CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214460Orig1s000
214461Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA ASSESSMENT AND EVALUATION

Application Numbers:	214460 & 214461
Supporting Document Number/s:	1, 2, 3, 5 & 17
CDER Receipt Date:	May 29th, June 30th, August 28th & October 7th, 2020, and January 11th, 2021
Applicant:	Chimerix, Inc.
Product:	TEMBEXA™ (Brincidofovir)
Pharmacologic Class:	Orthopoxvirus nucleotide analog DNA polymerase inhibitor
Indication:	The treatment of human smallpox disease in adult and pediatric patients, including neonates
Therapeutic area:	Anti-viral
Clinical Review Division:	Division of Antivirals (DAV)
Pharm/Tox Division	Division of Pharm/Tox for Infectious Diseases (DPT-ID)
Reviewers:	David McMillan, Ph.D., DABT Mark Seaton, Ph.D., DABT
Supervisor/Team Leader:	L. Peyton Myers, Ph.D., DABT
Project Manager:	Andrew Gentles, Pharm.D.
Reviewer Completion Date:	May 3, 2021

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDAs 214460 and 214461 are owned by Chimerix, Inc., or are data for which Chimerix, Inc., has obtained a written right of reference. Any information or data necessary for approval of NDAs 214460 and 214461 that Chimerix, Inc., does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of either NDA 214460 or 214461.

TABLE OF CONTENTS

GLOSSARY

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the plasma vs. time concentration curve
BCV	Brincidofovir
BUN	Blood urea nitrogen
CDV	Cidofovir
CDV-PP	Cidofovir diphosphate
CK	Creatine kinase
C_{max}	Maximum observed plasma concentration
ECTV	Ectromelia virus
GD	Gestation day
GI	Gastrointestinal
GLP	Good laboratory practice
IV	Intravenous
LD	Lactation day
NOAEL	No observed adverse effect level
NMT	Not more than
PBS	Phosphate-buffered saline
pfu	Particle-forming units
PK	Pharmacokinetics
PND	Postnatal day
PPND	Prenatal and postnatal development
RPXV	Rabbitpox virus
TK	Toxicokinetics
T_{max}	Time of maximum concentration

1. Executive Summary

1.1 Introduction

TEMBEXA™ (Brincidofovir; BCV; CMX001) is a lipophilic nucleotide analogue formed by covalently linking a lipid moiety to cidofovir (VISTIDE™; CDV; CMX021). The lipid moiety dictates the drug's distribution and pharmacokinetic (PK) properties in target organs, while the antiviral activity is contained within the nucleotide residue. The lipid conjugate is designed to utilize uptake pathways to achieve high intracellular concentrations of BCV. Once inside target cells, the lipid side chain of BCV is cleaved to yield free CDV. Conversion of CDV to the active antiviral agent, CDV diphosphate (CDV-PP), occurs via a two-step phosphorylation process catalyzed by intracellular anabolic kinases.

Cidofovir diphosphate (CDV-PP) exerts its antiviral effects by acting as a potent alternate substrate inhibitor of viral DNA synthesis. The small molecule nucleotide analog competitively inhibits incorporation of deoxycytidine triphosphate into viral DNA by viral DNA polymerase, and may selectively incorporate into viral DNA, thereby inhibiting viral DNA chain elongation.

The Applicant has submitted two marketing applications for BCV as an antiviral agent for treatment of human smallpox disease caused by variola virus in adult and pediatric patients. The proposed oral tablet dose, covered under NDA 214461, is 200 mg (2x100 mg tablets) once weekly for 2 doses (Days 1 and 8). The proposed BCV treatment regimen with the oral suspension, covered under NDA 214460, is 4 mg/kg for adult and pediatric patients weighing 10 to <48 kg, and 6 mg/kg for pediatric patients, including neonates, weighing <10 kg, up to a maximum of 200 mg once weekly for 2 weeks (on Days 1 and 8). The Applicant has submitted a complete nonclinical package of studies in mice, rats, rabbits, and nonhuman primates.

1.2 Brief Discussion of Nonclinical Findings

Efficacy/Primary Pharmacology Summary

The Applicant is seeking approval of BCV for treatment of smallpox disease under Animal Rule (21 CFR 314.610). Due to the infeasibility of evaluating BCV efficacy in humans or in a monkey model of variola virus infection, two surrogate animal models of orthopoxvirus disease using surrogate viruses were needed. Pivotal efficacy studies were therefore conducted in two animal models of orthopoxvirus disease, an ECTV model in mice and a RPXV model in rabbits. The monkey/monkeypox model was not used due to low intracellular concentrations of BCV metabolites in this species.

In the pivotal ECTV study #CMX001-VIR-044, 6- to 8-week-old mice were challenged with 200 pfu purified ECTV, strain Moscow (actual titer = 188-196 pfu), by intranasal aspiration on Day 1. BCV was administered in 3 doses spaced 48 hours apart (either 10, 5 and 5 mg/kg/dose or 20, 5 and 5 mg/kg/dose) by oral gavage starting on Days 5, 6, 7 or 8 post-challenge, corresponding to the timing of symptom onset. Both BCV regimens (10/5/5 and 20/5/5) were protective against ECTV-induced mortality, with survival highest in mice treated with 20/5/5 starting on Day 4 (84%) and Day 5 (75%). The lower regimen of 10/5/5 was also protective, with survival of 78% and 66% when started on Days 4 and 5, respectively. By comparison, survival in the

placebo group was 12.5%. No clear, drug-related differences in ECTV-induced secondary endpoints of clinical signs, body weights, viral loads were observed.

BCV efficacy in the RPXV model was established in the pivotal GLP study #CMX001-VIR-106. In this study, 13- to 16-week old rabbits were challenged intradermally on Day 0 with purified RPXV, strain Utrecht (target titer = 600 pfu, actual titer = 488 pfu). BCV was administered in 3 doses spaced 48 hours apart (20, 5 and 5 mg/kg/dose) by oral gavage starting 3, 4, 5 and 6 days post-challenge (Groups 1-4, respectively; placebo = Group 5), which corresponds to the timing of symptom onset. No clear, drug-related differences in RPXV-induced clinical signs, body weights, lesion counts or challenge site erythema/edema were observed. However, survival was 100%, 90%, 69%, 69%, and 29% in Groups 1-5, respectively, indicating that BCV was protective against RPXV-induced mortality, particularly in Groups 1 and 2. Viral loads also tended to be lower on Days 6 and 8 in Groups 1 and 2 relative to Group 5 (placebo). Body temperatures were significantly above concurrent controls in Groups 1, 2 and 4 on Days 6 and 7, in Group 4 on Days 8 and 9, and in Group 3 on Day 9.

Taken together, BCV was confirmed to be effective in reducing mortality in both the RPXV and ECTV models of orthopoxvirus disease. The studies reviewed were therefore considered to be sufficient to support approval of BCV for the treatment of smallpox infection and disease in humans via the Animal Rule pathway.

Toxicology Summary

The nonclinical toxicology of BCV has been evaluated in both *in vitro* and *in vivo* genotoxicity assays and safety pharmacology studies, and in single- and repeat-dose, oral and intravenous (IV) administration toxicology studies in mice, rats, rabbits, and cynomolgus monkeys. As a result of the mechanism of action (i.e., DNA chain-terminating activity of the nucleoside analogue), BCV-related nonclinical toxicities tended to affect rapidly dividing cell populations of the gastrointestinal (GI) epithelium, spermatogonia, and sebaceous glands (after IV administration). Nonclinical studies with BCV also demonstrated adverse effects on the developing fetus in rats and rabbits and a potent tumorigenic effect in rats.

Gastroenteropathy was dose limiting in rats and monkeys following daily oral dosing at systemic exposures less than the expected human exposure based on the recommended dose of TEMBEXA. In longer-term oral studies, animals were dosed less frequently to minimize the gastric and enteral effects. Gastric-related adverse events were observed in clinical trials. In repeat-dose studies up to 6 months in rats and 9 months in monkeys, when BCV was administered orally twice weekly to avoid gastric toxicity, or intravenously to increase systemic exposures, the primary toxic effects of treatment with BCV were on reproductive organs. Depletion of spermatogenesis and epididymal hypospermia were noted in rats, and testicular atrophy, epididymal, and seminal vesicle/prostate changes were observed in monkeys. In addition to effects on reproductive organs, palpable tissue masses were identified in BCV-treated rats as malignant mammary gland adenocarcinomas and squamous cell carcinomas. Findings in rats and monkeys were at systemic exposures less than the expected human exposure.

BCV was positive for increased structural chromosomal aberrations in the absence of metabolic activation in an *in vitro* assay and was carcinogenic in rats

following once- or twice-weekly oral and IV administration in 13-week and 26-week studies.

Treatment with BCV caused notable reductions in mating and/or fertility, and resulted in reduced maternal and fetal body weights, and decreased embryo viability in rats. In rabbits, decreased mean body weight and food consumption in dams, and BCV-related effects on intrauterine growth (lower mean fetal weights), survival (higher late resorptions) and morphology (external malformations and visceral and skeletal developmental variations) were noted. Findings in reproductive toxicology studies were associated with systemic exposures in rats and rabbits less than the expected human exposure.

The submitted studies represent a complete nonclinical toxicology package and the safety profile has been determined. With appropriate monitoring and precautions, the potential benefit outweighs the identified risks and it is recommended that BCV be approved as a treatment of human smallpox disease caused by variola virus in adult and pediatric patients.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted are sufficient to support approval.

1.3.2 Additional Nonclinical Recommendations

No recommendations at this time.

1.3.3 Labeling

Labeling is under review. Final labeling will be reflected in the approval package.

2. Drug Information

2.1 Drug

CAS Registry Number:	496765-79-8
Generic Name:	Brincidofovir (BCV)
Code Name:	CMX001
Chemical Name:	Phosphonic acid, [[(s)-2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl) ethoxy]methyl]mono[3-(hexadecyloxy)propyl] ester, (b) (4)
Molecular Formula/Weight:	(b) (4)
Structure:	

Pharmacologic Class: Orthopoxvirus nucleotide analog DNA polymerase inhibitor

2.2 Relevant INDs

INDs (b) (4) 65480 and 67681, and NDA 20638.

2.3 Drug Formulation

The free acid of BCV is a white to off-white powder. TEMBEXA™ is available as both 100 mg, film-coated, immediate release tablets (NDA 214461) and as a 10 mg/mL, aqueous-based, preserved oral suspension (NDA 214460). The compositions of both forms are presented in **Tables 1 & 2**.

Note: All tables and figures are excerpted from the Applicant's reports.

Table 1: Composition of the BCV 100 mg oral tablets

Ingredients	Function	Weight Composition (% w/w)	Quantity per Tablet (mg)
Brincidofovir	Active	(b) (4)	100.0
Silicified Microcrystalline Cellulose, NF/EP	(b) (4)		
Crospovidone, NF/EP			
Microcrystalline Cellulose, NF/EP			
Mannitol, USP/EP			
Colloidal Silicon Dioxide, NF/EP			
Magnesium Stearate, NF/EP			
(b) (4)			
Purified Water, USP/EP			
COATED TOTAL			
(b) (4)			

Table 2: Composition of the BCV 10 mg/mL oral suspension

Component Description	Function	Formula Composition (mg/mL)	Weight Composition (% w/w)[1]
[redacted]	Active	10.000	[redacted]
Microcrystalline Cellulose and Carboxymethyl Cellulose Sodium	[redacted]		
Xanthan Gum [redacted] USP/NF			
Simethicone 30% Emulsion, USP/NF			
Citric Acid Anhydrous, USP/NF			
Trisodium Citrate Anhydrous, USP/NF			
Sodium Benzoate, USP/NF			
Sucralose, Ph.Eur., USP/NF			
[redacted] Lemon Lime Flavor [redacted]			
Purified Water, USP/NF			
Total			

[redacted]

2.4 Comments on Novel Excipients

No novel excipients are included in the BCV drug product. All excipient exposures fall below maximum potencies listed for approved products in the FDA Inactive Ingredient Database.

2.5 Comments on Impurities/Degradants of Concern

[redacted] are impurities identified in the drug substance (DS) release specification, their respective limits are not more than (NMT) [redacted]%, NMT [redacted]%, and NMT [redacted]%. Compounds [redacted] contain [redacted] a structural alert for genotoxicity; however, the DS was negative in the Ames assay for mutagenicity. Therefore, [redacted] are appropriately controlled as non-mutagens. Toxicology studies have been provided for compounds [redacted] that support limits of NMT [redacted]%, NMT [redacted]%, and NMT [redacted]%, respectively in the DS. For [redacted] limits of NMT [redacted]% (oral suspension) and [redacted]% (tablet) are supported in the drug product.

2.6 Proposed Clinical Population and Dosing Regimen

TEMBEXA™ will be marketed as both an oral tablet and as an oral suspension for adults and pediatric patients <48 kg or otherwise unable to take solid medication. The proposed dose is 200 mg (2x100 mg tablets) once weekly for 2 doses. For the oral

suspension, the proposed dose is either 4 mg/kg for patients from 10 to <48 kg, or 6 mg/kg for patients <10 kg, up to a maximum of 200 mg once weekly for 2 weeks (on Days 1 and 8).

2.7 Regulatory Background

CDV was evaluated under INDs (b) (4) and 65480, and was approved under NDA 20638. The parent IND for BCV, IND 67681, was submitted on May 12th, 2005.

3. Studies Submitted

The Applicant submitted a complete nonclinical safety package including absorption, distribution, metabolism, and excretion studies, safety pharmacology studies, genotoxicity studies, single, and repeat-dose general toxicology studies in mice, rats, and monkeys, a carcinogenicity study in rats, and reproductive toxicology studies in rats and rabbits. Routes of administration included oral and intravenous. Reviews of pivotal studies are included in sections 6 through 10.

4. Pharmacology

4.1 Primary Pharmacology

BCV is a lipophilic nucleotide analogue formed by covalently linking a lipid moiety to cidofovir (CDV). The lipid moiety is responsible for the distribution and pharmacokinetic (PK) properties of the drug, while the antiviral activity is contained within the nucleotide residue. The lipid conjugate is designed to utilize uptake pathways to achieve high intracellular concentrations of BCV. Once inside target cells, the lipid sidechain of BCV is cleaved to yield free CDV. Conversion of CDV to the active antiviral agent, cidofovir diphosphate (CDV-PP), occurs via a two-step phosphorylation process catalyzed by intracellular anabolic kinases. CDV-PP exerts its antiviral effects by acting as a potent alternate substrate inhibitor of viral DNA synthesis.

The primary pharmacology studies encompass multiple proof-of-concept studies in multiple species. The primary species used to support variola virus efficacy in humans are the rabbit infected with RPXV and the mouse infected with ECTV. Although multiple studies were submitted, only the pivotal studies used to support efficacy were reviewed here. For further information, please refer to the Virology review by Dr. Patrick Harrington.

Mouse/Ectromelia (ECTV) Efficacy Studies

Study Title: Determination of the Potency and LD_{50} of Ectromelia virus-Moscow in the Mouse Model

Study no.:	2955-100029460
Study report location:	4.2.1.1
Study initiation date:	August 16th, 2013
Conducting laboratory and location:	(b) (4)

	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This study was conducted to determine the lethality of purified ECTV, strain Moscow, in BALB/c mice. This strain of ECTV was purified under study #2954-100029460. Six- to eight-week-old mice (8/sex/group) were challenged intranasally on Day 0 with ECTV at either 5, 50, 200, 500 or 1000 pfu (Groups 1-5, respectively). Actual titers for Groups 1-5 were 2.44, 27.5, 119.5, 292.5 and 700 pfu, respectively. All surviving animals were euthanized on Day 14 post-challenge. BCV was not administered in this study, and a sham control was not included. Endpoints included mortality, clinical signs, and body weights. Ketamine (100 mg/kg)/xylazine (10 mg/kg) was administered by IP injection prior to challenge and euthanasia. The euthanasia criteria were essentially identical to those used in natural history study #CMX000-VIR-111, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

ECTV-induced mortality was 62.5% (10/16 animals) in Group 1, 87.5% (14/16 animals) in Group 2, 93.8% (15/16 animals) in Group 3, and 100% in Groups 4 and 5. All deaths occurred within 6-13 days post-challenge, with median times of death of 192-260 hours (time of death decreased with increasing challenge doses). The LD_{50} and LD_{90} values for this ECTV strain were <2.44 and 32.10 pfu, respectively. Clinical signs included lethargy, ruffled fur, hunched posture, lacrimation, and ear and tail lesions. These symptoms appeared around Day 4 in Group 5, Day 5-8 in Groups 3 and 4, and Day 8-10 in Groups 1 and 2. Body weights also decreased by about 2-3 grams starting around Day 2 in Group 5, and around Day 6 in Groups 1-4. Weight decreased steadily prior to death in all animals, but recovered by roughly 3 grams in most surviving animals by Day 14. All findings were consistent with ECTV infection and disease in this species.

Study Title: Natural History Study of Intranasal Ectromelia virus Infection in the BALB/c Mouse Model

Study no.:	2956-100029460
Study report location:	4.2.1.1
Study initiation date:	September 27th, 2013
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This study was conducted to determine the disease progression following infection from purified ECTV, strain Moscow, in BALB/c mice. This strain of ECTV was purified under study #2954-100029460. Six- to eight-week-old mice were challenged intranasally on Day 0 with either sham (PBS) or ECTV at 200 pfu (actual titer = 125 pfu). All surviving animals were euthanized in groups every 12 hours post-challenge (2/sex/group) up to on 192 hours (Day 8) and on Day 14. BCV was not administered in this study. Endpoints included mortality, clinical signs, body weights, body temperatures, viral load in blood, oropharyngeal swabs and spleen and liver tissues, hematology, clinical chemistry, and gross/histopathology. Ketamine (100 mg/kg)/xylazine (10 mg/kg) was administered by IP injection prior to challenge and euthanasia. The euthanasia criteria were essentially identical to those used in natural history study #CMX000-VIR-111, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

No deaths or findings were observed in the sham-challenged groups. Mortality in the ECTV-infected groups was 100% by Day 14, with all animals succumbing between Day 7 and 10 (median time of death was 8.94 days). Clinical signs in the ECTV-challenged animals included lethargy, ruffled fur, hunched posture, lacrimation, abnormal breathing, labored breathing, clear discharge from and/or partial closure of one or both eyes, and ear and tail lesions. These symptoms appeared around Days 5-6 and peaked around Days 7-8, just prior to death. Body weights in the ECTV groups were decreased roughly 5 grams between Day 6 and the time of death. Body temperatures also decreased about 8 ºF on Day 9 in the ECTV groups. BUN was elevated in >50% of the ECTV-challenged animals, and AST and ALT were elevated in a much smaller fraction (3-15%), but generally no meaningful ECTV-related changes in hematology or clinical chemistry parameters were observed. Histopathologic lesions in the surviving animals included lymphoid necrosis in the white pulp of the spleen, which appeared 120 hours post-challenge. More extensive necrosis in the red and white pulp of the spleen as well as multifocal hepatocyte necrosis with viral inclusions in adjacent hepatocytes in the liver were also seen in the animals that died prematurely. All findings were consistent with ECTV infection in this species. ECTV viremia was also confirmed in the blood, oropharyngeal swabs, liver and spleen starting roughly 3-5 days post-challenge, and increased steadily through Day 8, prior to death. No endpoints were established as clear triggers to treat in this study.

Study Title: Assessment of the Ability to Identify Triggers to Treat in the ECTV Mouse Model

Study no.:	2957-100029460
Study report location:	4.2.1.1
Study initiation date:	October 30th, 2013
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study

Deviations: None which meaningfully affected study interpretation.

Summary

This study was conducted to evaluate the disease progression and identify clinical parameters that could be used for triggers to treat following disease onset following infection from purified ECTV, strain Moscow, in BALB/c mice. This strain of ECTV was purified under study #2954-100029460. Six- to eight-week-old mice (5-24/sex/group) were challenged intranasally on Day 0 with either sham (PBS) or ECTV at 200 pfu (actual titer = 98 pfu). All surviving animals were euthanized on Day 14 post-challenge. BCV was not administered in this study. Endpoints included mortality, clinical signs, body weights, body temperatures, viral load in blood, oropharyngeal swabs and spleen and liver tissues, hematology, clinical chemistry, and gross pathology. Ketamine (100 mg/kg)/xylazine (10 mg/kg) was administered by IP injection prior to challenge and euthanasia. The euthanasia criteria were essentially identical to those used in natural history study #CMX000-VIR-111, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

No deaths or findings were observed in the sham-challenged groups. Mortality in the ECTV-infected groups was 100% by Day 14, with all animals succumbing between Day 7 and 11 (median time of death was 8.20 days). Clinical signs in the ECTV-challenged animals included hunched posture, lacrimation, labored breathing, and ruffled fur. These symptoms appeared around Days 3-4 and peaked around Days 7-8 prior to death. Lethargy and lesions were also observed in some of the control animals, though the study director attributed the lesions in these animals to biting and scabbing rather than a dosing error. As a result, lesion data was not evaluated in this study. Body weights in the ECTV animals were decreased 2-3 grams between Day 6 and the time of death. Body temperatures in the ECTV groups fluctuated greatly between Days 8-11, but generally decreased up to 15-20 ºF from Day 8 to the time of death. Several fluctuations in clinical pathology parameters were observed in the ECTV-challenged animals, but no meaningful changes were observed that were also considered reliable triggers to treat. Gross pathology findings in the ECTV-challenged animals included discoloration of the liver and spleen only (histopathology was not evaluated). All findings were consistent with ECTV infection in this species. ECTV viremia was also confirmed in the blood, oropharyngeal swabs, liver and spleen starting roughly 4-5 days post-challenge, and increased steadily through at least Day 7, prior to death. Viremia was the only endpoint determined to be a reliable trigger to treat in this study.

Study Title: Determination of Lethality for Ectromelia Virus (ECTV) Strain Moscow in the Balb/c Mouse Model

Study no.: CMX000-VIR-107
Study report location: 4.2.1.1
Study initiation date: February 27th, 2017
Conducting laboratory and location: (b) (4)
GLP compliance: No

Drug, lot #, and % purity: No drug was used in this study
Deviations: None which meaningfully affected study interpretation.

Summary

This study was conducted to determine the lethality of purified ECTV, strain Moscow, in BALB/c mice. Eight-week-old mice (9/sex/group) were challenged intranasally on Day 0 with ECTV at either 1000 or 200 pfu (Groups 1 and 2, respectively; CMRX 3571 lot #032516-ECTV). Actual titers were 948 and 192 pfu for Groups 1 and 2, respectively. All surviving animals were euthanized on Day 21 post-challenge. BCV was not administered in this study, and a sham control was not included. Endpoints included mortality, clinical signs, and body weights. Whole blood, liver and spleen samples were collected at the time of euthanasia but were not analyzed. Ketamine (100 mg/kg)/xylazine (10 mg/kg) was administered by IP injection prior to challenge and euthanasia. The euthanasia criteria were essentially identical to those used in natural history study #CMX000-VIR-111, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

All Group 1 animals either died prematurely or were euthanized by Day 11 post-challenge, with a median time of death of 193.3 hours, or 8.1 days. Mortality in Group 2 was 78% (1 male and 3 females survived until Day 21), and the median time of death was 237.4 hours, or 9.9 days. The time of death was significantly longer in Group 2 compared to Group 1, but no significant difference in survival rate was observed. Clinical signs included lethargy, ruffled fur, hunched posture, lacrimation, clear discharge from one or both eyes, eye closure, and respiratory abnormalities. These symptoms appeared as soon as Day 1 in both groups, and peaked around Days 6 and 8 in Groups 1 and 2, respectively. Clinical signs were generally worse and appeared sooner in males vs. females. Body weights also decreased by about 3-4 grams starting around Day 6 in Group 1 and Day 7 in Group 2. Weight decreased steadily prior to death in all animals, but recovered by roughly 3 grams in the surviving Group 2 animals by Day 21. All findings were consistent with ECTV infection and disease in this species. An LD_{50} was not determined in this study, but the lethality of this ECTV strain was confirmed.

Study Title: Determination of LD_{50}/LD_{90} of Ectromelia Virus, CMRX 3571, in the Intranasal BALB/c Mouse Model

Study no.: CMX000-VIR-109
Study report location: 4.2.1.1
Study initiation date: August 18th, 2017
Conducting laboratory and location: (b) (4)
GLP compliance: No
Drug, lot #, and % purity: No drug was used in this study
Deviations: None which meaningfully affected study interpretation.

Summary

This study was conducted to determine the lethality of three different stocks of purified ECTV, strain Moscow, in BALB/c mice. Eight-week-old mice (9/sex/group) were challenged intranasally on Day 0 with one of three ECTV stocks (CMRX 3571 lot #032516-ECTV, (b) (4) ECTV-Mos lot #Jan 7 2015 KD, or BARDA 2954 lot #ECTV BARDA-ST-02) at 5, 50, 200, and 500 pfu, and 1000 pfu for CMRX 3571 and (b) (4) ECTV-Mos only. Actual titers were 5.5, 55.5, 246.5, 697.5 and 1580 pfu for CMRX 3571 (Groups 1-5), 2.42, 24.7, 107.3, 292.5 and 650 pfu for (b) (4) ECTV-Mos (Groups 6-10), and 4.7, 47, 203.3 and 477.5 pfu for BARDA 2954 (Groups 11-14). All surviving animals were euthanized on Day 21 post-challenge. BCV was not administered in this study, and a sham control was not included. Endpoints included mortality, clinical signs, body weights, and body temperature. Whole blood, liver and spleen samples were collected at the time of euthanasia but were not analyzed. Ketamine (80 mg/kg)/xylazine (5 mg/kg) was administered by IP injection prior to challenge and euthanasia. The euthanasia criteria were essentially identical to those used in natural history study #CMX000-VIR-111, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

For CMRX 3571 and (b) (4) ECTV-Mos, mortality was 77.8% (14/18 animals) at 5 pfu, and 100% at 50-1000 pfu. For BARDA 2954, mortality was 66.7% (12/18 animals) at 5 pfu, 88.9% (16/18 animals) at 50 pfu, and 100% at 200 and 500 pfu. The LD_{50} values for CMRX 3571, (b) (4) ECTV-Mos and BARDA 2954 were 1.73, 0.76, and 2.26 pfu, respectively. Similarly, the LD_{90} values for CMRX 3571, (b) (4) ECTV-Mos and BARDA 2954 were 14.17, 6.25, and 35.07 pfu, respectively. Median time of death was 182.54-244.13 hours for CMRX 3571, 185.58-261.44 hours for (b) (4) ECTV-Mos, and 191.32-265.36 hours for BARDA 2954. No significant differences in mortality or time of death were observed for any group.

Clinical signs included lethargy, tail lesions, ruffled fur, hunched posture, lacrimation, partial closure/swelling of one or both eyes, and respiratory abnormalities. These symptoms appeared within 24 hours post-challenge and peaked around 3-6 days post-challenge with all three ECTV stocks. Additionally, the time to symptom onset and peak were generally shorter in animals that were challenged with a higher titer of virus. Body weights also decreased by about 3-4 grams starting around Day 6 in all groups receiving CMRX 3571 and (b) (4) ECTV-Mos before recovering slightly in the surviving animals, particularly in those who received a lower challenge dose. For BARDA 2954, body weights were also decreased by 3-4 grams starting around Day 6 in animals receiving ≥50 pfu, but weights were largely unchanged in those receiving 5 pfu. Lastly, body temperatures were decreased by up to 13 ^{o}C starting around Day 7-8 with all ECTV stocks at titers ≥50 pfu, though temperatures were largely unchanged in those that received 5 pfu. Temperatures also recovered in all animals that survived ECTV challenge. All findings were consistent with ECTV infection and disease in this species.

Study Title: Natural History Study of Intranasal Ectromelia Virus (CMRX 3571) Infection in the BALB/c Mouse Model

Study no.: CMX000-VIR-111
Study report location: 4.2.1.1
Study initiation date: March 26th, 2018

Conducting laboratory and location:	(b) (4) [redacted]
GLP compliance:	Yes
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This study was conducted to characterize the disease progression of ECTV infection in 8-week-old mice. BCV was not administered in this study. The primary objective was to ensure that challenge with ECTV produced a robust, reproducible viremia and disease course that was consistent with prior studies of ECTV infection in this species. In addition, several clinical parameters were evaluated as potential early markers of ECTV infection that could be used as "triggers to treat" in follow-up efficacy studies. To qualify as triggers to treat, these parameters must have been specific for ECTV infection, unambiguous, reproducible and objective, and early enough in the disease course to allow intervention corresponding to the therapeutic window in human smallpox.

Survival in the ECTV-infected group was 15.7% by Day 21, with a median time of death of 8.6 days post-challenge. Clinical signs in the ECTV-challenged animals included rough hair coat, breathing abnormalities, lethargy, lacrimation, hunched posture, and moribundity. Decreased body weights and temperature and histopathologic lesions in the spleen and liver were observed in the ECTV-infected groups, though no gross, pustular rashes or lesions and no meaningful changes in clinical pathology parameters were observed in these animals. All findings were consistent with ECTV infection in this species. ECTV viremia was also confirmed in the blood, liver and spleen starting roughly 60 hours post-challenge. Of the endpoints evaluated, ECTV viremia was considered the most promising trigger to treat, being elevated starting roughly 2-3 days post-challenge.

Methods

Species/Strain:	BALB/c mice (b) (4) [redacted]
Number/Sex/Group:	2-6/sex/group
Age/Sexual Maturity:	8 weeks
Challenge Agent Info:	Ectromelia Virus (ECTV), Moscow strain
Challenge Agent Lot:	CMRX 3571, lot #032516-ECTV
Target Challenge Dose:	200 pfu/25 µL PBS (100 pfu/12.5 µL per naris)
Frequency of Dosing:	Single dose on Day 0
Route of Administration:	Intranasal administration via micropipette
Back Titrated Final Dose:	194 pfu
Comment on Study Design and Conduct:	ECTV challenge was conducted on Day 0. Animals were monitored through Day 21, though all were euthanized in groups according to the schedule in **Table 3**, from Day 2 (48 hours post-challenge) to Day 21 (504 hours post-challenge).

Table 3: Design of ECTV natural history study #CMX000-VIR-111

Post-Infection Day	Group #	Challenge Type	No of Animals (M/F)	Time of Scheduled Euthanasia (Hour)	Number of Mice Assessed[1] (M/F)		
					Viral Load and IL-4 Cytokine	Clinical Pathology[2]	Pathology[2]
0	All Groups	All Groups	All Animals	NA	NA	NA	NA
1				NA	NA	NA	NA
1.5				NA	NA	NA	NA
2	1	3571 ECTV	6/6	48	4/4	2/2	6/6
2	2	Sham	4/4	48	2/2	2/2	4/4
2.5	3	3571 ECTV	4/4	60	4/4	NA	4/4
2.5	4	Sham	2/2	60	2/2	NA	2/2
3	5	3571 ECTV	4/4	72	4/4	NA	4/4
3	6	Sham	2/2	72	2/2	NA	2/2
3.5	7	3571 ECTV	4/4	84	4/4	NA	4/4
3.5	8	Sham	2/2	84	2/2	NA	2/2
4	9	3571 ECTV	6/6	96	4/4	2/2	6/6
4	10	Sham	4/4	96	2/2	2/2	4/4
4.5	11	3571 ECTV	4/4	108	4/4	NA	4/4
4.5	12	Sham	2/2	108	2/2	NA	2/2
5	13	3571 ECTV	4/4	120	4/4	NA	4/4
5	14	Sham	2/2	120	2/2	NA	2/2
5.5	15	3571 ECTV	4/4	132	4/4	NA	4/4
5.5	16	Sham	2/2	132	2/2	NA	2/2
6	17	3571 ECTV	6/6	144	4/4	2/2	6/6
6	18	Sham	4/4	144	2/2	2/2	4/4
6.5	19	3571 ECTV	4/4	156	4/4	NA	4/4
6.5	20	Sham	2/2	156	2/2	NA	2/2
7	21	3571 ECTV	4/4	168	4/4	NA	4/4
7	22	Sham	2/2	168	2/2	NA	2/2
7.5	23	3571 ECTV	4/4	180	4/4	NA	4/4
7.5	24	Sham	2/2	180	2/2	NA	2/2

[1]Refer to Table 3 for details about sample collection for all assessments.
[2]Pathology included complete necropsy for all study animals and histopathology of liver and spleen for clinical pathology animals and animals that were unscheduled euthanized.

