used the same tumor data analysis methods used for the rat study in this mouse study

Sponsor's findings: The sponsor concluded that there were no statistically significant findings among males or females for tumor incidence.

3.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on December 3, 2019, via NDA214094/0000.

3.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 3 and 4 in the appendix for male and female mice, respectively. The intercurrent mortality data are given in Tables 13 and 14 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 15 and 16 in the appendix for male and female mice, respectively.

Reviewer's findings: This reviewer's analysis showed that the numbers (percents) of death were 1 (4%), 1 (4%), 1 (4%), 3 (12%), and 17 (68%) in male mice, and 0 (0%), 1 (4%), 3 (12%), 2

(8%), and 25 (100%) in female mice in vehicol control, low, medium, high dose groups and positive control group, respectively.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in either male or female mice. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in either male or female mice.

3.2.2. Tumor data analysis

The reviewer used the same tumor data analysis methods for the rat study in this mouse study.

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups, and between vehicle control and positive control are listed in Tables 17, 18, 19, and 20 in the appendix for male and female mice, respectively.

Adjustment for multiple testing: For the adjustment of multiple testing of dose response relationship for the transgenic mouse study in a submission with one chronic rat study and one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels $\alpha = 0.05$ for both common tumors and rare tumors for the mouse study. For pairwise, the same guidance document suggests the use of test levels $\alpha = 0.05$ for both common tumors and rare tumors for the mouse study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: The tumor types in Tables 5 and 6 below showed p-values less than or

equal to 0.05 in the tests for pairwise comparisons between vehicle and positive control groups for male mice and female mice, respectively.

Table 5: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
HEMOLYMPHORETICULA	LYMPHOMA, MALIGNANT	0/25 (25)	14/25 (22) <0.001
SKIN	PAPILLOMA	0/25 (25)	12/25 (18) <0.001
STOMACH	PAPILLOMA	1/25 (25)	16/25 (22) <0.001
	SQUAMOUS CELL CARCINOMA	0/25 (25)	5/25 (17) 0.0073

Table 6: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
HEMOLYMPHORETICULA	LYMPHOMA, MALIGNANT	0/25 (25)	18/25 (20) <0.001
SKIN	PAPILLOMA	0/25 (25)	10/25 (14) <0.001
STOMACH	PAPILLOMA	0/25 (25)	16/25 (19) <0.001
Skin	C_Papilloma+Carcinoma	1/25 (25)	12/25 (16) <0.001

Reviewer's findings: Based on the criteria of adjustment for multiple testing discussed in the mouse data analysis section, we make the following statistical conclusions:

1. For male mice, the pairwise comparisons between the vehicle control and the positive control showed a statistically significant increase in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Squamous cell carcinoma, stomach (P-value=0.0073<0.05)

2. For female mice, the pairwise comparisons between the vehicle control and the positive control showed statistically significant increases in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Combined tumor of Papilloma and Carcinoma, Skin (P-value<0.001)

4. Conclusion

In this submission the sponsor included reports of two animal carcinogenicity studies, one in Wistar Han [CrI:WI(Han)] rats and one in CByB6F1/Tg rasH2 hemizygous (transgenic) mice. These studies were intended to assess the carcinogenic potential of ORLADEYO (BCX7353), when administered orally by gavage at appropriate drug levels for 104 weeks in rats and 26 weeks in mice.

Rat Study: . Two hundred and forty Wistar Han [CrI:WI(Han)] rats of each sex were randomly assigned to the treated and vehicle control groups in equal size of 60 rats per group. The dose levels for treated groups were 3, 8 and 20 and mg/kg/day. The rats in the vehicle control group received the vehicle(deionized water).

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female rats. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female rats.

Tumor analysis:

1. For male rats, the tumor data analysis showed a statistically significant positive dose response relationship in incidences of the Hemangiosarcoma, Multicentric (P-value=0.0018<0.005), Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0001<0.005) and combined tumors of Hemangiosarcoma and Hemangioma in whole body (P-value=0.0044<0.005). The pairwise comparison of the Hemangiosarcoma in Lymph Node, Mesenteric (P-value=0.0049<0.01) between the vehicle control group and the 20 mg/kg/day group for male rats is significant at 0.01 level of significance. The pairwise comparison of the Hemangiosarcoma, Multicentric (p-value=0.0166) and) between the vehicle control group and the 20 mg/kg/day group for male rats is marginally significant at 0.01 level of significance. However, this tumor type for male rats is definitely significant at 0.05 level of significance in the joint test used by the ECAC but with the more appropriate levels of significance recommended in Lin and Rahman (2018, 2019).
2. For female rats, the tumor data analysis showed a statistically significant positive dose response relationship in incidence of the Sarcoma, endometrial stromal, Uterus

with cervix (P-value=0.0226<0.025).

Mouse Study: One hundred and twenty five hemizygous CByB6F1/Tg rasH2 hemizygous (transgenic) mice of each sex were randomly assigned to the treated, vehicle and positive control group in equal size of 25 mice per group. The dose levels for treated groups were 8, 20, and 50 mg/kg/day for males and females. The mice in the vehicle control group received the vehicle (Reverse Osmosis Deionized (RODI) Water). The study was designed to continue for up to 26 weeks for both sexes, however in accordance with study termination criteria, all surviving mice were sacrificed during Week 27. The mice in the positive control group received 75 mg/kg N-Nitrosomethylurea (NMU) in citrate-buffered saline (pH 4.5) once on Day 1 via intraperitoneal injection.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in either male or female mice. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in either male or female mice.

Tumor analysis:

1. For male mice, the pairwise comparisons between the vehicle control and the positive control showed a statistically significant increase in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Squamous cell carcinoma, stomach (P-value=0.0073<0.05)
2. For female mice, the pairwise comparisons between the vehicle control and the positive control showed statistically significant increases in incidence of Lymphoma, Hemolymphoreticula (P-value<0.001), Papilloma, Skin (P-value<0.001), Papilloma, Stomach (P-value<0.001) and Combined tumor of Papilloma and Carcinoma, Skin (P-value<0.001)

Zhuang Miao,

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NDA214094

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Archival NDA 214094

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5. Appendix

Table 7: Intercurrent Mortality Rate -Male Rats

Week	Vehicle Control 0 mg/kg/day (N=60)		3 mg/kg/day (N=60)		8 mg/kg/day (N=60)		20 mg/kg/day (N=60)	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	2	3.33	1	1.67	3	5.00	1	1.67
53 - 78	5	11.67	7	13.33	8	18.33	9	16.67
79 - 91	4	18.33	3	18.33	2	21.67	11	35.00
92 - 104	6	28.33	10	35.00	5	30.00	6	45.00
Ter. Sac.	43	71.67	39	65.00	42	70.00	33	55.00

Cum. %: Cumulative percentage except for Ter. Sac.

Table 8: Intercurrent Mortality Rate -Female Rats

Week	Vehicle Control 0 mg/kg/day (N=60)		3 mg/kg/day (N=60)		8 mg/kg/day (N=60)		20 mg/kg/day (N=60)	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	3	5.00	.	.	1	1.67	.	.
53 - 78	7	16.67	6	10.00	5	10.00	5	8.33
79 - 91	5	25.00	5	18.33	9	25.00	12	28.33
92 - 104	10	41.67	13	40.00	8	38.33	15	53.33
Ter. Sac.	35	58.33	36	60.00	37	61.67	28	46.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 9: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Male Rats

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.0897	0.5156	0.7847	0.0618
Homogeneity	Log-Rank	0.2221	0.5146	0.7843	0.0612

Table 10: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Female Rats

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.3000	0.7906	0.7391	0.2882

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Homogeneity	Log-Rank	0.4339	0.7894	0.7378	0.2850

Table 11: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
ADRENAL GLANDS	ADENOMA, CORTICAL, BENIGN	2/60 (52) 0.1751	3/60 (52) 0.5000	1/60 (50) 0.8713	4/60 (47) 0.2918
	CARCINOMA, CORTICAL, MALIGNANT	1/60 (53) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	GANGLIONEUROMA, BENIGN	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	PHEOCHROMOCYTOMA, BENIGN	1/60 (52) 0.5976	3/60 (52) 0.3088	4/60 (50) 0.1692	1/60 (47) 0.7267
	PHEOCHROMOCYTOMA, MALIGNANT	1/60 (52) 0.7335	0/60 (52) 1.0000	1/60 (50) 0.7426	0/60 (47) 1.0000
BRAIN	GLIOMA, MALIGNANT	1/60 (52) 0.7335	0/60 (52) 1.0000	1/60 (50) 0.7426	0/60 (47) 1.0000
	GRANULAR CELL TUMOR, BENIGN	1/60 (52) 0.4139	0/60 (52) 1.0000	0/60 (50) 1.0000	1/60 (47) 0.7267
	GRANULAR CELL TUMOR, MALIGNANT	0/60 (52) 0.1738	0/60 (52) NC	1/60 (50) 0.4902	1/60 (48) 0.4800
	OSTEOMA, BENIGN	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
COAGULATING GLANDS	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
EPIDIDYMIDES	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7995	2/60 (52) 0.2476	0/60 (50) NC	0/60 (47) NC
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
HARDERIAN GLANDS	ADENOMA, BENIGN	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
	FIBROSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
HEART	SCHWANNOMA, ENDOCARDIAL, MALIGNANT	0/60 (52) 0.2719	1/60 (52) 0.5000	1/60 (50) 0.4902	1/60 (47) 0.4747

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
KIDNEYS	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
LARGE INTESTINE, C	LEIOMYOMA, BENIGN	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
LARYNX	FIBROSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
LIVER	ADENOMA, HEPATOCELLULAR, BENIGN	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
LUNG	ADENOCARCINOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	CARCINOMA, CORTICAL, MALIGNANT	1/60 (53) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
LYMPH NODE, HEPATI	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
LYMPH NODE, MESENT	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
Lymph Node, Mesenteric	HEMANGIOSARCOMA, MALIGNANT	1/60 (52) 0.0001	1/60 (52) 0.7524	2/60 (51) 0.4851	9/60 (47) 0.0049
MAMMARY GLAND	FIBROADENOMA, BENIGN	0/60 (52) 0.4748	0/60 (52) NC	2/60 (50) 0.2378	0/60 (47) NC
MEDIASTINUM/PLE URA	SCHWANNOMA, MALIGNANT	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
MESENTERY/PERIT ONE	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7995	2/60 (52) 0.2476	0/60 (50) NC	0/60 (47) NC
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	SCHWANNOMA, MALIGNANT	1/60 (52) 0.9317	2/60 (52) 0.5000	0/60 (50) 1.0000	0/60 (47) 1.0000
MULTICENTRIC NEOPL	HEMANGIOMA, BENIGN	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	HEMANGIOSARCOMA, MALIGNANT	2/60 (52) 0.0018	2/60 (52) 0.6912	4/60 (51) 0.3298	9/60 (47) 0.0166

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
	LEUKEMIA, MALIGNANT	0/60 (52) 0.4748	0/60 (52) NC	2/60 (50) 0.2378	0/60 (47) NC
	LYMPHOMA, MALIGNANT	1/60 (53) 0.8899	3/60 (54) 0.3160	1/60 (51) 0.7427	0/60 (47) 1.0000
	SARCOMA, HISTIOCYTIC, MALIGNANT	0/60 (52) 0.0545	0/60 (52) NC	1/60 (50) 0.4902	2/60 (48) 0.2279
NOSE	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
PANCREAS	ADENOCARCINOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	ADENOMA, ACINAR CELL, BENIGN	1/60 (52) 0.4139	0/60 (52) 1.0000	0/60 (50) 1.0000	1/60 (47) 0.7267
	ADENOMA, ACINAR-ISLET CELL, BENIGN	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	ADENOMA, ISLET CELL, BENIGN	2/60 (52) 0.6997	2/60 (52) 0.6912	2/60 (50) 0.6763	1/60 (47) 0.8591
	CARCINOMA, ACINAR-ISLET CELL, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	CARCINOMA, ISLET CELL, MALIGNANT	0/60 (52) 0.4826	0/60 (52) NC	1/60 (50) 0.4902	0/60 (47) NC
	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
PARATHYROID GLANDS	ADENOMA, BENIGN	1/60 (52) 0.8613	2/60 (52) 0.5000	1/60 (50) 0.7426	0/60 (47) 1.0000
PITUITARY GLAND	ADENOMA, PARS DISTALIS, BENIGN	17/60 (56) 0.6571	23/60 (57) 0.1804	19/60 (52) 0.3167	16/60 (52) 0.5641
	ADENOMA, PARS INTERMEDIA, BENIGN	1/60 (52) 0.3635	0/60 (52) 1.0000	1/60 (50) 0.7426	1/60 (47) 0.7267
	GLIOMA, MALIGNANT	0/60 (52) 0.4826	0/60 (52) NC	1/60 (50) 0.4902	0/60 (47) NC
	PITUICYTOMA, PARS NERVOSA, BENIGN	1/60 (52) 0.9340	1/60 (52) 0.7524	0/60 (50) 1.0000	0/60 (47) 1.0000
PREPUTIAL GLANDS	CARCINOMA, SQUAMOUS CELL, MALIGNANT	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	PAPILLOMA, SQUAMOUS CELL, BENIGN	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
PROSTATE GLAND	ADENOCARCINOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	ADENOMA, BENIGN	1/60 (52) 0.7335	0/60 (52) 1.0000	1/60 (50) 0.7426	0/60 (47) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
Pancreas	C_Acinar cell Adenomas+mixed acinar islet cell adenomas	2/60 (52) 0.5670	0/60 (52) 1.0000	0/60 (50) 1.0000	1/60 (47) 0.8591
	C_Islet cell Adenomas+mixed acinar islet cell adenomas	2/60 (52) 0.6997	2/60 (52) 0.6912	2/60 (50) 0.6763	1/60 (47) 0.8591
SALIVARY GLAND, MA	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
SALIVARY GLAND, PA	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.0538	0/60 (52) NC	0/60 (50) NC	2/60 (47) 0.2228
SEMINAL VESICLES	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
SKELETAL MUSCLE	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.4851	0/60 (52) NC	1/60 (51) 0.4951	0/60 (47) NC
SKELETAL MUSCLE, B	GRANULAR CELL TUMOR, BENIGN	1/60 (52) 0.7335	0/60 (52) 1.0000	1/60 (50) 0.7426	0/60 (47) 1.0000
SKIN	ADENOMA, BASAL CELL, BENIGN	0/60 (52) 0.2923	1/60 (52) 0.5000	0/60 (50) NC	1/60 (47) 0.4747
	CARCINOMA, BASAL CELL, MALIGNANT	0/60 (52) 0.2719	1/60 (52) 0.5000	1/60 (50) 0.4902	1/60 (47) 0.4747
	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
	FIBROMA, BENIGN	1/60 (52) 0.5606	2/60 (52) 0.5000	2/60 (50) 0.4851	1/60 (47) 0.7267
	FIBROSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	KERATOACANTHOMA, BENIGN	1/60 (52) 0.0704	2/60 (52) 0.5000	2/60 (50) 0.4851	4/60 (47) 0.1511
	LIPOMA, BENIGN	1/60 (52) 0.4139	0/60 (52) 1.0000	0/60 (50) 1.0000	1/60 (47) 0.7267
	PAPILLOMA, SQUAMOUS CELL, BENIGN	1/60 (52) 0.8137	1/60 (52) 0.7524	1/60 (50) 0.7426	0/60 (47) 1.0000
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.0365	1/60 (52) 0.5000	1/60 (51) 0.4951	3/60 (48) 0.1070
	SCHWANNOMA, BENIGN	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
SMALL INTESTINE, J	ADENOCARCINOMA, MALIGNANT	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	LEIOMYOSARCOMA, MALIGNANT	0/60 (52) 0.4826	0/60 (52) NC	1/60 (50) 0.4902	0/60 (47) NC
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
SPLEEN	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
STOMACH, GLANDULAR	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
STOMACH, NONGLANDU	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2923	1/60 (52) 0.5000	0/60 (50) NC	1/60 (47) 0.4747
Skin	C_Basal cell Adenoma+Carcinoma	0/60 (52) 0.1733	2/60 (52) 0.2476	1/60 (50) 0.4902	2/60 (47) 0.2228
	C_Carcinoma squamous cell+keratoacanthoma+papilloma squamous c	2/60 (52) 0.0943	3/60 (52) 0.5000	3/60 (50) 0.4812	5/60 (47) 0.1784
TESTES	ADENOMA, LEYDIG CELL, BENIGN	4/60 (52) 0.5557	3/60 (52) 0.7816	4/60 (50) 0.6201	3/60 (48) 0.7475
	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
THYMUS	SCHWANNOMA, MALIGNANT	1/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
	THYMOMA, BENIGN	2/60 (52) 0.3736	1/60 (52) 0.8786	1/60 (50) 0.8713	2/60 (47) 0.6522
THYROID GLAND	ADENOMA, C-CELL, BENIGN	7/60 (52) 0.7087	11/60 (52) 0.2188	10/60 (50) 0.2679	6/60 (48) 0.6686
	ADENOMA, FOLLICULAR CELL, BENIGN	2/60 (52) 0.3248	3/60 (52) 0.5000	4/60 (51) 0.3298	3/60 (47) 0.4517
	CARCINOMA, C-CELL, MALIGNANT	0/60 (52) 0.4102	2/60 (52) 0.2476	0/60 (50) NC	1/60 (47) 0.4747
	CARCINOMA, FOLLICULAR CELL, MALIGNANT	2/60 (52) 1.0000	0/60 (52) 1.0000	0/60 (50) 1.0000	0/60 (47) 1.0000
TONGUE	FIBROSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
TOOTH/TEETH	ODONTOMA, BENIGN	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
TRACHEA	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
Thyroid Gland	C_Adenoma+carcinoma follicular cell	4/60 (52) 0.5439	3/60 (52) 0.7816	4/60 (51) 0.6313	3/60 (47) 0.7380
URETERS	OSTEOSARCOMA, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
URINARY BLADDER	MESOTHELIOMA, MALIGNANT	0/60 (52) 0.7413	1/60 (52) 0.5000	0/60 (50) NC	0/60 (47) NC
ZYMBAL'S GLAND	CARCINOMA, ZYMBALS GLAND, MALIGNANT	0/60 (52) 0.6030	1/60 (53) 0.5048	1/60 (50) 0.4902	0/60 (47) NC
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (52) 0.2376	0/60 (52) NC	0/60 (50) NC	1/60 (48) 0.4800
	SCHWANNOMA, MALIGNANT	0/60 (52) 0.2338	0/60 (52) NC	0/60 (50) NC	1/60 (47) 0.4747
Whold Body	C_Hemangiosarcoma+Hemangioma	3/60 (52) 0.0044	2/60 (52) 0.8187	4/60 (51) 0.4891	9/60 (47) 0.0410