Table 3, continued: Design of ECTV natural history study #CMX000-VIR-111

Post-Infection Day	Group #	Challenge Type	No of Animals (M/F)	Time of Scheduled Euthanasia (Hour)	Number of Mice Assessed[1] (M/F)		
					Viral Load	Clinical Pathology[2]	Pathology[2]
8	25	3571 ECTV	6/6	192	4/4	2/2	6/6
8	26	Sham	4/4	192	2/2	2/2	4/4
8.5	27	3571 ECTV	4/4	204	4/4	NA	4/4
8.5	28	Sham	2/2	204	2/2	NA	2/2
9	29	3571 ECTV	4/4	216	4/4	NA	4/4
9	30	Sham	2/2	216	2/2	NA	2/2
9.5	31	3571 ECTV	4/4	228	4/4	NA	4/4
9.5	32	Sham	2/2	228	2/2	NA	2/2
10	33	3571 ECTV	6/6	240	4/4	2/2	6/6
10	34	Sham	4/4	240	2/2	2/2	4/4
10.5	35	3571 ECTV	4/4	252	4/4	NA	4/4
10.5	36	Sham	2/2	252	2/2	NA	2/2
11	37	3571 ECTV	4/4	276	4/4	NA	4/4
11	38	Sham	2/2	276	2/2	NA	2/2
12	39	3571 ECTV	6/6	300	4/4	2/2	6/6
12	40	Sham	4/4	300	2/2	2/2	4/4
13	41	3571 ECTV	4/4	324	4/4	NA	4/4
13	42	Sham	2/2	324	2/2	NA	2/2
14	43	3571 ECTV	6/6	336	4/4	2/2	6/6
14	44	Sham	4/4	336	2/2	2/2	4/4
15-20	45-46	45-46	All Animals in Groups 45-46	NA	NA	NA	NA
21	45	3571 ECTV	8/8	504	Survivors	NA	Survivors
21	46	Sham	2/2	504	Survivors	NA	Survivors

[1]Refer to Table 3 for details about sample collection for all assessments.
[2]Pathology included complete necropsy for all study animals and histopathology of liver and spleen for clinical pathology animals and animals that were unscheduled euthanized.

Protocol-Related Discussion Points

- Anesthesia: Ketamine (80-100 mg/kg)/xylazine (5-10 mg/kg) was administered by IP injection prior to challenge, temperature chip implantation, blood draws and euthanasia.
- Blinding: None.

Euthanasia Criteria

Animals meeting the following criteria were euthanized:

- Moribund/persistent prostration and unresponsive to touch or external stimuli, which included a gentle prodding or placing the mouse on its back to determine if the animal could right itself.
- Any animal having > 25% weight loss (when compared to baseline) along with any concurrent severe sign of illness was euthanized. Animals meeting this level of weight loss but without a severe sign could be euthanized, if deemed necessary for humane reasons, following examination or consultation by the Study Veterinarian or designee. If an animal reached 30% weight loss, regardless of presence or absence of severe clinical signs, it was euthanized. The weight loss criteria were determined after consultation wit (b) (4) and analyses of the data presented in Gratz, et al. (Gratz et al., 2011).

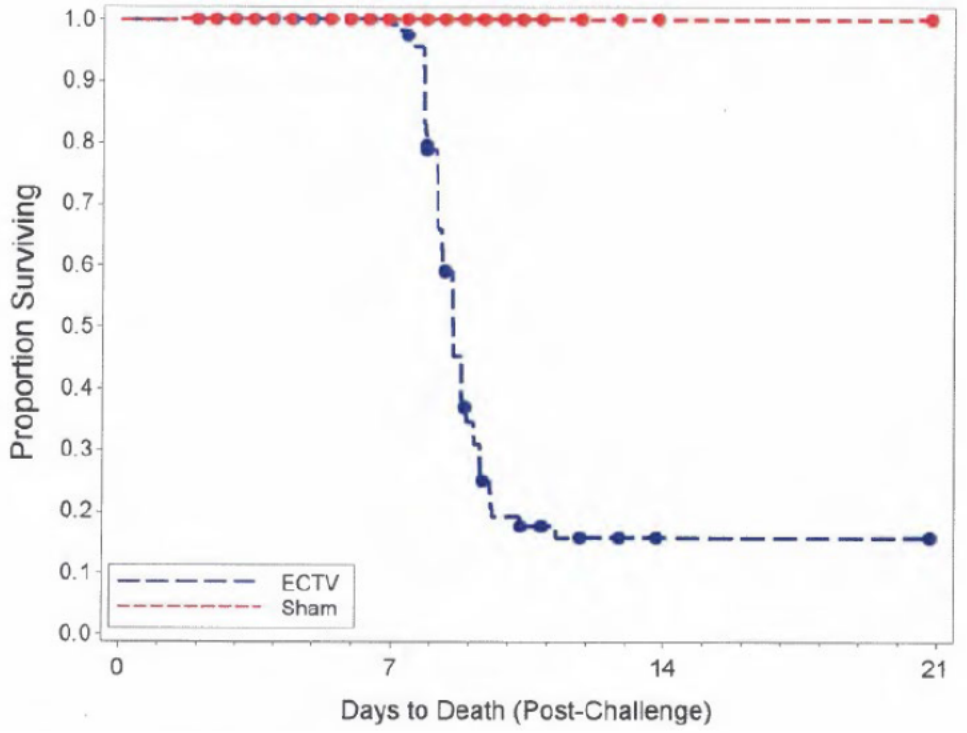
All unscheduled euthanasia adequately met these criteria.

Observations and Results

Mortality

Assessed at least twice daily. All sham-infected animals survived to their scheduled time points (up to Day 21). All ECTV-infected mice survived up to 180 hours post-challenge, but were either found dead or euthanized moribund starting 188 hours post-challenge. Median time of death was 8.6 days, and the median survival probability was 15.7% by Day 21. All unscheduled deaths were consistent with ECTV infection and disease. The Kaplan-Meier curve is presented in **Figure 1**.

Figure 1: Mortality curve for ECTV natural history study #CMX000-VIR-111



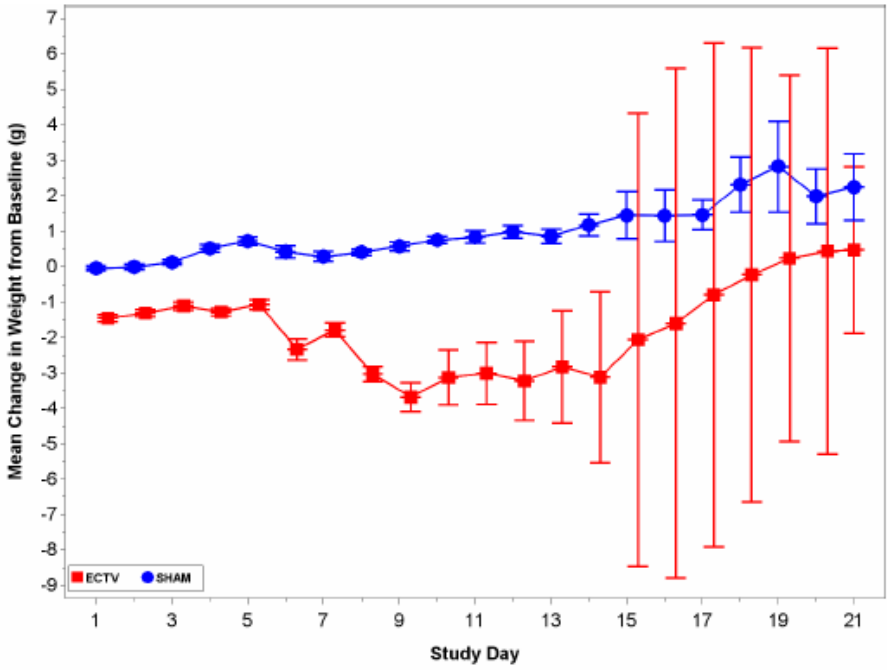
Clinical Signs

Assessed at least once daily from pre-challenge to Day 21. No notable findings were present in the sham-infected group. All ECTV-challenged animals exhibited signs of ECTV infection and disease (rough hair coat, breathing abnormalities, lethargy, lacrimation, hunched posture, and moribundity). These findings started as early as Day 2 post-infection, were present in all ECTV-infected animals by Day 7, and resolved in the surviving animals starting on Day 18. No pustular rashes or lesions were observed in any of the ECTV-infected animals.

Body Weights

Measured once daily from pre-challenge to Day 21. Body weights (presented in **Figure 2**) increased in the sham-infected group throughout the study, as expected. Body weights in the ECTV-challenged group, however, decreased sharply from Days 5 to 9, plateaued from Days 9 to 14, and recovered in the surviving animals through Day 21. Body weights in the surviving animals were significantly lower in the ECTV-infected animals relative to sham from Days 1-15 and 21.

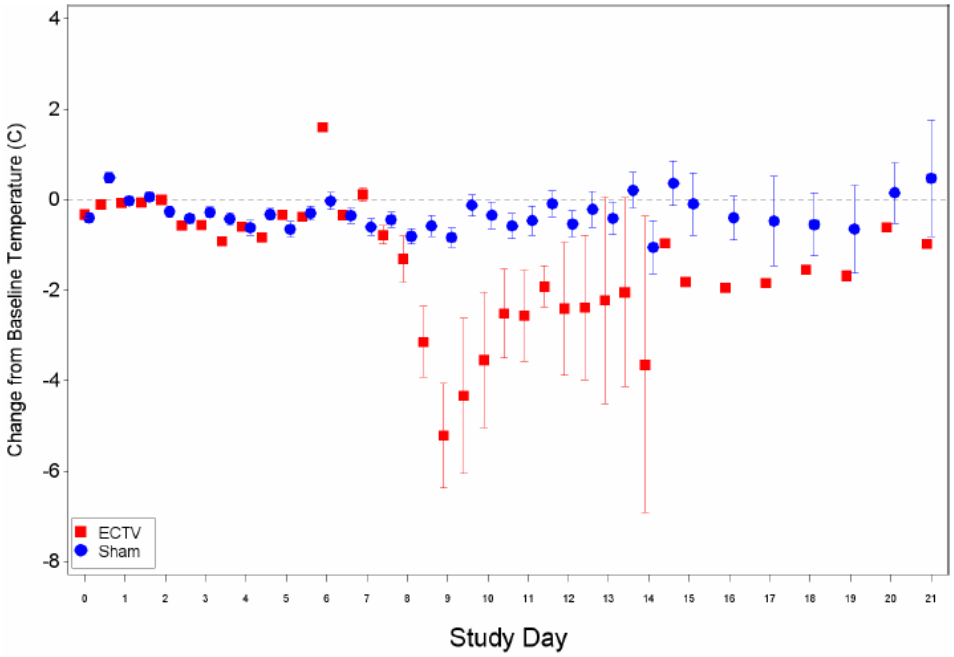
Figure 2: Body weights for ECTV natural history study #CMX000-VIR-111



Body Temperature

Temperature transponder chips were implanted in the shoulder of each animal prior to challenge. Temperatures were measured at least once daily pre-challenge to Day 21. Shoulder temperature changes from baseline are presented in **Figure 3**. No meaningful changes were observed in the sham-infected group. Temperatures in the ECTV-challenged mice increased briefly around Day 6 post-challenge before dropping sharply by ~5 ^{o}C through Day 9. Body temperatures then recovered partially through Day 11 and remained roughly 2^{o}C below sham in the surviving animals for the remainder of the study.

Figure 3: Temperature changes for ECTV natural history study #CMX000-VIR-111



Hematology & Clinical Chemistry

Blood samples were collected on Days 2, 4, 6, 8, 10, 12 and 14. Findings included increases in ALP, globulin, percent and total neutrophils, monocytes and basophils, and percent large unstained cells, and decreases in percent lymphocytes and sodium on Days 4 and/or 6. Given the difficulty with collecting blood samples in this model, the variability in the data, and the time associated with processing these samples, these endpoints were not considered reliable measures of ECTV progression and could not be used as triggers to treat.

IL-4 Production

Liver samples were collected every 12 hours post-challenge from Day 2 to 11, once daily from Day 11 to 14, on Day 21, and at the time of death from all animals euthanized prematurely or found dead. Spleen samples were collected on Days 2, 3, 4, 5 and 6, and at the time of death from all animals euthanized prematurely up to Day 7 post-challenge. IL-4 levels were measured by ELISA. IL-4 was increased relative to sham on Days 4 and 5, with highest levels detected on Day 5.

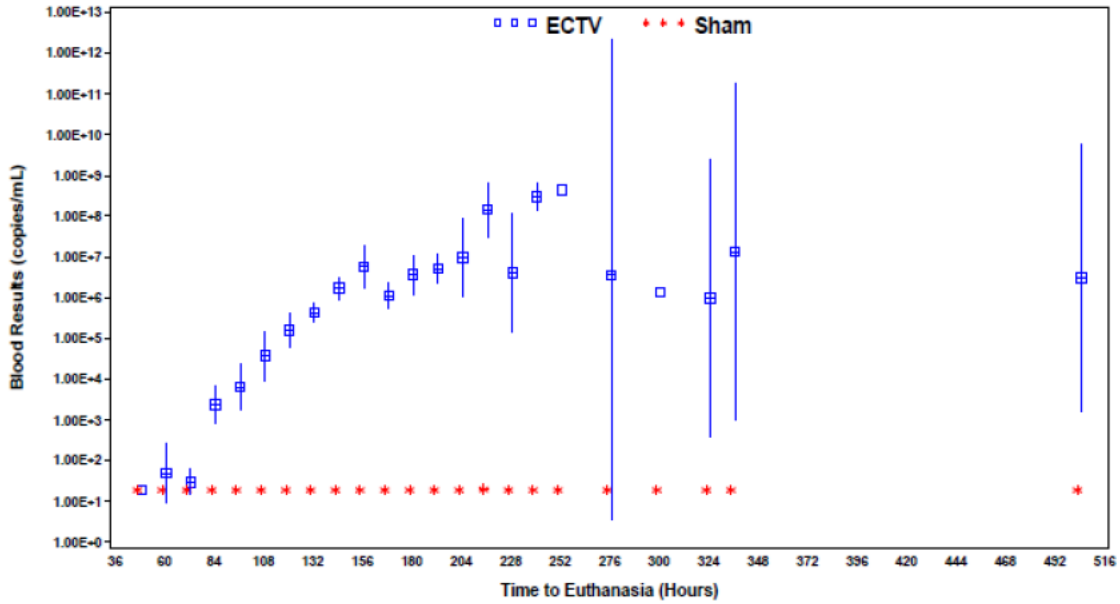
Anatomic Pathology

Gross pathology was evaluated in all animals at the time of necropsy. Liver and spleen tissues were collected for histopathology on Days 2, 4, 6, 8, 10, 12, 14 and at the time of death in all prematurely euthanized animals. Liver, spleen and lung tissues were collected on Days 21 but were not evaluated. No gross findings, including no ECTV lesions, were observed in any animal in this study. Mild to marked lytic necrosis were observed in the liver and spleen of the ECTV animals starting 142 hours post-infection. All ECTV animals that were euthanized moribund also displayed microscopic lesions in the liver and spleen. All findings were consistent with ECTV infection and disease in this species.

Viral Load

Blood, liver and spleen samples were collected every 12 hours post-challenge from Day 2 to 11, once daily from Day 11 to 14, on Day 21, and at the time of death from all animals euthanized prematurely or found dead. ECTV viral loads were confirmed by plaque assay and qPCR. Serum viral load, measured by qPCR, is presented in **Figure 6**. Viremia was confirmed in all ECTV-challenged animals starting around 60 hours post-challenge, and was present in all animals by hour 84, in the liver, spleen and whole blood, with highest levels appearing in the spleen. Viral levels also peaked around 180 hours post-challenge and remained elevated through the remainder of the study.

Figure 6: Whole blood viral load for ECTV natural history study #CMX000-VIR-111



Study Title: A Randomized, Blinded, Placebo-Controlled, Parallel Group Study of the Efficacy of Brincidofovir Treatment when Initiated 4, 5, 6, or 7 Days Post Challenge in BALB/c Mice Intranasally Inoculated with Ectromelia Virus (ECTV) Strain Moscow

Study no.:	CMX001-VIR-044
Study report location:	4.2.1.1
Study initiation date:	January 18th, 2018
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Brincidofovir (lot #CMX001-100; purity = 100%)
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This study was conducted to determine the efficacy of BCV in the treatment of ECTV-induced infection and disease. Survival over placebo was the primary endpoint, while clinical events including viral load (in whole blood, liver, and spleen), body temperature, and body weight were secondary.

In the primary endpoint of survival, BCV (20, 5 and 5 mg/kg spaced 48 hours apart) was protective against ECTV-induced mortality relative to placebo when administered starting on Days 4, 5, 6, and 7 with greatest survival benefit of 84% and 75% noted on days 4 and 5 post infection, respectively. Lower doses of 10, 5, and 5 mg/kg spaced 48 hrs apart on days 4, 5, and 6 also showed increased survival. However, survival on days 4 and 5 at this lower dose was only 78% and 66% respectively. No clear, drug-related differences in ECTV-induced secondary endpoints of clinical signs, body weights, viral loads were observed.

Overall, BCV was shown to be effective in the treatment of ECTV-induced infection and disease, particularly in the reduction of ECTV-induced mortality, under the conditions of this study.

Methods (Drug)

Doses:	First dose: 20 mg/kg or 10 mg/kg Second and third doses: 5 mg/kg
Frequency of Dosing:	Three doses spaced 48 hours apart
Number/Sex/Group:	16/sex/group
Dose volume:	10 µL/g
Formulation/Vehicle:	PBS, pH = 7.8-8.1
Route of administration:	Oral feeding via needleless syringe
Species/Strain:	BALB/c mice (b) (4)
Age/Sexual Maturity:	6-8 weeks
Comment on Study Design and Conduct:	ECTV challenge was conducted on Day 1. Drug was administered once every 48 hours for 3 total doses starting on Days 5, 6, 7 or 8. All surviving animals were euthanized on Day 43. See **Table 4** for more information.
Dosing Solution Analysis:	All samples met acceptance criteria

Methods (Pathogen Challenge)

Challenge Agent Info:	Ectromelia Virus (ECTV), Moscow strain
Challenge Agent Lot:	032516-ECTV
Target Challenge Dose:	200 pfu/25 µL PBS (100 pfu/12.5 µL per naris)
Frequency of Dosing:	Single dose on Day 1
Route of Administration:	Intranasal administration via micropipette
Back-Titrated Final Dose:	188-196 pfu

Table 4: Dosing regimen for ECTV study #CMX001-VIR-044

Arm	Group	n (M/F)	ECTV Challenge Day[a]	Blinded or Unblinded	BCV First/ Second/ Third/ Dose (mg/Kg)	BCV/ Placebo Dosing[b] Days								Blood, Liver, Spleen Collection Days	Number of Mice per Collection Time Point (M/F)
						Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12		
Efficacy Evaluation	1	16/16	1	Blinded	10 / 5 / 5	BCV	P	BCV	P	BCV	P	P	P	T[c]	16/16
	2	16/16	1	Blinded	10 / 5 / 5	P	BCV	P	BCV	P	BCV	P	P	T[c]	16/16
	3	16/16	1	Blinded	10 / 5 / 5	P	P	BCV	P	BCV	P	BCV	P	T[c]	16/16
	4	16/16	1	Blinded	20 / 5 / 5	BCV	P	BCV	P	BCV	P	P	P	T[c]	16/16
	5	16/16	1	Blinded	20 / 5 / 5	P	BCV	P	BCV	P	BCV	P	P	T[c]	16/16
	6	16/16	1	Blinded	20 / 5 / 5	P	P	BCV	P	BCV	P	BCV	P	T[c]	16/16
	7	16/16	1	Blinded	20 / 5 / 5	P	P	P	BCV	P	BCV	P	BCV	T[c]	16/16
	8	16/16	1	Blinded	0 / 0 / 0	P	P	P	P	P	P	P	P	T[c]	16/16
Viral Load	9	20/20	1	Unblinded	None	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3, 4, 5, 6, 7, T[d]	4/4
Resistance	10	7/7	1	Unblinded	20 / 5 / 5	N/A	BCV	P	BCV	P	BCV	P	N/A	8, 9, 10, 11, 12, 13, 16, T[e]	1/1
	11	7/7	1	Unblinded	20 / 5 / 5	N/A	P	BCV	P	BCV	P	BCV	N/A		1/1
	12	7/7	1	Unblinded	0 / 0 / 0	N/A	P	P	P	P	P	P	N/A		1/1

[a] Intranasal challenge for the first animal was initiated at 0900 ± 2 hours.

[b] Doses were administered by oral gavage. The first dose of each blinded treatment (BCV or placebo, P) for Groups 1 through 8 was administered on Day 5 (post-infection day 4 or PID4). The first dose of each unblinded treatment (BCV or P) for Groups 10 through 12 was administered on Day 6 (post-infection day 5 or PID5). Daily doses (blinded and unblinded) were administered at 1000 ± 2 hours.

[c] "T" refers to terminal for survivors on Day 43 (last day on study), unscheduled euthanasia or animals found dead. Blood was not to be collected from animals found dead; only liver and spleen were to be collected.

[d] "T" refers to unscheduled euthanasia or animals found dead. Blood was not to be collected from animals found dead; only liver and spleen were to be collected. NOTE: last day on study for Group 9 was Day 7.

[e] "T" refers to unscheduled euthanasia or animals found dead. Blood was not to be collected from animals found dead; only liver and spleen were to be collected. NOTE: last day on study for Groups 10, 11, and 12 was Day 16.

Protocol-Related Discussion Points

- Anesthesia: On Study Day 1, mice were anesthetized with ketamine 80 milligrams per kilogram (mg/kg) and xylazine 5 mg/kg by intraperitoneal (IP) injection to prepare mice for intranasal administration of ECTV as well as prior to administration of the temperature transponder.
- Blinding: Double Blind (sponsor, study director and staff were all blinded during study conduct).

Euthanasia Criteria

Animals meeting any of the following criteria were euthanized:

- Moribund/persistent prostration and unresponsive to touch or external stimuli, which included a gentle prodding or placing the mouse on its back to determine if the animal could right itself.
- Any animal having >25% weight loss (when compared to baseline) along with any concurrent severe sign of illness was euthanized. Animals meeting this level of weight loss but without a severe sign could be euthanized, if deemed necessary for humane reasons, following examination or consultation by the Study Veterinarian or designee. If an animal reached 30% weight loss, regardless of presence or absence of severe clinical signs, it was euthanized. The weight loss criteria were determined after consultation wit (b) (4) and analyses of the data presented in Gratz, *et al*. (Gratz *et al*., 2011).

These criteria were essentially identical to those used in the natural history studies and were considered acceptable.

Observations and Results

Mortality

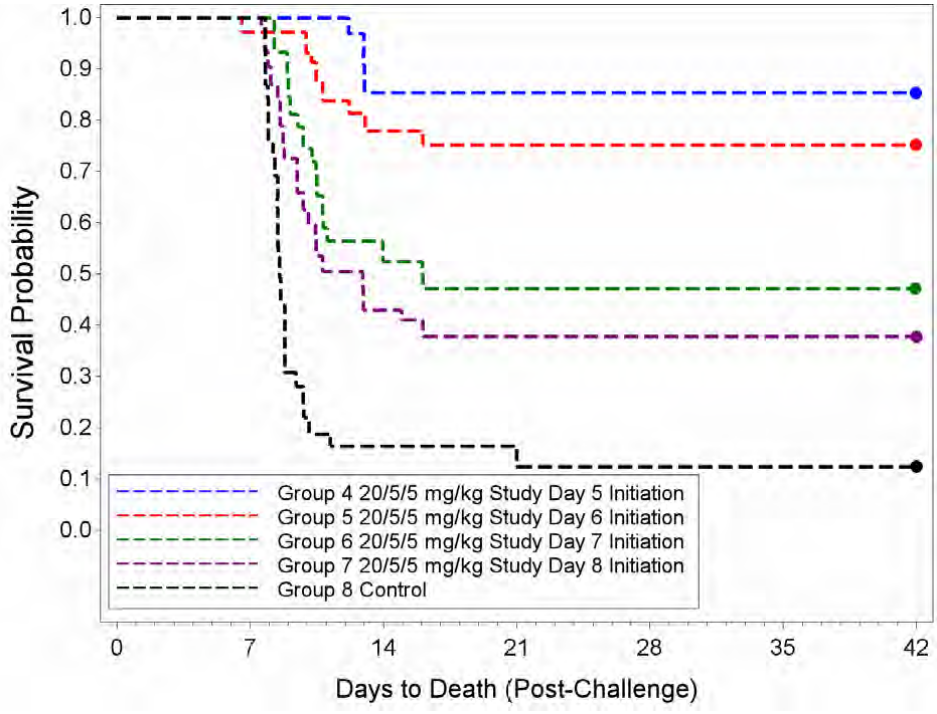
Assessed 2 times daily from Days 1-4 and 3 times daily from Days 5-12 then 2 times again from days 13-43. A summary of the deaths and the reasons for euthanasia are presented in **Table 5**. Mortality is also presented in the Kaplan-Meier curve in **Figure 7**. All euthanasia decisions were based on the euthanasia criteria and appeared to be valid. Survival was 84% for mice treated with 20/5/5 BCV starting on Day 4, 75% for those starting on Day 5, and 47% for those starting on Day 6. By comparison, survival in the placebo group (Group 8) was 12.5%.

Table 5: Mortality comparison for ECTV study #CMX001-VIR-044

Dose Regimen (mg/kg)	Group	N	Number Dead	Number Alive	Proportion of Survivors (95% Clopper-Pearson Confidence Interval)	One-Sided Boschloo's Test vs. Group 8	
						Unadjusted p-value	Stepdown Bonferroni Adjusted p-value
10/5/5	1	32	7	25	0.78 (0.60, 0.91)	<0.0001*	<0.0001*
	2	32	11	21	0.66 (0.47, 0.81)	<0.0001*	<0.0001*
	3	32	21	11	0.34 (0.19, 0.53)	0.0233*	0.0233*
0/0/0	8	32	28	4	0.13 (0.04, 0.29)		
Cochran-Armitage Trend Test					<0.0001*		
20/5/5	4	32	5	27	0.84 (0.67, 0.95)	<0.0001*	<0.0001*
	5	32	8	24	0.75 (0.57, 0.89)	<0.0001*	<0.0001*
	6	32	17	15	0.47 (0.29, 0.65)	0.0014*	0.0028*
	7	32	20	12	0.38 (0.21, 0.56)	0.0118*	0.0118*
0/0/0	8	32	28	4	0.13 (0.04, 0.29)		
Cochran-Armitage Trend Test					<0.0001*		

* Significant at the 0.025 level.

Figure 7: Mortality curve for the 20/5/5 regimen in ECTV study CMX-VIR-044



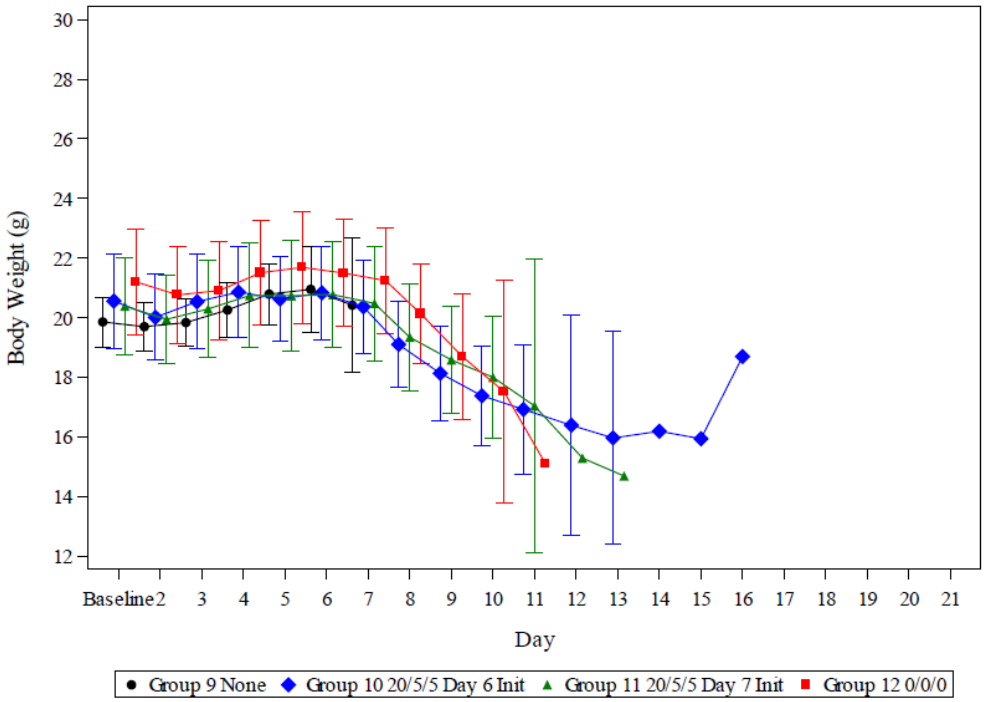
Clinical Signs

Assessed 2 times daily from Days 1-4, 3 times daily from Days 5-12, and 2 times daily from Days 13-43. ECTV-related clinical signs included exhibited rough coat, eye abnormalities, hunched posture, and/or breathing abnormalities. All observations progressively worsened in all groups throughout the study until the animals either recovered or were found dead/euthanized. No clear differences were noted between placebo and BCV-treated groups.

Body Weights

Assessed 2 times daily from Days 1-4 and 3 times daily from Days 5-12 then 2 times again from days 13-43. Body weights are presented in **Figure 8**. In all groups, body weights were decreased relative to baseline on roughly on Day 8 until euthanasia or end of study. No clear, dose-dependent differences were observed in among any of the groups in this study.

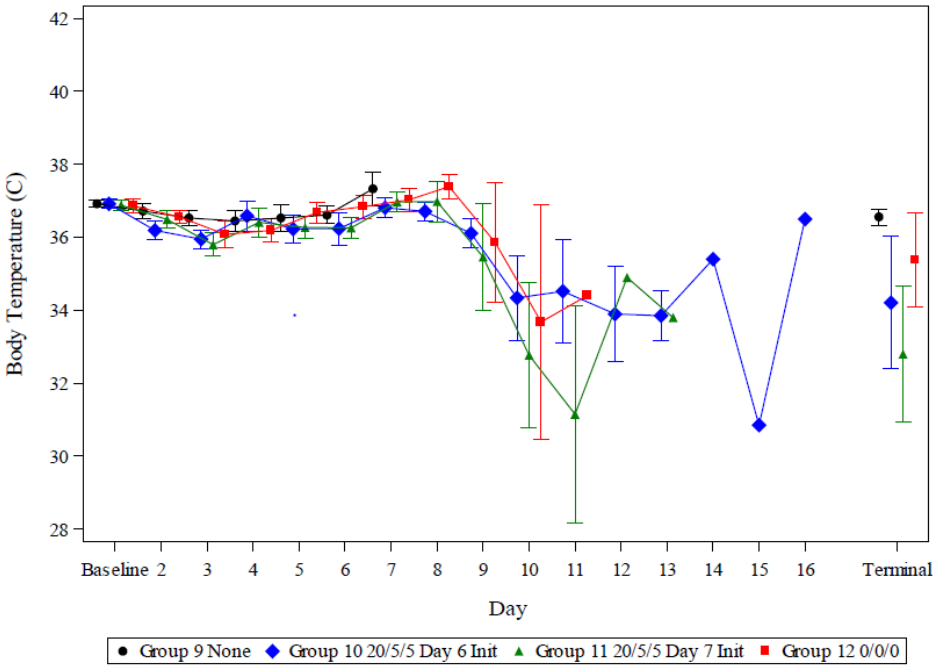
Figure 8: Body weights for ECTV study #CMX001-VIR-044



Body Temperature

Temperature transponder chips were implanted in the shoulders of each animal on Day -7 while under anesthesia. Temperatures were measured at least once daily (during pre-challenge and then daily while on study) until study completion. Body temperatures are presented in **Figure 9**. Temperatures were slightly increased with disease and then significantly decreased prior to death in animals that survived beyond Day 8.

Figure 9: Body temperatures for ECTV study #CMX001-VIR-044



Gross Pathology

Assessed at necropsy in all challenged animals found dead or euthanized. Gross findings consisted of ECTV-associated findings consistent with normal ECTV infection and disease. No drug-related findings were observed.

Viral Analysis

Although not a secondary endpoint, whole blood was collected for viral load analysis. Spleen and liver samples were also collected at termination for tissue viral load analysis. Results should be interpreted with caution as only terminal (i.e., for animals that were moribund euthanized) and Day 43 (i.e., for surviving animals at end of study) samples were collected and limited blood samples were collected from animals found dead. No trends were evident for viral load in whole blood or spleen in the efficacy evaluation groups. By four days post-infection, which corresponds to the first day of BCV treatment, the viral load animals tested (n=8) were PCR positive in whole blood, liver, and spleen indicating that ECTV disease progression was evident at the start of treatment.

Treatment Confirmation

PK for drug administration was not performed in this study. Blood samples were utilized for viral analysis. A separate PK study was performed (CMX001-VIR-071) to evaluate drug concentrations during ECTV disease.

Study Title: Determination of Single and Repeat Dose Pharmacokinetics of Brincidofovir and Cidofovir in Plasma and Concentrations of Cidofovir-Diphosphate in Peripheral Blood Mononuclear Cells Following Oral Administration of Brincidofovir in BALB/c Mice Infected with Ectromelia Virus via the Intranasal Route

Study no.:	CMX001-VIR-071
Study report location:	4.2.2.7
Study initiation date:	September 10th, 2018
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Brincidofovir (lot #CMX001-100; purity = 100%)
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This study was conducted to determine the pharmacokinetics (PK) of BCV and CDV in plasma and CDV-PP in peripheral blood mononuclear cells (PBMCs) from a single and repeat oral BCV dose in BALB/C mice infected with a lethal inoculum of ECTV strain Moscow via the intranasal (IN) route. The pharmacokinetics of BCV and CDV in the plasma of ECTV-infected mice were determined, as well as the intracellular concentrations of CDV-PP in the PBMCs of ECTV-infected mice. Please see the tables below for more information.

Methods (Drug)

Doses:	First dose: 20 mg/kg or 40 mg/kg Second and third doses: 10 mg/kg
Frequency of Dosing:	Three doses spaced 48 hours apart
Number/Sex/Group:	110/sex/group
Dose volume:	10 µL/g
Formulation/Vehicle:	PBS, pH = 7.8-8.1
Route of administration:	Oral feeding via needleless syringe
Species/Strain:	BALB/c (b) (4)
Age/Sexual Maturity:	6-8 weeks
Comment on Study Design and Conduct:	ECTV challenge was conducted on Day 1. Drug was administered once every 48 hours for 3 total doses starting on Days 3, 4, or 5. All surviving animals were euthanized on Day 43. See **Table 6** for more information.
Dosing Solution Analysis:	All samples met acceptance criteria

Methods (Pathogen Challenge)

Challenge Agent Info:	Ectromelia Virus (ECTV), Moscow strain
Challenge Agent Lot:	032516-ECTV
Target Challenge Dose:	200 pfu/25 µL PBS (100 pfu/12.5 µL per naris)

Frequency of Dosing: Single dose on Day 1
Route of Administration: Intranasal administration via micropipette
Back-Titrated Final Dose: 181-197 pfu

Table 6: Study #CMX001-VIR-071 dosing regimen

Group	n (M/F)[a]	ECTV Target Dose on Study Day 1 (PFU)	BCV Dosing Study Days	BCV Dose[b] (mg/kg)			Matrix Collected and Analyzed
				1st	2nd	3rd	
1	110/110	200	4, 6, 8	20	10	10	Plasma
2	110/110	200	5, 7, 9	40	10	10	Plasma
3	100/100	200	4, 6, 8	20	10	10	PBMCs
4	100/100	200	5, 7, 9	40	10	10	PBMCs

[a] An additional number of animals, 15/sex in Groups 1 and 2 and 10/sex in Groups 3 and 4, were challenged and treated to serve as replacements for study animals that succumb to disease prior to the scheduled timepoint. Blood was not collected from these additional animals unless blood was unable to be collected from study animals. These additional animals were bled in their place, as available.
[b] First BCV dose for Groups 1 and 3 was 72 (± 4 hrs) following challenge, with the second dose 48 ± 4 hrs after the first dose, and the third dose 48 ± 4 hrs after the second dose. First BCV dose for Group 2 and 4 was 96 (± 4 hrs) following challenge, with the second dose 48 ± 4 hrs after the first dose, and the third dose 48 ± 4 hrs after the second dose.

Protocol-Related Discussion Points

- Anesthesia: On Study Day 1, mice were anesthetized with ketamine (80 mg/kg)/xylazine (5 mg/kg) by intraperitoneal (IP) injection prior to intranasal ECTV challenge and temperature transponder implantation.
- Blinding: None.

Euthanasia Criteria

Animals meeting any of the following criteria were euthanized:

- Moribund/persistent prostration and unresponsive to touch or external stimuli, which included a gentle prodding or placing the mouse on its back to determine if the animal could right itself.
- Any animal having > 25% weight loss (when compared to baseline) along with any concurrent severe sign of illness was euthanized. Animals meeting this level of weight loss but without a severe sign could be euthanized, if deemed necessary for humane reasons, following examination or consultation by the Study Veterinarian or designee. If an animal reached 30% weight loss, regardless of presence or absence of severe clinical signs, it was euthanized. The weight loss criteria were determined after consultation with (b) (4) and analyses of the data presented in Gratz, *et al*. (Gratz *et al*., 2011).

These criteria were essentially identical to those used in the natural history studies and were considered acceptable.

Observations and Results

Mortality

All animals survived to planned euthanasia. Subgroups of animals within groups were sampled around the first or last dose of BCV as outlined in tables above.

Clinical Signs

Observed once on the afternoon of arrival, twice daily during quarantine through Day 8 (7 days post-challenge), and three times daily from Day 9 though the end of the study (euthanasia on Day 11). ECTV-related clinical signs included exhibited rough coat, eye abnormalities, hunched posture, and/or breathing abnormalities. Lacrimation also occurred toward end of study. All observations progressively worsened in all groups throughout the study until the animals were euthanized.

Body Weights

Assessed daily until end of study, and were used only to monitor weight loss for euthanasia purposes. None of the animals met euthanasia criteria on this study.

Gross Pathology

Assessed at necropsy in all challenged animals found dead or euthanized. Gross findings consisted of ECTV-associated findings consistent with normal ECTV infection and disease. No drug-related findings were observed.

Pharmacokinetics

Blood samples were collected 0, 0.5, 1, 1.5, 2, 4, 8, 12, 24 and 36 hours after the first and last doses, as well as 48 hours post-dose after the first dose. Pharmacokinetics and intracellular concentrations for both BCV, CDV and CDV-PP are presented in the following tables.

Table 7: Study #CMX001-VIR-071 BCV PK parameters after 1st dose

Group	Sex	C_{max} (ng/mL)	C_{max} /Dose ((ng/mL)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*ng/mL)	AUC_{last}/Dose ((h*ng/mL)/ (mg/kg))	AUC_{inf} (h*ng/mL)	AUC_{inf}/Dose ((h*ng/mL)/ (mg/kg))	$t_{1/2}$ (h)
1	Male	112	5.62	8.00	1049	52.4	1103	55.2	15.7
	Female	76.0	3.80	4.00	731	36.6	785	39.2	16.1
	Combined	72.9	3.64	4.00	898	44.9	953	47.7	16.2
2	Male	254	6.34	1.00	2533	63.3	2590	64.7	8.52
	Female	243	6.08	1.50	2333	58.3	NR	NR	NR
	Combined	219	5.47	4.00	2435	60.9	NR	NR	NR

NR = Not Reported.
Group 1: 20/10/10 mg/kg q48h Dosing Regimen. Group 2: 40/10/10 mg/kg q48h Dosing Regimen.

Table 8: Study #CMX001-VIR-071 BCV PK parameters after 3rd dose

Group	Sex	C_{max} (ng/mL)	C_{max} /Dose ((ng/mL)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*ng/mL)	AUC_{inf}/Dose ((h*ng/mL)/ (mg/kg))	$t_{1/2}$ (h)
1	Male	61.4	6.14	2.00	607	60.7	11.3
	Female	40.1	4.01	4.00	633	63.3	NR
	Combined	45.2	4.51	2.00	625	62.5	NR
2	Male	48.1	4.81	2.00	794	79.4	NR
	Female	31.8	3.18	1.50	466	46.6	NR
	Combined	34.0	3.40	1.50	631	63.1	NR

NR = Not Reported.
Group 1: 20/10/10 mg/kg q48h Dosing Regimen. Group 2: 40/10/10 mg/kg q48h Dosing Regimen.

Table 9: Study #CMX001-VIR-071 CDV PK parameters after 1st dose

Group	Sex	C_{max} (ng/mL)	C_{max} /Dose ((ng/mL)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*ng/mL)	AUC_{inf}/Dose ((h*ng/mL)/ (mg/kg))
1	Male	18.6	0.928	8.00	700	35.0
	Female	18.4	0.920	24.00	716	35.8
	Combined	17.4	0.868	24.00	707	35.4
2	Male	37.6	0.941	24.00	1451	36.3
	Female	35.3	0.882	8.00	1391	34.8
	Combined	35.6	0.891	12.00	1422	35.5

Group 1: 20/10/10 mg/kg q48h Dosing Regimen. Group 2: 40/10/10 mg/kg q48h Dosing Regimen.

Table 10: Study #CMX001-VIR-071 CDV PK parameters after 1st dose

Group	Sex	C_{max} (ng/mL)	C_{max} /Dose ((ng/mL)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*ng/mL)	AUC_{inf}/Dose ((h*ng/mL)/ (mg/kg))
1	Male	47.6	4.76	12.00	860	86.0
	Female	18.2	1.82	36.00	580	58.0
	Combined	31.8	3.18	12.00	726	72.6
2	Male	42.0	4.20	36.00	926	92.6
	Female	64.5	6.45	36.00	1097	110
	Combined	53.2	5.32	36.00	1019	102

Group 1: 20/10/10 mg/kg q48h Dosing Regimen. Group 2: 40/10/10 mg/kg q48h Dosing Regimen.

Table 11: Study #CMX001-VIR-071 CDV-PP concentrations in PBMCs

Group	Sex	Statistic	Preliminary Analysis Nominal Time			Secondary Analysis #1 Nominal Time		
			Dose 1 24 hours post-dose	Dose 1 48 hours post-dose	Dose 3 48 hours post-dose	Dose 1 24 hours post-dose	Dose 1 48 hours post-dose	Dose 3 48 hours post-dose
3	Male	N	6	6	6	4	6	5
		Mean	32.1	59.4	36.8	27.8	59.4	27.9
		SD	8.24	23.8	22.1	5.77	23.8	3.80
		CV%	25.6	40.0	60.1	20.7	40.0	13.6
		Min	20.2	31.3	22.2	20.2	31.3	22.2
		Median	32.0	58.6	29.3	28.7	58.6	28.8
		Max	43.8	89.6	81.4	33.8	89.6	32.2
	Female	N	6	6	5	5	5	3
		Mean	25.2	27.9	22.4	21.9	29.0	21.3
		SD	8.90	5.77	3.09	4.29	5.76	1.40
		CV%	35.3	20.7	13.8	19.6	19.9	6.61
		Min	16.3	22.6	19.8	16.3	22.7	19.8
		Median	23.0	26.4	21.4	21.6	28.2	21.4
		Max	41.6	36.7	27.6	27.6	36.7	22.6
	Combined	N	12	12	11	9	11	8
		Mean	28.7	43.7	30.3	24.6	45.6	25.4
		SD	8.94	23.3	17.5	5.60	23.4	4.53
		CV%	31.2	53.3	57.7	22.8	51.4	17.8
		Min	16.3	22.6	19.8	16.3	22.7	19.8
		Median	27.4	34.7	26.4	24.3	36.7	24.5
		Max	43.8	89.6	81.4	33.8	89.6	32.2
4	Male	N	6	6	6	6	6	6
		Mean	30.8	121	20.0	30.8	121	20.0
		SD	9.43	26.9	6.98	9.43	26.9	6.98
		CV%	30.6	22.2	35.0	30.6	22.2	35.0
		Min	18.0	91.1	13.0	18.0	91.1	13.0
		Median	29.0	119	18.2	29.0	119	18.2
		Max	42.4	164	32.6	42.4	164	32.6
	Female	N	6	6	6	6	5	6
		Mean	34.0	67.6	16.8	34.0	66.4	16.8
		SD	44.4	7.02	5.19	4.44	7.19	5.19
		CV%	13.1	10.4	30.9	13.1	10.8	30.9
		Min	28.2	55.7	8.38	28.2	55.7	8.38
		Median	35.5	69.6	17.9	35.5	69.0	17.9
		Max	39.4	74.2	22.2	39.4	74.2	22.2
	Combined	N	12	12	12	12	11	12
		Mean	32.4	94.5	18.4	32.4	96.4	18.4
		SD	7.23	33.8	6.10	7.23	34.7	6.10
		CV%	22.3	35.8	33.2	22.3	36.0	33.2
		Min	18.0	55.7	8.38	18.0	55.7	8.38
		Median	32.0	82.6	18.2	32.0	91.1	18.2
		Max	42.4	164	32.6	42.4	164	32.6

N=Sample size; Mean = Arithmetic mean; SD = Standard deviation; CV% = Arithmetic present coefficient of variation; Min = Minimum; Max = Maximum
Values expressed in pg/million cells.

Rabbit/Rabbitpox (RPXV) Efficacy Studies

Study Title: A Randomized Blinded Study of the Potency of Rabbitpox Virus in New Zealand White Rabbits Infected Via the Intradermal Route

Study no.:	CMX001-VIR-033
Study report location:	4.2.1.1
Study initiation date:	September 30th, 2011
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This study was conducted to evaluate the potency of purified RPXV, strain Utrecht, in New Zealand White rabbits. Nine-week-old rabbits (4/sex/group) were challenged intradermally on Day 0 with either test material at 30, 100, 300 or 900 pfu (Groups 1-4; RPXV lot 050310-ALS) or a positive control at 100 pfu (Group 5; RPXV reference lot 5-7-2008 from the (b) (4)). Actual titers were 15, 43, 117, 630 and 31 for Groups 1-5, respectively. All animals were euthanized on Day 21 post-challenge. BCV was not administered in this study. Endpoints included mortality, clinical signs, lesion counts, body weights, body temperatures, respiration rates, hematology, clinical chemistry, and viral load by qPCR. Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM or SC injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria were essentially identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

Mortality was 75% (6/8), 63% (5/8), 100% (8/8), 100% (8/8) and 50% (4/8) in Groups 1-5, respectively. Clinical signs included decreased food consumption, lethargy, wheezing, nasal flaring, labored/rapid/shallow/open mouth breathing, and discharge from nose and/or mouth, all of which are consistent with RPXV infection and disease. RPXV lesions appeared as early as Day 3, increased steadily in number through roughly Day 10, and were present in some animals up to Day 21. Body weights increased in all groups up to roughly Day 8 post-challenge, decreased from Day 8 to Day 13, and recovered in the surviving animals up to study conclusion on Day 21. Body temperatures also increased in all groups through roughly Day 6 post-challenge and remained elevated through roughly Day 10. Temperatures in Group 4 also decreased slightly below baseline on Days 10 and 12. Respiration rates fluctuated slightly up to Day 6 post-challenge before decreasing roughly 50% below baseline through the remainder of the study. No clear differences in clinical signs, body weight, body temperature, respiration rate, or lesion count were observed between groups. In

addition, fluctuations in multiple hematology and clinical chemistry parameters were observed, but no meaningful group-related changes were observed. All findings were consistent with RPXV infection in this species. RPXV viremia was also confirmed in the blood and nose starting roughly 8 days post-challenge and peaking around Day 10 before declining. Virus was still detectable in several Group 1 and 2 animals by study conclusion on Day 21.

While no clear relationships were observed between RPXV inoculum level and most of the parameters evaluated in this study, there was a statistically significant correlation between RPXV inoculum level and mortality. LD_{50} and LD_{90} values for the RPXV test material were identified as 8 pfu and 83 pfu, respectively. The potency of the test material was considered to be similar to that of the positive control (Group 5) and was considered adequate for use in the pivotal efficacy studies.

Study Title: A Randomized, Blinded, Placebo-Controlled, Parallel Group Study of the Safety, Tolerability, Efficacy, and Pharmacokinetics of CMX001 in New Zealand White Rabbits Intradermally Inoculated With a Lethal Inoculum of Rabbitpox Virus Strain Utrecht

Study no.:	CMX001-VIR-039
Study report location:	4.2.1.1
Study initiation date:	October 17th, 2012
Conducting laboratory and location:	(b) (4) [redacted]
GLP compliance:	No
Drug, lot #, and % purity:	CMX001 (lot #CMX001-DS-002, CMX001-DS-003, and CMX001-DS-004, purity not provided)
Deviations:	None which meaningfully affected study interpretation.

Summary

This non-GLP study was conducted to as a preliminary evaluation of BCV efficacy for the treatment of RPXV infection and disease. Seven-week-old New Zealand White rabbits were challenged intradermally on Day 0 with purified RPXV, strain Utrecht (target titer = 300 pfu, actual titer = 187 pfu). BCV was administered in 3 doses spaced 48 hours apart by oral gavage. The treatment regimen for this study is presented in **Table 12**. As stated in **Table 12**, the target minimum number of animals was 6/sex in Groups 1-4 and 9 males in Group 5, but the actual group sizes were 15 in Groups 1-3, 16 in Group 4, and 9 in Group 5. Groups 1-4 were blinded and were intended to evaluate efficacy, while Group 5 was not blinded and was intended to evaluate BCV pharmacokinetics. In Groups 1-4, treatment was initiated within 4 hours following the first observation of one or more RPXV lesions (Day 3-5 post-challenge). In Group 5, BCV was administered on Days 4, 6 and 8. All surviving animals were euthanized on Day 42 post-challenge. Endpoints included mortality, clinical signs, challenge site monitoring, lesion counts, body weights, body temperatures, respiration rates, gross pathology, viral load by qPCR, pharmacokinetics, and virology endpoints including resistance monitoring and anti-RPXV antibody production. Ketamine (22-50

mg/kg)/xylazine (3-10 mg/kg) was administered by IM or SC injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria were identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

Table 12: Dosing regimen for RPXV study #CMX001-VIR-039

Treatment Group	Number of Animals Randomized[a]	CMX001 Dose (mg/kg)	Timing of Dose Administration		
			First Dose	Second Dose	Third Dose
1	12 (6/sex)	5 + 5 + 5	At or within 4 hours of 1st observation of lesions (RD0)	At 1st observation of lesions plus 48 (± 4) hours (RD2)	At 1st observation of lesions plus 96 (± 4) hours (RD4)
2	12 (6/sex)	20 + 5 + 5			
3	12 (6/sex)	20 + 20 + 20			
4	12 (6/sex)	0 + 0 + 0 (vehicle)			
5 PK unblinded	9 males	20 + 5 + 5	Day 4 post infection (ID4)	48 (± 4) hours after first dose (ID6)	96 (± 4) hours after first dose (ID8)

[a] = Target minimum number of animals randomized per group.
Abbreviations: ID, Inoculation Day (Day all animals were simultaneously inoculated with RPXV in this study); RD, Randomization Day (unique for each animal based on first observation of lesions in that animal).

Survival following RPXV challenge was 7/15 (46.7%), 11/15 (73.3%), 12/15 (80.0%) and 4/16 (25%) in Groups 1-4, respectively. Treatment with BCV according to the 20/5/5 and 20/20/20 regimens (Groups 2 and 3, respectively) resulted in significantly greater survival relative to placebo (Group 4). Clinical signs included redness and swelling at the injection site and respiratory abnormalities including wheezing, nasal flaring and rales, all of which are consistent with RPXV infection and disease. Body weights generally increased in all Group 1-4 animals throughout the study despite RPXV infection, but body weight gain was generally significantly greater in Groups 1-3 relative to Group 4 (placebo). Body temperatures increased following RPXV challenge about 2-3 ^{o}F starting roughly 3 days post-infection and remained elevated through roughly Day 8 before decreasing about 2-3 ^{o}F for the remainder of the study. BCV animals, particularly in Group 3, exhibited slightly faster temperature recovery times relative to placebo (returning to pre-challenge values around Day 8-9 vs. Day 9-10 in Group 4), and also remained generally higher than in placebo through the remainder of the study. Respiratory rate was decreased through roughly Day 8 post-challenge before recovering in all groups, and fluctuated through the remainder of the study. No clear BCV-related effects on respiratory rate were observed. RPXV lesions appeared in all animals starting 3-5 days post-challenge and increased steadily in number through roughly Day 9. In general, lesion counts were significantly lower in Groups 1-3 (particularly Groups 2 and 3) relative to placebo. Gross pathology findings were generally limited to skin discoloration and lesions in the animals that died prematurely, and no BCV-related differences were noted. All findings were consistent with RPXV infection in this species. RPXV viremia was also confirmed in the blood starting roughly 3 days post-challenge and peaking around Day 5 in all groups. No differences between groups were observed with the exception of the day of euthanasia (roughly Day 42 in all

animals), which was significantly higher in Group 4 (placebo) relative to Groups 1-3. Pharmacokinetic parameters from Group 5 are also presented in **Table 13**.

Given the increased survival rates in Groups 2 and 3 relative to placebo (Group 4), BCV was shown to have a significant effect on RPXV-induced mortality. Additional analysis of these groups demonstrated that delaying initiation of treatment, which occurred between Days 3 and 5 post-challenge, negatively affected survival. In general, initiating treatment earlier in disease progression appeared to decrease RPXV-induced mortality. Initiation of treatment at the time of RPXV lesion appearance, as in this study, may not be the ideal treatment trigger. As a result, subsequent efficacy studies utilized different BCV treatment strategems.

Table 13: Study #CMX001-VIR-039 PK parameters

Dose Period	Dose (mg/kg)	Tmax (hr)	Cmax (pg/10^6 cells)	Tlast (hr)	Clast (pg/10^6 cells)	AUClast (pg*hr)/ 10^6 cells	AUC0-48 (pg*hr)/ 10^6 cells	AUCinf (pg*hr)/ 10^6 cells	t1/2elim (hr)
First dose	20	24	3.99	48	2.63	136	136	NC	NC
Last dose	5	48	14.5	168	0.60	795	393	821	30.0

Study Title: Determination of the Potency and LD_{50} of Rabbitpox virus Utrecht Strain in the Rabbit Model

Study no.: 2817 & 2818-100017958
Study report location: 4.2.1.1
Study initiation date: January 22nd, 2013
Conducting laboratory and location: (b) (4)
GLP compliance: No
Drug, lot #, and % purity: No drug was used in this study
Deviations: None which meaningfully affected study interpretation.

Summary

This non-GLP study was conducted to evaluate the potency of purified RPXV, strain Utrecht, propagated under study #2814-100017958. Nine-week-old New Zealand White rabbits (4/sex/group across 2 studies) were challenged intradermally on Day 0 with test material propagated under study #2814-100017958 at 3, 9, 27, 81 and 300 pfu (Groups 1-5). Actual titers were 4.66, 6.66, 20, 47.4 and 220 for Groups 1-5, respectively. All animals were euthanized on Day 14 post-challenge. BCV was not administered in this study. Endpoints included only mortality, clinical signs, and body weights. Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM or SC injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management,

but this was not explicitly stated. The euthanasia criteria were essentially identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

Three animals (1 male in Group 1, 1 female in Group 3, and 1 male in Group 4) were excluded from the study prior to challenge due to either gastric stasis or coccidia infection. Mortality among the RPXV-challenged animals was 75% in Group 2 and 100% in all other groups. The similarity in actual pfu (4.66 in Group 1 vs. 6.66 in Group 2) may account for the lack of a clear dose dependency. Given the high mortality across all groups, clear LD_{50} and LD_{90} values could not be established. Regardless, the LD_{50} and LD_{90} values were concluded to be ≤4.66 pfu and ≤220 pfu, respectively. Median time of death was 6.64 days in Group 5, 7.22-7.23 days in Groups 3 & 4, and 8.08-8.09 days in Group 1. Body weights remained largely unchanged relative to pre-challenge values, likely because of the edible supplementation, but two Group 5 females did lose roughly 8% of body weight prior to death on Day 6 post-challenge. Clinical signs included decreased food consumption, lethargy, splinting, nasal flaring, lacrimation, labored/rapid/shallow/open mouth breathing, bloody nasal discharge, and redness/swelling/scabbing at the inoculation site. All observed clinical signs were consistent with RPXV infection and disease, and were also observed in the two Group 2 animals that survived to study conclusion on Day 14.

Study Title: Natural History Study of Intradermal Rabbitpox Virus (Utrecht Strain) Infection in the Nine Week Old Rabbit Model

Study no.:	2819-100017958
Study report location:	4.2.1.1
Study initiation date:	April 10th, 2013
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This study was conducted to characterize the disease progression of RPXV infection in 9-week-old rabbits. BCV was not administered in this study. The primary objective was to ensure that challenge with RPXV produced a robust, reproducible viremia and disease course that was consistent with prior studies of RPXV infection in this species. In addition, several clinical parameters were evaluated as potential early markers of RPXV infection that could be used as "triggers to treat" in follow-up efficacy studies. To qualify as triggers to treat, these parameters must have been specific for RPXV infection, unambiguous, reproducible and objective, and early enough in the disease course to allow intervention corresponding to the therapeutic window in human smallpox.

All animals challenged with RPXV succumbed to illness within Days 6 to 10 post-challenge, while all sham-infected animals survived to study conclusion on Day 14.

RPXV-challenged animals also exhibited adverse clinical signs (decreased food consumption, lethargy, ataxia, eye closure, labored/rapid/open mouth breathing, nasal flaring, and discharge from nose and/or mouth), decreased body weight gain, changes in body temperature and respiratory rate, challenge site reactions, RPXV lesions, fluctuations in assorted hematology and clinical chemistry parameters, and gross pathology findings (necrosis, inflammation, hemorrhage and/or edema in the skin, tongue, lungs, spleen, testes/ovaries, liver, thymus and brain). All findings were consistent with RPXV infection in this species, and occurred roughly 1-5 days prior to death. RPXV viremia was also confirmed in the blood and nose starting roughly 3 days post-challenge.

Of the endpoints evaluated, body temperature, viremia and lesion count were considered the most promising as triggers to treat. Body temperature and serum viral load were elevated starting roughly 3 Days post-challenge (median time to fever and viremia onset were 2.8 and 3.0 days, respectively). RPXV lesions also appeared starting around Day 6 post-challenge, relatively later than fever and viremia onset and potentially too late for intervention given that mortality occurred between Days 6 and 10. In addition, no clear hematology or clinical chemistry parameters were identified as reliable treatment triggers.

Methods

Species/Strain:	NZW rabbits (b) (4)
Number/Sex/Group:	2-4/sex/group
Age/Sexual Maturity:	9 weeks
Challenge Agent Info:	Rabbitpox (RPXV), Utrecht strain
Challenge Agent Lot:	RPXV-(b) (4)-01
Target Challenge Dose:	300 pfu/200 µL PBS (150 pfu/100 µL per thigh)
Frequency of Dosing:	Single dose on Day 0
Route of Administration:	Bilateral intradermal injections in both thighs
Back Titrated Final Dose:	230 pfu
Comment on Study Design and Conduct:	RPXV challenge was conducted on Day 0. Animals were monitored through Day 14. See **Table 14** for more information.

Table 14: Design of RPXV natural history study #2819-100017958

Group	Infection Status	Number of Animals (M/F)	Blood & Buccal Swab Sampling Time Points[1]	Clinical Data Point (hrs)[2]	qPCR Whole Blood & Buccal Swab	Hematology and Clinical Chemistry
1	Infected	4/4	-6[4] days; 12, 60, & 108[3] hrs; M[4]/14[4] days	12 & 60	12, 60, & 108[3] hrs; M[4]/14[4] days	-6 days; 12 & 60 hrs; M/14 days
2	Sham-Infected	2/2	-6[4] days; 12, 60, & 108[3] hrs; M[4]/14[4] days	12 & 60	12, 60, & 108[3] hrs; M[4]/14[4] days	-6 days; 12 & 60 hrs; M/14 days
3	Infected	4/4	-6[4] days; 24, 72, & 120[3] hrs; M[4]/14[4] days	24 & 72	24, 72, & 120[3] hrs; M[4]/14[4] days	-6 days; 24 & 72 hrs; M/14 days
4	Sham-Infected	2/2	-6[4] days; 24, 72, & 120[3] hrs; M[4]/14[4] days	24 & 72	24, 72, & 120[3] hrs; M[4]/14[4] days	-6 days; 24 & 72 hrs; M/14 days
5	Infected	4/4	-6[4] days; 36 & 84 hrs; M[4]/14[4] days	36 & 84	36 & 84 hrs; M[4]/14[4] days	-6 days; 36 & 84 hrs; M/14 days
6	Sham-Infected	2/2	-6[4] days; 36 & 84 hrs; M[4]/14[4] days	36 & 84	36 & 84 hrs; M[4]/14[4] days	-6 days; 36 & 84 hrs; M/14 days
7	Infected	4/4	-6[4] days; 48 & 96 hrs; M[4]/14[4] days	48 & 96	48 & 96 hrs; M[4]/14[4] days	-6 days; 48 & 96 hrs; M/14 days
8	Sham-Infected	2/2	-6[4] days; 48 & 96 hrs; M[4]/14[4] days	48 & 96	48 & 96 hrs; M[4]/14[4] days	-6 days; 48 & 96 hrs; M/14 days

M= Moribund
[1] Blood draws were collected from the left ear (see DR-13543 and DR-13541, Appendix B). Cardiac puncture was used for euthanasia blood draws. Baseline blood draw was collected on Day -6. Remainder of sampling time points represented hours post- challenge time. Blood sampling time points occurred +/- 1 hour of the target draw time and were based on the individual animal's challenge time.
[2] See Table 2 for data collection. | [3] Only buccal swabs will be collected at this time point. | [4] Only blood will be collected at this time point.

Protocol-Related Discussion Points

- Anesthesia: Ketamine (22-50 mg/kg)/Xylazine (3-10 mg/kg) was administered by IM injection prior to challenge and euthanasia. Acepromazine (1-5 mg/kg) was administered by IM injection prior to blood draws and temperature chip implantation. The exact doses of anesthesia were recorded.
- Veterinary/Supportive Care: Soft, edible enrichment (apples, applesauce, yogurt and oats) was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated.
- Blinding: None.

Euthanasia Criteria

Animals meeting any of the following three (3) criteria were euthanized:

- Severe respiratory distress as assessed by clinical observations including open mouth breathing, audible rales or wheezing, nasal flaring and splinting.
- Moribund/persistent prostration and unresponsive to noxious stimuli.
- At least 2 of the following:
    - Respiration rate 75% lower or higher than the average rate observed during the pre-study period (confirmed by a second observation within 1 hour).
    - Body temperature less than 37.2°C (99°F) (confirmed by a second reading approximately 1 hour later).
    - Weight loss greater than 15% from pre-challenge weight.

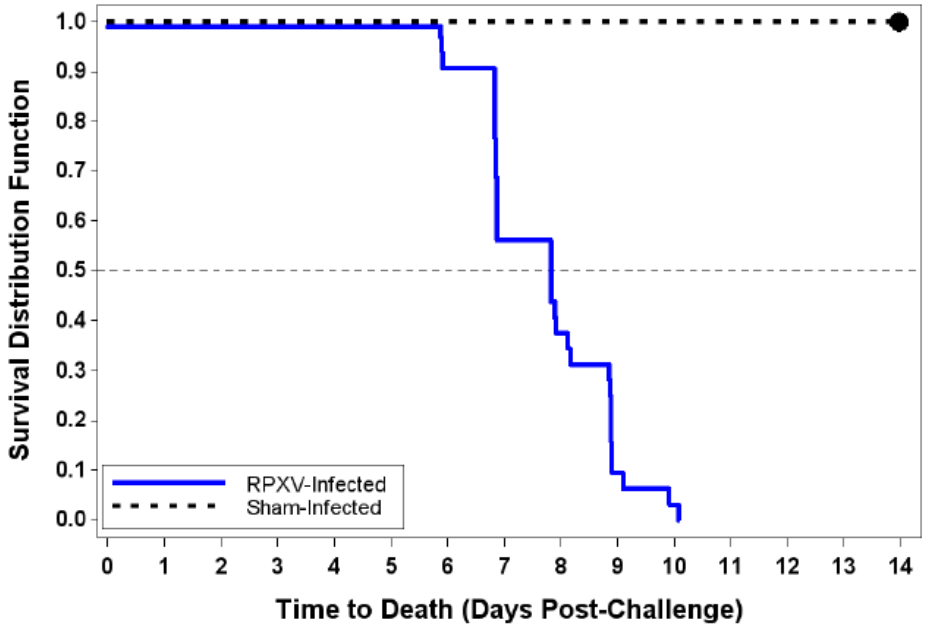
All unscheduled euthanasia adequately met these criteria.

Observations and Results

Mortality

Assessed at least once daily. All sham-infected animals survived to Day 14. Mortality in the RPXV-infected group was 100%, with all deaths occurring between Days 5 and 10. Median time of death was 7.8 days post-challenge, and the 95% confidence interval was 6.9 to 8.2 days post-challenge. The Kaplan-Meier curve is presented in **Figure 10**.

Figure 10: Mortality curve for RPXV natural history study #2819-100017958



Clinical Signs

Assessed at least once daily from pre-challenge to Day 14. No notable findings were present in the sham-infected group. All RPXV-challenged animals exhibited signs of RPXV infection and disease (decreased food consumption, lethargy, ataxia, eye closure, labored/rapid/open mouth breathing, nasal flaring, and discharge from nose and/or mouth). These findings generally started on Days 5 and 6 post-infection and preceded death by 1-2 days.