Table 12: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups- Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
ADRENAL GLANDS	ADENOMA, CORTICAL, BENIGN	2/60 (49) 0.3704	3/60 (51) 0.5191	4/60 (50) 0.3485	3/60 (49) 0.5000
	CARCINOMA, CORTICAL, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	MYELOLIPOMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	PHEOCHROMOCYTOMA, BENIGN	0/60 (49) 0.1841	0/60 (51) NC	1/60 (50) 0.5051	1/60 (49) 0.5000
BILE DUCT, EXTRAHE	CHOLANGIOCARCINOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
BRAIN	CARCINOMA, PARS DISTALIS, MALIGNANT	1/60 (49) 0.3864	0/60 (51) 1.0000	1/60 (50) 0.7576	1/60 (49) 0.7526
	EPENDYMOMA, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
	GLIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	GRANULAR CELL TUMOR, BENIGN	1/60 (49) 0.8296	1/60 (51) 0.7624	1/60 (50) 0.7576	0/60 (49) 1.0000
	GRANULAR CELL TUMOR, MALIGNANT	0/60 (49) 0.1061	1/60 (51) 0.5100	0/60 (50) NC	2/60 (49) 0.2474
CALVARIA	CARCINOMA, PARS DISTALIS, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
CLITORAL GLANDS	ADENOMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
DIAPHRAGM	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
EARS	SARCOMA (PRIMARY SITE UNKNOWN), MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
EYES	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
HARDERIAN GLANDS	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
HEART	SCHWANNOMA, ENDOCARDIAL, MALIGNANT	1/60 (49) 0.3864	0/60 (51) 1.0000	1/60 (50) 0.7576	1/60 (49) 0.7526
KIDNEYS	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
	MESOTHELIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
LARYNX	SARCOMA (PRIMARY SITE UNKNOWN), MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
LIVER	ADENOMA, HEPATOCELLULAR, BENIGN	0/60 (49) 0.7538	1/60 (51) 0.5100	0/60 (50) NC	0/60 (49) NC
	CHOLANGIOCARCINOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	CHOLANGIOMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
LUNG	ADENOCARCINOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	CARCINOMA, ISLET CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	CHOLANGIOCARCINOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
LYMPH NODE, MESENT	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
MAMMARY GLAND	ADENOCARCINOMA, MALIGNANT	4/60 (50) 0.9429	6/60 (52) 0.3959	5/60 (51) 0.5127	1/60 (49) 0.9704
	ADENOMA, BENIGN	2/60 (49) 0.5890	0/60 (51) 1.0000	0/60 (50) 1.0000	1/60 (49) 0.8789
	FIBROADENOMA, BENIGN	13/60 (51) 0.9984	17/60 (53) 0.3003	10/60 (51) 0.8283	4/60 (50) 0.9963
MESENTERY/PERIT ONE	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	MESOTHELIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SCHWANNOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
MULTICENTRIC NEOPL	HEMANGIOSARCOMA, MALIGNANT	4/60 (49) 0.0113	2/60 (51) 0.9067	1/60 (50) 0.9733	9/60 (50) 0.1245

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
	LYMPHOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
Mammary Gland	C_Adenocarcinoma+Adenoma	6/60 (50) 0.9399	6/60 (52) 0.6477	5/60 (51) 0.7490	2/60 (49) 0.9683
NERVE, TRIGEMINAL	CARCINOMA, PARS DISTALIS, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
NOSE	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
ORAL CAVITY	CARCINOMA, SQUAMOUS CELL, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
OVARIES	GRANULOSA CELL TUMOR, MALIGNANT	0/60 (49) 0.5000	0/60 (51) NC	1/60 (51) 0.5100	0/60 (49) NC
	LUTEOMA, BENIGN	0/60 (49) 0.7538	1/60 (51) 0.5100	0/60 (50) NC	0/60 (49) NC
	MESOTHELIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SCHWANNOMA, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
	SERTOLI CELL TUMOR, BENIGN	1/60 (49) 0.7487	0/60 (51) 1.0000	1/60 (50) 0.7576	0/60 (49) 1.0000
	SEX-CORD/STROMAL TUMOR, BENIGN	4/60 (49) 0.6471	1/60 (51) 0.9747	4/60 (50) 0.6539	2/60 (49) 0.8979
	THECOMA, BENIGN	0/60 (49) 0.7538	1/60 (51) 0.5100	0/60 (50) NC	0/60 (49) NC
PANCREAS	ADENOMA, ISLET CELL, BENIGN	0/60 (49) 0.4949	0/60 (51) NC	2/60 (50) 0.2525	0/60 (49) NC
	CARCINOMA, ISLET CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	MESOTHELIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SCHWANNOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
PITUITARY GLAND	ADENOMA, PARS DISTALIS, BENIGN	34/60 (53) 0.9994	38/60 (58) 0.5189	30/60 (55) 0.8872	19/60 (51) 0.9984
	CARCINOMA, PARS DISTALIS, MALIGNANT	1/60 (49) 0.1624	0/60 (51) 1.0000	1/60 (50) 0.7576	2/60 (49) 0.5000
SALIVARY GLAND, PA	ADENOCARCINOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	ADENOMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
SKIN	CARCINOMA, BASAL CELL, MALIGNANT	0/60 (49) 0.2500	0/60 (51) NC	0/60 (50) NC	1/60 (50) 0.5051
	CARCINOMA, SEBACEOUS CELL, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	FIBROMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	KERATOACANTHOMA, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	LIPOSARCOMA, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
	NEURAL CREST TUMOR, BENIGN	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	1/60 (49) 0.9403	1/60 (51) 0.7624	0/60 (50) 1.0000	0/60 (49) 1.0000
	SCHWANNOMA, MALIGNANT	1/60 (49) 0.7487	0/60 (51) 1.0000	1/60 (50) 0.7576	0/60 (49) 1.0000
SMALL INTESTINE, D	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
SMALL INTESTINE, I	LEIOMYOSARCOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
SMALL INTESTINE, J	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
SPINAL CORD	CARCINOMA, PARS DISTALIS, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
SPLEEN	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
STOMACH, GLANDULAR	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	LEIOMYOSARCOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	MESOTHELIOMA, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	SCHWANNOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
Skin	C_Carcinoma+Keratoacanthoma	1/60 (49) 0.1548	0/60 (51) 1.0000	0/60 (50) 1.0000	2/60 (50) 0.5077
THYMUS	SCHWANNOMA, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
	THYMOMA, BENIGN	4/60 (49) 0.5796	3/60 (51) 0.7982	6/60 (50) 0.3833	3/60 (49) 0.7822

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	3 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	8 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	20 mg/kg/day High (N=60) P-value - Vehicle vs. High
THYROID GLAND	ADENOMA, C-CELL, BENIGN	5/60 (49) 0.0902	5/60 (52) 0.6670	7/60 (51) 0.4087	9/60 (50) 0.2053
	ADENOMA, FOLLICULAR CELL, BENIGN	2/60 (49) 0.8270	0/60 (51) 1.0000	2/60 (50) 0.6990	0/60 (49) 1.0000
	CARCINOMA, C-CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	CARCINOMA, FOLLICULAR CELL, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
	SARCOMA (PRIMARY SITE UNKNOWN), MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
Thyroid	C_C cell Adenoma+Carcinoma	5/60 (49) 0.0507	5/60 (52) 0.6670	7/60 (51) 0.4087	10/60 (50) 0.1403
	C_Follicular cell Adenoma+Carcinoma	3/60 (49) 0.9159	0/60 (51) 1.0000	2/60 (50) 0.8252	0/60 (49) 1.0000
URINARY BLADDER	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
UTERUS WITH CERVIX	ADENOCARCINOMA, MALIGNANT	3/60 (49) 0.3258	1/60 (51) 0.9460	1/60 (50) 0.9437	3/60 (49) 0.6612
	CARCINOMA, SQUAMOUS CELL, MALIGNANT	1/60 (49) 0.4950	1/60 (51) 0.7624	2/60 (50) 0.5077	1/60 (49) 0.7526
	LEIOMYOMA, BENIGN	1/60 (49) 0.7487	0/60 (51) 1.0000	1/60 (50) 0.7576	0/60 (49) 1.0000
	LEIOMYOSARCOMA, MALIGNANT	0/60 (49) 0.2936	1/60 (51) 0.5100	1/60 (50) 0.5051	1/60 (49) 0.5000
	POLYP, ENDOMETRIAL STROMAL, BENIGN	8/60 (51) 0.5441	12/60 (53) 0.2582	9/60 (52) 0.5176	9/60 (51) 0.5000
	POLYP, GLANDULAR, BENIGN	0/60 (49) 0.6244	1/60 (51) 0.5100	1/60 (50) 0.5051	0/60 (49) NC
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.0226	0/60 (51) NC	2/60 (51) 0.2576	3/60 (49) 0.1211
	SARCOMA, UNDIFFERENTIATED, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
	SCHWANNOMA, MALIGNANT	1/60 (49) 1.0000	0/60 (51) 1.0000	0/60 (50) 1.0000	0/60 (49) 1.0000
VAGINA	CARCINOMA, SQUAMOUS CELL, MALIGNANT	0/60 (49) 0.4975	0/60 (51) NC	1/60 (50) 0.5051	0/60 (49) NC
	SARCOMA, ENDOMETRIAL STROMAL, MALIGNANT	0/60 (49) 0.2462	0/60 (51) NC	0/60 (50) NC	1/60 (49) 0.5000
ZYMBAL'S GLAND	CARCINOMA, ZYMBALS GLAND, MALIGNANT	1/60 (50) 0.1534	0/60 (51) 1.0000	0/60 (50) 1.0000	2/60 (50) 0.5000
Whold Body	C_Hemangiosarcoma+Hemangioma	4/60 (49) 0.0113	2/60 (51) 0.9067	1/60 (50) 0.9733	9/60 (50) 0.1245

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Table 13: Intercurrent Mortality Rate -Male Mice

Week	Vehicle 0 mg/kg/day (N=25)		8 mg/kg/day (N=25)		20 mg/kg/day (N=25)		50 mg/kg/day (N=25)		Positive (N=25)	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	.	.	.	.	.	.	.	.
14 - 26	1	4.00	1	4.00	1	4.00	3	12.00	17	68.00
Ter. Sac.	24	96.00	24	96.00	24	96.00	22	88.00	8	32.00

Cum. %: Cumulative percentage except for Ter. Sac.

Table 14: Intercurrent Mortality Rate -Female Mice

Week	Vehicle 0 mg/kg/day (N=25)		8 mg/kg/day (N=25)		20 mg/kg/day (N=25)		50 mg/kg/day (N=25)		Positive (N=25)	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	.	.	1	4.00	.	.	3	12.00
14 - 26	.	.	1	4.00	2	12.00	2	8.00	22	100.00
Ter. Sac.	25	100.00	24	96.00	22	88.00	23	92.00	.	.

Cum. %: Cumulative percentage except for Ter. Sac.