Challenge Site Assessment

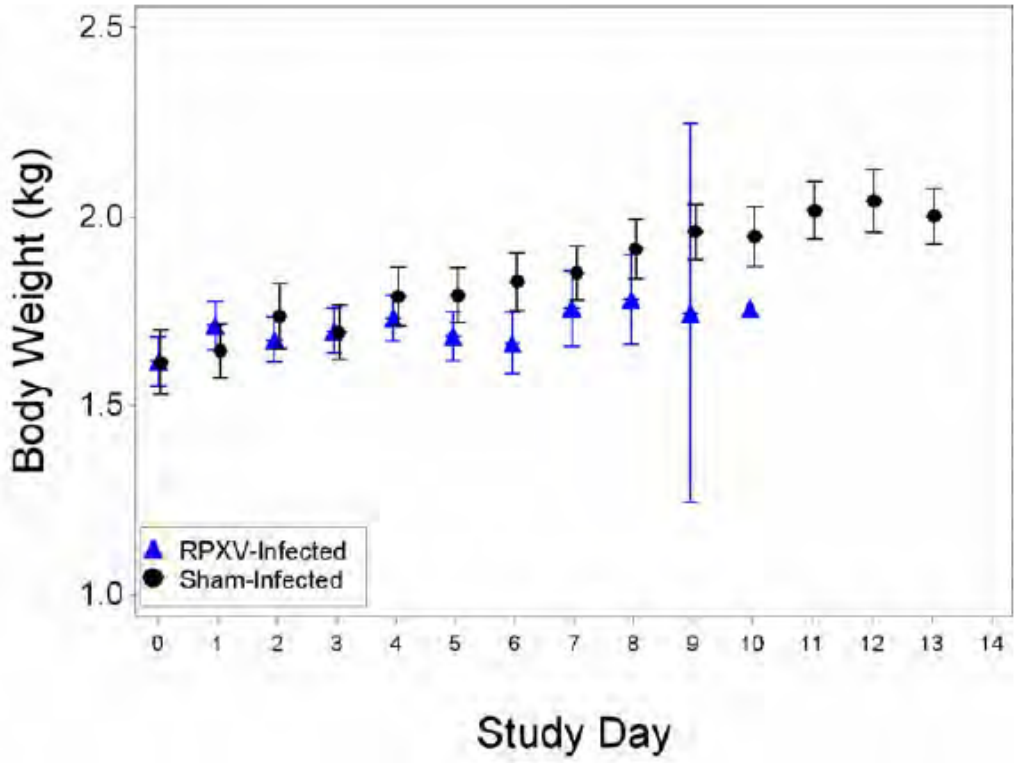
Assessed pre-challenge, 12, 24, 36, 48, 60, 72, 84 and 96 hours post-challenge, and once daily from Day 5 to Day 14. There were no findings in the sham-infected group. RPXV-challenged animals exhibited erythema and edema starting 36 hours post-challenge, which progressed to scabbing and/or necrosis that subsequently resolved in all animals by Days 5-6 post-challenge.

Body Weights

Measured once daily from pre-challenge to Day 14. Body weights (presented in **Figure 11**) steadily increased in the sham-infected group throughout the study, as expected. However, body weights plateaued in the RPXV-challenged group relative to sham starting around Day 4. As the study director noted, RPXV-infected animals

generally lose weight post-challenge. The observed plateau in body weight is likely due to the supplemental enrichment provided to these animals. Body weights were significantly lower in the RPXV-infected animals at Day 10 post-challenge, just prior to death.

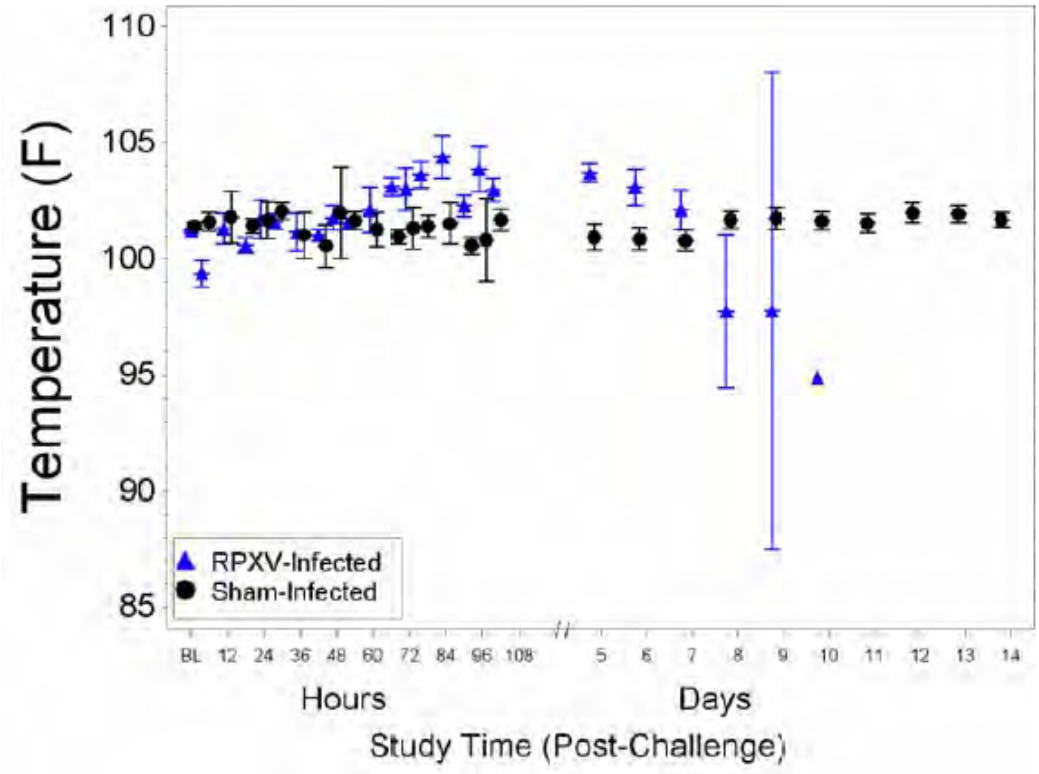
Figure 11: Body weights for RPXV natural history study #2819-100017958



Body Temperature

Temperature transponder chips were implanted in the shoulder/back and rump/hip of each animal prior to challenge. Temperatures were measured at least once daily pre-challenge to Day 14. Shoulder temperature changes are presented in **Figure 12**. No notable differences in shoulder and rump temperatures were observed. Likewise, as expected, no changes were noted in the sham-infected group. Temperatures were significantly increased by ~1-3 ^{o}F in the RPXV-infected group on Days 3 to 6 post-challenge before decreasing more than 3 ^{o}F (significantly below baseline) on Days 8 to 10, and below 90 ^{o}F in several animals on Day 9 just prior to death. Median time to fever onset was 2.8 days post-challenge. Given the early onset in temperature change relative to RPXV challenge and mortality, body temperature was considered a viable marker of early RPXV infection.

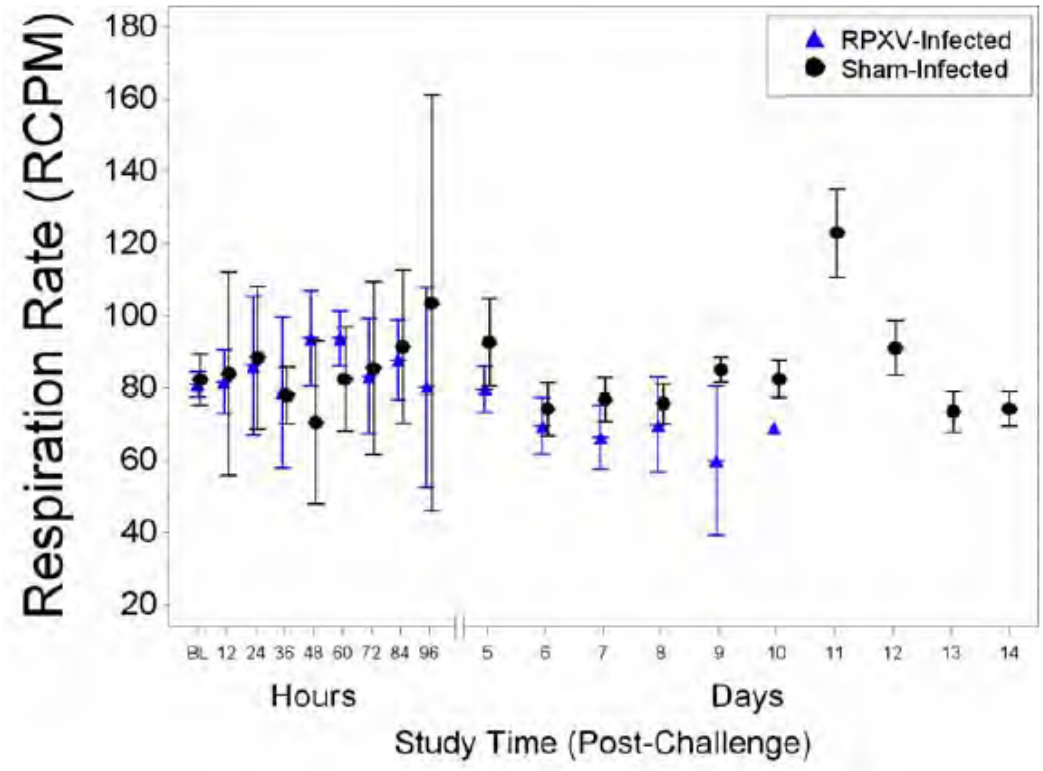
Figure 12: Temperature changes for natural history study #2819-100017958



Respiration Rates

Measured pre-challenge, 12, 24, 36, 48, 60, 72, 84 and 96 hours post-challenge, and once daily from Day 5 to Day 14. Respirations rates, presented in **Figure 13**, were increased 60 hours post-infection, and decreased on Days 6 to 9, relative to sham. The only significant difference was the decrease on Day 9, however, just prior to death in the few surviving animals. Qualitative changes also included nasal flaring and rapid/labored breathing in 41% of the RPXV-infected animals starting around Day 7. Given the variability in respiration rate and late onset relative to challenge and death, this parameter was not considered a viable marker of early RPXV infection.

Figure 13: Respiration rates for RPXV natural history study #2819-100017958



Hematology

Blood samples were collected pre-challenge, 12, 24, 36, 48, 60, 72, 84 and 96 hours post-challenge, and on Day 14. The following were observed:

- Significant decreases in hemoglobin, hematocrit and mean corpuscular volume, and increases in mean corpuscular hemoglobin concentration at most post-challenge time points relative to the sham-infected group.
- Decreased platelet counts at 12, 24 and 60 hours post-challenge, and increased mean platelet volume was increased 24 hours post-challenge, in the RPXV-infected group (also increased at several time points in the sham-infected group).
- Increased neutrophils in the RPXV-treated group at 36, 60 and 72 hours post-challenge, and decreased monocytes at several time points in both RPXV- and sham-infected groups.

In an attempt to identify potential hematology parameters as markers of RPXV infection, a statistical assessment was conducted in which “abnormal” was defined as being outside of the animal’s baseline plus or minus two times the standard deviation for all of the animals at baseline. The change in hematocrit was considered abnormal in 31% of the RPXV-infected animals, though several abnormal parameters were identified in the sham-infected group as well. Given that less than less than a third of the RPXV-infected animals displayed this abnormal finding, that this parameter is not specific for the infectious agent, and there were several abnormal findings in the sham-infected group as well, hematology parameters were not recommended as indicators of infection in the RPXV model.

Clinical Chemistry

Blood samples were collected pre-challenge, 12, 24, 36, 48, 60, 72, 84 and 96 hours post-challenge, and on Day 14. The following were observed:

- Decreased albumin, globulin and total protein levels were observed in RPXV-infected group relative to sham up to 48 hours post-challenge.
- Decreased BUN and creatinine levels in both RPXV- and sham-challenged groups at all study times.
- Increased potassium levels 24 hours post-challenge, and decreased calcium levels at several time points, in the RPXV-infected group relative to sham.
- Increased CRP levels in the RPXV-infected group relative to sham 60, 84 and 96 hours post-challenge.

As with the hematology parameters, an attempt was made to identify potential clinical chemistry parameters of RPXV infection. Except for CRP, "abnormal" was defined as being outside of the animal's baseline plus or minus two times the standard deviation for all of the animals at baseline. "Abnormal" CRP was defined as a measurement greater than the limit of detection. Several abnormal changes were noted in the RPXV-challenged group, but similar changes were also noted in sham, including CRP. As such, no clinical chemistry parameter was identified as a possible indicator of early RPXV infection.

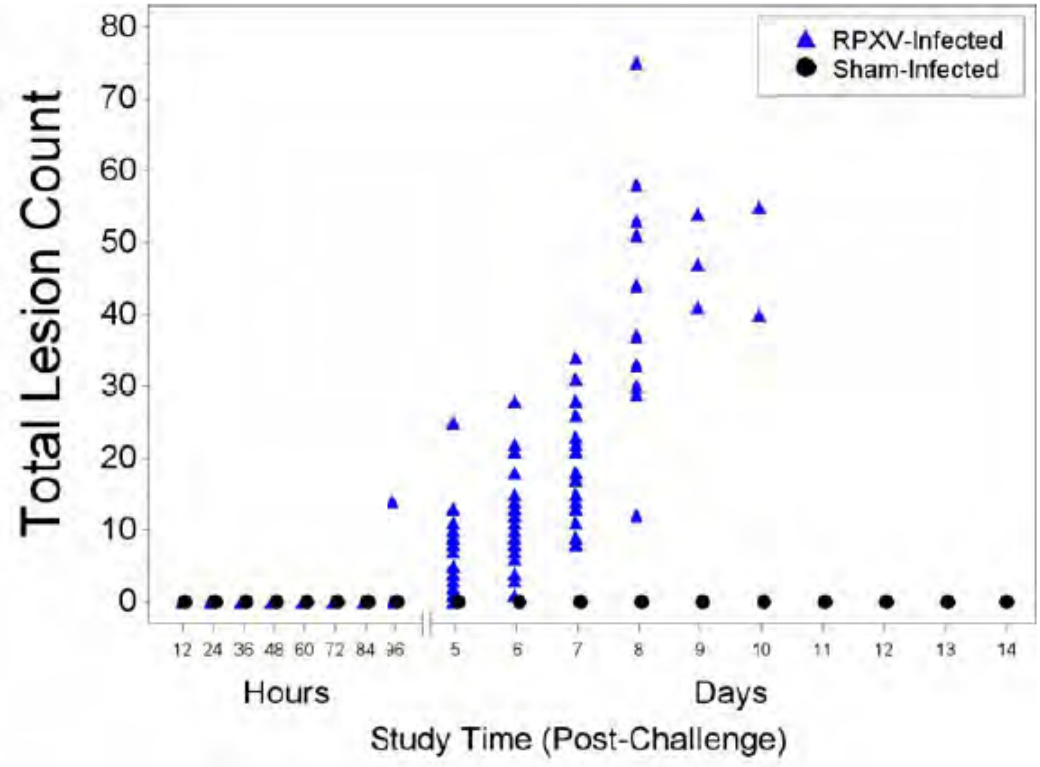
Gross Pathology

Evaluated at necropsy on Day 14 and following all premature deaths. RPXV-associated findings included necrosis, inflammation, hemorrhage and/or edema in the skin, tongue, lungs, spleen, testes/ovaries, liver, thymus and brain. All findings were consistent with prior studies of RPXV infection.

Lesion Score

Assessed 12, 24, 36, 48, 60, 72, 84 and 96 hours post-challenge, and once daily from Day 5 to Day 14. Total lesion counts are presented in **Figure 14**. All RPXV-challenged animals exhibited lesions between Days 6 and 10 post-challenge, with most appearing on the back. Half of the RPXV group also exhibited ear lesions, and 9 in the RPXV group had lesions on the nose, mouth and eyes. Given that lesions appeared relatively late in infection relative to mortality, lesion score was not considered a viable marker of early RPXV infection.

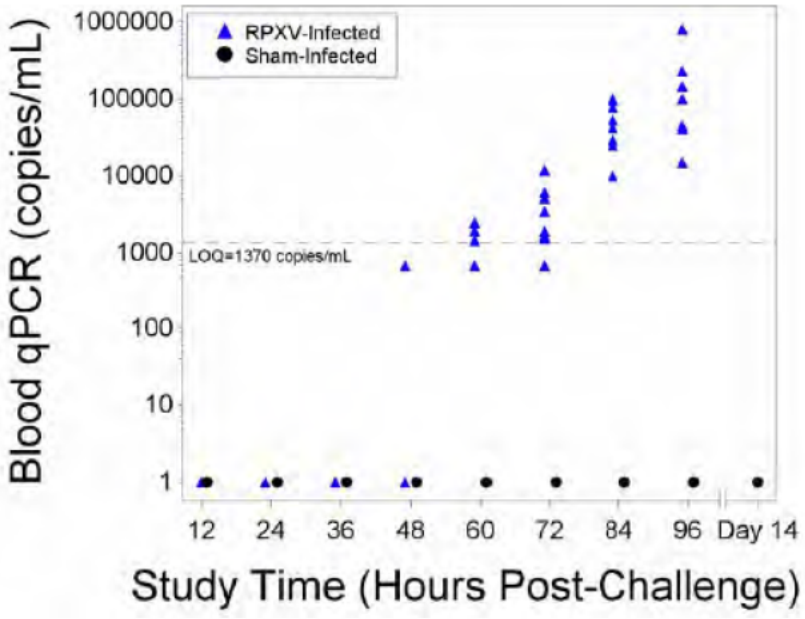
Figure 14: Total lesion count for RPXV natural history study #2819-100017958



Viral Load

Blood samples and buccal swabs were collected pre-challenge, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours post-challenge, and on Day 14. RPXV viral loads, as measured as gene copies of the RPXV HA gene, were confirmed by qPCR. Serum viral load is presented in **Figure 15**. Viremia was confirmed starting around 60 hours post-challenge, and was present in all animals by hour 84. Buccal samples also tested positive for viremia, but were slightly delated, with most animals testing positive around 96-120 hours post-challenge. All individual tissues tested from RPXV-challenged animals (spleen, kidney, heart, lung, liver, brain, skin, tongue and ear) were also positive for RPXV viremia on Day 14.

Figure 15: Serum viral load for RPXV natural history study #2819-100017958



Study Title: Characterization of the Intradermal Rabbitpox Model/Natural History in the Six Month New Zealand White Rabbit

Study no.:	2820-100017958
Study report location:	4.2.1.1
Study initiation date:	April 9th, 2013
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This study was conducted to characterize the disease progression of RPXV infection in 6-month-old New Zealand White rabbits. Eighteen rabbits with implanted venous ports were divided into Group A (7 males/5 females) and Group B (2 males/2 females). Group A animals were challenged intradermally with 1000 pfu of RPXV, strain Utrecht (actual titer = 734 pfu) on Day 0. Group B animals were challenged with sterile PBS. BCV was not administered, and the study was not blinded. Endpoints included mortality, clinical signs, challenge site monitoring, lesion counts, body weights, body temperatures, respiration rates, hematology, clinical chemistry, gross pathology, and viral load by qPCR. Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM or SC injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria

were identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

The primary objective of this study was to ensure that challenge with RPXV produced a robust, reproducible viremia and disease course in 6-month old animals that was consistent with prior studies of RPXV infection in this species, and to address the robustness of the model relative to what was seen in much younger, 9-week old animals (study #2819-100017958). In addition, several clinical parameters were evaluated as potential early markers of RPXV infection that could be used as "triggers to treat" in follow-up efficacy studies. To qualify as triggers to treat, these parameters must have been specific for RPXV infection, unambiguous, reproducible and objective, and early enough in the disease course to allow intervention corresponding to the therapeutic window in human smallpox.

All RPXV-challenged animals succumbed to illness within Days 6 to 10 post-challenge, while all sham-infected animals survived to study conclusion on Day 14. Clinical signs included decreased food consumption, lethargy, wheezing, nasal flaring, labored/rapid/shallow/open mouth breathing, and discharge from nose and/or mouth, all of which are consistent with RPXV infection and disease. Challenge sites in all RPXV animals were swollen from 48-60 hours post-challenge through death in all but one animal. RPXV lesions appeared starting 96 hours post-challenge in 4 animals, and appeared in all animals by Day 6. Body weights in the RPXV group were significantly decreased on Days 1-8 post-challenge relative to baseline. Body temperatures in the RPXV-infected animals were increased from 60 hours to 5 days post-challenge, after which temperatures decreased below baseline from Days 8 to 10, just prior to death. Respiration rates were increased in the RPXV-challenged animals 12 hours post-challenge and were decreased from hour 48 until death. Fluctuations in multiple hematology and clinical chemistry parameters were observed, but no meaningful changes relative to controls were observed. Gross pathology findings in the RPXV-challenged animals included (necrosis, inflammation, hemorrhage and/or edema in the skin, challenge sites, lung, spleen, testes/ovaries, liver and brain). All findings were consistent with RPXV infection in this species, and occurred roughly 1-5 days prior to death. RPXV viremia was also confirmed in the blood and nose starting roughly 2 days post-challenge.

Of the endpoints evaluated, body temperature, viremia and lesion count were considered the most promising as triggers to treat. Body temperature and serum viral load were elevated starting 2.5 Days post-challenge. RPXV lesions also appeared starting around Day 4-5 post-challenge, relatively later than fever and viremia onset and potentially too late for intervention given that mortality occurred between Days 6 and 10. In addition, no clear hematology or clinical chemistry parameters were identified as reliable treatment triggers.

Study Title: Blinded Study to Assess Ability to Identify Triggers to Treat in the ID Rabbitpox Model

Study no.: 2923-100017958
Study report location: 4.2.1.1
Study initiation date: June 25th, 2013

Conducting laboratory and location:	[redacted] (b) (4)
GLP compliance:	No
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This non-GLP study was conducted to evaluate body temperature (fever) and viremia as measured by qPCR in the blood as reliable indicators of disease onset following RPXV challenge. If considered reliable, these endpoints could have been used a triggers to initiate treatment with BCV in the subsequent efficacy studies. Fever was defined as a temperature (ºF) reading ≥2 standard deviations above baseline in each animal. Nine-week-old New Zealand White rabbits (4/sex/group) were challenged intradermally on Day 0 with either purified RPXV from study #2814-100017956 (target titer = 300 pfu, actual titer = 173 pfu) or sham (PBS). All surviving animals were euthanized on Day 14 post-challenge. BCV was not administered in this study. Endpoints included mortality, clinical signs, challenge site monitoring, lesion counts, body weights, body temperatures, respiration rates, hematology, clinical chemistry, gross pathology, and viral load by qPCR. Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria were essentially identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

All RPXV-challenged animals succumbed to disease by roughly Day 9 post-challenge (median time of death = 6.9 days). Clinical signs included decreased food consumption, lacrimation, nasal discharge and flaring, mucoid stools, and respiratory abnormalities, all of which are consistent with RPXV infection and disease. Body weight and body weight change generally plateaued in the RPXV-infected animals throughout the study, likely due to the edible supplementation provided to prevent RPXV-induced weight loss. Body temperatures in the RPXV-challenged group increased about 2-3 ºF starting roughly 60 hours post-infection and remained elevated through Day 6 before decreasing about 2-3 ºF below baseline prior to death around Day 8. Lesions appeared in all RPXV-infected animals starting around 96 hours post-challenge and increased steadily in number up to the time of death around Day 8. Fluctuations in respiratory rate were observed in both RPXV- and sham-infected groups, and no clear RPXV-related changes were observed relative to controls. Fluctuations in multiple hematology and clinical chemistry parameters were observed in the RPXV-infected animals, including decreases in RBC and platelet levels, hemoglobin and hematocrit starting roughly 72-96 hours post-challenge, but no statistically significant changes relative to sham were observed. All findings were consistent with RPXV infection in this species. RPXV viremia was also confirmed in the blood starting roughly 48 hours post-challenge and

peaking around Day 5 (120 hours post-challenge) before declining. Viremia was also confirmed in the RPXV-challenged animals that displayed a significant increase in body temperature prior to death.

As previously stated, the intention of this study was to determine whether body temperature and viremia were reliable indicators of RPXV disease onset in this species. Body temperature was significantly increased relative to pre-challenge and sham values from about 60 hours to 6 days post-challenge, and median time to onset of abnormality (as defined as 2 standard deviations above baseline) was 3 days. Likewise, viremia was clearly increased in RPXV-infected animals starting 2 days post-challenge. No meaningful changes in either endpoint were observed in the sham-infected group. Both body temperature and viremia were therefore considered reliable indicators of RPXV disease onset in this model, and could potentially be used as triggers to treat in the subsequent BCV efficacy studies.

Study Title: A Second, Randomized, Blinded, Study of the Potency of Rabbitpox Virus in New Zealand White Rabbits Infected via the Intradermal Route and Evaluation of Secondary Pox Lesions

Study no.:	CMX001-VIR-038
Study report location:	4.2.1.1
Study initiation date:	March 12th, 2014
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	No drug was used in this study
Deviations:	None which meaningfully affected study interpretation.

Summary

This study was conducted to evaluate the potency of purified RPXV, strain Utrecht, in New Zealand White rabbits. Design of this study was similar to the preceding potency study #CMX001-VIR-033, though lower RPXV titers were evaluated. Nine-week-old rabbits (4/sex in Groups 1-3, 6/sex in Group 4) were challenged intradermally on Day 0 with RPXV (lot 050310-ALS) at 3 pfu (Group 1), 30 (Group 2) or 300 pfu (Groups 3 & 4). Actual titers were 0.666, 8.66, 96.8 and 102.2 for Groups 1-4, respectively. All animals were euthanized on Day 21 post-challenge. BCV was not administered in this study. Endpoints included mortality, clinical signs, lesion counts, body weights, body temperatures, respiration rates, and viral load in whole blood and pox lesions by qPCR. Ketamine (~35 mg/kg)/xylazine (~5 mg/kg) was administered by IM injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria were essentially identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

Mortality was 50% in Group 1 and 100% in Groups 2-4. Clinical signs included decreased food consumption, respiratory abnormalities, anorexia, lethargy, and discharge from the nose and/or mouth, all of which are consistent with RPXV infection and disease. RPXV lesions appeared starting on Day 6, increased steadily in number through roughly Day 10. Lesions resolved in Group 1 by Day 11, while they were still present in Groups 2-4 up to the time of death around Day 14. Body weight change generally plateaued in all animals through at least Day 6 post-challenge. In Group 1, body weight change remained stagnant until Day 11 before increasing through study conclusion, which was attributed to recovery of the surviving animals. Weights in Groups 2-4 declined steadily from Day 6 up to death on roughly Day 14. Body temperatures also increased in all animals through roughly Day 5 post-challenge, after which temperatures either returned to or dropped below baseline. Group 1 temperatures returned to pre-challenge levels around roughly Day 10. Temperatures in Groups 2 and 3 were below baseline from Day 7 up to the time of death. Group 4 temperatures dropped on Day 7, were elevated again from Days 8 to 11, and steadily decreased leading up to the time of death. Respiration rates increased slightly up to Day 5 post-challenge before decreasing 20-50% below baseline in most animals through the remainder of the study. Group 1 rates recovered by Day 11 in the surviving animals. Group 2 rates increased sharply from Days 9-11 just prior to death. Other than the 50% recovery and resolution of most findings by roughly Day 11 in Group 1, no clear differences in any of the parameters evaluated were observed between groups. All findings were consistent with RPXV infection in this species. RPXV viremia was also confirmed in the blood and nose starting roughly 4 days post-challenge and peaking around Day 7 before declining. Virus was still detectable in several Group 1 animals on Day 20 shortly before study conclusion.

As observed in study #CMX001-VIR-033, there was a statistically significant correlation between RPXV inoculum level and survival. Mortality results from both potency studies were combined to calculate more accurate LD_{50} and LD_{90} values of 0.6 pfu and 46 pfu, respectively. The potency of the test material was considered adequate for use in the pivotal efficacy studies.

Study Title: A Randomized, Blinded, Placebo-Controlled, Parallel Group Study of the Efficacy of Immediate or Delayed Treatment with Brincidofovir in New Zealand White Rabbits Intradermally Inoculated with Rabbit Pox Virus Strain Utrecht

Study no.:	CMX001-VIR-041
Study report location:	4.2.1.1
Study initiation date:	February 27th, 2015
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Brincidofovir (lot #CMX001-097, purity = 100%)
Deviations:	Serious issues regarding the dosing and exposures for several time points in both the control group and treatment groups were unresolved and impacted data quality and

integrity. This study was deemed supportive only and could not be considered pivotal. Please refer to the following summary for more information.

Summary

This was the first of two studies conducted to definitively determine the efficacy of BCV in the treatment of RPXV-induced infection and disease. Survival over placebo was the primary endpoint, while clinical events including viremia, body temperature, body weight and respiration rate were secondary. Thirteen- to eighteen-week-old New Zealand White rabbits were challenged intradermally on Day 0 with purified RPXV, strain Utrecht (target titer = 300 pfu, actual titer = 316-350 pfu). BCV was administered in 3 doses spaced 48 hours apart by oral gavage. The design of this study is presented in **Table 15**. As stated in **Table 15**, the target minimum number of animals was 12/sex/group, but the actual group sizes were 28 in Groups 1 and 29 in Groups 2-5. All groups were double-blinded. BCV treatment was initiated within 4 hours following verification of fever, defined as 1.5ºF above baseline (14-71 hours post-challenge with a median of 62 hours). All surviving animals were euthanized on Day 42 post-challenge. Endpoints included mortality, clinical signs, challenge site monitoring, lesion counts, body weights, body temperatures, respiration rates, gross pathology, viral load by qPCR, and virology endpoints including resistance monitoring and plaque reduction neutralization titer. Please refer to the virology review by Dr. Pat Harrington for more information. Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM injection prior to challenge and euthanasia. Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated. The euthanasia criteria were identical to those used in natural history study #2819-100017958, and were considered acceptable. All unscheduled euthanasia adequately met the established criteria.

Table 15: Dosing regimen for RPXV study #CMX001-VIR-041

Group	Target Randomized	BCV Dose (mg/kg)	Timing of BCV and Placebo (p) Administration Per Blinded Kit (Day Post Randomization, RD)							
			RD0	RD1	RD2	RD3	RD4	RD5	RD6	RD7
1 (0 hr; immediate)	24 (12/sex)	20/5/5	BCV	p	BCV	p	BCV	p	p	p
2 (+24 hrs)	24 (12/sex)	20/5/5	p	BCV	p	BCV	p	BCV	p	p
3 (+48 hrs)	24 (12/sex)	20/5/5	p	p	BCV	p	BCV	p	BCV	p
4 (+72 hrs)	24 (12/sex)	20/5/5	p	p	p	BCV	p	BCV	p	BCV
5 (Placebo)	24 (12/sex)	0/0/0	p	p	p	p	p	p	p	p

Survival following RPXV challenge was 28/28 (100.0%), 27/29 (93.1%), 27/29 (93.1%), 20/29 (68.9%), and 14/29 (48.3%) in Groups 1-5, respectively, with death

occurring 8-10 days post-challenge. Treatment with BCV therefore resulted in significantly greater survival relative to placebo (Group 5), and survival increased the sooner treatment was initiated. Clinical signs included decreased food consumption, lethargy, lacrimation, nasal discharge, and respiratory abnormalities, all of which are consistent with RPXV infection and disease. No clear BCV-related changes in clinical signs were observed relative to placebo. Body weights plateaued in Group 1 and 2 animals and decreased in Groups 3-5 roughly 10 days post-challenge, after which weights increased steadily in all surviving animals through the remainder of the study. BCV treatment starting up to 24 hours post-randomization therefore prevented RPXV-associated weight loss, particularly in Group 1. As noted above, body temperatures increased following RPXV challenge about 2-3 ^{o}F starting roughly 62 hours, or roughly 2.5 days post-infection and remained elevated through roughly Day 7-10 before decreasing about 2-3 ^{o}F for the remainder of the study. BCV-treated animals exhibited slightly faster temperature recovery times in all groups relative to placebo (returning to pre-challenge values around Day 7 vs. Day 10 in Group 4). Respiratory rates also decreased in all groups through roughly Day 11 post-challenge before recovering, though the greatest decreases in respiratory rate were noted in Groups 4 and 5. BCV therefore appeared to alleviate RPXV-associated decreases in respiratory rate when administered up to 48 hours post-randomization. RPXV lesions appeared in all groups, though the number of animals with lesions was decreased the sooner BCV was administered relative to challenge. Lesions were seen in all animals except for 7 Group 1 animals, 7 Group 2 animals, and 2 Group 3 animals (all Group 4 animals developed lesions). By comparison, all placebo (Group 5) animals developed lesions except 2 which were euthanized moribund before lesions were observed. Gross pathology findings were generally limited to RPXV lesions in almost all animals, and no meaningful BCV-related differences were noted. All findings were consistent with RPXV infection in this species. RPXV viremia was also confirmed in the blood and nose starting roughly 1-2 days post-randomization and peaking around 6 days post-randomization in all groups. The timing of peak viral load was unchanged among groups, but the level of viremia was decreased relative to placebo (Group 5) in all BCV treatment groups, particularly in Group 1.

The results of this study suggest that BCV treatment protected against RPXV-induced mortality and changes in body temperature, respiratory rate and lesion count. BCV efficacy was also significantly improved the sooner that treatment was initiated relative to symptom onset.

Additional comments regarding Study CMX001-VIR-041:

The analysis of blood samples collected to confirm drug exposure in the BCV-treated animals, and absence of drug in placebo, unexpectedly identified the presence of BCV in several placebo samples, and a lack of drug in several samples from “treated” animals. The root cause of this error could neither be identified nor remedied despite multiple investigations. As a result of the data quality and integrity issues, this study was considered supportive of BCV efficacy only and could not be considered pivotal. This study was therefore repeated (see CMX001-VIR-106).

Study Title: A Randomized, Blinded, Placebo-Controlled, Parallel Group Study of the Efficacy of Brincidofovir Treatment when Initiated 3, 4, 5, or 6 Days Post Challenge in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus Strain Utrecht

Study no.:	CMX001-VIR-106
Study report location:	4.2.1.1
Study initiation date:	September 28th, 2018
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Brincidofovir (lot #CMX001-100; purity = 100%)
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This was the second of two GLP studies conducted to determine the efficacy of BCV in the treatment of RPXV-induced infection and disease, and of the two was considered the pivotal study. Survival over placebo was the primary endpoint, while clinical events including viremia, body temperature, body weight and respiration rate were secondary. No clear, drug-related differences in RPXV-induced clinical signs, body weights, lesion counts or challenge sites erythema/edema were observed. However, BCV (20, 5 and 5 mg/kg spaced 48 hours apart) was protective against RPXV-induced mortality relative to placebo when administered starting on Days 3, 4, 5 and 6, with the greatest survival benefit of 100% and 90% noted in Groups 1 (treated Days 3, 5 and 7 post-challenge) and 2 (treated on Days 4, 6 and 8 post-challenge), respectively. Viral loads also tended to be lower on Days 6 and 8 in Groups 1 and 2 relative to Group 5 (control). Body temperatures were also significantly above concurrent controls in Groups 1, 2 and 4 on Days 6 and 7, in Group 4 on Days 8 and 9, and in Group 3 on Day 9. Overall, BCV was shown to be effective in the treatment of RPXV-induced infection and disease, particularly in the reduction of RPXV-induced mortality, under the conditions of this study.

Methods (Drug)

Doses:	First dose: 20 mg/kg Second and third doses: 5 mg/kg
Frequency of Dosing:	Three doses spaced 48 hours apart
Number/Sex/Group:	15/sex/group
Dose volume:	1 mL/kg
Formulation/Vehicle:	(b) (4) Formulation, pH = 8.1
Route of administration:	Oral feeding via needleless syringe
Species/Strain:	NZW rabbits (b) (4)
Age/Sexual Maturity:	13-16 weeks
Comment on Study Design and Conduct:	RPXV challenge was conducted on Day 0. Drug was administered once every 48 hours for 3 total doses starting on Days 3, 4, 5 or 6. All surviving

animals were euthanized on Day 42. See **Table 16** for more information.

Dosing Solution Analysis: All samples met acceptance criteria

Methods (Pathogen Challenge)

Challenge Agent Info: Rabbitpox (RPXV), Utrecht strain
Challenge Agent Lot: 050310-ALS
Target Challenge Dose: 600 pfu/200 µL PBS (300 pfu/100 µL per thigh)
Frequency of Dosing: Single dose on Day 0
Route of Administration: Bilateral intradermal injections in both thighs
Back-Titrated Final Dose: 488 pfu. The lower actual titer was attributed to that of a single challenge group (Group C), which was 272 pfu, or 45% of the targeted challenge dose. All other groups ranged from 528-562 pfu, or 88-94% of the targeted dose. Despite the lower titer in Group C, no clear differences in mortality were noted relative to the other groups, indicating that did not impact interpretation of the study.

Table 16: Study #CMX001-VIR-106 dosing regimen

Group	Planned Number of Animals	Actual Number of Animals	Challenge	Timing of BCV and Control Article Administration Per Blinded Kit							
			Study Day 0	Study Day 3	Study Day 4	Study Day 5	Study Day 6	Study Day 7	Study Day 8	Study Day 9	Study Day 10
			Target Challenge Dose	Blinded Dosing Kit Dose Vial 1	Blinded Dosing Kit Dose Vial 2	Blinded Dosing Kit Dose Vial 3	Blinded Dosing Kit Dose Vial 4	Blinded Dosing Kit Dose Vial 5	Blinded Dosing Kit Dose Vial 6	Blinded Dosing Kit Dose Vial 7	Blinded Dosing Kit Dose Vial 8
1	30 (15/sex)	29 (14 M, 15 F)	600 PFU	**BCV (20 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	Control Article	Control Article
2	30 (15/sex)	29 (15 M, 14 F)		Control Article	**BCV (20 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	Control Article
3	30 (15/sex)	29 (14 M, 15 F)		Control Article	Control Article	**BCV (20 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article
4	30 (15/sex)	29 (15 M, 14 F)		Control Article	Control Article	Control Article	**BCV (20 mg/kg)**	Control Article	**BCV (5 mg/kg)**	Control Article	**BCV (5 mg/kg)**
5	30 (15/sex)	28 (14 M,14 F)		Control Article	Control Article	Control Article	Control Article	Control Article	Control Article	Control Article	Control Article

Protocol-Related Discussion Points

- Anesthesia: Ketamine (22-50 mg/kg)/Xylazine (3-10 mg/kg) was administered by IM or SC injection prior to challenge and euthanasia. Acepromazine (1-5 mg/kg) was administered by IM injection prior to blood draws and temperature chip implantation. The exact doses of anesthesia were recorded.
- Veterinary/Supportive Care: Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated.
- Blinding: Double Blind (sponsor, study director and staff were all blinded during study conduct).

Euthanasia Criteria

Animals meeting any of the following three (3) criteria were euthanized:

- Severe respiratory distress as assessed by clinical observations or morbidity and moribundity checks including open mouth breathing and/or forced abdominal respirations.
- Moribund, persistent prostration, seizures, and/or unresponsive to external stimuli (e.g., gentle prodding by hand).
- Any animal(s) meeting at least two (2) of the following criteria were euthanized:
    - Respiration rate 75% lower or higher than the average observed during the baseline period (confirmed by second respiration rate measurement 1 hr ± 10 min later).
    - Body temperature less than 37.2^{o}C from either chip (confirmed by a second temperature measurement 1 hr ± 10 min later).
    - Weight loss greater than 15% from pre-challenge (Day 0) weight.

Note: For respiration rate and body temperature, the second confirmatory measurement was not required if that was the only euthanasia criteria met at that time point.

These criteria were essentially identical to those used in the pivotal natural history studies and were considered acceptable. All unscheduled euthanasia adequately met these criteria.

Observations and Results

Mortality

Assessed three times daily from Days 4 to 11, and once daily from Days 12 to 42. A total of 6 animals were excluded from the study prior to challenge. The reasons for exclusion of each of these animals are presented in **Table 17**.

Table 17: Study #CMX001-VIR-106 animals excluded/not challenged

Animal ID	Sex	Challenge Cohort	Reason Animal was Excluded/Not Challenged
29	F	CDA	Animal euthanized in consultation with staff veterinarian as not being suitable for study per inclusion criteria.
50	F	CDB	Animal euthanized in consultation with staff veterinarian as not being suitable for study per inclusion criteria.
75	M	CDC	Animal died prior to challenge immediately after anesthesia (see Appendix B, Investigation Report INV-S-201811-006556).
97	M	CDD	Animal euthanized after being found with a broken leg.
137	F	CDE	Animal found dead.
135	M	CDE	Animal euthanized in consultation with staff veterinarian as not being suitable for study per inclusion criteria.

Of the animals challenged, a total of 14 were found dead and 27 were euthanized prior to study completion. A summary of the deaths and the reasons for euthanasia are presented in **Tables 18 and 19**, respectively. Mortality is also presented in the Kaplan-Meier curve in **Figure 16**. All euthanasia decisions were based on the euthanasia criteria and appeared to be valid. Survival was 100% for those treated with BCV starting on Day 3, 90% for those starting on Day 4, 69% for those starting on Days 5 and 6, and 29% for placebo.

Figure 16: Mortality curve for RPXV study #CMX001-VIR-106

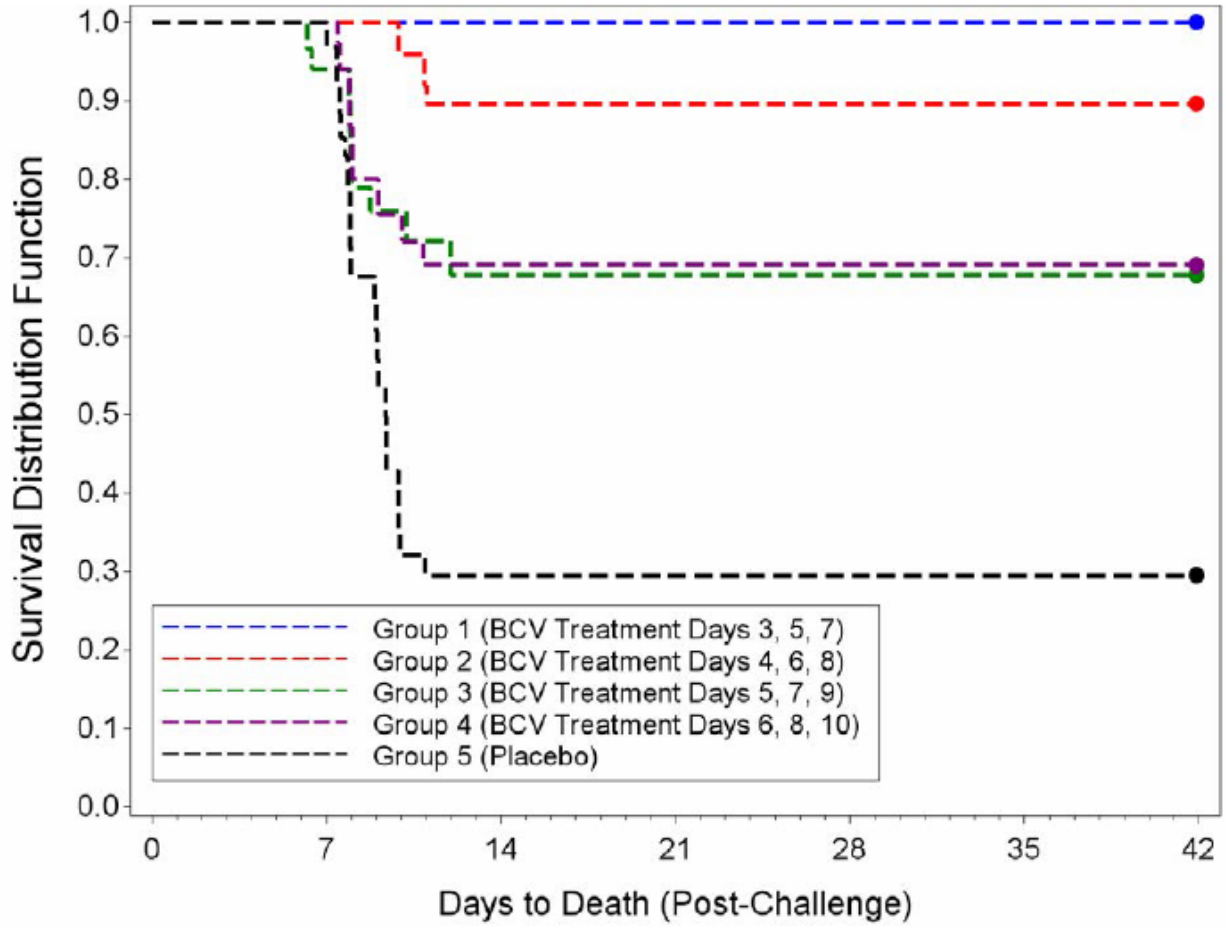


Table 18: Study #CMX001-VIR-106 summary of mortality

Group	BCV Regimen^	Number of Animals Challenged	Number of Animals Found Dead	Number of Animals Euthanized	Total Number of Animals Died
1	3, 5, 7	29	0	0	0
2	4, 6, 8	29	0	3	3
3	5, 7 , 9	29	3	6	9
4	6, 8, 10	29	3	6	9
5	Placebo	28	8	12	20

^ Study days on which the first, second and third dose of the 20/5/5 (q48hr) BCV treatment regimen was administered.

Table 19: Study #CMX001-VIR-106 reasons for euthanasia

Group	BCV Regimen*	Animal ID	Reason for Euthanasia^
2	4, 6, 8	42	Body Weight and Temperature Euthanasia Criteria Met
2		76	Open Mouth Breathing, Nasal Flaring
2		121	Body Weight and Temperature Euthanasia Criteria Met
3	5, 7, 9	1	Unresponsive, Moribund, Forced Abdominal Respiration
3		16	Prostrate, Moribund
3		24	Body Weight and Temperature Euthanasia Criteria Met
3		43	Nasal Flaring, Open Mouth Breathing
3		48	Labored Breathing, Nasal Flaring, Unresponsive
3		111	Body Weight and Temperature Euthanasia Criteria Met
4	6, 8, 10	40	Moribund, Prostrate, Forced Abdominal Respiration
4		56	Open Mouth Breathing, Forced Abdominal Respiration
4		66	Prostrate, Unresponsive, Respiratory Distress
4		101	Open Mouth Breathing
4		141	Open Mouth Breathing, Forced Abdominal Respiration
4		150	Open Mouth Breathing
5	Placebo	13	Open Mouth Breathing
5		21	Prostrate, Forced Abdominal Respiration
5		22	Unresponsive, Moribund, Forced Abdominal Respiration
5		31	Body Weight and Temperature Euthanasia Criteria Met
5		33	Prostrate, Shallow Breathing, Unresponsive
5		41	Prostrate, Unresponsive, Labored Breathing
5		59	Open Mouth Breathing, Forced Abdominal Respiration
5		80	Respiratory Distress
5		100	Forced Abdominal Respiration, Wheezing, Nasal Flaring
5		115	Body Weight and Temperature Euthanasia Criteria Met
5		128	Unresponsive, Moribund, Forced Abdominal Respiration
5		131	Unresponsive, Moribund, Respiratory Distress

* Study days on which the first, second and third dose of the 20/5/5 (q48hr) BCV treatment regimen was administered.
^ Note: Not all abnormal clinical observations which were observed are summarized in this table. This table highlights the specific criteria which were used as the criteria for euthanasia.

Clinical Signs

Assessed twice daily from pre-challenge to Day 3, three times daily from Days 4 to 11, and twice daily from Days 12 to 42. RPXV-related clinical signs included stool abnormalities, nasal discharge, reduced food consumption, lacrimation, and lethargy, and started as early as Day 3. All observations progressively worsened in all groups throughout the study until the animals either recovered or were found dead/euthanized. For those that succumbed to RPXV infection, clinical signs progressed to severe respiratory abnormalities (audible rales or wheezing, nasal flaring, splinting, and open mouth breathing) prior to death. No clear differences were noted between placebo and BCV-treated groups.

Lesion Score

The whole bodies of each animal were assessed for RPXV lesions daily from pre-challenge to Day 42. Lesions appeared in all groups starting on Day 2 and resulted

around Day 33, with most appearing from Days 5 to 20. No clear, drug-related differences in lesion count were observed.

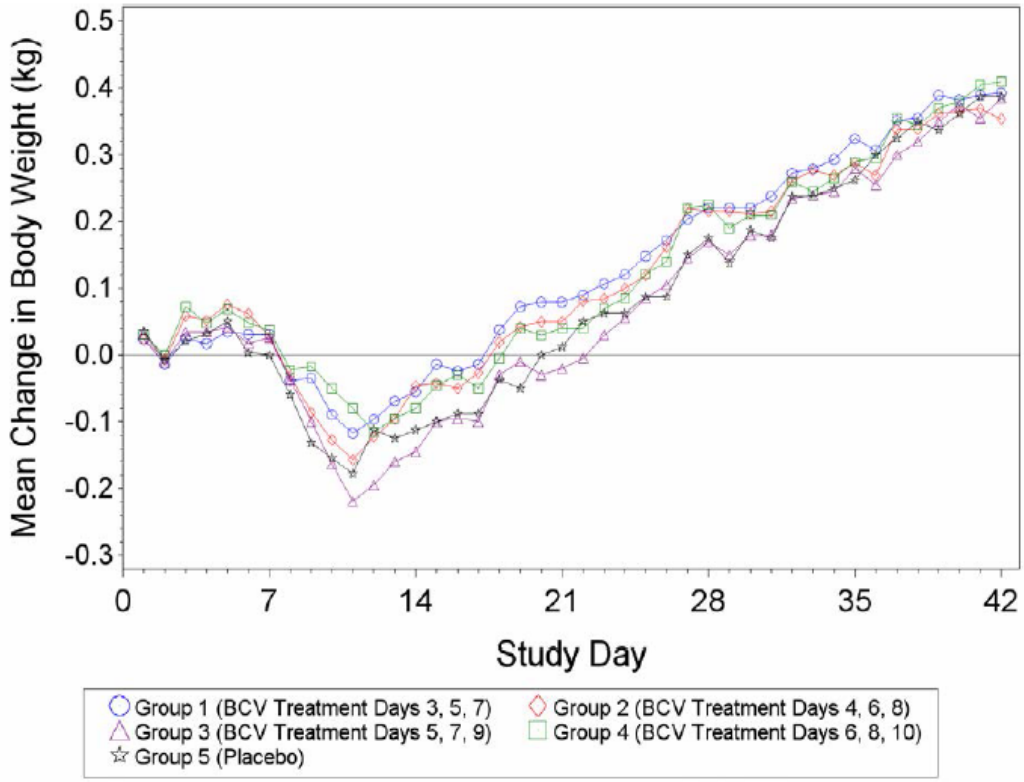
Challenge Site Assessment

The RPXV challenge sites were assessed for erythema and edema in each animal once daily from pre-challenge to Day 42. Erythema and edema were scored from 0 (no erythema/swelling) to 4 (severe erythema/swelling). Mild to moderate erythema/edema were noted in all groups starting on Day 2, and increased to severe on Days 4 to 7 before starting to resolve. Animals who were euthanized or found dead generally had higher erythema/edema scores than those who survived to Day 42. No clear, drug-related differences in erythema or edema were observed.

Body Weights

Measured once daily from pre-challenge to Day 42. Body weight changes are presented in **Figure 17**. In all groups, body weights were decreased relative to baseline on Days 8 to roughly Day 18, after which weights increased steadily for the remainder of the study. No clear, dose-dependent differences were observed in among any of the groups in this study.

Figure 17: Body weight changes for RPXV study #CMX001-VIR-106

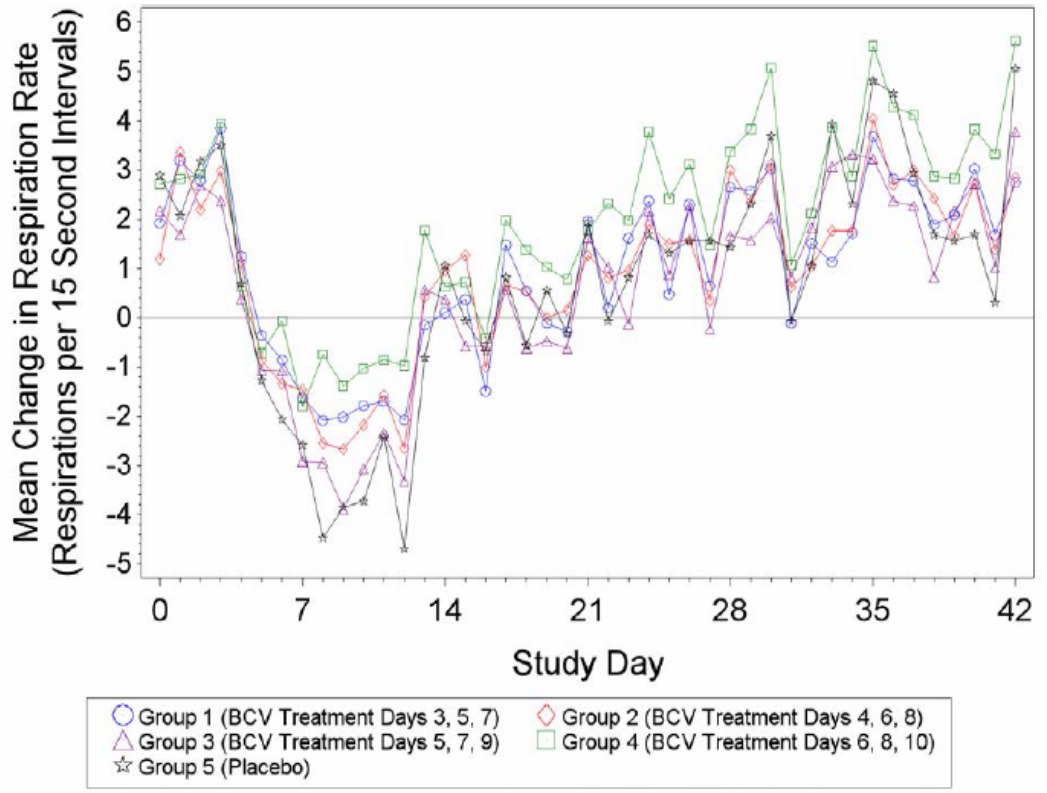


Respiration Rates

Assessed qualitatively by visual inspection once daily prior to dosing and handling (while animals were at rest) from pre-challenge to Day 3, three times daily from

Days 4 to 11, and once daily from Days 12 to 42. Additional observations included audible rales or wheezing, nasal flaring, splinting, and open mouth breathing. Mean changes in respiration rate are presented in **Figure 18**. In all groups, respiration appeared to elevated above baseline on Days 1 to 3, drop below baseline on Days 5 to 13, and were elevated again from Day 14 through most of the remainder of the study. No clear, dose-dependent differences were observed among any of the groups in this study.

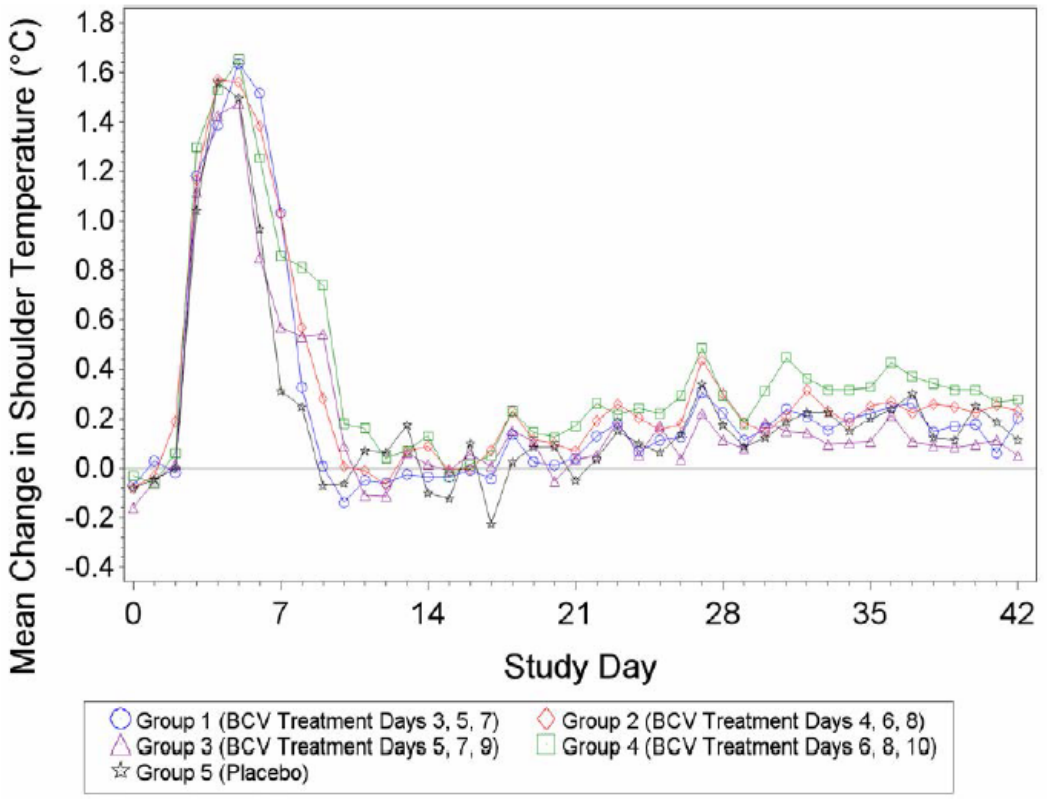
Figure 18: Respiration rate changes for RPXV study #CMX001-VIR-106



Body Temperature

Temperature transponder chips were implanted in the shoulder/back and rump/hip of each animal prior to challenge. Temperatures were measured at least once daily pre-challenge to Day 3, three times daily from Days 4 to 11, and once daily from Days 12 to 42. Shoulder temperature changes are presented in **Figure 19**. Temperatures were significantly increased above baseline in all animals from Days 3 to 6 and on Day 27 (likely incidental on Day 27). On Days 6 and 7, the increases in Groups 1, 2 and 4 were significantly higher than in (control) Group 5. The temperatures in Group 4 were also increased above concurrent control levels on Days 8 and 9, and in Group 3 on Day 9.

Figure 19: Shoulder temperature changes for RPXV study #CMX001-VIR-106



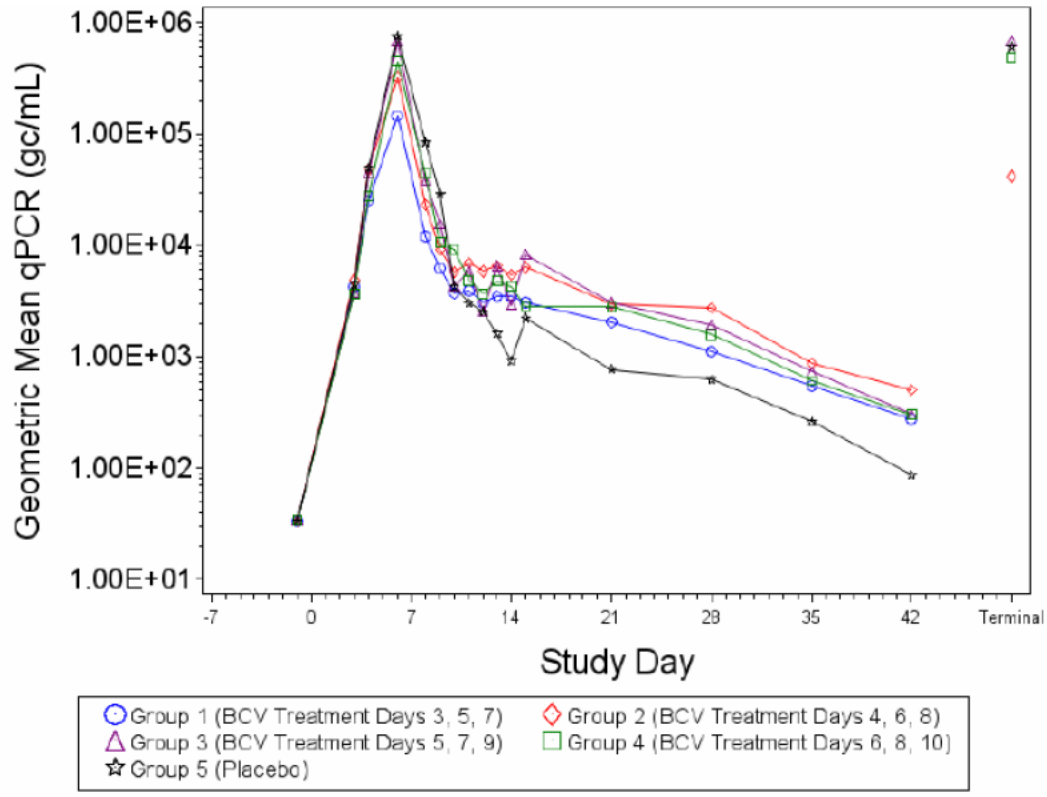
Gross Pathology

Assessed at necropsy in all challenged animals found dead or euthanized. Gross findings consisted of RPXV-associated lesions at the challenge sites and on the dorsum, and red/white/mottled foci on the tongue, skin, lips and nose in several animals, all of which are consistent with normal RPXV infection and disease. No drug-related findings were observed.

Serum Viral Load

Blood samples were collected pre-challenge and on Days 3 to 6, 8, 10, 12, 14, 21, 28, 35 and 42, and RPXV viral loads were confirmed by plaque assay and qPCR. RPXV levels as measured by qPCR are presented in **Figure 20**. Viral loads on Days 6 and 8 were significantly higher in control Group 5 than in Groups 1 or 2. Conversely, viral loads were higher in Groups 1, 2 and 4 relative to Group 5 on Day 14, and were higher in Group 2 relative to Group 5 on Day 28. Overall, the mean maximum values in Group 5 were significantly higher than in Groups 1 and 2.

Figure 20: Serum viral load for RPXV study #CMX001-VIR-106



Treatment Confirmation

Blood samples were collected pre-challenge and on Days 3 to 6, 8, 10, 12, 14, 21, 28, 35 and 42. Toxicokinetics were not evaluated in this study, but serum drug levels were measured by mass spectrometry to confirm the presence of drug. The presence of BCV was confirmed in all samples that were expected to contain drug. Likewise, no BCV was detected in all samples which were expected to be negative except for Group 4 animal #124. Drug was detected in this sample on Day 5, one day prior to its first scheduled dose. According to the study director, this was likely the result of contamination as drug levels were only slightly above the level of detection. This animal also succumbed to RPXV infection on Day 7, indicating that BCV was not protective against disease in this case.

Study Title: A Single and Repeat Oral Dose Pharmacokinetic Study of Brincidofovir in Plasma and Concentrations of Cidofovir-Diphosphate in Peripheral Blood Mononuclear Cells of New Zealand White Rabbits Intradermally Infected with Rabbitpox Virus Strain Utrecht

Study no.: CMX001-VIR-122
Study report location: 4.2.2.7
Study initiation date: October 17th, 2019
Conducting laboratory and location: (b) (4) [redacted]

GLP compliance:	Yes
Drug, lot #, and % purity:	Brincidofovir (lot #CMX001-100; purity = 100%)
Deviations:	None which meaningfully affected study interpretation.

Key Findings

This study was conducted to evaluate the pharmacokinetics of BCV in plasma and the intracellular metabolite, CDV-PP, in PBMCs following single or repeat doses of BCV in rabbits following a lethal RPXV challenge. Pharmacokinetic parameters for BCV and CDV-PP are presented below.