Table 15: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control, Positive Control and Vehicle Control -Male Mice

Test	Statistic	P_Value				
		Vehicle vs Treated Groups Dose Response	Vehicle vs. Low	Vehicle vs. Med	Vehicle vs. High	Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.2462	0.9885	0.9885	0.2874	<.0001
Homogeneity	Log-Rank	0.5283	0.9885	0.9885	0.2974	<.0001

Table 16: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control, Positive Control and Vehicle Control --Female Mice

Test	Statistic	P_Value				
		Vehicle vs Treated Groups Dose Response	Vehicle vs. Low	Vehicle vs. Med	Vehicle vs. High	Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.1289	0.2390	0.0395	0.0935	<.0001

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Homogeneity	Log-Rank	0.3289	0.3173	0.0770	0.1531	<.0001

Table 17: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups- Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	8 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	20 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	50 mg/kg/day High (N=25) P-value - Vehicle vs. High
gland, harderian	adenocarcinoma	0/25 (25) 0.4949	0/25 (25) NC	1/25 (25) 0.5000	0/25 (24) NC
	adenoma	1/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000
Gland Harderian	C_adenocarcinoma+adenoma	1/25 (25) 0.7475	0/25 (25) 1.0000	1/25 (25) 0.7551	0/25 (24) 1.0000
hemolymphoreticular tissue	lymphoma, malignant	0/25 (25) 0.7475	1/25 (25) 0.5000	0/25 (25) NC	0/25 (24) NC
lung	bronchioloalveolar adenoma	1/25 (25) 0.6818	1/25 (25) 0.7551	2/25 (25) 0.5000	0/19 (18) 1.0000
skin	hemangiosarcoma	0/25 (25) 0.4949	0/25 (25) NC	1/25 (25) 0.5000	0/25 (24) NC
	papilloma	0/25 (25) 0.2424	0/25 (25) NC	0/25 (25) NC	1/25 (24) 0.4898
spleen	hemangiosarcoma	0/25 (25) 0.2446	2/25 (25) 0.2449	0/25 (25) NC	2/25 (24) 0.2347
stomach	papilloma	1/25 (25) 0.6186	0/25 (25) 1.0000	0/25 (25) 1.0000	1/25 (24) 0.7449
	squamous cell carcinoma	0/25 (25) 0.7475	1/25 (25) 0.5000	0/25 (25) NC	0/25 (24) NC
testis	hemangiosarcoma	2/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000
Whole Body	C_Hemangiosarcoma	2/25 (25) 0.4947	2/25 (25) 0.6954	1/25 (25) 0.8827	2/25 (24) 0.6798

Table 18: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
ESOPHAGUS	PAPILLOMA	0/25 (25)	2/24 (16) 0.1463
GLAND, HARDERIAN	ADENOCARCINOMA	0/25 (25)	1/25 (15) 0.3750
	ADENOMA	1/25 (25)	2/25 (16) 0.3340
Gland Harderian	C_adenocarcinoma+adenoma	1/25 (25)	3/25 (16) 0.1562
HEMOLYMPHORETICULA	LYMPHOMA, MALIGNANT	0/25 (25)	14/25 (22) <0.001
KIDNEY	ADENOMA	0/25 (25)	1/25 (15) 0.3750
LUNG	BRONCHIOLOALVEOLAR ADENOMA	1/25 (25)	2/25 (16) 0.3340
SKIN	KERATOACANTHOMA	0/25 (25)	1/25 (16) 0.3902
	PAPILLOMA	0/25 (25)	12/25 (18) <0.001
SMALL INTESTINE, D	ADENOMA	0/25 (25)	1/24 (15) 0.3750
SMALL INTESTINE, J	ADENOCARCINOMA	0/25 (25)	1/20 (12) 0.3243
SPLEEN	HEMANGIOSARCOMA	0/25 (25)	3/25 (16) 0.0525
STOMACH	ADENOCARCINOMA	0/25 (25)	1/25 (15) 0.3750
	PAPILLOMA	1/25 (25)	16/25 (22) <0.001
	SQUAMOUS CELL CARCINOMA	0/25 (25)	5/25 (17) 0.0073
TESTIS	HEMANGIOSARCOMA	2/25 (25)	0/25 (15) 1.0000
TRACHEA	PAPILLOMA	0/25 (25)	1/23 (14) 0.3590
URINARY BLADDER	PAPILLOMA	0/25 (25)	2/25 (16) 0.1463
Whole Body	C_Hemangiosarcoma	2/25 (25)	3/25 (16) 0.2907

Table 19: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups- Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	8 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	20 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	50 mg/kg/day High (N=25) P-value - Vehicle vs. High
BODY CAVITY, NASAL	SQUAMOUS CELL CARCINOMA	1/25 (25) 0.4356	0/25 (24) 1.0000	0/25 (24) 1.0000	1/25 (24) 0.7449
GLAND, HARDERIAN	ADENOMA	2/25 (25) 0.9799	3/25 (24) 0.4800	0/25 (24) 1.0000	0/25 (24) 1.0000
LUNG	BRONCHIOLOALVEOLAR ADENOMA	3/25 (25) 0.4654	0/25 (24) 1.0000	1/25 (24) 0.9403	2/25 (25) 0.8257
	BRONCHIOLOALVEOLAR CARCINOMA	0/25 (25) 0.6160	1/25 (25) 0.5000	1/25 (24) 0.4898	0/25 (24) NC
Lung	C_bronchioloalveolar adenoma+Carcinoma	3/25 (25) 0.5542	1/25 (25) 0.9451	2/25 (24) 0.8129	2/25 (25) 0.8257
SKIN	HEMANGIOSARCOMA	0/25 (25) 0.2551	0/25 (24) NC	0/25 (24) NC	1/25 (25) 0.5000
	PAPILLOMA	0/25 (25) 0.7423	1/25 (24) 0.4898	0/25 (24) NC	0/25 (24) NC
	SQUAMOUS CELL CARCINOMA	1/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000
SPLEEN	HEMANGIOSARCOMA	0/25 (25) 0.2645	3/25 (24) 0.1099	0/25 (24) NC	2/25 (24) 0.2347
Skin	C_Papilloma+Carcinoma	1/25 (25) 0.9356	1/25 (24) 0.7449	0/25 (24) 1.0000	0/25 (24) 1.0000
Whole Body	C_Hemangiosarcoma	0/25 (25) 0.1228	3/25 (24) 0.1099	0/25 (24) NC	3/25 (25) 0.1173

Table 20: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control -Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=25) P-value - Vehicle vs. Positive
BODY CAVITY, NASAL	SQUAMOUS CELL CARCINOMA	1/25 (25)	0/25 (9) 1.0000
GLAND, HARDERIAN	ADENOMA	2/25 (25)	1/24 (8) 0.5784
GLAND, MAMMARY	ADENOCARCINOMA	0/25 (25)	1/25 (9) 0.2647
HEMOLYMPHORETICULA	LYMPHOMA, MALIGNANT	0/25 (25)	18/25 (20) <0.001
KIDNEY	MESENCHYMAL TUMOR, MALIGNANT	0/25 (25)	1/25 (9) 0.2647
LUNG	BRONCHIOLOALVEOLAR ADENOMA	3/25 (25) 0.0020	5/25 (12) 0.0549
Lung	C_bronchioloalveolar adenoma+Carcinoma	3/25 (25)	5/25 (12) 0.0549
SKIN	BASAL CELL TUMOR, MALIGNANT	0/25 (25)	2/25 (10) 0.0756
	PAPILLOMA	0/25 (25)	10/25 (14) <0.001
	SQUAMOUS CELL CARCINOMA	1/25 (25)	2/25 (10) 0.1902
SPLEEN	HEMANGIOSARCOMA	0/25 (25)	1/25 (9) 0.2647
STOMACH	PAPILLOMA	0/25 (25)	16/25 (19) <0.001
	SQUAMOUS CELL CARCINOMA	0/25 (25)	2/25 (10) 0.0756
Skin	C_Papilloma+Carcinoma	1/25 (25)	12/25 (16) <0.001
UTERUS	ENDOMETRIAL STROMAL POLYP	0/25 (25)	2/25 (10) 0.0756
VAGINA	PAPILLOMA	0/25 (25)	1/25 (9) 0.2647
	SQUAMOUS CELL CARCINOMA	0/25 (25)	1/25 (10) 0.2857
Whole Body	C_Hemangiosarcoma	0/25 (25)	1/25 (9) 0.2647

Figure 1: Kaplan-Meier Survival Functions for Male Rats

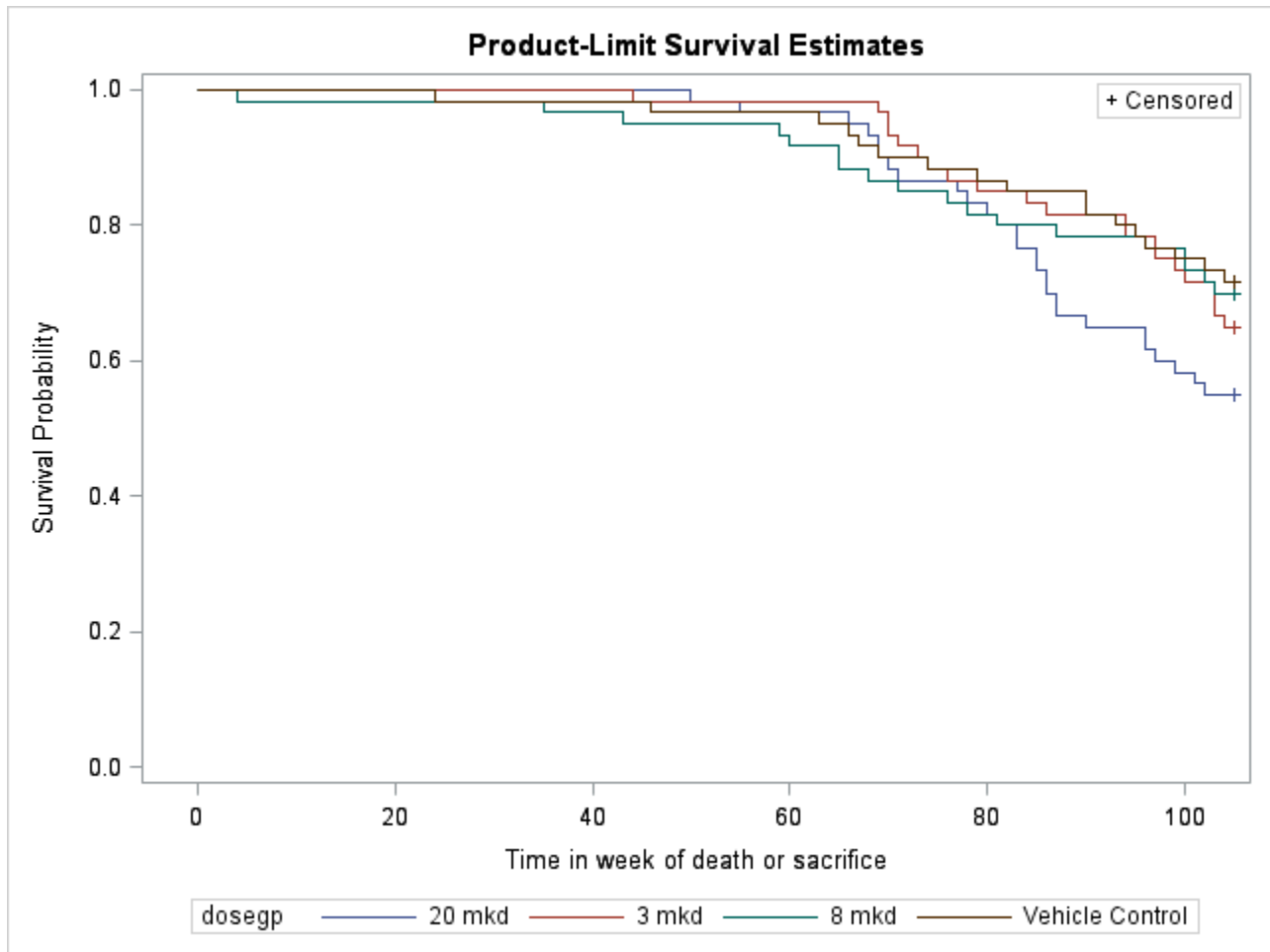


Figure 2: Kaplan-Meier Survival Functions for Female Rats

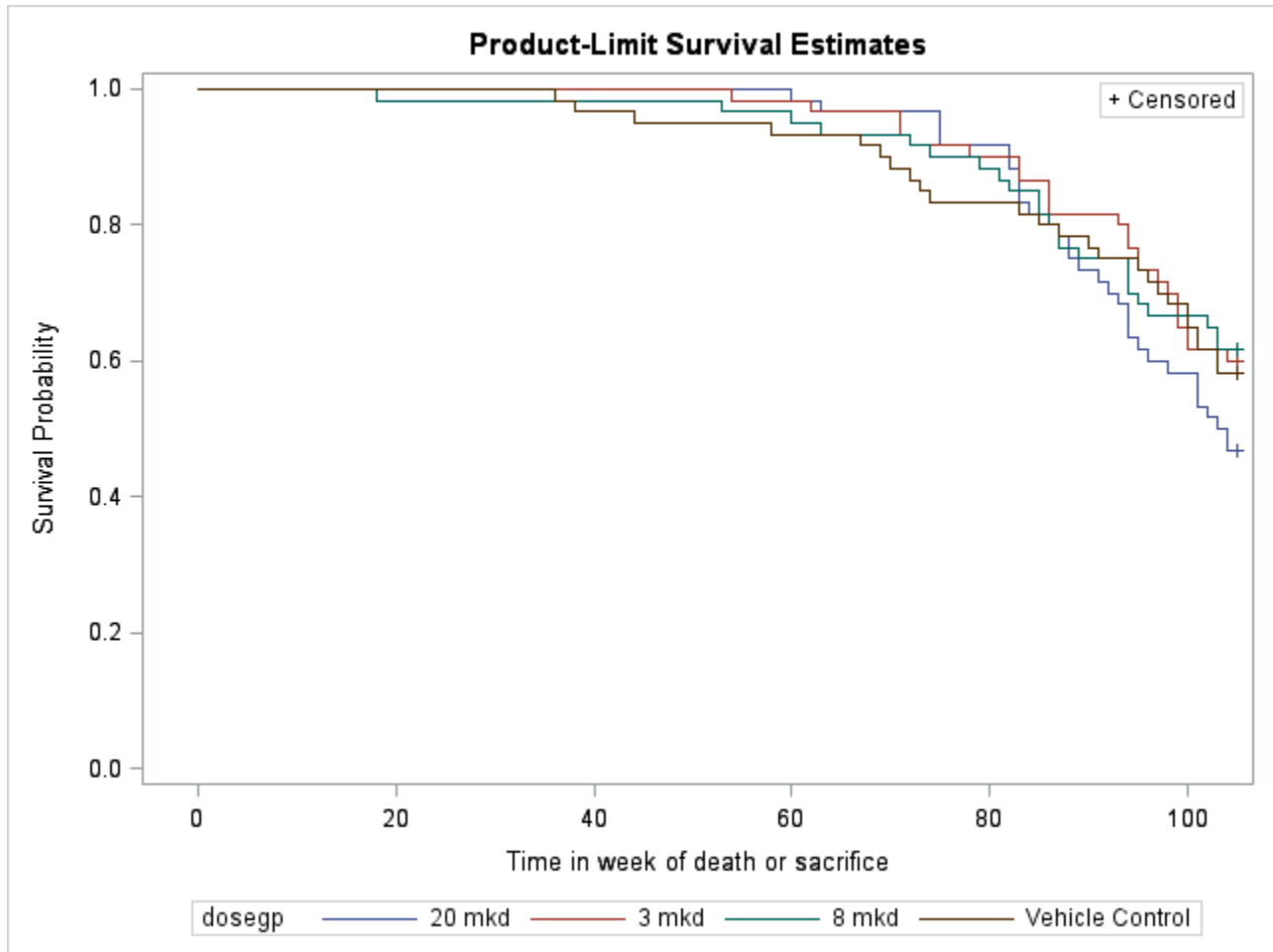


Figure 3: Kaplan-Meier Survival Functions for Male Mice

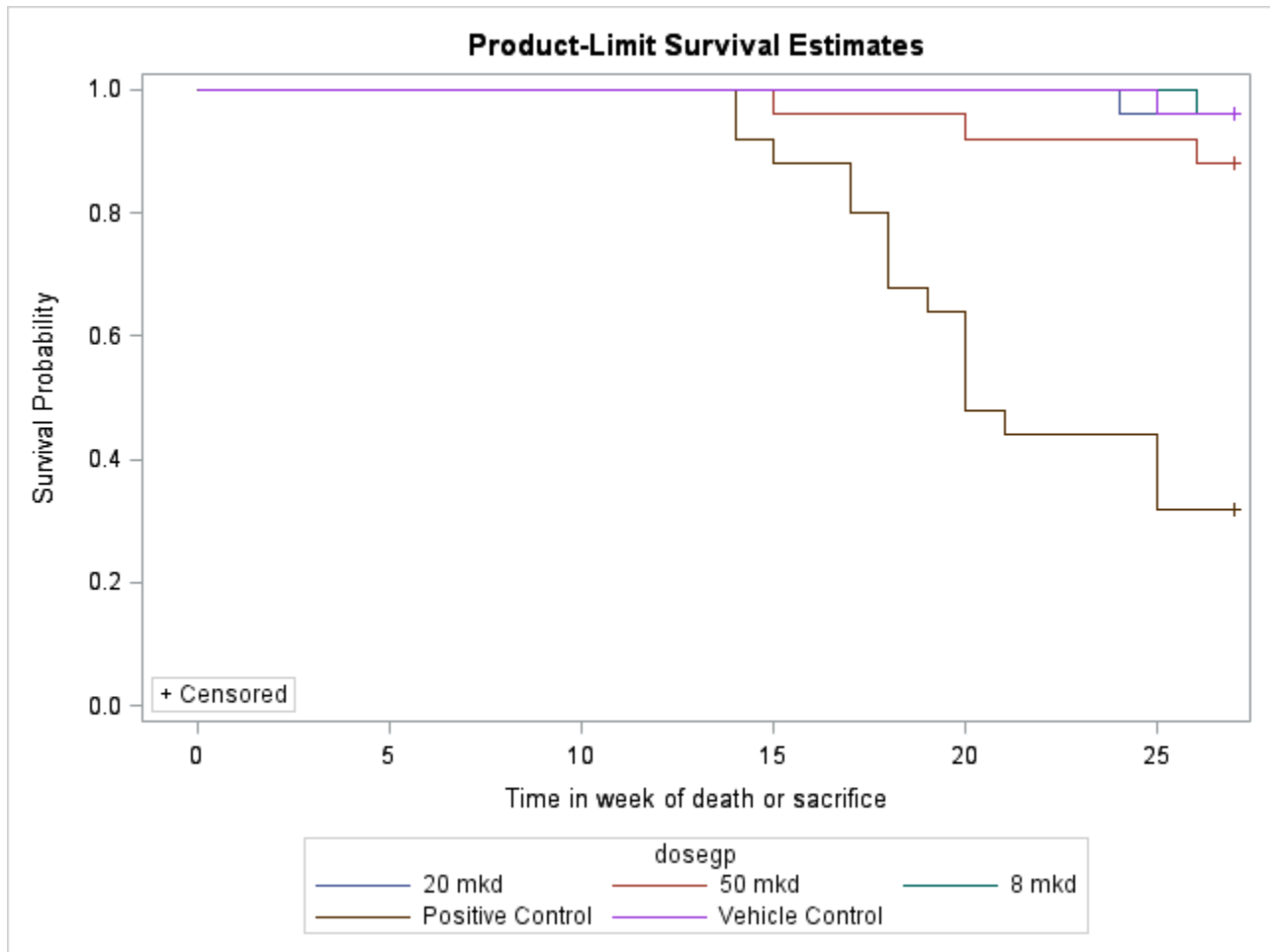
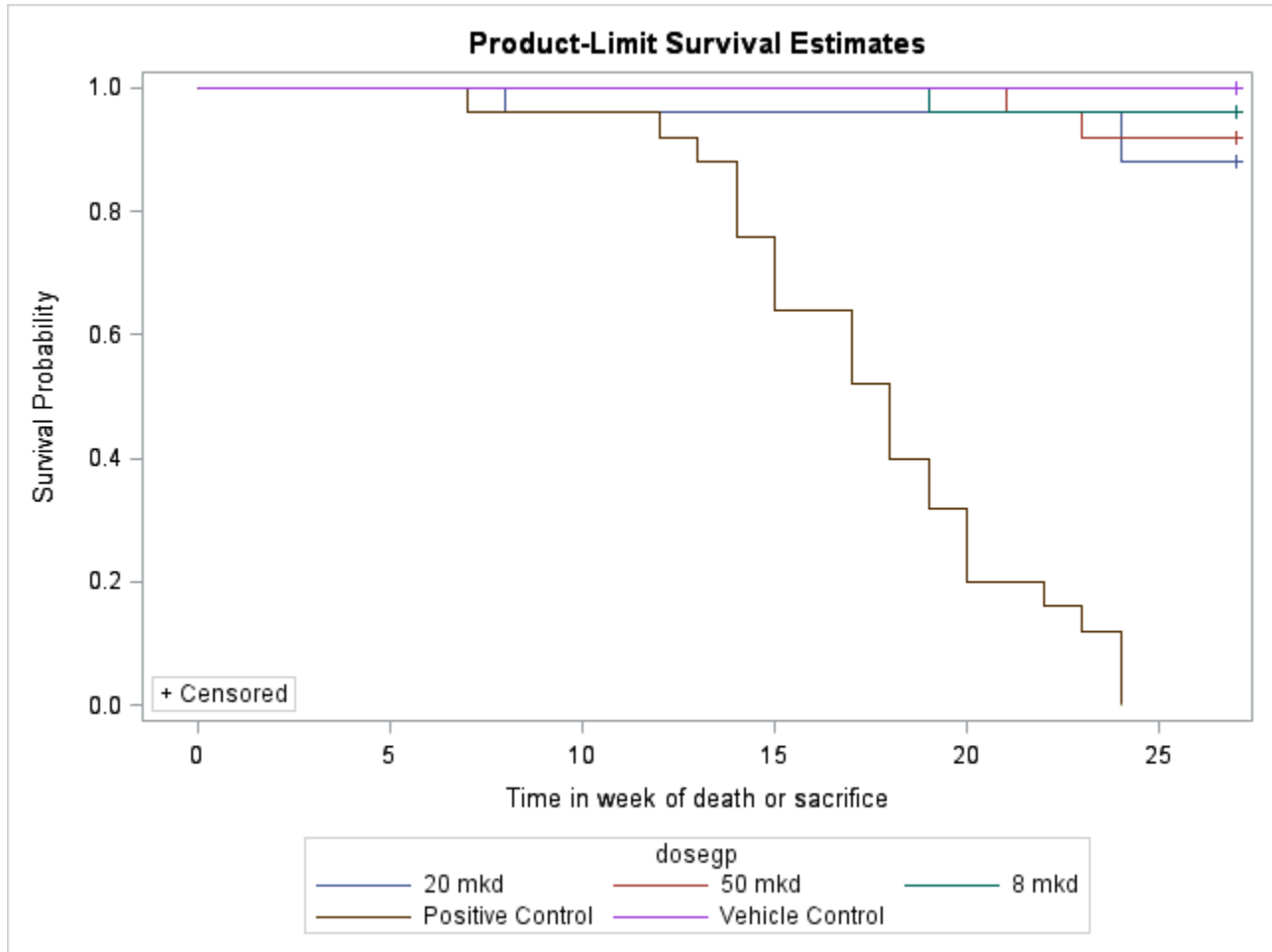


Figure 4: Kaplan-Meier Survival Functions for Female Mice



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Concur with review.

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 214094
Supporting document/s: 0001
Applicant's letter date: November 27, 2019
CDER stamp date: December 3, 2019
Product: ORLADEYO (berotralstat, BCX7353)
Indication: Hereditary Angioedema (HAE)
Therapeutic area: Critical care medicine
Applicant: BioCryst Pharmaceuticals
Review Division: Division of Pulmonary, Allergy and Critical Care
Reviewer: Luqi Pei, PhD
Supervisor/Team Leader: Carol Galvis, PhD
Division Director: Sally Seymour, MD
Project Manager: Nina Ton, PharmD

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This nonclinical review evaluates the carcinogenicity potential of berotralstat (BCX7353) in animal studies. Berotralstat is a kallikrein inhibitor being developed as a therapy for the prevention of hereditary angioedema (HAE) attacks in adult and adolescent patients. The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in rats and a 26-week alternative animal model in mice (Reports 806-161 and 20088905, respectively). Results showed that berotralstat was carcinogenic in rats, but not in mice.

1.2 Brief Discussion of Nonclinical Findings

The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in Wistar rats and a 26-week study in B6F1 Tg.rasH2 mice. Rats (60/sex/dose) were dosed by oral gavage with 0 (vehicle: deionized water), 3, 8, and 20-mg/kg berotralstat once daily for 104 weeks. Mice (25/sex/dose) were dosed by oral gavage 0 (vehicle: deionized water), 8, 20, and 50-mg/kg berotralstat once daily for 26 weeks. The male rats showed evidence of tumorigenicity as discussed below. No evidence of tumorigenicity was found in mice or female rats.

The male rats showed statistically significant, dose-related increases in the incidence of hemangiosarcomas (HS) in the mesenteric lymph nodes (Table 1). In pairwise comparisons between the high dose (HD) and controls (C), only the increase in the HS incidence in the mesenteric lymph nodes reached statistical significance ($p < 0.01$); test for HS in the whole body reached a marginal significance ($p=0.017$); test for the HS and hemangioma in combination in the whole body did not reach statistical significance ($p=0.041$). The trend analyses for all the above tumor findings were significant ($p < 0.005$).

Table 1: Incidence of Hemangiosarcoma in Male Rats

Tumor, organ	Sex	Incidence (n = 60)				p value	
		0	3	8	20	Pairwise ^a	Trend
Hemangiosarcoma (HS), total	M	2	2	4	9 (15.0%)	0.0166	0.002
Mesenteric lymph node	M	1	1	2	9 (15.0%)	0.0049	0.0001
Skin/adipose tissues	M	1	1	2	0	- ^b	-
HS + hemangioma, total	M	3	2	4	9 (15.0%)	0.041	0.004

a. Pairwise comparison between HD and C groups.

b. (-), not calculated.

It is a common practice in the evaluation of tumorigenesis to conduct statistical analysis of the HS and hemangioma in the whole body because these tumors are generally present in multiple organs and have similar mechanism of tumorigenesis. Berotralstat induced HS specifically in the mesenteric lymph node in males. The organ and tumor specificity of berotralstat may render it more appropriate to conduct a tissue specific analysis of tumorigenesis.

The mechanism of tumorigenesis for berotralstat remains unclear. The applicant claims that the mechanism for berotralstat-induced tumorigenesis in rats is not relevant for humans. However, the Division doesn't agree with this claim at this point based on the available data.

The Executive Carcinogenicity Assessment Committee (Exec. CAC) of the Center evaluated the above findings in a meeting held on March 10, 2020 [see meeting minutes, DARRTS ID# 2576054]. The Committee concluded the following:

1. Both rat and mouse studies were adequate to evaluate the carcinogenicity potential of berotralstat.
2. Berotralstat tested positive for evidence of carcinogenicity in male rats. The 20-mg/kg/day berotralstat dose for 2-years caused statistically significant increases in the incidence of hemangiosarcomas in the mesenteric lymph node in males. No increases in the incidence of this tumor were observed at ≤ 8 mg/kg/day. Relevance of the tumorigenicity data in rats to humans is unknown.
3. Berotralstat tested negative for tumorigenicity in both sexes in mice and females in rats.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 1809010-52-3 (dihydrochloride)

Generic name: Berotralstat

Code Name: BCX7353

Chemical Name: (+)-1-(3-(aminomethyl)phenyl)-N-(5-((3-cyanophenyl)(cyclopropyl-methylamino)methyl)-2-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide dihydrochloride

Molecular Formula/Mol. Weight: $C_{30}H_{26}F_4N_6O \cdot 2HCl$ / 635.48

Pharmacologic Class: Kallikrein inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

BLA 125277, Kalbitor, a recombinant human plasma kallikrein inhibitor, for HAE
IND 135058, berotralstat (BCX7353), for HAE prophylaxis
IND 142879, berotralstat (BCX7353), intended for the treatment of HAE attacks

2.3 Drug Formulation

Capsules containing (b) (4)-mg berotralstat dihydrochloride (150-mg free base), (b) (4)-mg pregelatinized starch, (b) (4)-mg crospovidone, (b) (4)-mg colloidal silicon dioxide, and (b) (4)-mg magnesium stearate.

2.6 Proposed Clinical Population and Dosing Regimen

Prevention of hereditary angioedema (HAE) in patients 12 years of age and older. The proposed clinical dose of berotralstat is 150 mg (base), (b) (4)

2.7 Regulatory Background

BioCryst Pharmaceuticals filed NDA 214094 seeking market registration of berotralstat for the prevention of HAE in adult and adolescent patients on December 3, 2019. The development program of the application was completed in IND 135038.

The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in rats and a 26-week study in B6F1 Tg.RasH2 mice (Reports #806-161 and #20088905, respectively). The Agency concurred with the dose selection of the protocol of the 26-week study in transgenic mice on December 14, 2017. The Applicant did not seek the Agency's input on the dose selection of the rat study protocol.

3 Studies Submitted

3.1 Studies Reviewed

Study No	Description	Location
20088905	26-week oral carcinogenicity study in cbyb6f1/tg.rash2 mice	4.2.3.4.1
806-161	104-week oral oncogenicity study in rats	4.2.3.4.1

3.3 Previous Reviews Referenced

Application	Reviewer	Date	DARRTS#	Review content
IND 135058	Luqi Pei	07/19/2017	4126737	IND nonclinical safety review
IND 135058	Dong Zhao	12/14/2017	4195500	Mouse carcinogenicity study protocol

4 Pharmacology

Berotralstat is a small molecule kallikrein inhibitor. Kallikrein is a plasma enzyme responsible for the production of bradykinin, a vasodilatory agent. Kallikrein cleaves and releases bradykinin from high-molecular-weight keningen. Bradykinin binds to the

bradykinin B2 receptor and results in dilation of blood vessel, fluid leakage, and edema. Berotralstat inhibited purified plasma human kallikrein ($IC_{50} = 5.83$ nM).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Berotralstat was quickly absorbed after oral administration in animals. Plasma drug levels increased generally in proportion to the administered doses. There were no significant differences in plasma drug levels between sexes. The half-life was 18.5 and 22.7 hr in rats and monkeys, respectively. The oral bioavailability was 33% and 45% in rats and monkeys, respectively. The protein binding capacity was high in humans, rats, and mice (98.7-99.8%) but lower in monkeys and rabbits (74.1-81.9%). The tissues showing the highest maximum concentrations of radioactivity included liver, kidney medulla, spleen, adrenal gland(s), kidney(s), lungs, and pituitary gland. Metabolism of berotralstat was low across species. About 61% of orally administered berotralstat was excreted as unchanged in rats. Less than 10% of berotralstat was metabolized when it was incubated with human cryopreserved hepatocytes for 360 minutes.

5.2 Toxicokinetics

Bioaccumulation of BCX7353 occurred in rats, but not in monkeys. Table 2 presents plasma drug AUC levels in rats and monkeys in chronic toxicity studies. In rats, berotralstat AUCs in week 26 were approximately 3 times the AUCs at week 1. The monkeys showed no apparent difference in AUCs between Weeks 1 and 39.

Table 2: Toxicokinetics of Berotralstat in Rats and Monkeys

Berotralstat (mg/kg/day)	AUC ₀₋₂₄ ng.h/mL ^a									
	Rat (Study #806-101)				Monkey					
					Study #8001292			Study #806-102		
	1	2.5	8	20	2.5	7.5	20	30	55	80
Week 1	-	101	786	3190	213	868	3310	7700	7350	10200
Week 13	46.1	190	1550	7090	196	790	2955	7650	11400	20000
Week 26	56.1	297	2030	9710	230	791	3110	-	-	-
Week 39	-	-	-	-	240	559	2880	3950	7780	13500

a. Source: eCTD Section 2.6.7, p37, 59, and 62.

7 Genetic Toxicity

Berotralstat tested negative in the Ames test, a chromosomal aberration assay in human peripheral blood lymphocytes in vitro, and an in vivo micronucleus assay in rats.