Methods (Drug)

Doses:	First dose: 20 mg/kg Second and third doses: 5 mg/kg
Frequency of Dosing:	Single dose or 3 doses spaced 48 hours apart
Number/Sex/Group:	6/sex/group
Dose volume:	1 mL/kg
Formulation/Vehicle:	(b) (4) (w/v) and (b) (4) (w/v) in dH_2O, pH = 7.8-8.1
Route of administration:	Oral feeding via needleless syringe
Species/Strain:	NZW rabbits (b) (4)
Age/Sexual Maturity:	16 weeks
Comment on Study Design and Conduct:	RPXV challenge was conducted on Day 1. Drug was administered either once on Day 5 or once every 48 hours for 3 total doses on Days 5, 7 and 9. All surviving animals were euthanized on Day 14. See **Table 20** for more information.
Dosing Solution Analysis:	All samples met acceptance criteria

Methods (Pathogen Challenge)

Challenge Agent Info:	Rabbitpox (RPXV), Utrecht strain
Challenge Agent Lot:	050310-ALS
Target Challenge Dose:	600 pfu/200 µL PBS (300 pfu/100 µL per thigh)
Frequency of Dosing:	Single dose on Day 1
Route of Administration:	Bilateral intradermal injections in both thighs
Back-Titrated Final Dose:	244 pfu

Table 20: Study #CMX001-VIR-106 dosing regimen

Group	Number of Rabbits	RPXV Challenge Day	BCV Treatment Regimen	Timing of Oral BCV administration[1] (Study Day)			Blood sample collection schedule relative to BCV treatment			
							Analytical Samples Collected for Assay (6/sex/timepoint) [After 20mg/kg BCV Dose #1]		Analytical Samples Collected for Assay (6/sex/timepoint) [After 5mg/kg BCV Dose #3]	
				20mg/kg BCV (Dose #1)	5mg/kg BCV (Dose #2)	5mg/kg BCV (Dose #3)	Plasma[2] (Hrs)	PBMC[3] (Hrs)	Plasma[2] (Hrs)	PBMC[3] (Hrs)
1	12 (6M/6F)	Day 1	20 mg/kg	Day 5			0.5, 1, 2, 4, 12	12, 24, 48, 72, 120		
2	12 (6M/6F)	Day 1	20/5/5 mg/kg (q48hrs)	Day 5	Day 7	Day 9			0, 0.5, 1, 2, 4, 12	0, 24, 48, 72, 120

M=male; F=female; RPXV= Rabbit pox virus; BCV= Brincidofovir; mg/kg= Milligrams per kilogram; Hrs= Hours; q48hrs= Dose every 48 hrs; mL=Milliliter
[1] Oral administration at 1 mL/kg dose volume
[2] Approximately 0.5 mL of whole blood obtained per timepoint; approximately 2.0 mL whole blood obtained at end of study (Day 14) for retention.
[3] Approximately 4 mL of whole blood obtained per timepoint

Protocol-Related Discussion Points

- Anesthesia: Ketamine (22-50 mg/kg)/xylazine (3-10 mg/kg) was administered by IM injection prior to challenge and euthanasia. Acepromazine (1-6 mg/kg) was administered by IM injection prior to blood draws. The exact doses of anesthesia were recorded.
- Veterinary/Supportive Care: Soft, edible enrichment was provided and documented for post-challenge animals displaying signs of reduced food consumption and/or difficulty eating. Anesthetics were presumably not used for pain management, but this was not explicitly stated.
- Blinding: None.

Euthanasia Criteria

Animals meeting any of the following three (3) criteria were euthanized:

- Severe respiratory distress as assessed by clinical observations or morbidity and moribundity checks, including open mouth breathing and/or forced abdominal respirations.
- Moribund, persistent prostration, convulsions (seizures), and/or unresponsive to external stimuli (e.g., gentle prodding by hand).
- Weight loss greater than 20% from pre-challenge (Day 1) weight.

These criteria were essentially identical to those used in the pivotal natural history studies and were considered acceptable. No unscheduled euthanasia were performed in this study.

Observations and Results

Mortality

Assessed twice daily. One Group 1 animal was found dead approximately 10 days post-challenge. All other animals survived to the end of the study.

Clinical Signs

Assessed twice daily. From Day 4 through the end of the study, animals exhibited discolored skin, skin ulcers on the hind limb, eye abnormalities, respiratory abnormalities, nose and eye discharge, low food consumption, and skin lesions, all of which were consistent with RPXV disease.

Body Weights

Measured on Days 1, 5, 7 and 9. Body weights increased relative to pre-challenge values in both groups, suggesting a protective effect of BCV, but a true drug-related effect cannot be determined due to the lack of a concurrent control group.

Viremia

Whole blood samples were collected pre-dose on Days 1 and 5, and viral load was confirmed by qPCR. As expected, RPXV viremia was not detected on Day 1, but was confirmed in all animals on Day 5.

Toxicokinetics

Plasma and PBMC samples were collected according to the schedules presented in **Tables 21 and 22**. BCV levels were measured in the plasma and CDV-PP levels were measured intracellularly in PBMCs. Pharmacokinetic data for BCV and CDV-PP are presented in **Tables 23 and 24**, respectively.

Table 21: Study #CMX001-VIR-106 plasma collection schedule

Group	n (M/F)	First BCV Dose On Study Day 5 (mg/kg)	Last BCV Dose On Study Day 9 (mg/kg)	Plasma PK Sample Collection Time-Points Following Designated BCV Dose[a] (Time in Hours Post-Dose)					
				0[b]	0.5	1	2	4	12
				Window for Sample Collections (± X minutes)					
				60	5	10	10	10	30
1	6/6	20			✓	✓	✓	✓	✓
2	6/6	20	5	✓	✓	✓	✓	✓	✓

✓ = sample was collected;
[a] Samples collected after the first BCV dose for Group 1 and after the last BCV dose (Dose #3) for Group 2;
[b] 0 hr samples were collected prior to last dose (Dose #3) in Group 2

Table 22: Study #CMX001-VIR-106 PBMC collection schedule

Group	n (M/F)	First BCV Dose On Study Day 5 (mg/kg)	Last BCV Dose On Study Day 9 (mg/kg)	PBMC Sample Collection Time-Points Following Designated BCV Dose[a] (Time in Hours Post-Dose)					
				0[b]	12	24	48	72	120
				Window for Sample Collections (± X minutes)					
				60	30	60	60	60	60
1	6/6	20			✓	✓	✓	✓	✓
2	6/6	20	5	✓		✓	✓	✓	✓

✓ = sample was collected;
[a] Samples collected after the first BCV dose for Group 1 and after the last BCV dose (Dose #3) for Group 2;
[b] 0 hr samples were collected prior to last dose (Dose #3) in Group 2

Table 23: Study #CMX001-VIR-106 BCV plasma PK parameters

Group	Sex	C_{max} (ng/mL)	C_{max} /Dose ((ng/mL)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*ng/mL)	AUC_{last}/Dose ((h*ng/mL)/ (mg/kg))
1	Male	149	7.47	NC	584	29.2
	Female	220	11.0	NC	834	41.7
	Combined	185	9.23	NC	709	35.5
2	Male	12.9	2.58	NC	62.1	12.4
	Female	17.6	3.52	NC	86.6	17.3
	Combined	15.2	3.05	NC	74.3	14.9

Table 24: Study #CMX001-VIR-106 CDV-PP PBMC PK parameters

Group	Sex	C_{max} (pg/million cells)	C_{max} /Dose ((pg/million cells)/ (mg/kg))	T_{max} (h)	AUC_{last} (h*pg/million cells)	AUC_{last}/Dose ((h*pg/million cells)/ (mg/kg))
1	Male	7.08	0.354	NC	365	18.2
	Female	6.64	0.332	NC	358	17.9
	Combined	6.86	0.343	NC	362	18.1
2	Male	5.72	1.14	NC	403	80.7
	Female	6.62	1.32	NC	423	84.6
	Combined	6.17	1.23	NC	413	82.7

4.3 Safety Pharmacology

The effect of BCV on hERG channel current was measured at concentrations of 0 (vehicle control), 0.01 (low), 0.1 (mid) and 1.0 (high) μM using whole cell patch clamp techniques and human embryonic kidney cells that express the hERG channel. Effects on the hERG channel were calculated by measuring inhibition of peak tail current. BCV inhibited hERG currents by 0.0%, -7.8% and -24.9% at the low, mid and high doses, respectively. Under the conditions of this study, BCV inhibited the hERG channel current and thus has potential to prolong QT intervals. Effects on cardiac parameters were not noted in nonclinical studies or clinical trials.

In telemetered male dogs, a single IV injection of BCV at dose levels of 0 (vehicle controls) 4 (low), 10 (mid) or 25 mg/kg (high) caused increases in systolic blood pressure, diastolic blood pressure and mean arterial blood pressure, with the most notable effects at 60-90 minutes post-dose.

In rats, a single IV injection of BCV (40 mg/kg) reduced dynamic lung compliance (56% and 45% at 2 and 3 minutes, respectively) and tidal volume (2 to 5 minutes post-dose, with peak reduction of 23%). BCV also significantly increased respiratory rate (35%, 87%, 57%, 45% and 32% increases at 1, 2, 3, 4 and 5 minutes, respectively) and minute volume (52%, 35% and 23% at 2, 3 and 4 minutes, respectively). In a 28-day IV infusion study in rats, administration of BCV resulted in intermittent (generally following infusion) rapid breathing or irregular breathing at ≥4 mg/kg/dose.

BCV did not produce effects on behavior or body temperature in male rats following a single oral gavage dose of BCV.

5 Pharmacokinetics/ADME

5.1 PK/ADME

Nonclinical Pharmacokinetics

In nonclinical studies, BCV and CDV exposure (C_{max} and AUC values) tended to increase with increasing dose, but the increase was less than dose-proportional. Exposure in males and females was not notably different (i.e., <2-fold difference). In rats, systemic exposures were approximately 10% of systemic exposures in humans given a single 200 mg oral dose of BCV. The IV route of administration in rats resulted in clinically-relevant BCV exposures. BCV exposures exceeded the C_{max} and AUC in humans following a single 200 mg oral dose by approximately 5-fold and 2-fold, respectively.

Absorption

The absolute bioavailability of BCV in mice and rats following oral administration was estimated to be >30% and 8%, respectively. In monkeys, the absolute bioavailability of BCV was 0.7% when administered as a solution and 0.3% when administered as a capsule. Clinically, bioavailability was determined to be 16.8% when research subjects were administered an oral suspension.

Distribution

Protein binding was assessed in CD-1 mice, SD rats, NZW rabbits, beagle dogs, mini pigs, cynomolgus monkeys, and human plasma. BCV binding to plasma proteins was high (>98%). A study in mice revealed significant and sustained levels of [^{14}C]-BCV-derived radioactivity in liver and small intestine following oral administration of a dose that was reported to be efficacious in a mouse model of smallpox. The tissues with the highest concentrations (in decreasing order) were small intestine, stomach, liver, urinary bladder, cecum, kidney cortex, large intestine, kidney medulla, and adrenal gland. The data suggested the possibility of significant first pass metabolism or extraction by the liver. Correspondingly, BCV, but not its metabolites, was a substrate for liver uptake transporters OATP1B1 and OATP1B3. No parent BCV and low levels of CDV were detected in plasma of monkeys given BCV by the oral route. Following IV dosing, BCV disappeared rapidly from blood in monkeys, while CDV increased to only modest levels.

Metabolism

BCV was extensively metabolized in mice, rats, rabbits, monkeys, and humans after oral or IV administration. No unchanged BCV was detected in urine or feces after either route. Metabolism of BCV followed two primary pathways: i) hydrolysis to form CDV, and ii) oxidation.

BCV is metabolized by hydrolysis of the phosphoester bond to form CDV. One enzyme involved in BCV hydrolysis is acid sphingomyelinase. CDV is subsequently phosphorylated to form CDV-PP. BCV is also hydroxylated at the terminal carbon by CYP4F2, followed by subsequent CYP-mediated oxidations and multiple cycles of fatty acid beta-oxidation. The major inactive metabolites formed via these pathways are

CMX103 (3-hydroxypropyl ester of CDV) and CMX064 (4-(3-propoxy)butanoic acid ester of CDV).

Studies with liver homogenates and hepatocytes from multiple species confirmed that BCV is rapidly metabolized in rats, and less rapidly metabolized in mice, rabbits and humans. CDV was the major metabolite in human hepatocyte cultures, not CMX064 which was the major metabolite in cultured hepatocytes from mice, rats, rabbits and cynomolgus monkeys. Ten metabolites of BCV were identified following oral and IV administration to cynomolgus monkeys, with CMX064 identified as the major metabolite.

Proposed pathways of BCV metabolism, and relative exposures to BCV and metabolites in humans, rats, and monkeys are presented in **Figure 21** and **Table 25**.

Figure 21: Proposed metabolic pathways for BCV

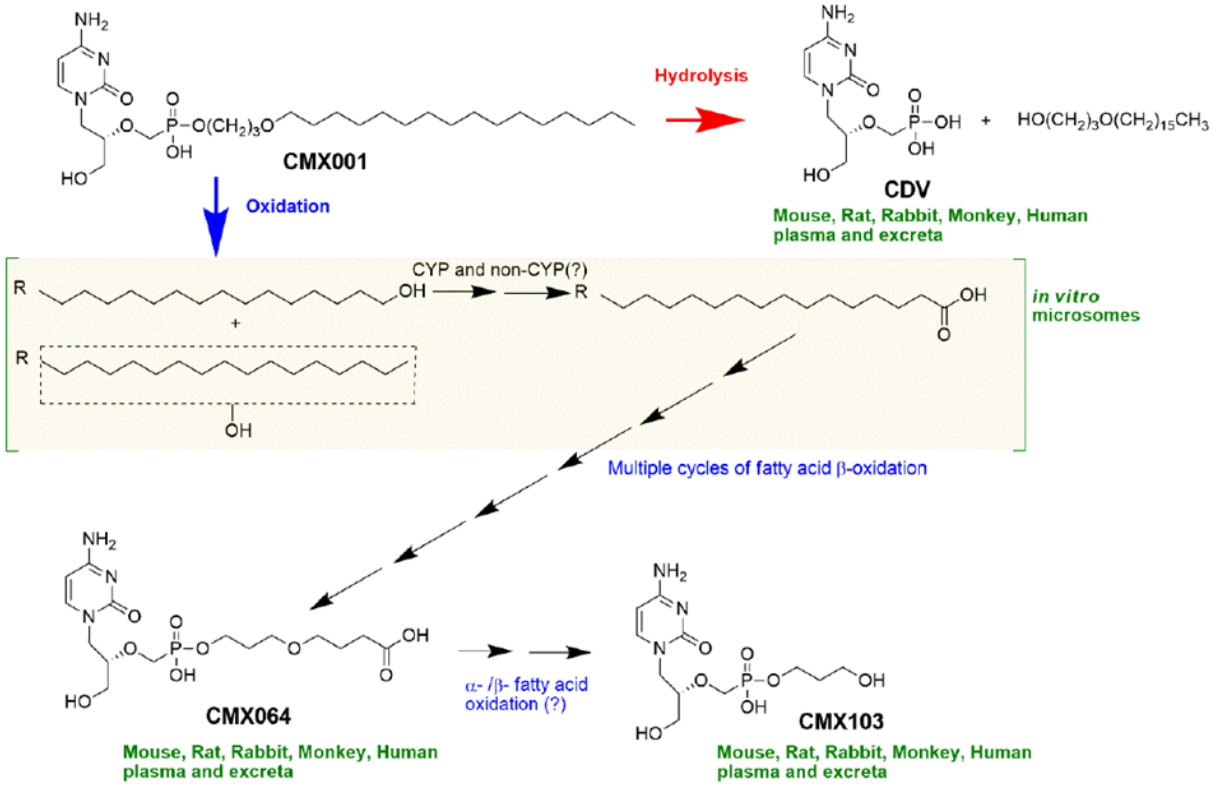


Table 25: Comparison of exposure to BCV and metabolites in humans, rats and monkeys

Species (sex)	Dose	Dose Regimen	BCV	CMX103	CMX064	CDV
			End of Study AUC (ng*h/mL plasma)[a]			
Human[b] (male)	200 mg	Single	3826	3271	2084	1661
Rat[c] (M+F)	15 mg/kg/dose (MTD)	Repeat twice weekly for 13 weeks	956	1633	359	1138
Monkey[d] (male)	4 mg/kg/dose (NOAEL)	Repeat twice weekly for 13 weeks	1.0	53.9	3263	238
			Exposure Ratios			
Rat:Human		Repeat twice weekly for 13 weeks	0.25	0.50	0.17	0.69
Monkey:Human (4 mg/kg)		Repeat twice weekly for 13 weeks	0.0003	0.02	1.6	0.14

a AUClast value at end of study for rats and monkeys; AUCinf for human. Plasma concentrations of analytes measured by LC-MS/MS.
b Study CMX001-112
c Study CMX001-TX-021; values are for male and female animals combined.
d Study CMX001-TX-022

Elimination

In all nonclinical species studied, an oral dose of BCV was excreted primarily via the fecal route. Urinary excretion was also a significate route of elimination (see **Table 26**). BCV, CMX064 and CMX103 were not substrates for kidney transporters OAT1 or OAT3. CDV uptake was mediated by OAT1, but not OAT3. Nephrotoxicity associated with IV administration of CDV was not observed following BCV administration, likely due to the lack of renal uptake of BCV by OAT transporters. As noted above, BCV, but not its metabolites, was a substrate for liver uptake transporters OATP1B1 and OATP1B3.

Table 26: Excretion of total radioactivity following oral or IV administration of [^{14}C]CMX001

		Mean ± SD Percent of Dose (%)			
Species	Sex	Urine	Feces	Total [a]	Study
CD-1 Mouse (oral)	Male	31.1 ± 4.25	39.2 ± 2.11	84.4 ± 1.27	CMX001-NCA-029
	Female	27.2 ± 2.73	41.0 ± 6.41	83.4 ± 3.27	
SD Rat (oral)	Male	40.1 ± 3.31	48.4 ± 0.0752	95.6 ± 0.521	CMX001-NCA-051
	Female	31.3 ± 0.793	59.6 ± 1.30	96.0 ± 0.464	
	Male (BDC)	30.4 ± 1.95	57.0 ± 2.27 [Bile: 7.99 ± 1.03]	99.2 ±2.82	
	Male (BDC w/ ABT)	27.6 ± 2.61	55.1 ± 2.81 [Bile: 2.84 ± 1.23]	90.0 ± 0.785	
SD Rat (IV)[b]	Male	51.2 ± 3.8%	42.2 ± 2.0%	93.5 ± 1.8%	CMX001-NCA-066
	Male (BDC)	43.6 ± 2.4	1.1 ± 0.1 [Bile: 33.9 ± 1.9]	78.6 ± 4.2	CMX001-NCA-068
Cynomolgus Monkey (oral)	Male	24.2 ± 3.77	53.6 ± 0.642	93.2 ± 0.206	CMX001-NCA-052
	Female	24.8 ± 7.84	58.7 ± 3.81	94.8 ± 1.35	
Human (oral)	Male	50.7 ± 5.30	40.4 ± 4.96	91.1 ± 0.76	CMX001-112

a. Total includes: Mice: urine, feces, plasma, carcass, cage wash, and packed cells. Rats: urine, feces, cage wash/wipe, expired $C0_2$/volatiles, and carcass. Monkeys: urine, feces, and cage debris/wash/wipe. Human: urine and feces.
b. Abbreviated collection period of 0- 24h for BDC animals (donor bile supplementation) in Study CMX001-NCA-068.

6. General Toxicology

6.1 Single-Dose Toxicity

Study Title: CMX001: An Extended Single Dose Toxicity and Toxicokinetic Study of CMX001 Given to Rats via Oral Administration

Study no.: CMX001-TX-011
Study report location: 4.2.3.1
Study initiation date: April 26, 2004
Conducting laboratory and location: (b) (4) [redacted]
Duration: Single Dose
GLP compliance: Y
Drug, lot #, and % purity: CMX001 (lot #CMX001-5 and CMX001-8, purity = 97.51% and 92.7%, respectively)
Target Organ+: SYSTEM, GASTROINTESTINAL TRACT

Summary

In this study, groups of male and female rats (8 animals/sex/group) received a single oral gavage (10 mL/kg) dose of CMX001 at dose levels of 0 (vehicle control, purified water), 30 (low), 100 (mid), 300 (high) or 1000 (highest). On Day 2, the first batch of animals (4 rats/sex/group) was sacrificed and on Day 15 remaining rats were sacrificed for detailed necropsy.

Nineteen early deaths at the mid, high and highest doses on Days 5-9 were attributed to enteritis. Minimal enteritis with single cell necrosis evident as pyknotic and karyorrhectic bodies among the epithelial cells lining, mucosal crypt, or glands at the base of small intestinal villi was noted for all treated rats necropsied on Day 2. At the low dose on Day 2, enteritis was the only finding observed histopathologically. Enteritis was not seen on Day 15 in animals from the low dose group, indicating the reversibility of the toxicity at the low dose.

A NOAEL was not defined in this study. A single dose of the test article that produced minimal enteritis in rats was 30 mg/kg. At 30 mg/kg dose level, based on the body surface area factor, an equivalent dose in humans would be 4.87 mg/kg or 292 mg/day (60 kg person). The exposures (steady state AUC_{0-24} and C_{max}) in rats were 5.56 (CMX001) and 1.70 (CDV) µg.hr/ml, and 0.42 (CMX001) and 0.93 (CDV) µg/mL, respectively. Exposures to BCV in animals were less than exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

6.2 Repeat-Dose Toxicity

6.2.1 Study Title: CMX001: A 14-Day Oral Toxicity and Toxicokinetic Study of CMX001 in CD-1 Mice

Study no.:	CMX001-TX-001
Study report location:	4.2.3.2
Study initiation date:	January 20, 2004
Conducting laboratory and location:	(b) (4)
Duration:	2
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	CMX001 (lot #CMX001-5, purity = 97.51%)
Target Organ+:	SYSTEM, GASTROINTESTINAL TRACT

Key Findings

Fifteen of 24 animals in the high dose group and 1/24 animals from the mid-dose group died or were sacrificed moribund. Microscopic examination determined that the cause of the mortality was moderate to severe enteritis primarily affecting the ileum and jejunum. The incidence of enteritis was dose related: 0/24 (low), 10/24 (mid) and 24/24 (high). Lesions apparently related to drug administration included distended/enlarged gallbladder, distended ileum, fluid-filled intestines, distended and fluid-filled jejunum, and stomach distended with ingesta/dried ingesta.

Based on results of the study, minor effects seen at the low dose were not considered adverse, so that 4 mg/kg/day is defined as the NOAEL. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 0.32 mg/kg/day (19.46 mg/day for a 60 kg person). Exposures (steady state AUC and C_{max}) of CDV were 44.9 (male) and 63.7 (female) ng.hr/mL and 8.89 (male) and 11.4 (female) ng/mL in mice. Exposures to BCV in animals were less than exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 4, 20 & 100 mg/kg
Frequency of dosing:	Once daily
Number/Sex/Group:	12/sex/group
Dose volume:	5 mL/kg
Formulation/Vehicle:	Purified water
Route of administration:	ORAL GAVAGE
Species:	MOUSE
Strain:	CD1(ICR)
Age / Sexual Maturity:	6-7 weeks
Comment on Study Design and Conduct:	Due to mortality and morbidity, all surviving high-dose mice were euthanized on Day 9 (males) and 8 (females) and mid-dose mice were euthanized on Days 11 (males) and 8 (females). All control and low-dose animals were euthanized on Day 14.
Dosing Solution Analysis:	All samples met acceptance criteria

Observations and Results

Mortality

Assessed once daily. The distribution and occurrence of unscheduled deaths of main study and satellite animals are shown in **Tables 27 & 28**. Frequent clinical signs of toxicity noted prior to death included decreased activity, dehydration, chemosis, cool to touch, rough haircoat, hunched posture and squinting. Due to mortality and morbidity of surviving mice, all remaining high-dose mice were sacrificed on Day 9 (males) and 8 (females) and mid-dose mice were sacrificed on Days 11 (males) and 8 (females) in order to preserve tissues for histological evaluation.

Table 27: Main study group unscheduled deaths from the 14-day mouse tox study

Groups	Day 7/6 Male/female	Day 8/7 Male/female	Day 9/8 Male/female	Day 10/9 Male/female	Day 11/10 Male/female
Controls	0/0	0/0	0/0	0/0	0/0
Low	0/0	0/0	0/0	0/0	0/0
Mid	0/0	0/0	0/0	0/0	0/1
High	2/0	7/4	2/0	0/0	0/0

Table 28: Satellite group unscheduled deaths from the 14-day mouse tox study

Groups	Day 7 Male/female	Day 8 Male/female	Day 9 Male/female	Day 10 Male/female
Low	0/0	0/0	0/0	0/0
Mid	0/0	0/0	0/0	0/1
High	1/2	4/8	0/1	0/0

Clinical Signs

Assessed once daily. The signs were observed beginning Days 7-10 and included decreased activity, hunched posture, dehydration, chemosis, cool to touch, rough haircoat and squinting. Post-dosing observations were recorded within 1 hr of dose administration and these included cage biting, decreased activity, hunched posture and squinting (mid and high).

Body Weights

Measured once weekly and at necropsy. A statistically significant decrease in body weight was recorded during Week 1 for male and female mice (high) and for females (mid). Week 2 determinations of body weight, body weight gain and food consumption were not possible in the mid and high dose groups due to the early sacrifice of these groups. A statistically significant decreased body weight gain was recorded during Week 1 for all mice (mid or high).

Food Consumption

Measured once weekly. Statistically significant reduced food intake was recorded in Week 1 for all mice (high).

Hematology

Blood samples were collected just prior to necropsy. Since blood samples were collected (mid and high) prior to the control group animals, statistical analysis was not conducted for group mean hematology values from mid- and high-dose group. There was a marked increase in erythrocyte count, hemoglobin levels and hematocrit in moribund mice in the high-dose group that was attributed to the enteritis and dehydration produced by the test article. In the low-dose animals, decreased mean cell volume of erythrocytes in males and decreased platelets in females were significantly different from the controls.

Clinical Chemistry

Blood samples were collected just prior to necropsy. Due to early deaths and poor condition of the surviving animals in the high dose group, samples were collected from only two mice. These samples were pooled. In the mid-dose group mice, samples were collected prior to the control samples so that statistical analysis was not conducted for group mean chemistry values. Decreased mean creatinine values were noted in males from the low dose group.

Gross Pathology

Evaluated at necropsy. An incidence of gross observations for the main study is presented in **Table 29**. Pale raised areas (Peyer's patches) were noted grossly in the ileum of two mice (controls) and several mice in treated groups. There was no supportive evidence noted microscopically for this finding.

Table 29: Gross pathology findings from the 14-day mouse tox study

Gross finding	Vehicle control	Low dose	Mid dose	High dose
Gallbladder-distended/enlarged	0	2	7	12
Ileum-distended	0	0	1	0
Intestines-fluid filled	0	0	0	5
Jejunum-distended with fluid	0	0	1	0
Stomach-distended with ingesta/dried ingesta	0	0	0	19

Organ Weights

Measured at necropsy. Because the necropsies of the mid-dose and high-dose mice were conducted prior to that of the control group animals, statistical analysis was not conducted for group mean organ weight from the mid- and high-dose animals. Statistically significant increased brain weights and decreased testis weights were noted for the males in the low-dose group.

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Eleven of 12 males in the high dose group were found dead or were sacrificed moribund on Days 7, 8 or 9. The one survivor was also sacrificed and necropsied on Day 9. Four females from the high dose group were found dead or sacrificed moribund on Days 7, and 8. Survivors were necropsied on Day 8. The mid-dose females, 1 dead and 11 survivors, were necropsied on Day 10, and the mid-dose males, all survived until necropsy on Day 11. All low-dose and control animals survived until the scheduled necropsy on Day 14.

Mortality and moribundity were attributed to enteritis, primarily affecting the ileum and jejunum. The incidence of enteritis increased with the administered dose, with 0/24, 10/24 and 24/24 mice affected at the low, mid and high doses, respectively. Moderate to severe enteritis with extensive loss of mucosal epithelium and/or inflammatory cellular infiltration into the lamina propria occurred in the ileum of 12/12 males and 12/12 females from the high dose group.

Moderate enteritis with lesser epithelial loss and inflammation was evident for most ileum and several jejunum sections in mice from the mid-dose group.

Slight enteritis with some epithelial alteration, cytoplasmic vacuolation or cellular flattening, was noted in the duodenum of some mice in the high dose group, and in the jejunum or ileum from some mice in the mid-dose group. Enteritis was accompanied by

thymic cortical lymphoid depletion. In mice from the high dose group, the lymphoid depletion was moderate with an essential absence of lymphocytes in the cortical stroma, whereas the depletion from spleens was less complete. Gross distention of stomachs and some gallbladders for high dose mice also attributed to the enteritis.

Toxicokinetics

Blood samples were collected on Days 1 and 14 at 0, 1, 2, 5, 8, 12 and 24 hr post-dose. Toxicokinetic data is shown in **Tables 30 & 31**. The peak plasma concentrations for CMX001 were achieved within 1 to 5 hr following dose administrations (mid) and 2 to 8 hr for the high dose. For CMX001, C_{max} and AUCs increased approximately proportionally with the increasing dose from mid to high in both genders (CMX001 was not detected in plasma from low dose-group animals. No gender differences were observed with the C_{max} and AUC values for CMX001 on both Days 1 and 8. For CDV, C_{max} and AUC increased approximately proportionally with the increasing dose from 4 to 20 and from 20 to 100 mg/kg/day in both genders and on both Days 1 and 14.

Table 30: BCV TK data from the 14-day mouse tox study

Group; dosage (mg/kg/day)		$AUC_{(0\text{-last})}$ (ng*hr/ml)		Cmax (ng/ml)	
		drug days		drug days	
		1	14	1	14
low 4	male	Nd	nd	Nd	nd
	female	Nd	nd	nd	nd
mid 20	male	877	988	88.3	196
	female	1335	1210	177	242
High 100	male	7547	14629	552	3389
	female	7114	4787	615	784

Table 31: CDV TK data from the 14-day mouse tox study

Group; dosage (mg/kg/day)		$AUC_{(0\text{-last})}$ (ng*hr/ml)		Cmax (ng/ml)	
		drug days		drug days	
		1	14	1	14
low 4	male	7.98	44.9	3.99	8.89
	female	Nd	63.7	nd	11.4
mid 20	male	342	604	20	70
	female	286	591	18.7	57.8
High 100	male	990	3522	54.4	459
	female	939	3313	57.4	477

6.2.2 Study Title: A 3-Dose Study of Brincidofovir by Intravenous Infusion in Rats with a 6-Month Recovery Period

Study no.:	CMX001-TX-102
Study report location:	4.2.3.2
Study initiation date:	January 10, 2018
Conducting laboratory and location:	(b) (4)
Duration:	3
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	Brincidofovir (CMX001; lot #170002, purity = 99.9%)
Target Organ+:	TESTIS

Key Findings

Test article-related mild, bilateral germ cell depletion in the testis at 15 mg/kg/dose was characterized by depletion or loss of early spermatocytes and/or pachytene spermatocytes in scattered seminiferous tubules, particularly at late stages of spermatogenesis. Minimal tubular atrophy was observed in animals from the 15 mg/kg/dose group following a 6-month recovery period. The NOAEL was defined as 4 mg/kg. On Day 15, exposures (AUC) in rats at 4 mg/kg were 1100 ng.hr/mL. Exposures at 15 mg/kg (AUC_{last} = 4408 ng.hr/mL) were approximately equivalent to exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 4 & 15 mg/kg
Frequency of dosing:	Once weekly on Days 1, 8 and 15
Number/Sex/Group:	7/main group, 3-12/satellite group
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) for injection
Route of administration:	INTRAVENOUS
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age / Sexual Maturity:	11 weeks
Comment on Study Design and Conduct:	Animals were euthanized on either Day 29, 120 or 209.
Dosing Solution Analysis:	All samples met acceptance criteria.

Observations and Results

Mortality

Animals were observed for general health/mortality and moribundity twice daily. One animal dosed with 4 mg/kg was humanely euthanized and 13 rats dosed with 15

mg/kg were humanely euthanized due to deterioration of the animals' tails during IV dosing. Early deaths are presented in **Table 32**.

Table 32: Early deaths and euthanasia from the 3-dose rat tox study

Group No.	Test Material	Dose Level (mg/kg/dose)	Number of Animals by Necropsy Day			
			Unscheduled	Day 29	Day 120	Day 209
1	Control Article	0	0	7	-	-
2	Brincidofovir	4	1	6	7	7
3	Brincidofovir	15	13	2	2	4

- = Not applicable.

Clinical Signs

Cage side observations were performed at least once daily. Detailed clinical observation was performed weekly. In rats dosed with 4 and 15 mg/kg, observations related to injection site deterioration were noted. In rats dosed with 15 mg/kg, pink urine (1/21), red liquid material (9/21) and increased vocalization (2/21) were noted between Day 1 and 29.

Body Weights

Animals were weighed individually at least once weekly. Rats dosed with 15 mg/kg had reduced body weight gain (80% of controls) to Day 28. The effect on body weight gain was apparently reversed at later timepoints during the recovery period.

Food Consumption

Food consumption was quantitatively measured weekly. There were no toxicologically significant differences in food consumption.

Hematology

Blood samples were collected on Days 29, 120 and 209, and prior to unscheduled euthanasia when possible. Changes in white blood cell counts at 15 mg/kg were not considered to be toxicologically significant because values remained within the range of historical controls.

Clinical Chemistry

Blood samples were collected on Days 29, 120 and 209, and prior to unscheduled euthanasia when possible. Changes in creatine kinase at 15 mg/kg were not considered to be toxicologically significant because values remained within the range of historical controls.

Urinalysis

Urine samples were collected on Days 29, 120 and 209. There were no toxicologically significant findings from urinalysis.

Gross Pathology

Evaluated at necropsy. No drug-related findings were observed.

Organ Weights

Measured at necropsy. Only the brain, epididymis, prostate, seminal vesicles and testes were weighed, which was considered adequate for the purposes of this study. No drug-related changes were observed.