8 Carcinogenicity

8.1 Two-Year Rat Study (806-161)

Study title: BCX7353: A 104-Week Oral Oncogenicity Study in Rats

<i>Study no.:</i>	806-161
<i>Study report location:</i>	4.2.3.4.1
<i>Conducting laboratory and location:</i>	(b) (4)
<i>Date of study initiation:</i>	September 1, 2016
<i>GLP compliance:</i>	Yes
<i>QA statement:</i>	Yes
<i>Drug, lot #, and % purity:</i>	BCX7353.2HCL, 0000007963, 99.1%
<i>CAC concurrence:</i>	No

Key Study Findings

- Wistar rats dosed by oral gavage with 0 (deionized water), 3, 8, or 20-mg/kg/day berotralstat for 104 weeks showed dose-related increases in the incidence of the following malignant tumors: hemangiosarcoma (HS) in the mesenteric lymph nodes in males and females, undifferentiated sarcoma in the skin in males, and endometrial stromal sarcoma in the uterus in females.
- Only the increase in the incidence of HS in the mesenteric lymph node in HD males reached statistical significance in both the trend test ($p < 0.005$) and pairwise analyses ($p < 0.01$).
- Pairwise analysis for HS in HD males (whole body) was considered marginally significant ($p=0.0166$), although the trend analysis was significant ($p < 0.005$).
- Pairwise comparison for HS and hemangioma in combination in the whole body was not statistically significant ($p=0.041$), although the trend analysis was significant ($p < 0.005$).
- Pairwise analysis for neither undifferentiated sarcoma in the skin in males nor endometrial stromal sarcoma in the uterus in females was statistically significant.
- The HD group showed decreases in mean body weights (by approximately 17.6% and 13.5% in males and females, respectively). These rats also showed audible breathing in the clinical observations. The audible breathing was apparently due to presence of excrete (exudate) in the nasal passage.
- See Table 6 below for the incidence of the above tumors.

Adequacy of Carcinogenicity Study

This study is considered adequate. See minutes of the March 10, 2020 Exec CAC meeting (DARRTS ID 4576064). The duration of treatment (i.e., 104 weeks) is adequate. The dose selection seems to be appropriate. The high dose of 20 mg/kg/day was one-third to half of the lethal dose of 40 – 60 mg/kg/day. [see Footnote 1 on page 10]. Dose-related responses in both neoplastic and non-neoplastic findings were observed.

Neoplastic responses were discussed above in the Key Study Finding section. Non-neoplastic findings are listed in Table 7. Briefly, treatment-related lesions were observed in several organs and tissues in the HD group: adrenal glands, kidney, lung, lymph nodes, skeletal muscle, intestine, and spleen. The lesions were mostly cell vacuolations.

The Agency did not concur with the dose selection of berotralstat for the 2-year rat study because the applicant did not seek the Agency's input on the dose selection. However, the study results as discussed in the review indicated that the dose-selection was appropriate.

Appropriateness of Test Model

The model is appropriate. This strain of rats was used in the repeat-dose toxicity studies of berotralstat. The rats showed dose and temporal effects of berotralstat in oral studies up to 26 weeks in treatment duration.

Evaluation of Tumor Findings

Berotralstat is an epigenetic carcinogen in rats. Male rats dosed with 20-mg/kg/day berotralstat (HD dose) orally for 2 years showed statistically significant increases in the incidence of HS in the mesenteric lymph node (Tables 6 and 7). Hemangiosarcoma is a common malignant tumor in rats.

Dr. Zhuang Miao conducted the statistical analysis of the data. A draft review was provided to the DPARP nonclinical team on February 14, 2020. Dr. Miao conducted the following two tests in the review: trend test and pairwise comparison. The review uses the following criteria below to define a positive result:

- 1) Trend test alone: 0.005 and 0.025 for common and rare tumors, respectively
- 2) Pairwise comparison alone: 0.01 and 0.05 for common and rare tumors, respectively.
- 3) Joint tests (trend + pairwise): respective p values for common and rare tumors are 0.005 and 0.025 in trend test and 0.05 and 0.1 in pairwise comparisons

HS is considered a common tumor in rats. Only HS in the mesenteric lymph node in high-dose male rats met all 3 criteria above and was considered positive.

Pairwise comparison for the incidence of HS between the HD and C groups in whole body was marginally significant ($p=0.017$). Pairwise comparison for the incidence of HS and hemangioma in combination between the HD and Control groups was insignificant ($p=0.041$), although trend analysis showed positive dose responses ($p < 0.005$).

There were also dose-related increases in the incidence of HS in HD females, undifferentiated sarcoma in the skin in males, and endometrial stromal sarcoma in the uterus in females. None of these increases reached statistical significance in pairwise comparison. Skin sarcoma and endometrial stromal sarcoma were considered rare tumors for this analysis.

The HD group showed decreases in mean body weight, audible breathing, and presence of excrete (exudate) in the nasal passage. It is unclear whether these observations had any major impact on the tumorigenicity of berotralstat in rats.

Methods

<i>Doses:</i>	0, 3, 8, and 20 mg/kg/day (Table 3) The above dose schedule excluded another group - extra high dose (EH) group that was terminated prematurely at Week 28 (day 191). ¹
<i>Frequency of dosing:</i>	Daily
<i>Dose volume:</i>	2.5 mL/kg
<i>Route of administration:</i>	Oral gavage
<i>Formulation/Vehicle:</i>	Aqueous solution/ deionized water
<i>Basis of dose selection:</i>	Mortality, the HD was 1/3 to half of the lethal dose
<i>Species/Strain:</i>	Rat, Wistar Han [CrI:WI(Han)]
<i>Number/Sex/Group:</i>	60
<i>Age:</i>	7.5 weeks on day 1 of dosing
<i>Animal housing:</i>	paired
<i>Paradigm for dietary restriction:</i>	No dietary restriction
<i>Dual control employed:</i>	No
<i>Interim sacrifice:</i>	No
<i>Satellite groups:</i>	5 and 12/sex/dose in the control and BCX7353 treatment groups for TK analysis. The TK animals were sacrificed after blood sampling on day 182.
<i>Deviation from study protocol:</i>	Not remarkable

¹ The review excludes the extra-high dose (EH, Group 5) group from the evaluation and discussions of the carcinogenicity results of BCX7353. This is to enhance the clarity of the discussion and evaluation of the study results. The EH group primarily provides information that supports the dose selection of the carcinogenicity study design. This group was terminated for humane reasons prematurely in Week 28 (Day 191). By the time of termination of the animals, the group mean body weights was decreased by 16.2% and 8.7% in males and females, respectively. Five of the females died. The dosing regimen was 60 and 40 mg/kg/day for weeks 1 – 17 and 18 – 27, respectively. No data relevant to tumorigenicity was obtained from any of the animals.

Animals in this EH group generally showed clinical observations similar to that of the HD group. These included decreases in body weight and food consumption, salivation, thin appearance and audible breathing. Microscopic changes, however, were unremarkable because the findings were generally less severe and with lower incidence.

Table 3: Design of the 2-Year Rat Carcinogenicity Study

Group	Code	Berotralstat		No. of Rat (n/sex/group)	
		Dose (mg/kg/day)	Concentration (mg/mL)	Carcinogenicity	TK
1	C	0 (water)	0	60	5
2	LD	3	1.2	60	12
3	MD	8	3.2	60	12
4	HD	20	8.0	60	12

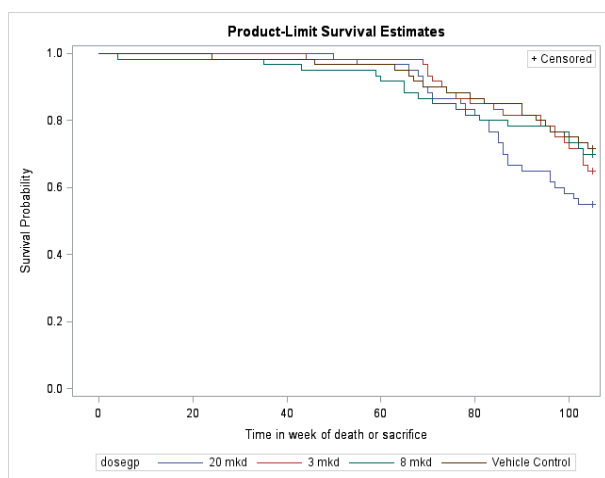
OBSERVATIONS AND RESULTS

Mortality

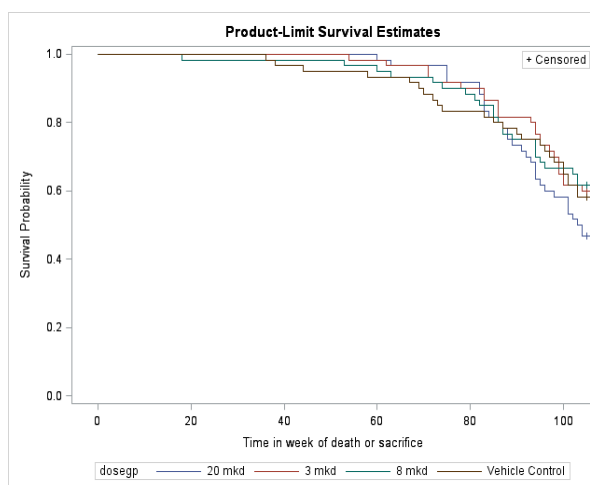
The HD group in males and females showed higher incidence of unscheduled deaths (Table 4). The respective number of deaths in the C, LD, MD, and HD groups was 17, 21, 18, and 26 in males, and 25, 24, 23, and 32 in the females. The respective survival rate in the HD group and remaining groups was 55% and 65 – 70% in males, and 47% and 58 – 62% in females. In short, the HD group showed mortality rate at least 10 – 12% higher than other groups. None of the deaths were considered HS-related.

Table 4: Mortality in the 2-Year Rat Carcinogenicity Study

Berotralstat (mg/kg/day)	Male (n = 60/group)				Female (n = 60/group)			
	0	3	8	20	0	3	8	20
Died after dosing	0	0	0	1	0	0	0	0
Euthanized in extremis	10	20	15	23	24	23	22	28
Found dead	7	1	3	3	1	1	1	4
Survivors at terminal necropsy	43	39	42	33	35	36	37	28
Percent of survival	71.7%	65.0%	70.0%	55.0%	58.3%	60.0%	61.7%	46.7%



Male



Female

Fig. 1: Kaplan-Meier survival curves of the 2-year Carcinogenicity study in rats

Clinical Signs

The HD group showed increases in incidence of audible breathing. The respective incidence of audible breathing in the C, LD, MD, and HD groups (observations/animals) was 5/4, 1/1, 1/1, and 1160/47 in males and 3/3, 3/3, 2/2 and 470/45 in females.

Body Weights

The HD group showed decreases in body weights (Table 5 and Fig 2). The HD males and females showed decreases of 17.6% and 13.5% in mean body weight (vs. control) at the end of the study.

Table 5: Body Weights in 2-Yr Rat Study

Time (day)	Male				Female			
	Bdwt (g)	Changes from the control (%)			Bdwt (g)	Changes from the control (%)		
		Control	LD	MD		HD	Control	LD
7	259.8	-0.19%	0.80%	0.38%	174.8	0.20%	0.56%	0.13%
91	419.6	2.06%	2.66%	0.01%	247.0	-1.67%	-0.47%	-1.65%
175	485.1	1.67%	0.63%	-3.56%	268.1	-2.01%	-1.13%	-2.19%
371	572.9	2.00%	1.25%	-6.22%	311.1	-3.73%	-2.97%	-7.04%
567	634.9	1.39%	1.13%	-10.21%	353.1	-4.13%	-3.97%	-10.30%
707	662.4	0.33%	-1.36%	-17.64%	370.7	-3.48%	-2.92%	-13.51%

The decreases in body weights became visually apparent starting at approximately week 52.

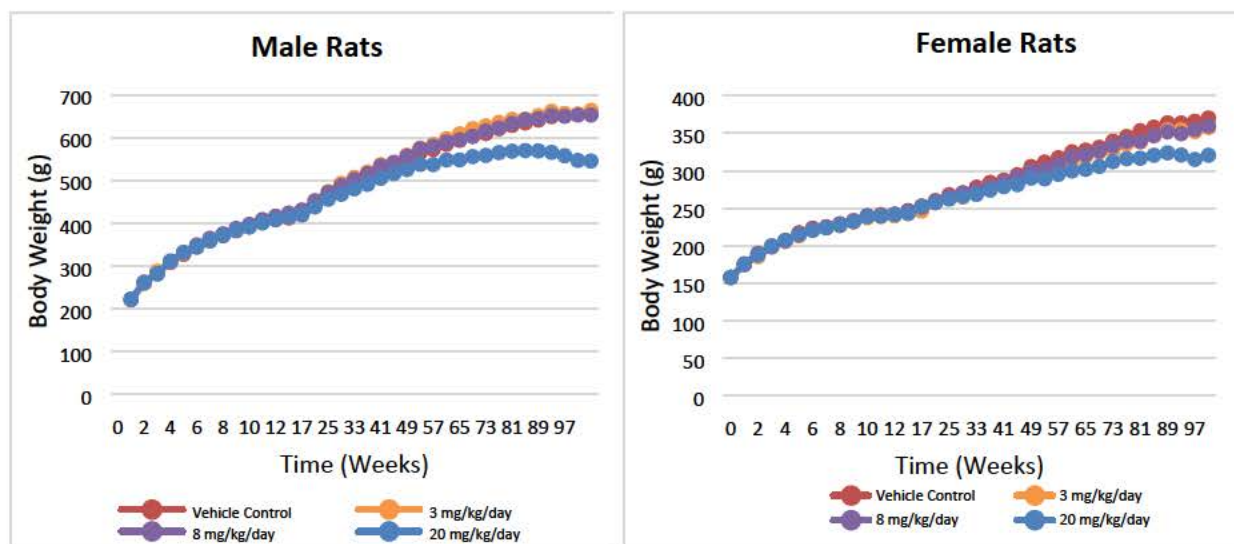


Fig. 2: Mean body weights - Time course of the 2-year Carcinogenicity study in rats

Feed Consumption

There were no apparent treatment-related effects on food consumption.

Histopathology

Peer Review

Yes. Keith Nelson, DVM, PhD, conducted a peer review of the pathology report.

Neoplastic Findings

Table 6 presents notable neoplastic findings in the 2-year study. Appendices B and C (provided by Dr. Zhuang Miao, the Statistical Reviewer) list the overall tumor incidence in each sex in the study. Dose-related increases in the incidence of the following tumors were observed: HS in multiple organs (mostly in the mesenteric lymph nodes) in both males and females, undifferentiated carcinoma in the skin in males, and endometrial stromal carcinoma in the females.

Table 6: Notable Tumor Findings in 2-Year Rat Study

Berotralstat (mg/kg/day)		Incidence (N = 60/group)				P value ^a	
		0	3	8	20	Trend	Pairwise
HS (HS), whole body	M	2	2	4	9 (15.0%)	0.002 ^b	0.0166
HS, mesenteric lymph node	M	1	1	2	9 (15.0%)	0.0049	0.0001
HS + hemangioma	M	3	2	4	9 (15.0%)	0.004	0.041
HS (HS), whole body	F	4	2	1	9 (15.0%)	0.067	0.125
Uterus: sarcoma, endometrial stromal ^a	F	0	0	2	3 (5.0%)	0.023	0.121
Skin: sarcoma, undifferentiated	M	0	1	1	3 (5.0%)	0.037	0.107

a. From draft statistical review completed by Dr. Zhuang Miao on February 14, 2020.

b. Highlights indicate that at least one of the statistical analysis criteria for a positive result was met.

Note that the p values listed in Table 6 differ from the sponsor's submission. These differences were due to differences in analytical methods. See the draft statistical review completed by Dr. Zhuang Miao on February 14, 2020.

Hemangiosarcomas (HS): Table 7 presents the distribution of HS in the study. The mesenteric lymph node is the primary location of HS. It accounted for 89% (16/18) of the tumors in the HD group. Five (5/18) rats with HS either died or were sacrificed prematurely; none of the deaths, however, were attributed to HS by the pathologist.