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Histopathology was limited to a select panel of tissues – epididymides, prostate, seminal vesicles, and testes. This was considered adequate for the intended purposes of the study (i.e., to determine if effects of treatment on male rat reproductive organs are reversible after 6 months). At the Day 29 sacrifice, mild germ cell depletion was noted in both animals administered 15 mg/kg. At the terminal sacrifice following a 6-month recovery phase, there was minimal, bilateral tubular atrophy in 3/4 rats from the 15 mg/kg/dose group. Atrophy was limited to a few scattered tubules, indicating incomplete recovery of the testicular toxicity finding. All findings are presented in **Tables 33-35**.

Table 33: Day 29 histopathology data from the 3-dose rat tox study

	Males		
Group	**1**	**2**	**3**
Dose (mg/kg/dose)	**0**	**4**	**15**
No. Animals Examined	**7**	**6**	**2**
Testis (No. Examined)	7	6	2
Depletion, Germ cell	(0)[a]	(0)	(2)
Minimal	0	0	0
Mild	0	0	2

[a] Numbers in parentheses represent the number of animals with the finding.

Table 34: Day 120 histopathology data from the 3-dose rat tox study

	Males		
Group	**1**	**2**	**3**
Dose (mg/kg/dose)	**0**	**4**	**15**
No. Animals Examined	**0**	**7**	**2**
Testis (No. Examined)	0	7	2
Depletion, Germ cell	(0)[a]	(0)	(2)
Minimal	0	0	2

[a] Numbers in parentheses represent the number of animals with the finding.

Table 35: Day 209 histopathology data from the 3-dose rat tox study

	Males		
Group	**1**	**2**	**3**
Dose (mg/kg/dose)	**0**	**4**	**15**
No. Animals Examined	**0**	**7**	**4**
Testis (No. Examined)	0	7	4
Atrophy, Tubular	(0)[a]	(0)	(3)
Minimal	0	0	3

[a] Numbers in parentheses represent the number of animals with the finding.

Toxicokinetics

Blood samples were collected on Days 1 and 15 pre-dose and 1, 2, 4, 8, 12, 24 and 48 hours post-dose (2 hours post-dose only in controls). Toxicokinetic data are presented in **Tables 36 & 37**.

Table 36: Day 1 BCV TK data from the 3-dose rat tox study

Treatment (mg/kg/dose)	Cmax (ng/mL)	Cmax/Dose ((ng/mL)/(mg/kg))	Tmax (h)	Clast (ng/mL)	Tlast (h)	AUClast (h*ng/mL)	AUClast/Dose ((h*ng/mL)/(mg/kg))
4	579	145	1.00	0.340	12.00	1303	326
15	2440	163	2.00	2.54	24.00	4467	298

Treatment (mg/kg/dose)	AUCinf (h*ng/mL)	AUCinf/Dose ((h*ng/mL)/(mg/kg))	AUCextrap (%)	λz (1/h)	t½elim (h)	CL (mL/h/kg)	Vz (mL/kg)	Vss (mL/kg)
4	1303	326	0.0388	0.672	1.03	3070	4570	3120
15	4485	299	0.393	0.144	4.80	3340	23200	5290

Table 37: Day 15 BCV TK data from the 3-dose rat tox study

Treatment (mg/kg/dose)	Cmax (ng/mL)	Cmax/Dose ((ng/mL)/(mg/kg))	Tmax (h)	Clast (ng/mL)	Tlast (h)	AUClast (h*ng/mL)	AUClast/Dose ((h*ng/mL)/(mg/kg))
4	585	146	1.00	1.29	12.00	1145	286
15	1620	108	1.00	0.717	48.00	4082	272

Treatment (mg/kg/dose)	AUCtau (h*ng/mL)	AUCtau/Dose ((h*ng/mL)/(mg/kg))	λz (1/h)	t½elim (h)	CL (mL/h/kg)	Vss (mL/kg)
4	NR	NR	NR	NR	NR	NR
15	4088	273	0.116	5.99	3670	9140

6.2.3 Study Title: CMX001: A 28-Day Intravenous Infusion Toxicity Study in Rats with a 14-Day Recovery Period

Study no.: CMX001-TX-034
Study report location: 4.2.3.2
Study initiation date: November 5, 2015
Conducting laboratory and location: (b) (4)
Duration: 28
Duration Units: days
GLP compliance: Y
Drug, lot #, and % purity: CMX001 (lot #CMX001-097, purity = 99.8%)
Target Organ+: TESTIS
Target Organ+: EPIDIDYMIS
Target Organ+: SYSTEM, GASTROINTESTINAL TRACT
Target Organ+: SKIN

Key Findings

CMX001-related findings were present in the male reproductive tract (testes, epididymides and seminal vesicles) and the intestinal tract (duodenum, jejunum, and/or ileum) in animals dosed with ≥4 mg/kg/dose. Sebaceous gland atrophy was noted in males and females in the 15 mg/kg dose group. Based on testicular germ cell depletion in males at ≥4 mg/kg/dose and moderate intestinal single cell crypt necrosis in one female at 15 mg/kg/dose, the NOAEL was considered to be 1 mg/kg/dose in males and 4 mg/kg/dose for in females.

On Day 29, systemic BCV exposures at the NOAEL for males (1 mg/kg) were 0.1-times the exposures in humans following the clinical dose (200 mg). At 15 mg/kg, systemic BCV exposures (AUC_{last} = 9893 ng.hr/mL) were approximately 2-times the exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 1, 4 & 15 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	10/sex/main group, 5/sex/recovery group, 3/sex/satellite group
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) for injection
Route of administration:	INTRAVENOUS
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age / Sexual Maturity:	Approximately 10 weeks
Comment on Study Design and Conduct:	Doses were administered twice per week via 2-hour intravenous infusion using implanted femoral catheters on a total of 9 occasions over 29 days. All animals were euthanized on either Day 29 (main study group) or 33 (recovery group).
Dosing Solution Analysis:	Group 2 (low dose; 0.05 mg/mL nominal) dose group samples were consistently below 90% of the nominal concentration.

Observations and Results

Mortality

Animals were observed in their cages twice daily for mortality and morbidity. Two rats, one from the control group and one from the 1 mg/kg dose group, died early. These deaths were not considered to be related to BCV administration. One rat from the 15 mg/kg dose group was euthanized on Day 23. No microscopic evaluation was conducted in this animal, so the cause of death could not be determined.

Clinical Signs

Evaluated twice daily. Intermittent signs included rapid or irregular breathing, hunched posture, and decreased activity. There were no drug-related clinical observations during the recovery period.

Body Weights

Measured pretreatment, one day prior to each dose during the treatment period, and weekly during the recovery period. No drug-related changes.

Food Consumption

Measured weekly beginning one week prior to treatment. Decreases in food consumption at all dose levels did not impact body weight or weight gain. The effects on food consumption were not present during the recovery period.

Ophthalmoscopy

Evaluated pretreatment and at the end of dosing and recovery. No drug-related findings.

Hematology

Blood samples were collected at the end of treatment and recovery. No drug-related changes.

Clinical Chemistry

Blood samples were collected at the end of treatment and recovery. No drug-related changes.

Urinalysis

Urine samples were collected at the end of treatment and recovery. No drug-related changes.

Gross Pathology

Evaluated at necropsy. Animals were euthanized by exsanguination following isoflurane inhalation. There were no dose-related gross observations.

Organ Weights

Measured at necropsy. Dose-related, minimal to mild decreases in testes weights were noted in males administered ≥4 mg/kg/dose (see **Tables 38 & 39**). Lower testes weights correlated microscopically with germ cell depletion. Males administered 15 mg/kg/dose had minimally lower epididymal weights without microscopic correlation. At the end of the recovery period, the decreases in testes weights in males administered ≥4 mg/kg/dose was exacerbated due to sperm depletion secondary to the loss of germ cells noted at the end of the dosing period.

Table 38: Organ weight changes at the end of the treatment period (% difference relative to controls) from the 28-day rat IV tox study

Group/sex	2M	3M	4M	2F	3F	4F
Dose (mg/kg/dose)	1	4	15	1	4	15
Testes						
Absolute weight (%)	-	-9[a]	-24[a]	NA	NA	NA
vs. body weight (%)	-	-3	-16[a]	NA	NA	NA
vs. brain weight (%)	-	-9[a]	-22[a]	NA	NA	NA
Epididymides						
Absolute weight (%)	-	-	-14[a]	NA	NA	NA
vs. body weight (%)	-	-	-6	NA	NA	NA
vs. brain weight (%)	-	-	-13[a]	NA	NA	NA

[a]Statistically significant difference between mean values for CMX001-treated and control groups.
- = not CMX00-related; NA = Not applicable

Table 39: Organ weight changes following a 14-day recovery period (% difference relative to controls) from the 28-day rat IV tox study

Group/sex	2M	3M	4M	2F	3F	4F
Dose (mg/kg/dose)	1	4	15	1	4	15
Testes						
Absolute weight (%)	-	-21[a]	-42[a]	NA	NA	NA
vs. body weight (%)	-	-26[a]	-40[a]	NA	NA	NA
vs. brain weight (%)	-	-18[a]	-41[a]	NA	NA	NA

[a]Statistically significant difference between mean values for CMX001-treated and control.
- = not CMX00-related; NA = Not applicable

Histopathology

Adequate Battery: Yes

Peer Review: No

Testes: Minimal to slight (4 mg/kg), and slight to moderate (15 mg/kg) bilateral germ cell depletion was present in the testes (see **Table 40**). The severity grading generally reflected the number of cell types (spermatogenesis stages) affected by the depletion. CMX001-related findings at 15 mg/kg/dose also included minimal luminal cell debris in the epididymides and minimally increased epithelial apoptosis in the seminal vesicles. The latter change was occasionally accompanied by an increase in mitotic figures. Minimally increased epithelial apoptosis was also present in 4 males administered 4 mg/kg/dose. Following the 14-day recovery period, microscopic findings were still present in the testes and/or epididymides in males at ≥4 mg/kg/dose.

Table 40: Histopathology findings in the male reproductive tract at the end of the treatment phase from the 28-day rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined	10 [a]	10	10	10	NA	NA	NA	NA
Testes								
Depletion, Germ Cell								
Minimal	-	-	6	-	NA	NA	NA	NA
Slight	-	-	3	5	NA	NA	NA	NA
Moderate	-	-	-	5	NA	NA	NA	NA
Epididymides								
Cell Debris, Luminal								
Minimal	-	-	-	9	NA	NA	NA	NA
Seminal Vesicles								
Apoptosis, Epithelial								
Minimal	-	-	4	8	NA	NA	NA	NA
Mitotic Figures, Epithelial								
Minimal	-	-	-	4	NA	NA	NA	NA
Total Animals Represented	0	0	10	10	NA	NA	NA	NA

[a] Incidence includes one unscheduled death (Animal No. 1005).
- = Finding not present. NA = not applicable

GI tract: Dose-dependent, minimal to mild single cell necrosis of the crypt, occasionally accompanied by minimal hyperplasia, was noted in the small intestine (duodenum, jejunum, and/or ileum) in males and females at ≥4 mg/kg/dose. Minimal single cell necrosis was also present in the glandular epithelium of the large intestine (cecum and/or colon) in males and females at 15 mg/kg/dose (see **Table 41**). Following the 14-day recovery period there was complete recovery of all microscopic findings in the intestinal tract observed at the end of dosing.

Table 41: Histopathology findings in the intestinal tract from the end of the treatment period from the 28-day rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined	10[c]	10	10	10	10	11[d]	10	10
Small intestine[a]								
Necrosis, Epithelium, Crypts								
Minimal	-	-	8	2	-	-	9	3
Slight	-	-	1	8	-	-	-	5
Moderate	-	-	-	-	-	-	-	1
Hyperplasia, Epithelium, Crypts								
Minimal	-	-	5	2	-	-	-	5
Large intestine[b]								
Necrosis, Epithelium, glands								
Minimal	-	-	-	9	-	-	-	4
Total Animals Represented	0	0	9	10	0	0	9	9

[a,b] Incidence of severity grades represent highest grade recorded for duodenum, jejunum and ileum[a] and for cecum and colon[b].
[c] Incidence includes one unscheduled death (Animal No. 1005). Duodenum was autolytic in this animal and not readable.
[d] Incidence includes one unscheduled death (Animal No. 2595).
- = Finding not present.

Skin: Sebaceous gland atrophy (slight to marked) was present in males and females at 15 mg/kg/dose. Marked severity indicated absence of sebaceous glands microscopically (see **Table 42**). Following the 14-day recovery period there was complete recovery of all microscopic findings in the sebaceous glands observed at the end of dosing.

Table 42: Histopathology findings in the skin at the end of the treatment period from the 28-day rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined	10[a]	10	10	10	10	11[b]	10	10
Atrophy, Sebaceous Glands								
Slight	-	-	-	3	-	-	-	5
Moderate	-	-	-	3	-	-	-	3
Marked	-	-	-	4	-	-	-	2
Total Animals Represented	0	0	0	10	0	0	0	10

[a] Incidence includes one unscheduled death (Animal No. 1005).
[b] Incidence includes one unscheduled death (Animal No. 2595).
- = Finding not present.

Bone marrow: At the 15 mg/kg/dose, a few recovery males and females had minimal to slight decreased cellularity in the bone marrow, a finding which was not observed at the end of the dosing period (see **Table 43**).

Table 43: Histopathology findings in bone marrow following a 14-day recovery period from the 28-day rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined	5	5	5	5	5	4	5	5
Cellularity, Decreased[a]								
Minimal	-	-	-	-	-	-	-	2
Slight	-	-	-	3	-	-	-	-
Total Animals Represented	0	0	0	3	0	0	0	2

[a] Incidence of severity grades represent highest grade recorded for sternal or femoral bone marrow.
- = Finding not present.

Toxicokinetics

Blood samples were collected on Days 1 and 29 pre-dose and 1, 2, 8, 24 and 48 hours post-dose (2 and 24 hours post-dose only in the control group). Toxicokinetic parameters are presented in **Tables 44 & 45**. By Day 29, the systemic exposures (AUC) of BCV in rats at 15 mg/kg (9893 ng.hr/mL) were 2 times greater than the exposures observed in humans at the clinical dose (200 mg).

Table 44: Day 29 BCV TK data from the 28-day rat IV tox study

Treatment	Statistic	C_{max} (ng/mL)	C_{max}/Dose (kg*ng/mL/mg)	T_{max} (hr)	C_{last} (ng/mL)	T_{last} (hr)	AUC_{last} (hr*ng/mL)	AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))
1 mg/kg	N	6	6	6	6	6	6	6
	Mean	298	298	NC	126	NC	410	410
	SD	130	130	NC	88.2	NC	190	190
	CV%	43.7	43.7	NC	69.9	NC	46.4	46.4
	Min	52.6	52.6	1.00	1.12	2.00	73.9	73.9
	Median	324	324	1.00	144	2.00	434	434
	Max	421	421	2.00	229	8.00	653	653
4 mg/kg	N	6	6	6	6	6	6	6
	Mean	1200	301	NC	2.06	NC	2759	690
	SD	297	74.3	NC	0.519	NC	851	213
	CV%	24.7	24.7	NC	25.2	NC	30.8	30.8
	Min	935	234	1.00	1.38	8.00	2119	530
	Median	1160	290	1.00	2.14	8.00	2549	637
	Max	1700	425	2.00	2.67	48.00	4415	1104
15 mg/kg	N	5	5	5	5	5	5	5
	Mean	4500	300	NC	10.9	NC	9893	660
	SD	742	49.5	NC	4.14	NC	1840	122
	CV%	16.5	16.5	NC	37.9	NC	18.6	18.6
	Min	3550	237	1.00	7.75	8.00	8001	533
	Median	4460	297	1.00	8.90	8.00	9528	635
	Max	5600	373	1.00	18.0	8.00	12928	862

NC = Not calculated; For the 1 mg/kg dose group, AUC_{last} values were calculated using only 2 quantifiable data points.

Table 45: Day 29 CDV TK data from the 28-day rat IV tox study

Treatment	Statistic	C_{max} (ng/mL)	T_{max} (hr)	C_{last} (ng/mL)	T_{last} (hr)	T_{lag} (hr)	AUC_{last} (hr*ng/mL)
1 mg/kg	N	6	5	5	5	5	6
	Mean	7.81	NC	8.31	NC	NC	38.6
	SD	4.18	NC	1.90	NC	NC	28.9
	CV%	53.5	NC	22.9	NC	NC	75.0
	Min	0	2.00	5.24	2.00	0	0
	Median	8.41	2.00	8.61	8.00	1.00	43.0
	Max	12.5	8.00	10.4	8.00	1.00	74.8
4 mg/kg	N	6	6	6	6	6	6
	Mean	20.4	NC	8.76	NC	NC	399
	SD	9.41	NC	1.69	NC	NC	248
	CV%	46.2	NC	19.3	NC	NC	62.3
	Min	10.6	8.00	6.47	24.00	0	195
	Median	20.0	8.00	8.92	24.00	0	347
	Max	37.3	8.00	10.7	48.00	1.00	883
15 mg/kg	N	5	5	5	5	5	5
	Mean	41.3	NC	8.05	NC	NC	1068
	SD	16.0	NC	1.04	NC	NC	327
	CV%	38.8	NC	13.0	NC	NC	30.6
	Min	23.6	2.00	6.99	48.00	0	732
	Median	44.0	8.00	7.81	48.00	0	1117
	Max	60.9	8.00	9.40	48.00	0	1473

NC = Not calculated; For the 1 mg/kg dose group, AUC_{last} values were calculated using only 2 quantifiable data points.
N = 5 in for the 15 mg/kg dose group because animal 4107 was euthanized prior to the collection of TK samples on Day 29.

6.2.4 Study Title: BCV: A 13-Week (Once/Twice Weekly) Intravenous Infusion Toxicity and Toxicokinetic Study in Rats with A 28-Day Recovery Period

Study no.: CMX001-TX-037
Study report location: 4.2.3.2
Study initiation date: September 14, 2016
Conducting laboratory and location: (b) (4)
Duration: 13
Duration Units: weeks
GLP compliance: Y
Drug, lot #, and % purity: Brincidofovir (CMX001; lot #CMX001-098, purity = 100.1%)
Target Organ+: TESTIS
Target Organ+: EPIDIDYMIS
Target Organ+: SYSTEM, GASTROINTESTINAL TRACT
Target Organ+: GLAND, ZYMBAL

Key Findings

CMX001-related findings were present in the testes (epididymides and seminal vesicles) and the intestinal tract (duodenum, jejunum, and/or ileum) in animals dosed

with ≥4 mg/kg/dose. At the end of the recovery period, neoplasms were observed sporadically in female rats from all dose groups and male rats from the 15 mg/kg dose group.

The NOAEL could not be identified in the females due to the neoplasms at ≥1 mg/kg/dose (BIW or QW). The NOAEL was considered to be 1 mg/kg/dose (BIW) in males, due to the adverse seminiferous tubular atrophy in the testes at ≥4 mg/kg/dose and the neoplasms at 15 mg/kg/dose (QW). The associated TK parameters were C_{max} = 287 ng/mL and AUC_{last} = 551 ng.hr/mL. At the NOAEL for male rats, the systemic exposures (AUC_{last}) were approximately 0.12-times the exposures in humans at the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 1, 4 & 15 mg/kg
Frequency of dosing:	Twice weekly (1 & 4 mg/kg) or once weekly (15 mg/kg)
Number/Sex/Group:	5-10/sex/main group, 4-5/sex/recovery group, 3-9/sex/satellite group
Dose volume:	20 mL/kg
Formulation/Vehicle:	(b) (4) in Water for Injection
Route of administration:	INTRAVENOUS
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age / Sexual Maturity:	Approximately 13 weeks
Comment on Study Design and Conduct:	Doses were administered once or twice per week via 2-hour intravenous infusion using implanted femoral catheters.
Dosing Solution Analysis:	All samples met acceptance criteria.

Observations and Results

Mortality

Animals were observed in their cages twice daily for mortality and morbidity. There were 17 unscheduled deaths among male and female toxicity animals that were administered BCV. These animals were either found dead or euthanized for welfare reasons from Day 35 to Day 92. There were 2 animals from the 15 mg/kg/dose group that were euthanized for welfare reasons during the recovery period (1 male on recovery Day 11 and 1 female on recovery Day 20). Early deaths were generally related to infected catheters. The incidence of the deaths, which was greater at ≥4 mg/kg/dose, suggests there may have been an influence of BCV and/or formulation; however, this is not clear.

Clinical Signs

Detailed observations were performed weekly during the dosing and recovery periods. Clinical observations were considered to be secondary to

inflammation/abscess at the injection site due to infection. Observations consisted of swollen/firm areas (ventrally, dorsally, or in limbs), impaired locomotion/abnormal gait, hunched posture, paleness, rapid/labored breathing, and distended abdomen, generally present in early sacrifice or found dead animals, with the greatest incidence at ≥4 mg/kg/dose in both sexes.

Body Weights

Animals were weighed twice pretest, and weekly during the dosing and recovery periods. There were no dose-related changes in body weight.

Food Consumption

Food consumption was measured (weighed) weekly, beginning one week prior to treatment. There were no dose-related changes in food consumption.

Ophthalmoscopy

All animals were examined pretest. All surviving animals in groups 1 and 4 were examined at end of dosing and end of recovery. There were no dose-related effects on ophthalmoscopic parameters.

Neurobehavioral Evaluations

Neurobehavioral evaluations were performed during Week 13 and during the last week of the recovery period. There were no dose-related changes in performance of the functional observational battery.

Hematology

Blood samples were collected at the end of treatment and recovery. There were no dose-related effects on hematologic parameters that were considered to be adverse. An apparent regenerative response (increases in reticulocytes) suggests that minor red cell decreases may be due to hemolysis and/or blood loss. There were no dose-related effects on coagulation.

Clinical Chemistry

Blood samples were collected at the end of treatment and recovery. BCV-related clinical chemistry changes in male rats administered 15 mg/kg/QW were limited to a 4.4-fold increase over controls in alkaline phosphatase activity. ALP remained increased 1.4-fold compared with controls following the recovery period.

Urinalysis

Urine samples were collected at the end of treatment and recovery. There were no drug-related effects on urinary parameters.

Gross Pathology

Animals were euthanized by exsanguination following isoflurane inhalation. Testes in males administered ≥4 mg/kg/dose BCV were observed to be small and/or soft in nearly all animals. These findings correlated with marked to severe bilateral

seminiferous tubular atrophy in the testes and almost complete lack of sperm in the epididymides.

Organ Weights

Testis and epididymis weights are presented in **Table 46**. BCV-related organ weight differences were limited to lower testes and/or epididymal weights at ≥1 mg/kg/dose. At ≥4 mg/kg/dose, lower weights correlated with marked to severe bilateral seminiferous tubular atrophy and almost complete lack of sperm in the epididymis, with only residual sperm remaining in the caudal tail. At the end of the recovery period testes weights in males treated with BCV were similar to testes weights recorded at the end of the dosing period, suggesting little or no recovery after 28 days.

Table 46: Organ weight changes from the 13-week rat IV tox study

Group/sex Dose (mg/kg/dose)	2M 1	3M 4	4M 15	2F 1	3F 4	4F 15
Testes						
Absolute weight (%)	-11[a]	-59[a]	-57[a]	NA	NA	NA
vs. body weight (%)	-6	-57[a]	-54[a]	NA	NA	NA
vs. brain weight (%)	-10[a]	-58[a]	-56[a]	NA	NA	NA
Epididymides						
Absolute weight (%)	-	-25[a]	-30[a]	NA	NA	NA
vs. body weight (%)	-	-23[a]	-27[a]	NA	NA	NA
vs. brain weight (%)	-	-23[a]	-30[a]	NA	NA	NA

[a]Statistically significant difference between mean values for BCV-treated and control groups.

- = Not BCV-related; NA = Not applicable

Histopathology

Adequate Battery: Yes

Peer Review: No

Testes: Minimal to slight (4 mg/kg), and slight to moderate (15 mg/kg) bilateral germ cell depletion was present in the testes. The severity grading generally reflected the number of cell types (spermatogenesis stages) affected by the depletion. Drug-related findings at 15 mg/kg/dose also included minimal luminal cell debris in the epididymides and minimally increased epithelial apoptosis in the seminal vesicles. The latter change was occasionally accompanied by an increase in mitotic figures. Minimally increased epithelial apoptosis was also present in 4 males administered 4 mg/kg/dose (see **Table 47**). Following the 28-day recovery period, microscopic findings were still present in the testes and/or epididymides in males at ≥4 mg/kg/dose.

Table 47: Histopathology findings in the male reproductive tract at the end of the treatment period from the 13-week rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined [a]	10	10	10	10	NA	NA	NA	NA
Testes								
Atrophy, Tubular, Bilateral								
Marked	-	-	1	2	NA	NA	NA	NA
Severe	-	-	7	8	NA	NA	NA	NA
Degeneration/Atrophy, Tubular, Bilateral								
Minimal	-	-	1	-	NA	NA	NA	NA
Depletion, Germ Cell, Bilateral								
Minimal	-	3	-	-	NA	NA	NA	NA
Total Animals	0	3	9	10				
Epididymides								
Sperm, Absent, Bilateral								
Finding present [b]	-	-	8	10	NA	NA	NA	NA
Cribriform Change, Bilateral								
Slight	-	-	6	10	NA	NA	NA	NA
Moderate	-	-	2	-	NA	NA	NA	NA
Cell Debris, Luminal, Bilateral								
Minimal	-	-	1	-	NA	NA	NA	NA
Total Animals	0	0	9	10				

[a] Incidence includes unscheduled decedents.
[b] Finding not given severity grading but reported as being present.
- = Finding not present. NA = not applicable

GI tract: Dose-dependent, minimal to slight single cell necrosis of the crypt epithelium, occasionally accompanied by minimal hyperplasia, was noted in the small intestine (duodenum, jejunum, and/or ileum) in males and females at ≥1 mg/kg/dose. The finding tended to be in multiple segments at 15 mg/kg/dose. Following the 28-day recovery period there was complete recovery of all microscopic findings in the intestinal tract and sebaceous glands observed at the end of dosing.

Skin: Sebaceous gland atrophy (slight to marked) was present in males and females at 15 mg/kg/dose. Marked severity indicated absence of sebaceous glands microscopically. Following the 28-day recovery period there was incomplete recovery of microscopic findings in the sebaceous glands observed at the end of dosing. Adenocarcinoma of the Zymbal's gland was present in one male administered 15 mg/kg (see **Tables 48 & 49**).

Table 48: Histopathology findings in the skin at the end of the treatment period from the 13-week rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined [a]	10	10[b]	10	10	10	10	10	10
Skin								
Atrophy, Sebaceous Glands								
Slight	-	-	-	1	-	-	-	-
Moderate	-	-	-	4	-	-	-	7
Total Animals	0	0	0	5	0	0	0	7
Zymbal's Gland, SGBE								
Atrophy, Sebaceous Glands								
Minimal	-	-	-	1	-	-	-	3
Slight	-	-	-	2	-	-	-	3
Moderate	-	-	-	3	-	-	-	-
Total Animals	0	0	0	6	0	0	0	6

[a]Incidence includes unscheduled deaths.
[b] Zymbal's gland and SGBE were missing in Animal No. 2045.
- = Finding not present.

Table 49: Histopathology findings in the skin following a 28-day recovery period from the 28-day rat IV tox study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/dose)	0	1	4	15	0	1	4	15
Number of tissues examined [a]	5	5	5	5	5	5	5	5
Skin								
Atrophy, Sebaceous Glands								
Slight	-	-	-	1	-	-	-	-
Moderate	-	-	-	-	-	-	-	1
Total Animals	0	0	0	1	0	0	0	1
Zymbal's Gland, SGBE								
Atrophy, Sebaceous Glands								
Minimal	-	-	-	-	-	-	-	1
Slight	-	-	-	1	-	-	-	-
Cyst, Duct								
Minimal	-	-	-	-	-	-	1	1
Slight	-	-	-	2	-	-	1	-
Moderate	-	-	1	-	-	-	-	-
Hyperplasia								
Minimal	-	-	1	2	-	-	-	-
Slight	-	-	-	-	-	-	1	1
Adenocarcinoma								
Present				1				
Total Animals	0	0	2	4	0	0	3	3

[a]Incidence includes unscheduled deaths.
- = Finding not present.

Neoplasias: At the end of the 13-week dosing period, one male at 15 mg/kg/dose had an adenoma in the parathyroid. One female at 15 mg/kg/dose had a mammary adenocarcinoma. In addition to the neoplasia described at the end of the 13-week dosing period, one female in the 1 mg/kg dose group had an adenoma in the parathyroid, two females in the 4 mg/kg dose group and 1 female administered 15 mg/kg/dose had a mammary adenocarcinoma. One male in the 15 mg/kg dose group had a carcinoma of the Zymbal's gland. Since neoplasms are uncommon in rats of this age and strain, the tumors present at multiple sites in rats dosed with BCV in this study are likely BCV-related.

Toxicokinetics

Blood samples were collected on Days 1 and 92 pre-dose and 1, 2, 4, 8, 12, 24 and 48 hours post-dose for the determination of plasma concentrations of CMX001 and the CMX001 metabolite, CDV (CMX021). Toxicokinetic parameters are presented in Tables **50 & 51**. Exposures tended to increase proportionate to dose, there was no significant accumulation between Day 1 and 92, and there were no significant (i.e., >2-fold) gender-related differences in exposures.

Table 50: Day 92 BCV TK data from the 13-week rat IV tox study

Treatment (mg/kg)	C_{max} (ng/mL)	C_{max}/Dose ((ng/mL)/(mg/kg))	T_{max} (h)	T_{last} (h)	AUC_{0-12} (h*ng/mL)	AUC_{last} (h*ng/mL)	AUC_{last}/Dose ((h*ng/mL)/(mg/kg))
1	208	208	2	48	478	554	554
4	928	232	1	48	1855	2025	506
15	4990	333	2	24	9142	9328	622

Groups 2 (1 mg/kg) and 3 (4mg/kg) received two doses per week. Group 4 (15 mg/kg) received one dose per week.

Table 51: Day 92 CDV TK data from the 13-week rat IV tox study

Treatment (mg/kg)	C_{max} (ng/mL)	C_{max}/Dose ((ng/mL)/(mg/kg))	T_{max} (h)	T_{last} (h)	AUC_{last} (h*ng/mL)	AUC_{last}/Dose ((h*ng/mL)/(mg/kg))	AUC_{inf} (h*ng/mL)	$t^{1/2}_{elim}$ (h)	M/P C_{max} Ratio	M/P AUC_{last} Ratio
1	8.27	8.27	4	24	89.7	89.7	96.7	5.70	0.0455	0.288
4	20.2	5.04	4	24	276	69.0	330	8.08	0.0392	0.288
15	41.3	2.75	4	48	992	66.2	NR	NR	0.0190	0.239

NR = Not Reported
Groups 2 (1 mg/kg) and 3 (4mg/kg) received two doses per week. Group 4 (15 mg/kg) received one dose per week.
M/P = Metabolite/Parent ratios are corrected for molecular weight.

6.2.5 Study Title: A Thirteen-Week GLP Oral Toxicity Study of CMX001 in Rats with Twice Weekly Dosing

Study no.: CMX001-TX-021
Study report location: 4.2.3.2
Study initiation date: January 2, 2008

Conducting laboratory and location:	(b) (4)
Duration:	13
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	CMX001 (lot #AA-020112-B-3-4 Crude 2 #1, purity = 97.5%)
Target Organ+:	TESTIS

Key Findings

Palpable tissue masses were identified in several BCV-treated rats during the 12-week post-treatment recovery phase. Microscopic findings of malignant mammary gland adenocarcinomas were observed in females administered 1 mg/kg BCV and higher and in one male administered 15 mg/kg. Squamous cell carcinomas were observed in one male and one female administered 15 mg/kg.

Small, flaccid testes, decreased testes and epididymal weights, and decreased spermatogenesis and hypospermia were observed in male rats given 15 mg/kg BCV twice weekly for 13 weeks. These findings did not reverse during a 12-week post-treatment recovery period.

Based on these findings, the NOAEL of BCV administered to rats twice weekly by oral gavage for 13 weeks at dosages of 0 (vehicle control), 1, 4 or 15 mg/kg/dose, was 4 mg/kg in males, and was not identified in females.

Plasma samples were obtained for determination of concentrations of CMX001 (BCV) and metabolites CMX021 (CDV) and CMX064 (major inactive metabolite) after a single dose (Day 1) and at steady state (Week 13).