Table 7: Incidence of HS in the 2-Year Rat Study

Organ/tissue	Incidence of HS (n = 60/group)							
	Male				Female			
	C	LD	MD	HD	C	LD	MD	HD
Lymph node, mesenteric	1	1	2	9	3	1	1	7
Skin	-	1	2	-	-	-	-	-
Adipose tissue	1	-	-	-	1	-	-	-
Mesentery/peritoneum	-	-	-	-	-	-	-	1
Muscle, skeletal	-	-	-	-	-	-	-	1
Total	2	2	4	9	4	2	1	9

There was no apparent sex difference in the HS incidence between these early deaths/sacrifice. Two (of 9) males either died or were sacrificed (Rats #4007 and #4024 on days 714 and 670, respectively). Three of the 7 females with HS in the lymph nodes

were sacrificed pre-terminally (Rats #4506, #4517, and #4523 on days 633, 572, and 594, respectively).

Skin tumors: Three HD males (#4013, #4033, and #4053) showed undifferentiated carcinoma in the skin. These rats were sacrificed on Days 730 (terminal), 489 (moribund), and 601 (moribund). The report identified these tumor as “mortality-independent” primary tumors. None of these rats had HS. The statistical analysis showed p values of 0.045 for trend test and 0.107 for pairwise comparison between the HD and control. This tumor type did not meet any criterion for a positive result.

Uterine tumors: Two MD and three HD females showed endometrial stromal sarcoma. The tumor was the cause of death in 4 of the 5 rats (2 in both MD and HD). The 2 MD rats (#3522 and #3548) were sacrificed on Days 601 and 606, respectively. The 2 HD rats (#4511 and #4520) were sacrificed on days 722 and 713, respectively. The remaining HD rat (#4528) was sacrificed as scheduled. This tumor type did not meet any criterion for a positive result.

Non-Neoplastic

Table 8 summarizes notable non-neoplastic findings. Treatment-related lesions were observed in the following organs and tissues: adrenal glands, kidney, lung, lymph nodes, skeletal muscle, intestine, and spleen. The most common finding is vacuolated or foamy macrophages (adrenals, lymph nodes, intestine, and spleen) and is observed mostly in the HD group. Other findings included accumulation of alveolar macrophages and exudate in the nasal cavity in both sexes and myofiber degeneration of the skeletal muscles in males.

Table 8: Non-neoplastic Findings in the 2-Year Rat Study

Organ/findings	Seve- -rity	Incidence (n = 60/group)							
		Male				Female			
		C	LD	MD	HD	C	LD	MD	HD
Adrenal gland: vacuolation, cortical	Total	1	- ^a	-	51	-	1	-	53
Macrophage, foamy, pigmented		-	-	2	33	-	-	3	35
Kidney: tubular vacuolation	Total	1	-	-	22	1	-	2	14
Min		-	-	-	-	-	-	-	4
Mild		-	-	-	18	-	-	2	9
Mod		1	-	-	4	1	-	-	1
Larynx: degeneration/necrosis myofiber	Total	1	-	2	19	-	-	-	10
Min		1	-	2	13	-	-	-	7
Mild		-	-	-	4	-	-	-	3
Mod		-	-	-	2	-	-	-	-
Lung: macrophage, alveolar	Total	13	21	10	40	16	16	19	47
Min		12	20	9	29	12	16	16	39
Mild		1	1	1	9	4	-	3	8
Mod		-	-	-	2	-	-	-	-
Lymph node: histiocytosis, sinus mesenteric	Total	1	19	35	59	8	14	38	60
Min		1	19	19	2	6	13	29	2
Mild		-	-	12	17	2	1	9	28
Mod		-	-	4	40	-	-	-	28
Mandibular	Min	-	-	-	5	-	-	-	1
Nose: degen./necrosis, epithelium	Total	-	-	1	1	-	-	-	-
Exudate, luminal (min- mod)	Total	2	2	8	23	1	1	4	25
Proliferation	Total	-	-	-	1	-	-	-	-
Skeletal muscle: degeneration/ Necrosis, myofiber	Total	-	2	4	21	1	-	-	5
Min		-	1	3	14	1	-	-	4
Mild		-	1	1	7	-	-	-	1
Small intestine: macrophage, foamy	Total	-	-	-	26	-	-	-	2
Spleen: macrophage, vacuolated	Total	2	-	-	46	-	-	-	44

a. (-), absence of finding.

Toxicokinetics: Table 9 presents berotralstat exposure data in rats in the first year. Both mean AUC and C_{max} were dose-proportional in week 1 but supra proportional to dose in weeks 13 and 52. The exposures were the highest in week 52. Data indicate that berotralstat bioaccumulates in rats.

Table 9: Toxicokinetics of Berotralstat in Rats

Berotralstat (mg/kg/day)		AUC ₀₋₂₄ ng.h/mL ^a				C _{max} (ng/mL)			
		3	8	20	60	3	8	20	60
Week 1	M	70.3	485	2270	6530	6.01	40.1	190	491
	F	96.7	611	3400	8470	10.1	62.7	258	614
	M/F	83.5	548	2830	7500	8.0	51.4	224	525
Week 13	M	392	2040	8470	33700	32.4	115	506	2100
	F	432	1880	9480	35800	31.0	118	711	2200
	M/F	412	1960	8980	34700	31.7	106	552	2150
Week 52	M	775	2620	13200	-	43.8	143	787	-
	F	503	2820	9680	-	46.8	219	706	-
	M/F	639	2720	11500	-	44.8	181	689	-

a. Source: eCTD Section 2.6.7, p. 96.

Dosing Solution Analysis: Dosing solutions were analyzed in weeks 1, 4, 13, 19, 26, 39, 52, 53, 65, 78, 91, and 104. Berotralstat concentrations ranged from 86.1% to 105.1% of the target concentration, which is considered adequate.

8.2 Transgenic Mouse Study (20088905)

Study title: BCX7353: A 26-Week Oral Oncogenicity Study in Tg.rasH2 mice

Study no.: 20088905
 Study report location: 4.2.3.4.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 14, 2018
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BCX7353.2HCL, 0000038590, 99.8%
 CAC concurrence: Yes

Key Study Findings

B6F1 Tg.RasH2 mice dosed orally with ≤ 50-mg/kg/day berotralstat for 26 weeks did not show any evidence of carcinogenicity. Mice were dosed by oral gavage with 0 (vehicle: deionized water), 8, 20, and 50-mg/kg/day berotralstat for 26 weeks. There were no significant dose-related increases in the incidence of any tumors.

Adequacy of Carcinogenicity Study

This study was adequate. The study design was routine for transgenic models. The treatment duration was 26 weeks. There were 25 mice/sex/dose. The Agency concurred with the dose selection. See nonclinical review completed by Dr. Dong Zhao on December 14, 2017 (DARRTS ID 4195500).

Appropriateness of Test Models

The model is appropriate. The Executive CAC reviewed the model and dose selection in the December 14, 2017 meeting. See nonclinical review completed by Dr. Dong Zhao on December 14, 2017 (DARRTS ID 4195500).

Evaluation of Tumor Findings

B6F1 Tg.RasH2 mice dosed by oral gavage with up to 50-mg/kg/day berotralstat for 26 weeks showed no dose-related increases in the incidence of any tumors. A positive control (75 mg/kg/day N-nitroso-N-methylurea, or NMU) group showed increases in the incidence of tumors typical of the treatment in this model. Berotralstat was considered negative for carcinogenicity in the Tg.RasH2 mouse model.

The HD male group treated with berotralstat showed a higher incidence of HS in the spleen (2/25), but the C and HD females showed similar incidences (3/25 and 2/25, respectively). The finding is not considered significant or treatment-related.

Many tissues in 6 HD males were not examined because tissues were missing. The missing tissues included pituitary, thymus, thyroid/parathyroid, adrenals, lungs, trachea, gallbladder, urinary bladder, rectum, mesenteric lymph nodes, prostate, epididymides, and larynx. See Protocol deviation. The lack of tumor information in these organs would not probably affect the validity of the study results and the overall interpretation of study findings.

Methods

<i>Doses:</i>	0, 8, 20, and 50 mg/kg/day, see Table 10.
<i>Frequency of dosing:</i>	Daily
<i>Dose volume:</i>	2.5 mL/kg
<i>Route of administration:</i>	Oral gavage
<i>Formulation/Vehicle:</i>	Aqueous solution/ deionized water The NMU concentration in the PC group was 7.5-mg/mL in citrate-buffered saline (ph4.5).
<i>Basis of dose selection:</i>	Mortality, the HD was 1/3 of the lethal dose
<i>Species/Strain:</i>	Mice, B6F1Tg rasH2 hemizygous
<i>Number/Sex/Group:</i>	25
<i>Age:</i>	7.5 weeks on day 1 of dosing
<i>Animal housing:</i>	paired
<i>Paradigm for dietary restriction:</i>	No dietary restriction
<i>Dual control employed:</i>	No
<i>Interim sacrifice:</i>	No

<i>Satellite groups:</i>	5 and 12/sex/dose in the control and berotralstat treatment groups for TK analysis.
<i>Exec. CAC concurrence on dose selection:</i>	Yes, the Committee evaluated the dose selection of the study on December 12, 2017. The Committee recommended berotralstat doses of 0, 8, 20, and 50 mg/kg/day. The Sponsor accepted the Committee's recommendation.
<i>Deviation from study protocol:</i>	A significant number of organs/tissues were missing for unknown reasons. The report states: "Several tissues (pituitary, thymus, thyroid/parathyroid, adrenals, lungs, trachea, gallbladder, urinary bladder, rectum, mesenteric lymph nodes, prostate, epididymides, and larynx) from 6 males administered 50 mg/kg berotralstat were inadvertently lost during histology processing."

Table 10: Design of the Berotralstat Carcinogenicity Study in Transgenic Mice

Group	Treatment	Dose (mg/kg/day)	No. of mice (n/sex)	
			Main (transgenic)	TK (non-transgenic)
1	0 (VC) ^a	0	25	12
2	Berotralstat	8	25	36
3		20	25	36
4		50	25	36
5		NMU (PC)	75	15
6	Sentinel	-. ^b	10	5

a. NMU = N-Nitroso-N-methylurea (by intraperitoneal injection), PC = positive control, VC= vehicle control (deionized water).

b. (-), not applicable.

OBSERVATION AND RESULTS

Mortality

Not remarkable. There were no significant differences in mortality between the vehicle control and the berotralstat treatment groups. The number of mice that survived to the terminal sacrifice was 22 - 25 out of 25 in the control and berotralstat treatment groups, respectively, Table 11. Four animals in the berotralstat treatment groups (1-HD M, 2 MD Fs, and 1 HD F) died of gavage accidents. Berotralstat treatment groups showed no apparent increases in tumor-related deaths.

Table 11: Mortality in Transgenic Mouse Carcinogenicity Study

Berotralstat (mg/kg/day)	Male (n = 25/group)					Female (n = 25/group)				
	0	8	20	50	NMU	0	8	20	50	NMU
No. death/euthanasia (total)	1	1	1	1	17	-	1	3	2	25
No of found dead	-	-	-	-	4	-	1	1	-	5
No. of euthanasia	1	1	1	3	13	-	-	2	2	20
Survivors at terminal necropsy	24	24	24	22	8	25	24	22	23	-
Percent of survival (%)	96	96	96	88	32	100	96	88	92	-

Significant mortality occurred in the positive control group; 0 – 8 (of 25) in PC groups survived the study. Most of PC animals died of malignant lymphoma in the hemolymphoreticular tissues (12 and 18 in males and females, respectively).

Clinical Signs: There were no apparent treatment related effects on clinical signs.

Body Weights: Both HD males and females showed decreases in body weights (Table 12). The decrease in mean body weights was 14.4% and 9.5% in males and females (vs. control) at the end of study.

Table 12: Body Weights in 26-week study in Mice

Time (Day)	Male				Female			
	Control (g)	Changes from C (%)			Control (g)	Changes from C (%)		
		LD	MD	HD		LD	MD	HD
1	26.2	0.26%	-0.81%	-1.07%	20.4	-0.88%	-0.78%	-1.88%
29	27.3	1.61%	-1.92%	-3.29%	22.4	0.82%	-0.32%	-5.57%
92	30.7	-0.94%	-7.95%	-9.74%	24.4	-0.76%	-4.08%	-8.48%
182	35.4	-3.40%	-11.41%	-14.38%	26.5	-0.75%	-6.92%	-9.54%

Feed Consumption

There were no apparent treatment-related effects on food consumption.

Histopathology

Peer Review

Yes. Keith Nelson, DVM, PhD, conducted a peer review of the pathology report.

Neoplastic Findings

There were no significant, dose-related increases in any tumors in the berotralstat treatment groups (Tables 13 and 14). The PC group showed marked increases in tumor incidences in both males and females. The tumors in the PC groups included squamous cell papilloma and malignant lymphomas.

Table 13: Neoplastic Findings in Transgenic Mice - Males

Test material	Male (n = 25/group)				
	Water ^a	Berotralstat			NMU
	0	8	20	50	75
	Group 1	2	3	4	5
Adenoma, Harderian gland	1	-	-	-	2
Adenocarcinoma, Harderian gland	-	-	1	-	1
Ameloblastoma, tooth, head	1	-	-	-	-
Adenoma, Kidney	-	-	-	-	1
Bronchioloalveolar adenoma, lung	1	1	2	-	2
HS, spleen	-	-	-	2	3
Skin	-	-	1	-	-
Testis	2	-	-	-	-
Keratoacanthoma, skin	-	-	-	-	1
Adenoma, duodenum	-	-	-	-	1
Adenocarcinoma, jejunum	-	-	-	-	1
Stomach	-	-	-	-	1
Lymphoma, malignant ^b	--	1	-	-	14
Papilloma, squamous cell, ear	-	-	-	--	1
Esophagus	--	-	-	-	2
Skin	-	-	-	1	12
Stomach	1	-	-	1	16
Papilloma, trachea	-	-	-	-	1
Transitional cell, urinary bladder	-	1	-	-	2
Squamous cell carcinoma, stomach	-	-	-	-	5

a. Water (deionized), NMU = N-Nitrosomethylurea (positive control), and (-) = no finding.

b. Lymphoma in hemolymphoreticular tissue.

Table 14: Neoplastic Findings in Transgenic Mice - Females

Test material	Female (n = 25/group)				
	W ^a	Berotralstat			PC
	0	8	20	50	75
Group	1	2	3	4	5
Basal cell tumor, malignant, skin	-	-	-	-	2
Adenoma, bronchioloalveolar lung	3	-	1	2	5
Carcinoma, bronchioloalveolar, lung	-	1	1	-	-
Adenocarcinoma, mammary gland	-	-	-	-	1
Endometrial stromal polyp, uterus	-	-	-	-	2
HS, spleen	-	3	-	2	1
Skin	-	-	-	1	-
Lymphoma, malignant ^b	-	-	-	-	18
Mesenchymal tumor, malignant, kidney	-	-	-	-	1
Papilloma, squamous cell, ear	-	-	-	-	1
Skin	-	1	-	-	10
Stomach	-	-	-	-	16
Vagina	-	-	-	-	1
Carcinoma, squamous cell, nasal cavity	1	-	-	1	-
Bone, mandible	-	-	-	-	1
Pinna	-	-	-	-	1
Skin	1	-	-	-	2
Stomach	-	-	-	-	2
Vagina	-	-	-	-	1

a. W = RODI Water, BCX = berotralstat, PC = Positive Control (NMU = N-Nitrosomethylurea), and (-) = no finding.

b. Lymphoma in hemolymphoreticular tissue.

Non-Neoplastic

Non-neoplastic findings were noticed in the nasal cavity, eye, liver, lymph nodes, and spleen (Table 15). The findings in the nose included inflammation in all berotralstat treatment groups in both sexes and erosion/ulceration of respiratory epithelium in the HD males. Findings in the eyes (dysplasia), liver (inflammation and hepatocellular atrophy), lymph nodes (decreases in cellularity) and spleen (decreases in cellularity) were minimal in severity, low in incidence or occurred in only one sex. They also lacked significant dose-response relationship.

Table 15: Non-neoplastic Findings in Transgenic Mice

Organ/ tissue	Finding	Incidence (n = 25) ^a							
		Male				Female			
		C	LD	MD	HD	C	LD	MD	HD
Nasal cavity	Erosion/ulcer, resp. epithelium	-	1	-	3 ^b	-	-	1	-
	Inflammation, neutrophilic	1	9	7	13 ^b	-	10	7	11
Eye	Dysplasia, focal; multifocal	-	-	-	4	1	-	-	2
Liver	Atrophy, hepatocellular, focal/diffused	-	-	-	3	-	1	2	2
	Inflammation, mixed cell, multifocal	-	-	-	-	-	-	2	2
Lymph node									
Mandibular	Decreased cellularity, lymphoid	-	-	-	2	-	1	2	1
Mesenteric	Decreased cellularity, lymphoid	-	-	-	2	-	1	2	1
Spleen	Decreased cellularity, lymphoid	-	-	-	2	-	1	2	1
	Increased hematopoiesis	-	-	1	-	-	-	1	2 ^b

a. The severity of lesions is minimal for all unless specified.

b. Including 1 animal with moderate lesion in severity.

Dosing Solution Analysis: Dosing solutions were analyzed in weeks 1, 4, 13, and 26. Berotralstat concentrations ranged from 83.9% to 110% of the target concentration.

Toxicokinetics

Toxicokinetics of berotralstat was studied in satellite groups in non-transgenic mice. Blood samples were collected at hours 0, 1, 2, 4, 8, and 24 post dosing on days 1 and 182 (n = 3 mice/time point). Table 16 presents AUC and C_{max} data. Both mean AUC and C_{max} were dose-proportional in week 1 but supra proportional to dose in week 26. It is unknown whether the data reflect the plasma drug levels in the transgenic mice.

Table 16: Toxicokinetics of Berotralstat in Mice

Berotralstat (mg/kg/day)		AUC ₀₋₂₄ ng.h/mL ^a			C _{max} (ng/mL)		
		8	20	50	8	20	50
Male	1	2100	5670	16100	231	678	1880
	182	2280	8090	23100	164	647	2880
Female	1	3280	7000	19000	443	714	2430
	182	4690	14100	31800	535	1120	2970

a. Source: eCTD 2.6.7, Section 10.1 (page 90)

11 Integrated Summary and Safety Evaluation

The carcinogenic potential of berotralstat was evaluated in a traditional 2-yr bioassay in rats and a 26-week alternative assay in a transgenic mouse model. The respective berotralstat dose in the LD, MD, and HD groups was 3, 8, and 20 mg/kg/day in rats and 8, 20, and 50 mg/kg/day in mice. There were 60 and 25 animals/sex/dose in rats and Tg.rasH2 transgenic mice, respectively. The results showed that berotralstat was carcinogenic in male rats, but not in female rats, nor in transgenic mouse. Relevance of the tumorigenicity data in rats to humans is unknown.

The carcinogenicity of berotralstat in male rats was demonstrated by dose-related increases in the incidence of HS in the mesenteric lymph node (incidence: 1, 1, 2, and 9 in the C, LD, MD, and HD groups, respectively). Only the increase in the HS incidence in the HD group reached statistical significance ($p < 0.01$ in pairwise comparison). The increase in the HS incidence in the whole body in the HD group reached statistically marginal significance in pairwise comparison ($p = 0.017$). Both were positive in the trend analysis ($p < 0.005$).

The Applicant considers berotralstat an epigenetic carcinogen in rats. The review agrees with the Applicant. The review, however, disagrees with the Applicant about the relevance of the tumorigenic findings to humans. The Exec. CAC agreed with the review. See minutes of the Exec. CAC meeting dated March 10, 2020 (DARRTS ID# 4576054).

The applicant contends that the HS finding is irrelevant to humans and of no safety concern for the proposed clinical use of the drug. Section 13.1 of the proposed labeling states “[t]his finding is considered rodent specific”. The Applicant argues that their conclusion was based on the mechanism of tumorigenesis of HS in rodents. Specifically, HS in rats is secondary to hypoxia in rats, which will not happen in humans receiving berotralstat treatment. There is evidence for and against this argument.

Primary evidence in support of the hypoxia hypothesis is a paper by Cohen et al. published in 2009 [*Toxicol Sci*, 2009;111(1)4-18]. This is a review article (referred to as the Cohen paper hereafter) discussing the mechanism of HS formation associated with non-genotoxic compounds in rodents and the relevance of the HS in rodents to humans. Both genotoxic and non-genotoxic compounds can induce HS in rodents (mostly in mice). The mechanism of carcinogenesis of HS differs between genotoxic and non-genotoxic compounds. Non-genotoxic compounds can produce HS in mice secondary to tissue hypoxia. Such compounds include pesticides, industrial chemicals, and pharmaceuticals. Pharmaceuticals include “calcium channel blockers, antipsychotics, PDE-5 inhibitors, DPP-4 inhibitors, antiarrhythmics, gonadotropin receptor antagonists, antisense compounds, nitric oxide releasers, hemolytic compounds, and vascular endothelial growth factor (VEGF) inducers.”

The Cohen paper concludes that hypoxia and macrophage activation is a common mechanism of action of non-genotoxic drug-induced HS in rodents. The paper states the carcinogenesis appears to “be a convergence of multiple initiating events (e.g., hemolysis, decreased respiration, adipocyte growth) leading to either dysregulated angiogenesis and/or erythropoiesis that results from hypoxia and macrophage activation. These events lead to the release of angiogenic growth factors and cytokines that stimulate endothelial cell proliferation, which, if sustained, provide the milieu that can lead to HS formation.”

The Cohen paper discussed in depth the hypoxia-related HS induced by two pharmaceuticals: pregabalin and troglitazone. Pregabalin (Lyrica, NDA 21-446) caused HS in the tissues where spontaneous HS are commonly observed in mice: liver, spleen,

and bone marrow in B6C3F1 and CD1 mice, but not any tissue in rats.² The paper states: “The precipitating event in pregabalin-associated HS development in mice appears to be decreased respiratory function, including decreased respiration rate and decreased minute volumes, along with alterations in acid-base balance, gradually shifting to a relative alkalotic state.” This eventually leads to local tissue hypoxia.

Troglitazone (Rezulin, withdrawn from the market) produced primarily HS in the skin (subcutaneous adipose tissue) in mice, but not rats. Troglitazone produced local tissue hypoxia in mice “due to the inability of angiogenesis to keep pace with growth of the fat pad. The local tissue hypoxia results in [hypoxia-inducible factor] HIF-1a activation which can activate VEGF secretion and/or macrophage activation”.

The Applicant argues that HS in rats treated with berotralstat was also a result of hypoxia, which occurred because of narrowing of the nasal airway passage and breathing difficulty. Narrowing of nasal airway passage was demonstrated by the presence of exudate in the nasal cavity in berotralstat -treated rats in microscopic examinations. The respective incidence of the presence of luminal exudate in nasal cavity in the C, LD, MD, and HD groups (out of 60) was 2, 2, 8, and 23 in males and 1, 1, 4, 25 in females. Breathing difficulty was demonstrated by audible breathing. The respective incidence of audible breathing in the C, LD, MD, and HD groups (observations/animals) was 5/4, 1/1, 1/1, and 1160/47 in males and 3/3, 3/3, 2/2, and 470/45 in females.

The review agrees with the Applicant that the HD rats showed breathing difficulty, which suggest the presence of hypoxia. Rodents are obligated nose-breathing animals. Presence of exudate in the nasal passage may impede the airflow and causing breathing difficulty which is indicated by the observation of audible breathing. The difficulty of breathing may not indicate necessarily tissue hypoxia in rats.

There are also factors arguing against applying the hypoxia hypothesis to the interpretation of the berotralstat study results in rats. This includes differences in animal species, tumor locations, and drug classes. Table 17 presents pharmaceuticals that induce HS in the Cohen paper. None of these drugs induced HS in rats. Regarding to tumor locations, the Cohen paper reported that HS “most frequently occur in liver, spleen, heart, or subcutaneous adipose tissue”, which is consistent with the findings observed with these drugs. Further, mice have higher spontaneous incidence of HS than rats or humans because they have lower antioxidant levels (e.g., Vitamin E) than rats and humans. The high spontaneous background rate may contribute to their enhanced susceptibility to developing HS.

However, berotralstat does not belong to any of the HS-inducing drug classes. The rat has low spontaneous HS rates. Further, rats and mice differ in organs/tissues with HS. Hypoxia could have been present in rats treated with high dose of berotralstat, but the

² Section 5 of the Label of pregabalin states: “In standard preclinical *in vivo* lifetime carcinogenicity studies of LYRICA, an unexpectedly high incidence of hemangiosarcoma was identified in two different strains of mice [see *Nonclinical Toxicology* (13.1)]. The clinical significance of this finding is unknown.”

hypoxia hypothesis alone cannot fully explain the mechanism of HS formation in rats. The lymph node specificity of HS in rats could be related to the high local concentration of the parent compound and metabolites, alone or in combination.

Table 17: Non-genotoxic HS-Inducing Pharmaceuticals in Mice ^a

Drug (NDA#) ^b	Species ^c	Primary pharmacology	Potential MOA
Entecavir (21797)	Mouse	Antivirals—nucleoside analogs	Unknown
Fenretinide (NA)	Mouse	PRAR agonist	Unknown—PPAR-c–like MOA since RAR forms a heterodimer with PPAR-c receptor?
Pregabalin (21446)	Mouse	α2δ subunit of voltage gated calcium - analgesic, anxiolytic, and antiseizure	Decreased respiration leading to local tissue hypoxia ^f
Olanzapine (020592)	Mouse	5-HT ₂ /D ₂ antagonist - antipsychotic	Unknown
Troglitazone (20720)	Mouse	PPAR-c agonist - type II diabetes mellitus	Dysregulated angiogenesis leading to local tissue hypoxia
Tadalafil (22332)	None ^d	PDE-5 inhibitor - erectile dysfunction	Unknown
Vildagliptin	Mouse ^e	DDP-4 inhibitor - type II diabetes mellitus	Dysregulated angiogenesis - inhibitor of angiogenesis

- Extracted and reconstructed from Cowen et al, *Toxicol Sci*, 2009:111(1):4-18. Industrial solvents and insecticides (i.e., 2-butoxyethanol, carbaryl, and *p*-chloroaniline) are excluded.
- NDA numbers are added for easy reference of the nonclinical data in the product labeling.
- Animal species are added for clarity.
- The product label of tadalafil states the drug “not carcinogenic to rats or mice”.
- “An increased incidence of mammary adenocarcinomas and HS was observed with a no-effect dose of 500 mg/kg (59-fold human exposure) and 100 mg/kg (16-fold human exposure), respectively. The increased incidence of these tumors in mice is considered not to represent a significant risk to humans based on the lack of genotoxicity of vildagliptin and its principal metabolite, the occurrence of tumors only in one species and the high systemic exposure ratios at which tumors were observed.” ---
https://www.ema.europa.eu/en/documents/product-information/galvus-epar-product-information_en.pdf
- Highlights indicate drugs that cause apparent hypoxia.

There is insufficient evidence to support the Applicant’s argument that HS in the mesenteric lymph node associated with berotralstat treatment in male rats is a species-specific finding. Thus, relevance of the carcinogenic finding in rats to humans is unknown at present time.

12 Appendix/Attachments

- Minutes of the December 12, 2017, Exec CAC meeting
- Tumor Incidence Table in Male Rats
- Tumor Incidence Table in Female Rats
- Minutes of the March 10, 2020, Exec CAC meeting

Appendix A. Minutes of the December 12, 2017, Exec CAC meeting.

Executive CAC Protocol Minutes

Date of Meeting: December 12, 2017

Committee: Karen Davis Bruno, PhD, OND IO, Chair
Paul Brown, PhD, OND IO, Member
Tim McGovern, PhD, OND IO, Member
Ron Wange, PhD, OND IO, Member
John Leighton, PhD, DHOT, Alternate Member
Andrew Goodwin, PhD, DPARP, Pharm Tox Supervisor
Dong Zhao, PhD, DPARP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submissions in Electronic Format - Standardized Study Data (December 2014) and the latest Study Data Technical Conformance Guide.

IND# 135058

Drug Name: BCX7353

Sponsor: Biocryst Pharmaceuticals, Inc.

Background

Biocryst Pharmaceuticals is developing BCX7353, a small molecule kallikrein inhibitor, as an oral treatment of hereditary angioedema (HAE) under IND 135058. Kallikrein is a plasma enzyme that regulates the production of bradykinin. Bradykinin, as a vasodilatory agent, binds to the bradykinin B2 receptor and results in dilation of blood vessel, fluid leakage, and edema. BCX7353 was negative in the *in vitro* bacterial mutagenicity (Ames test), *in vitro* mammalian cell mutagenicity (human peripheral blood lymphocytes mutation assay) studies, and *in vivo* rat bone marrow micronucleus study. In the present submission, the sponsor submitted a Special Protocol Assessment request (SD 13 received 11/14/2017) for a carcinogenicity study evaluating BCX7353 in RasH2 transgenic (Tg.RasH2) mice.

Tg.rasH2 Mouse Carcinogenicity Study Dose Selection

BCX7353 dose selection is supported by the results of a 4-week toxicity study in Tg.RasH2 wildtype littermate mice (Study 20088903) that evaluated oral gavage doses of 0 (vehicle of water), 25, 50 or 125 mg/kg/day. The maximal tolerable dose (MTD) has been identified as 50 mg/kg/day based on mortalities, clinical signs and body weight decreases at 125 mg/kg/day and slight decreases in body weight gain at 50 mg/kg/day.

Biocryst is proposing dose levels of 0, 8, 20 and 60 mg/kg/day for the 6-month carcinogenicity study in Tg.RasH2 hemizygous mice. The sponsor's proposed high-dose was set at one-half the lethal dose of 125 mg/kg/day.

Executive CAC Recommendations and Conclusions

Tg.rasH2 Mouse

- The Committee recommended doses of 0, 8, 20, and 50 mg/kg/day for males and females with the high dose based on providing approximately one-third the exposure of the lethal dose of 125 mg/kg in the 28-day mouse toxicology study.
- The mid- and low-doses for both sexes were selected based on adequate spacing of AUC.
- The Committee noted that hematology at the end of the study is not needed for FDA.
- The Committee noted that the use of toxicokinetic dose groups is not needed for the FDA.
- The sponsor should conduct histopathological examination in all dose groups.

Karen Davis Bruno, PhD
Chair, Executive CAC

Appendix B. Tumor Incidence Table in Male Rats

Organ/ tissue name	Tumor name	Incidence (n = 60)			
		0 mg	3 mg	8 mg	20 mg
Adrenal glands	Adenoma, cortical	2	3	1	4
	Carcinoma, cortical	1	0	0	0
	Ganglioneuroma	0	1	0	0
	Pheochromocytoma	1	3	4	1
	Pheochromocytoma	1	0	1	0
Brain	Glioma	1	0	1	0
	Granular cell tumor	1	0	0	1
	Granular cell tumor	0	0	1	1
	Osteoma	0	0	0	1
Coagulating glands	Mesothelioma	0	1	0	0
Epididymis	Mesothelioma	0	2	0	0
	Osteosarcoma	0	0	0	1
Harderian glands	Adenoma	0	0	0	1
	Fibrosarcoma	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
Heart	Schwannoma, endocardial	0	1	1	1
Kidneys	Sarcoma, undifferentiated	0	0	0	1
L. intestine, cecum	Leiomyoma	1	0	0	0
Larynx	Fibrosarcoma	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
	Schwannoma	0	0	0	1
Liver	Adenoma, hepatocellular	0	1	0	0
	Sarcoma, undifferentiated	0	1	0	0
	Adenocarcinoma	0	0	0	1
	Carcinoma, cortical	1	0	0	0
	Osteosarcoma	0	0	0	1
Lung	Sarcoma, undifferentiated	0	0	0	1
Lymph Node, hepatic LN, Mesenteric	Schwannoma	0	0	0	1
	Sarcoma, undifferentiated	0	1	0	0
	Hemangiosarcoma	1	1	2	9
Mammary gland	Fibroadenoma	0	0	2	0
Mediastinum/pleural	Schwannoma	1	0	0	0
Mesentery/ peritoneal	Mesothelioma	0	2	0	0
	Osteosarcoma	0	0	0	1
	Sarcoma, undifferentiated	0	1	0	0
	Schwannoma	1	2	0	0
Multicentric neoplasm	Hemangioma	1	0	0	0
	Hemangiosarcoma	2	2	4	9
	Leukemia	0	0	2	0
	Lymphoma	1	3	1	0
	Sarcoma, histiocytic	0	0	1	2
Nose	Carcinoma, squamous cell	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
Pancreas	Adenocarcinoma	0	0	0	1
	Adenoma, acinar cell	1	0	0	1
	Adenoma, acinar-islet cell	1	0	0	0
	Adenoma, islet cell	2	2	2	1

	Carcinoma, acinar-islet cell	0	1	0	0
	Carcinoma, islet cell	0	0	1	0
	Mesothelioma	0	1	0	0
	Osteosarcoma	0	0	0	1
Parathyroid glands	Adenoma	1	2	1	0
Pituitary gland	Adenoma, pars distalis	17	23	19	16
	Adenoma, pars intermedia	1	0	1	1
	Glioma	0	0	1	0
	Pituicytoma, pars nervosa	1	1	0	0
Preputial glands	Carcinoma, squamous cell	1	0	0	0
	Papilloma, squamous cell	0	0	0	1
Prostate gland	Adenocarcinoma	0	0	0	1
	Adenoma	1	0	1	0
	Mesothelioma	0	1	0	0
	Osteosarcoma	0	0	0	1
Pancreas	Acinar cell adenomas/acinar isle	2	0	0	1
	Islet cell adenomas/mixed acinar islet	2	2	2	1
Salivary gland	Schwannoma	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
	Schwannoma	0	0	0	2
Seminal vesicles	Mesothelioma	0	1	0	0
	Osteosarcoma	0	0	0	1
Skeletal muscle	Sarcoma, undifferentiated	0	0	1	0
	Granular cell tumor	1	0	1	0
Skin	Adenoma, basal cell	0	1	0	1
	Carcinoma, basal cell	0	1	1	1
	Carcinoma, squamous cell	0	0	0	1
	Fibroma	1	2	2	1
	Fibrosarcoma	0	0	0	1
	Keratoacanthoma	1	2	2	4
	Lipoma	1	0	0	1
	Papilloma, squamous cell	1	1	1	0
	Sarcoma, undifferentiated	0	1	1	3
	Schwannoma	0	0	0	1
	Schwannoma	0	0	0	1
S. intestine, jejunum	Adenocarcinoma	1	0	0	0
	Leiomyosarcoma	0	0	1	0
	Schwannoma	0	1	0	0
Spleen	Mesothelioma	0	1	0	0
Stomach, glandular	Sarcoma, undifferentiated	0	1	0	0
	Schwannoma	0	0	0	1
Non-glandular	Sarcoma, undifferentiated	0	1	0	1
Skin	Basal cell adenoma + carcinoma	0	2	1	2
	Carcinoma squamous cell + keratoacanthom	2	3	3	5
Testes	Adenoma, Leydig cell	4	3	4	3
	Mesothelioma	0	1	0	0
Thymus	Schwannoma	1	0	0	0
	Thymoma	2	1	1	2
Thyroid gland	Adenoma, c-cell	7	11	10	6
	Adenoma, follicular cell	2	3	4	3

	Carcinoma, c-cell	0	2	0	1
	Carcinoma, follicular cell	2	0	0	0
Tongue	Fibrosarcoma	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
Tooth/teeth	Odontoma	0	0	0	1
Trachea	Schwannoma	0	0	0	1
Thyroid gland	Adenoma + carcinoma, follicular cell	4	3	4	3
Ureters	Osteosarcoma	0	0	0	1
Urinary bladder	Mesothelioma	0	1	0	0
Whole body	Hemangiosarcoma + hemangioma	3	2	4	9
Zymbal's gland	Carcinoma	0	1	1	0
	Sarcoma, undifferentiated	0	0	0	1
	Schwannoma	0	0	0	1

Appendix C. Tumor Incidence Table in Female Rats

Organ/ tissue name	Tumor name	Incidence (n = 60)			
		0 mg	3 mg	8 mg	20 mg
Adrenal glands	Adenoma, cortical	2	3	4	3
	Carcinoma, cortical	1	0	0	0
	Carcinoma, squamous cell	0	0	0	1
	Myelolipoma	0	0	0	1
	Pheochromocytoma	0	0	1	1
Bile duct, extrahepatic	Cholangiocarcinoma	1	0	0	0
Brain	Carcinoma, pars distalis	1	0	1	1
	Ependymoma	0	0	1	0
	Glioma	0	0	0	1
	Granular cell tumor	1	1	1	0
	Granular cell tumor	0	1	0	2
Calvaria	Carcinoma, pars distalis	0	0	1	0
Clitoral glands	Adenoma	0	0	0	1
Diaphragm	Sarcoma, undifferentiated	0	0	0	1
Ears	Sarcoma (primary site unknown)	0	0	0	1
Eyes	Leiomyosarcoma	1	0	0	0
Harderian glands	Leiomyosarcoma	1	0	0	0
Heart	Schwannoma, endocardial	1	0	1	1
Kidneys	Carcinoma, squamous cell	0	0	0	1
	Mesothelioma	0	0	0	1
	Sarcoma (primary site unknown)	0	0	0	1
Larynx	Sarcoma (primary site unknown)	0	0	0	1
Liver	Adenoma, hepatocellular	0	1	0	0
	Cholangiocarcinoma	1	0	0	0
	Cholangioma	0	0	0	1
	Sarcoma, endometrial stromal	0	0	0	1
	Lung	Adenocarcinoma	1	0	0
Lung	Carcinoma, islet cell	0	0	0	1
	Carcinoma, squamous cell	0	0	0	1
	Cholangiocarcinoma	1	0	0	0
	Sarcoma, endometrial stromal	0	0	0	1
	LN, mesenteric	Carcinoma, squamous cell	0	0	0
Mammary gland	Adenocarcinoma	4	6	5	1
	Adenoma	2	0	0	1
	Fibroadenoma,	13	17	10	4
Mesentery/ peritoneal	Adenocarcinoma + adenoma	6	6	5	2
	Carcinoma, squamous cell	0	0	0	1
	Leiomyosarcoma	1	0	0	0
	Mesothelioma	0	0	0	1
	Sarcoma, endometrial stromal	0	0	0	1
	Sarcoma, undifferentiated	0	0	0	1
	Schwannoma	1	0	0	0
Multicentric neoplasm	Hemangiosarcoma	4	2	1	9
	Lymphoma	0	0	0	1
Nerve, trigeminal	Carcinoma, pars distalis	1	0	0	0
Nose	Carcinoma, squamous cell	0	0	0	1
Oral cavity	Carcinoma, squamous cell	1	0	0	0
Ovaries	Granulosa cell tumor	0	0	1	0

	Luteoma	0	1	0	0
	Mesothelioma	0	0	0	1
	Schwannoma	0	0	1	0
	Sertoli cell tumor	1	0	1	0
	Sex-cord/stromal tumor	4	1	4	2
	Thecoma,	0	1	0	0
Pancreas	Adenoma, islet cell,	0	0	2	0
	Carcinoma, islet cell	0	0	0	1
	Carcinoma, squamous cell	0	0	0	1
	Leiomyosarcoma	1	0	0	0
	Mesothelioma	0	0	0	1
	Schwannoma	1	0	0	0
Pituitary gland	Adenoma, pars distalis,	34	38	30	19
	Carcinoma, pars distalis	1	0	1	2
Salivary gland, pa	Adenocarcinoma	1	0	0	0
	Adenoma,	0	0	0	1
Skin	Carcinoma, basal cell	0	0	0	1
	Carcinoma, sebaceous cell	1	0	0	0
	Fibroma,	0	0	0	1
	Keratoacanthoma,	0	0	0	1
	Liposarcoma	0	0	1	0
	Neural crest tumor,	0	0	0	1
	Sarcoma, undifferentiated	1	1	0	0
	Schwannoma	1	0	1	0
	Carcinoma + Keratoacanthoma	1	0	0	2
S. intestine, d	Carcinoma, squamous cell	0	0	0	1
Jejunum	Leiomyosarcoma	0	0	0	1
	Leiomyosarcoma	1	0	0	0
	Sarcoma, undifferentiated	0	0	0	1
Spinal cord	Carcinoma, pars distalis	0	0	0	1
Spleen	Sarcoma, undifferentiated	0	0	0	1
Stomach, gland.	Carcinoma, squamous cell	0	0	0	1
	Leiomyosarcoma	1	0	0	0
	Mesothelioma	0	0	0	1
	Sarcoma, undifferentiated	1	0	0	0
	Schwannoma	1	0	0	0
Thymus	Schwannoma	0	0	1	0
	Thymoma,	4	3	6	3
Thyroid gland	Adenoma, c-cell,	5	5	7	9
	Adenoma, follicular cell,	2	0	2	0
	Carcinoma, c-cell	0	0	0	1
	Carcinoma, follicular cell	1	0	0	0
	Sarcoma (primary site unknown),	0	0	0	1
	Adenoma + Carcinoma	5	5	7	10
	Follicular cell Adenoma + Carcinoma	3	0	2	0
Urinary bladder	Carcinoma, squamous cell	0	0	0	1
	Sarcoma, endometrial stromal	0	0	0	1
Uterus/cervix	Adenocarcinoma	3	1	1	3
	Carcinoma, squamous cell	1	1	2	1
	Leiomyoma	1	0	1	0

	Leiomyosarcoma	0	1	1	1
	Polyp, endometrial stromal	8	12	9	9
	Polyp, glandular	0	1	1	0
	Sarcoma, endometrial stromal	0	0	2	3
	Sarcoma, undifferentiated	0	0	0	1
	Schwannoma	1	0	0	0
Vagina	Carcinoma, squamous cell	0	0	1	0
	Sarcoma, endometrial stromal	0	0	0	1
Whole body	Hemangiosarcoma + hemangioma	4	2	1	9
Zymbal's gland	Carcinoma, zymbal's gland	1	0	0	2

Appendix D. Minutes of the March 10, 2020, Exec CAC Meeting dated.

Executive CAC Final Study Minutes

Date of Meeting: March 10, 2020

Committee: Karen Davis Bruno, PhD, OND IO, Chair
Paul Brown, PhD, OND IO, Member
Tim McGovern, PhD, OND IO, Member
Ron Wange, PhD, OND IO, Member
Federica Basso, PhD, DMEP, Alternate Member
Carol Galvis, PhD, DPARP, Pharm/Tox Team Leader
Luqi Pei, PhD, DPARP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

Application Type and Number(s): NDA 214094

Drug Name: Berotralstat (BCX7353)

Sponsor: BioCryst Pharmaceuticals

Background

BioCryst Pharmaceuticals filed NDA 214094 on December 3, 2019 seeking marketing approval of berotralstat, a kallikrein inhibitor, for the prevention of hereditary angioedema in adult and adolescent patients. The carcinogenicity potential of berotralstat was evaluated in a 2-year traditional bioassay in rats and in a 26-week study in transgenic mice (Reports #806-161 and #20088905, respectively). The Agency concurred with the dose selection of the protocol of the 26-week study in transgenic mice on December 14, 2017.

Mouse Carcinogenicity Study

B6F1 Tg.rasH2 mice (25/sex/dose) were dosed by oral gavage 0 (vehicle: deionized water), 8, 20, and 50-mg/kg berotralstat once daily for 26 weeks. No evidence of tumorigenicity was found in either sex. A positive control (75 mg/kg/day N-nitroso-N-methylurea) group showed increases in the incidence of tumors typical of the treatment in this model.

Rat Carcinogenicity Study

Rats (60/sex/dose) were dosed by oral gavage with 0 (vehicle: deionized water), 3, 8, and 20-mg/kg berotralstat once daily for 104 weeks. The male rats showed statistically significant, dose-related increases in the incidence of hemangiosarcomas (HS) in the mesenteric lymph nodes (Table 1). In pairwise comparisons between the high dose (HD) and controls (C), only the increase in the HS incidence in the mesenteric lymph nodes reached statistical significance ($p < 0.01$); the test for HS in the whole body reached a marginal significance ($p=0.017$); the test for the HS and hemangioma in combination in the whole body did not reach statistical significance ($p=0.041$). The trend analyses for all the above tumor findings were significant ($p < 0.005$).

The high dose rats in the study showed clinical signs of labored and audible breathing during the study. Microscopic examination revealed presence of nasal exudate and sinus histiocytosis in the mesenteric lymph node. These findings suggest potential presence of tissue hypoxia in these rats.

The relevance of the HS findings associated with berotralstat treatment in rats to human is unknown. Cohen et al [*Toxicol Sci*, 2009;111(1)4-18] reported that HS and hemangiomas associated with non-genotoxic chemicals in rodents may be attributed to hypoxia. Hypoxia that appeared present in the high dose in rats may play a significant role in the carcinogenesis of berotralstat, but tissue specificity of the finding suggests that other factors may also play important roles. These factors may include higher exposures of the tissue to the parent compound, metabolites, and/or drug impurities. Overall, relevance of the HS finding to humans remains unknown.

Table 18: Incidence of Hemangiosarcoma in Male Rats

Tumor, organ	Sex	Incidence (n = 60)				p value	
		0	3	8	20	Pairwise ^a	Trend
Hemangiosarcoma (HS), total	M	2	2	4	9 (15.0%)	0.0166	0.002
Mesenteric lymph node	M	1	1	2	9 (15.0%)	0.0049	0.0001
Skin/adipose tissues	M	1	1	2	0	- ^b	-
HS + hemangioma, total	M	3	2	4	9 (15.0%)	0.041	0.004

a. Pairwise comparison between HD and C groups.

b. (-), not calculated.

Executive CAC Conclusions

Mouse study:

- The Committee concluded the carcinogenicity study is adequate and negative for drug-related neoplasms in males and females.

Rat study:

- The Committee concluded the carcinogenicity study is adequate.
- The Committee concluded that berotralstat is positive for drug-related neoplasms in male rats. The high dose of berotralstat (20 mg/kg/day) caused a statistically significant increase in hemangiosarcoma in male rats in the mesenteric lymph node. ExecCAC criteria for statistical significance were not reached for hemangiosarcoma in all tissues or for hemangiosarcoma and hemangioma combined in all tissues.
- The Committee concluded that the mechanism of carcinogenesis of berotralstat in rats remains unclear and that the relevance of berotralstat-induced tumorigenesis in rats to humans is unknown.
- The Committee concluded that berotralstat tested negative for drug-related neoplasms in female rats.

Karen Davis Bruno, PhD
Chair, Executive CAC

APPEARS THIS WAY ON ORIGINAL

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/

LUQI PEI
03/24/2020 01:27:13 PM

CAROL M GALVIS
03/24/2020 01:31:38 PM
I concur.