The week 13 $AUC_{(0-\infty)}$ values (males and females combined) at the NOAEL (4 mg/kg/day) for BCV, CDV, and CMX064 were 208.9, 484.8, and 48.39 ng.hr/mL, respectively. The associated combined-sex Week 13 $AUC_{(0-\infty)}$ values at 1 mg/kg/day for BCV was 28.8 ng.hr/mL. CMX021 and CMX064 were not detected in the plasma. Exposures to BCV in animals were less than exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 1, 4 & 15 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	10/sex/main group, 5/sex/recovery group
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) in purified water
Route of administration:	ORAL GAVAGE
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age / Sexual Maturity:	8-9 weeks

Comment on Study Design and Conduct:	Main study animals were euthanized on Week 13. Recovery animals were euthanized on Week 25.
Dosing Solution Analysis:	All samples met acceptance criteria

Observations and Results

Mortality

Assessed twice daily. One male animal that had received 15 mg/kg was found dead during the recovery period (Day 146). Prior to the death, clinical observations for this animal included respiratory crackles/rales, dyspnea, staining around mouth, unkempt appearance, anogenital staining, material around eyes, nose/nares staining and absent feces. Gross findings included decreased testicular size, and testicles appearing flaccid, stomach ulcers and distension of the duodenum, jejunum, ileum and cecum and discolored (dark red) lung.

The testicular findings corresponded microscopically with decreased spermatogenesis. There was no microscopic correlation with the stomach ulcers or distension, and the lung discoloration corresponded microscopically with pulmonary vascular congestion. Lymphoid depletion was also observed microscopically, likely related to stress.

Because treatment with BCV was well tolerated in this and other high dose rats during the dosing phase, because the death occurred significantly after termination of dosing, and further because all other high dose rats survived to the scheduled termination with no signs of morbidity, this death was considered unrelated to treatment with BCV.

Clinical Signs

Assessed at least twice daily. One male and 1 female in the 1 mg/kg group, 3 females in the 4 mg/kg group, and 1 male and 4 females in the 15 mg/kg group, were remarkable for the appearance of palpable masses during the recovery period. The recovery period was from Day 91 through Day 179, with the first mass observed in a 15 mg/kg female on Day 144 (during Week 8 of the recovery phase; the 21st week after initiation of dosing). The masses were generally located in the axillary region, but also were observed in the neck, thorax, abdomen, eyelid, head and mouth regions. No masses were observed during the administration period.

Body Weights

Measured pretreatment, twice weekly, and at necropsy. Decreased mean body weight was observed at the high dose (15 mg/kg) during the dosing phase of the study beginning during Week 2 and continuing through termination of dosing. Prior to termination of dosing (Day 88 – male; Day 89 - female), the body weight of males and females in the 15 mg/kg group was decreased 7% and 5%, respectively, compared with controls. Reversibility in the body weight decrease was observed in females during the recovery phase; however, the body weight of males in the 15 mg/kg group remained decreased about 8.0% compared with control males at termination of the recovery phase. The body weight of females in the 15 mg/kg group was comparable with female control animals at the termination of the recovery phase. Test article-related effects on

body weight change generally corresponded with effects on body weights described above.

Food Consumption

Measured once weekly. Decreased food consumption was observed at 15 mg/kg during the dosing phase of the study beginning during Week 2 and continuing through termination of dosing. Prior to termination of dosing (Days 77-84), mean food consumption in males in the 15 mg/kg group was decreased 10.2% compared with control males. At termination of the recovery phase, mean food consumption in males in the 15 mg/kg group was decreased 16.2% compared with males in the control group. Female group means were similar to control group means during the dosing portion and the recovery period.

Ophthalmology

Evaluted pretreatment and at necropsy. No drug-related changes.

Hematology

Blood samples were collected on Weeks 6 and 12 and at the end of recovery. At the recovery phase necropsy, 1 male and 1 female from the high dose had slightly increased white blood cell counts of 17.10 and 15.14, respectively, compared with mean control values of 9.40 (males) and 5.57 (females). The absolute neutrophil counts in the 1 male and 1 female were 7.90 and 7.28, respectively, compared with mean control values of 2.77 (males) and 1.28 (females). Palpable masses were previously observed in both animals during Week 10 of the recovery phase. The masses were diagnosed as malignant mammary gland adenocarcinoma and squamous cell carcinoma, which are the likely cause of the elevations. One male administered the low dose was remarkable for an elevated white blood cell count of 17.24 and an elevated absolute neutrophil count of 9.19 at termination of the recovery phase. A 1.0 to 1.5 cm mass observed at necropsy was confirmed by histopathology to be an abscess, which was judged to be the likely cause of the elevations.

Coagulation

Blood samples were collected at necropsy. No drug-related changes.

Clinical Chemistry

Blood samples were collected on Weeks 6 and at necropsy. No drug-related changes.

Urinalysis

Urine samples were collected at necropsy. No drug-related changes.

Gross Pathology

Evaluated at necropsy. At the necropsy conducted at termination of the dosing phase, 9 of 10 male rats in the 15 mg/kg group were remarkable for a decreased testes size. Similarly, at the necropsy conducted at termination of the recovery phase, all 4 surviving male rats in the 15 mg/kg group were remarkable for testes that were decreased in size and flaccid in appearance compared with control males. Masses

were observed in 1 male and 1 female in the 1 mg/kg group, 3 females in the 4 mg/kg group, and 1 male and 5 females in the 15 mg/kg group during the necropsy at the termination of the recovery phase. The masses correlated with malignant mammary gland adenocarcinomas in 1 of 5 low-dose, 3 of 5 mid-dose and 3 of 5 high-dose females and 1 of 4 high dose males. Squamous cell carcinoma was also diagnosed in 1 male and 1 female in the 15 mg/kg group. Two masses (one in a male that had received 1 mg/kg and one in a female that had received 15 mg/kg) were diagnosed as abscesses during microscopic examination.

Organ weights

Measured at necropsy. At termination of the dosing phase, decreased testicular and epididymal weights were observed in the 15 mg/kg BCV group compared with values for control males. Group mean differences in testicular and epididymal weights were -48% and -18%, respectively. Following a 12-week recovery phase, the mean decreases in testicular and epididymal weights in the 15 mg/kg males were -66% and -28%, respectively compared with values for control males.

Histopathology

Adequate Battery: yes
Peer Review: yes

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections were performed on protocol-specified tissues. All specified tissues from animals in the high dose and control groups were examined microscopically, including gross lesions. Gross lesions, epididymides, testes, and tissues in the GI tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum) from all rats in the low and mid-dose groups were also evaluated.

One male receiving 15 mg/kg was found dead during Week 8 of the recovery phase. There was no microscopic correlate to macroscopically-observed stomach ulcers or distension of the duodenum, jejunum, ileum and cecum. Pulmonary vascular congestion was observed microscopically, which correlated with discolored lungs at necropsy. Lymphoid depletion, commonly associated with stress, was also observed. Decreased spermatogenesis correlated with macroscopic findings of decreased testicular size and testicles appearing flaccid. All other animals in all groups survived until the scheduled necropsy.

After the 13-week treatment regimen, minimal to moderate depletion of spermatogenesis was evident in the testes of all 15 mg/kg male rats. Epididymal hypospermia was slight/mild to moderate in several of the affected rats. After the 12-week recovery period, spermatogenesis depletion and epididymal hypospermia were more extensive in the 15 mg/kg males with no evidence of reversibility. No BCV-related change was apparent in testes or epididymides of male rats given 1 or 4 mg/kg for 13 weeks, nor were any changes observed following a 12-week recovery phase.

No histomorphologic tissue changes were apparent in the 15 mg/kg female rats after the 13-week dosing phase. Palpable tissue masses were identified in several BCV- treated rats during the 12-week post-treatment recovery phase. During histopathological evaluation, the masses were diagnosed as malignant mammary gland adenocarcinomas in 1/5 low-dose, 3/5 mid-dose and 3/5 high-dose females, and 1/4

high-dose males. Squamous cell carcinomas were also noted for the high-dose male and one of the high-dose females. Other masses that had been identified by palpation were diagnosed by histopathology as hair follicle trichoepithelioma, abdominal mesenteric lipoma and two abscesses.

Common microscopic findings similarly distributed among high-dose and control rats included kidney nephropathy for most, focal inflammatory cellular infiltrate in livers for some, and unilateral focal seminiferous tubule hypoplasia for a few rats. Modest inflammation in the esophagus or stomach for a few control and treated rats had no apparent dose relationship and was attributed to the dosing procedures. Those findings and the few other microscopic findings were considered incidental and unrelated to treatment with BCV.

Toxicokinetics

Blood samples were collected on Day 1 at 0.5, 1, 2, 4, 10, 24, 36, 48, 60 and 72 hours post-dose and on Week 13 (after the second to last dose) pre-dose and 0.5, 1, 2, 4, 10, 24, 36, 48 and 60 hours post-dose. Toxicokinetic data are presented in **Tables 52 & 53**. Peak plasma concentrations and systemic exposure to BCV increased greater than in proportion to dose on both Day 1 and Week 13 with evidence of slight accumulation after repeated dosing. While there was variability on Day 1, both the C_{max} and AUC for CDV increased less than in proportion to dose during Week 13 with little or no accumulation after repeated dosing. Peak plasma concentrations (C_{max}) and systemic exposure (AUC) to CMX064 increased more than in proportion to dose on both Day 1 and Week 13; however, systemic exposure decreased significantly during Week 13 compared with Day 1, suggesting the possibility of enhanced elimination with repeated administration. At steady state (Week 13), the bulk of systemic exposure on a molar basis was to CDV (about 60%) followed by BCV (about 22% to 28%) and lastly to CMX064 (about 7 to 12%).

Table 52: BCV TK parameters from the 13-week rat oral tox study

Dose (mg/kg)	Week	Sex	Rsq	t1/2elim (hr)	Tlag (hr)	Tmax (ng/mL)	Cmax (ng/mL)	Tlast (hr)	Clast (ng/mL)	AUClast (ng*hr/mL)	AUCinf (ng*hr/mL)	AUC%Extr (%)	AUClast / Dose (ng*hr/mL) / (mg/kg)
1	1	Female	0.98	3.6	0.0	0.5	2.91	10	0.37	10.9	12.8	14.9	10.9
		Male	0.98	3.2	0.0	1.0	2.39	10	0.36	11.9	13.6	12.1	11.9
		M+F	0.98	3.4	0.0	0.5	2.06	10	0.36	11.4	13.2	13.4	11.4
	13	Female	0.77	5.8	0.0	0.5	2.35	10	0.69	13.8	19.6	29.5	13.8
		Male	NC	NC	0.0	2.0	1.88	10	1.09	13.2	NC	NC	13.2
		M+F	NC	NC	0.0	4.0	1.81	10	0.89	13.6	NC	NC	13.6
4	1	Female	0.90	7.4	0.0	2.0	15.9	24	1.64	126	143	12.3	31.4
		Male	0.98	5.8	0.0	2.0	12.3	24	0.85	114	121	5.9	28.5
		M+F	0.99	6.6	0.0	2.0	14.1	24	1.25	121	133	9.0	30.3
	13	Female	1.00	7.9	0.0	2.0	17.6	24	2.04	161	184	12.6	40.2
		Male	0.96	5.9	0.0	2.0	14.3	24	1.53	191	204	6.4	47.7
		M+F	0.99	6.8	0.0	2.0	16.0	24	1.79	177	194	9.0	44.2
15	1	Female	0.99	5.4	0.0	4.0	92.7	36	0.63	623	628	0.8	41.5
		Male	0.71	10.2	0.0	4.0	75.5	60	0.91	770	784	1.7	51.3
		M+F	0.89	16.1	0.0	4.0	84.1	60	0.46	706	716	1.5	47.1
	13	Female	0.94	6.4	0.0	2.0	168	48	0.84	1044	1052	0.7	69.6
		Male	0.66	11.1	0.0	2.0	127	60	2.79	877	922	4.9	58.5
		M+F	0.81	8.2	0.0	2.0	147	60	1.39	956	973	1.7	63.8

Grey cells indicate that due to either Rsq or %Extrapolated, reported t1/2elim and AUCinf should be used with caution
NC: Not calculated due to insufficient data
BLQ: <1.00 ng/mL CMX001

Table 53: CDV TK parameters from the 13-week rat oral tox study

Dose (mg/kg)	Week	Sex	Rsq	t1/2elim (hr)	Tlag (hr)	Tmax (ng/mL)	Cmax (ng/mL)	Tlast (hr)	Clast (ng/mL)	AUClast (ng*hr/mL)	AUCinf (ng*hr/mL)	AUC%Extr (%)
1	1	Female	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
		Male	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
		M+F	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
	13	Female	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
		M+F	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
		Male	NC	NC	NC	ND	BLQ	ND	BLQ	NC	NC	NC
4	1	Female	NC	NC	2.0	10.0	9.01	36	3.58	242	NC	NC
		Male	NC	NC	4.0	10.0	8.94	36	1.73	175	NC	NC
		M+F	0.85	15.1	2.0	10.0	8.97	36	2.65	209	267	21.6
	13	Female	0.87	10.1	1.0	10.0	13.5	48	1.72	366	391	6.4
		Male	NC	NC	2.0	10.0	9.86	36	7.35	241	NC	NC
		M+F	0.79	7.4	1.0	10.0	11.7	48	0.86	317	326	2.8
15	1	Female	0.91	18.2	0.5	10.0	39.8	60	5.92	1139	1295	12.0
		Male	0.99	19.2	1.0	10.0	26.4	72	3.81	960	1065	9.9
		M+F	0.96	11.9	0.5	10.0	33.1	72	1.91	1065	1097	3.0
	13	Female	0.96	12.8	0.0	10.0	39.2	60	4.57	1320	1405	6.0
		Male	0.98	21.4	0.0	10.0	27.7	60	7.47	947	1178	19.6
		M+F	0.99	16.9	0.0	10.0	33.5	60	6.02	1138	1285	11.4

Grey cells indicate that due to either Rsq or %Extrapolated, reported t1/2elim and AUCinf should be used with caution
NC: Not calculated due to insufficient data
BLQ: <5.00 ng/mL CMX021

6.2.6 Study Title: 14-Day Oral Toxicity and Toxicokinetic Sample Collection Study of CMX001 in Cynomolgus Monkeys with a 6 Week Reversibility Period

Study no.: CMX001-TX-002
Study report location: 4.2.3.2
Study initiation date: April 8, 2021
Conducting laboratory and location: (b) (4)
Duration: 2
Duration Units: weeks
GLP compliance: Y
Drug, lot #, and % purity: CMX001 (lot #CMX001-8, purity = 92.7%)
Target Organ+: SYSTEM, GASTROINTESTINAL TRACT

Key Findings

There were unscheduled deaths in the high dose group: 2 females on Day 12, and 1 female and 2 males on Day 13 were found dead. All five deaths were attributed to drug-related gastropathy and enteropathy. Changes in the gastric mucosa (gastropathy) were present in all treated monkeys that were found dead or were sacrificed early during the study. Enteropathy was most pronounced in the posterior portion of the small intestine and in all regions of the large intestine and was most severe in animals from the high dose group of animals. Microscopic changes in the adrenal gland (decreased vacuolization) and thymus (lymphoid depletion) were noted in the high-dose group.

Based on results of the study, a NOAEL was not identified in monkeys. At the low dose, 4 mg/kg/day, based on the body surface area factor, an equivalent dose in humans would be 1.33 mg/kg/day or 80 mg/day for a 60 kg person. At the low dose, exposures (steady state AUC_{0-24} and C_{max}) of CMX001 were 17.6 (male) and 12.7 (female) ng.hr/mL and 6.52 (male) and 8.3 (female) ng/mL, respectively. For CDV at the low dose, exposures (steady state AUC_{0-24} and C_{max}) were 613 (male) and 597 (female) ng.hr/mL and 33.2 (male) and 38.4 (female) ng/mL, respectively. Exposures to BCV in animals were less than exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 4, 10 & 25 mg/kg
Frequency of dosing:	Once daily
Number/Sex/Group:	2/sex/main group, 1/sex/recovery group (recovery was assessed at the mid- and high doses only)
Dose volume:	5 mL/kg
Formulation/Vehicle:	Purified water
Route of administration:	ORAL GAVAGE
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	5-8 years
Comment on Study Design and Conduct:	Surviving animals were euthanized on either Day 16 (main study group) or Day 58 (recovery group).
Dosing Solution Analysis:	All samples met acceptance criteria

Observations and Results

Mortality

Assessed at least once daily. There were 5 unscheduled deaths in the high dose group: 2 females on Day 12 and 1 female and 2 males on Day 13 were found dead. All five deaths were attributed to drug-related gastropathy and enteropathy. The dosing of the lone surviving male in the high dose group was terminated after Day 14 due to the animal's poor health. This animal and two animals (one male and one female) from the mid-dose group were observed for reversibility during a 6-week post treatment period.

Clinical Signs

Assessed at least once daily. Clinical signs associated with administration of low, mid and high doses included one or more of the followings: hunched posture, foamy vomitus, and liquid, nonformed or absent feces. During the post-treatment recovery period, intended to assess reversibility of findings, the liquid feces and/or nonformed feces were not observed for the mid-dose animals. For males in the high dose group, liquid and/or nonformed feces were observed through Day 34 (Day 20 of post-treatment period).

Body Weights

Measured twice daily and at necropsy. For female animals in the high dose group and male monkeys at all dose levels, body weights were decreased slightly beginning Day 8 through the terminal sacrifice, when compared with Day 1 body weights. All monkeys in the recovery group gained weight after termination of dosing. Body weight of the high dose male equated to his Day 1 body weight at about 4 weeks after cessation of the treatment.

Ophthalmology

Evaluated pretreatment and during Week 2. No drug-related changes.

Cardiology

Evaluated pretreatment and during Week 2. There were no EKG findings and the QT intervals were normal.

Clinical Pathology

Blood samples were collected pretreatment, on Day 10, and at necropsy. There were no toxicologically-relevant changes in low and mid-dose group animals in hematology, coagulation, urine chemistry or urinalysis parameters. At the high dose, small changes in clinical chemistry values consistent with gastrointestinal toxicity occurred. Serum total protein, albumin, globulin, sodium and chloride concentrations were decreased in individual animals on Days 10 and 13.

Hematological changes in females from the high dose group consisted of statistically significant increases in neutrophil count in one female on Day 10, and decreased lymphocyte counts in all females on Day 10 and 13.

Minimal to mild increases in serum urea nitrogen and creatinine concentrations occurred in one male and all females from the high dose group on Days 10 and 13. Turbid, brown urine containing minimally to mildly increased protein, occult blood, and bacteria occurred in two males from the high dose group on Day 13. Urinalysis was not performed on the high dose females.

Monkeys administered mid- and high doses and assessed after a post-treatment recovery period had hematology, coagulation, clinical chemistry, urine chemistry and urinalysis values that were comparable to the pretreatment values.

Gross Pathology

Evaluated at necropsy. Drug-related macroscopic lesions were present in the stomach and/or large intestine in monkeys in each treated group (low, mid and high). Dark red areas were noted in the stomach of one male and one female at the mid-dose and in one male at the low dose. Dark liquid/material was present in the colon of one male each from the mid and high dose groups. A red area at the ileocecal junction and a red raised area in the mucosa of the cecum of a male from the low dose group corresponded to severe enteropathy in the ileum and moderately severe changes in the large intestine. The presence of dark raised areas in the wall of the large intestine corresponded to the presence of parasitic granulomas.

During the recovery phase, a dark red area was noted in the fundic region of the stomach in one male administered the high dose. No other gross changes were seen in these animals.

Organ Weights

Measured at necropsy. No drug-related changes.

Histopathology

Adequate Battery: yes
Peer Review: yes

Drug-related, histo-morphologic changes were present in the gastrointestinal tract (gastropathy and enteropathy) in treated monkeys at all doses and resulted in the unscheduled death of five monkeys at the high dose level. The changes consisted of necrosis, regeneration and disorganization in the gastrointestinal mucosa. Changes in the gastric mucosa (gastropathy) were present in all treated monkeys that were unscheduled deaths or sacrificed during the study. Enteropathy was most pronounced in the posterior portion of the small intestine, and in all regions of the large intestine, and was most severe in the high dose group of animals.

Microscopic changes in the adrenal gland (decreased vacuolization) and thymus (lymphoid depletion) were noted in the high dose animals.

In the 3 animals sacrificed on Day 58, the dark red area noted grossly in the fundic region of the stomach in one male from the high dose group correlated histopathologically with erosion and hemorrhage in the mucosa. The cardiac and pyloric regions of the stomach and intestinal sections were unremarkable. Drug-related, histo-morphologic changes were not present in the mid-dose animals.

Toxicokinetics

Blood samples were collected on Days 1 and 15 pre-dose and 1, 2, 5, 8, 12 and 24 hr post-dose. Mean toxicokinetic parameters are shown in **Tables 54 & 55**. Dose-related increases of CMX001 C_{max} and AUC were observed in monkeys administered the low and mid-doses for 15 days. Depending on the dose levels, the C_{max} values of CMX001 occurred within 1.0 to 3.5 hr following drug administration. There were insufficient data to draw a conclusion on gender differences in CMX001 exposures. Some evidence of CMX001 accumulation after multiple dosing was observed for the mid-dose group on Day 15.

CDV is the active metabolite of CMX001. Dose related increases of CDV C_{max} and AUC after CMX001 administration were seen in both male and female monkeys. The mean terminal half-lives of CDV ranged from 10.3 to 31.5 hrs. Accumulation of CDV was evident after multiple dosing on Day 15.

CDV C_{max} and AUC values were greater than those of CMX001. The AUC ratio of CDV to CMX001 on Day 1 ranged from 3.8 to 40.8, and on Day 15 ranged from 5.9 to 65.7. The high ratios suggested rapid conversion of CMX001 to CDV in cynomolgus monkeys.

Table 54: BCV TK data from the 14-day monkey oral tox study

Group; dosage (mg/kg/day)		AUC_{0-24} (ng*hr/ml)		Cmax (ng/ml)	
		drug days		drug days	
		1	15	1	15
Low 4	male	4.19	17.6	4.19	6.52
	female	7.32	12.7	7.32	8.3
Mid 10	male	25.7	131	9.66	31.2
	female	9.55	70.9	9.55	25.7
High 25	male	336	376	65.7	51.6
	female	72.9	83.5	17.5	18

Table 55: CDV TK data from the 14-day monkey oral tox study

Group; dosage (mg/kg/day)		AUC_{0-24} (ng*hr/ml)		Cmax (ng/ml)	
		drug days		drug days	
		1	15	1	15
Low 4	male	218	613	11.8	33.2
	female	188	597	9.57	38.4
Mid 10	male	377	1694	24.4	111
	female	384	1150	21.8	78.7
High 25	male	963	2203	49.1	115
	female	1029	5488	65.6	323

6.2.7 Study Title: A 13-Week Oral Toxicity and Toxicokinetic Study of CMX001 in Monkeys with a 12-Week Post-Treatment Period

Study no.: CMX001-TX-022
Study report location: 4.2.3.2
Study initiation date: March 11, 2008
Conducting laboratory and location: (b) (4)
Duration: 13
Duration Units: weeks
GLP compliance: Y

Drug, lot #, and % purity: CMX001 (lot #AA-020112-Batch-01-2007, purity 97.5%)

Key Findings

The only observations in this study were minor, but statistically significant, elevations in ALT values in monkeys given 4 or 15 mg/kg BCV. Because the increases were not associated with any macroscopic or microscopic changes and because the elevations were fully reversible after cessation of dosing, the NOAEL for BCV was 15 mg/kg/dose when administered orally twice per week to cynomolgus monkeys under the conditions of this study.

The associated combined-sex Week 13 $AUC_{(0-\infty)}$ values at the NOAEL for BCV, CMX021, and CMX064 were 81.4, 1658, and 10630 ng.hr/mL, respectively. Exposures to BCV in animals were less than exposures in humans following the clinical dose (200 mg). The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 1, 4 & 15 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	4/sex/main group, 2/sex/recovery group (recovery assessed in control and high-dose groups only)
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) in purified water
Route of administration:	ORAL GAVAGE
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	3-6 years
Comment on Study Design and Conduct:	Main study animals were euthanized on Week 13. Recovery animals were euthanized on Week 25. The overall immaturity of reproductive tissues examined in this study precluded definitive assessment of reproductive toxicity.
Dosing Solution Analysis:	All samples met acceptance criteria.

Observations and Results

Mortality

Assessed twice daily. There were no unscheduled deaths.

Clinical signs

Assessed twice daily. No drug-related observations.

Body weights

Measured twice weekly. No drug-related changes.

Food consumption

Evaluated qualitatively daily. No drug-related changes.

Ophthalmoscopy

Evaluated pretreatment and prior to necropsy. No drug-related findings.

Electrocardiography

Evaluated pretreatment, 3-5 hours post-dose on Day 1 and during the last week of dosing, and during the last week of recovery. There were no drug-related findings.

Hematology, Coagulation and Urinalysis

Blood and urine samples were collected pretreatment, during Week 6, and prior to necropsy. No drug-related changes.

Clinical Chemistry

Blood and urine samples were collected pretreatment, during Week 6, and prior to necropsy. ALT was dose-dependently increased in males and females during Week 6 and at termination. These increases were statistically significant in males at 4 mg/kg/dose at both intervals and in both sexes at 15 mg/kg/dose at both intervals. There was no progression in elevated ALT levels from Week 6 to termination. The increased ALT values were 1.5-3, and 3 to 4-fold higher than controls in the 4 and 15 mg/kg groups, respectively, and resolved fully during the recovery period. AST also exhibited sporadic statistical increases relative to controls in males or females, but values remained within expected ranges and/or were comparable to respective pretest values.

Gross Pathology

Evaluated at necropsy. No drug-related findings.

Organ Weights

Measured at necropsy. At 15 mg/kg/dose, males had a non-significant decrease in thymus weight compared to controls.

Histopathology

Adequate Battery: yes
Peer Review: yes

The overall immaturity of reproductive tissues examined in this study precluded definitive assessment of test article effect on them. One female administered 4 mg/kg had the tongue missing from microscopic observation. One male at 15 mg/kg/dose had the rectum, prostate gland, seminal vesicles, and urinary bladder missing from microscopic observation. Numerous animals in both male and female groups had parathyroid glands that were not examined. All microscopic findings in these monkeys were typical of those seen at this facility in monkeys of this species, sex, and age and were considered incidental to administration of test article.

Toxicokinetics

Blood samples were collected pre-dose and 0.5, 1, 2, 4, 8, 12, 24, 36, 48, and 72 hours post-dose on Days 1 and 88. The toxicokinetic parameters for the parent compound (CMX001; BCV) and the two metabolites CMX021 (CDV), CMX064 (major

inactive metabolite), and minor inactive metabolites CMX103, CMX104, CMX105, and CMX106 are summarized in **Table 56**. There were no gender differences in systemic exposures to BCV and the two key metabolites, CDV and CMX064; therefore, combined-sex toxicokinetic parameters and summarized in the table. There was no evidence of accumulation throughout the 13-week study. Exposures to the parent compound, CMX001 (BCV), were considerably lower than the two metabolites. Systemic exposures increased in a dose-proportional manner on Day 1 and Week 13.

Table 56: BCV and metabolite TK data from the 13-week monkey oral tox study

C_{max} and AUC_{∞} Exposures at 15 mg/kg/dose During Week 13 for Males and Females, Combined			
CMX001 DOSE (mg/kg/day)	ANALYTE	C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (hr*ng/mL)
15	CMX001	11.4	81.4
	CMX021	37.0	1658
	CMX064	932	10630
	CMX103	31.6	NC
	CMX104	81.4	1692
	CMX105	51.2	1296
	CMX106	43.8	1035
NC=Non-calculated due to insufficient data.			

6.2.8 Study Title: A 26- and 39-Week Oral Gavage Toxicity and Toxicokinetic Study of CMX001 in Cynomolgus Monkeys Followed by a 26-Week Post-Treatment Period

Study no.: CMX001-TX-027
Study report location: 4.2.3.2
Study initiation date: June 1, 2010
Conducting laboratory and location: (b) (4)
Duration: 39
Duration Units: weeks
GLP compliance: Y
Drug, lot #, and % purity: CMX001 (lot #09-338-002, batch CMX001-068, purity = 98.8%)
Target Organ+: KIDNEY
Target Organ+: TESTIS

Key Findings

Minimal or mild karyomegaly of renal tubular epithelial cells was noted in 1 of 4 middle dose (15 mg/kg) males and 2 of 4 high dose (25 mg/kg) monkeys (both sexes). In all treated males, dose-related testicular, epididymal, and seminal vesicle/prostate changes were observed. In high-dose females, changes were noted in menstrual cycle (extended cycles, decreased number of cycles) and decreased body weight.

No NOAEL for male cynomolgus monkeys was identified in this study. The NOAEL for female cynomolgus monkeys was 15 mg/kg, corresponding to a mean C_{max} value (both sexes) of 17.37 ng/mL and a mean AUC value (both sexes) of 63.74 ng.hr/mL. The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127).

Methods

Doses:	0, 5, 15 & 25 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	4/sex/interim group, 4/sex/main group, 2/sex/recovery group
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) in purified water
Route of administration:	NASOGASTRIC
Species:	MONKEY
Strain:	CYNOMOLGUS
Age / Sexual Maturity:	4-11 years
Comment on Study Design and Conduct:	Animals were euthanized on either Day 183 (interim group), 270-271 (main group), or 465 (recovery group). The presence of testicular masses was not determined due to apparent deficiencies in technical staff training. The missing data does not invalidate other findings.
Dosing Solution Analysis:	All samples met acceptance criteria.

Observations and Results

Mortality

One female (15 mg/kg) was found dead on Day 98 (Week 14). The animal was discovered cold, with mouth/nose full of foam-like substance. Previous clinical observations included loss of fur (Days 86 – 98) and soft feces (Day 4). Normal food consumption was noted prior to death, and no decrease in body weight was observed.

At necropsy, no BCV-related gross or microscopic changes were observed. A mild blood staining on the peri-oral skin, severe and diffuse dark-red discoloration of the left caudal lobe of the lung, a distention with liquid ingesta and/or gas in the stomach (severe), jejunum (mild), and ileum (mild), and liquid contents and gas in the colon (moderate) were seen in this animal. Microscopic changes included moderate congestion, mild edema and minimal hemorrhage in the alveoli of the lung, mild hemorrhage in the mesenteric lymph nodes, and moderate hemorrhage (artifact) in the adventitia of the aorta. The cause of death could not be determined for this animal by gross and microscopic examinations. The distention with liquid and gas in the

gastrointestinal tract was related to autolysis. Although the cause of death could not be positively established, there is no evidence suggesting that it was related to BCV administration.

One high dose female was euthanized on Day 407 (137 days after the last dose) following signs of chronic renal failure and nonregenerative anemia that did not respond to veterinary intervention. During Week 52, increased creatinine (4.4 mg/dL; normal value is approximately 1 mg/dL), a slight increase (approximate 2-fold) in blood urea nitrogen, and moderately decreased red blood cells, hemoglobin and hematocrit were indicative of renal insufficiency. A 2-fold increase in amylase (not associated with an increase in lipase) was noted. Blood (3+) and protein (1+) were detected in urine collected at the same time point. The increased creatinine, blood urea nitrogen, amylase, and decreased indicators of red blood cell mass persisted through Week 58. Mild pale discoloration of the kidneys was observed macroscopically at necropsy. Histopathologically, renal effects included mild karyomegaly of renal tubular epithelial cells, moderate atrophy of tubular epithelium, mild dilatation of renal tubules with protein casts, minimal neutrophil infiltration, and hemorrhage in renal tubules, mild edema in the interstitium, and mild mononuclear cell infiltration.

Clinical Signs

Cage-side clinical observations were performed twice-daily for each animal, beginning on Day -14. Detailed clinical observations were performed once during acclimation and once weekly throughout the study, immediately following dose administration. No BCV-related changes were identified during clinical observations.

Body Weights

Body weights were assessed for each animal twice during acclimation and twice weekly throughout the study. There was no effect of treatment with BCV on the mean body weights of males and in females in Groups 1-3 during the 26-week evaluation period. In females (high dose), during the 26-week period, occasional body weight decreases exceeding 10% of baseline (from last acclimation time point) and requiring veterinary intervention occurred in 4 of 10 high dose females. The body weight decreases correlated with sporadic decreases in food consumption seen over the first 26 weeks of the study. Food supplements were provided to all 4 monkeys and their body weights subsequently increased.

Over the 9-month evaluation period, there was no effect of BCV administration on mean body weights of male monkeys. Overall, control and low dose (5 mg/kg) females experienced consistent body weight gain from Day 1 through the dosing and post-treatment recovery period.

Middle dose females appeared to gain weight at a slower rate than that of control or low dose females, and high dose females showed minimal decreases in body weight.

Food Consumption

A qualitative evaluation of food consumption was made once daily. Decreased food consumption was seen sporadically in 3 of 10 high dose females during the dosing period (through terminal necropsy).

Ophthalmoscopy

Ophthalmology examinations were performed on each animal once during acclimation (Days -32, -31, -26, and -25), once during Week 25 (Day 171) or Week 26 (Days 177 and 180), and once during Week 38 (Day 261). There were no BCV-related ophthalmic findings.

Electrocardiography

ECG parameters were recorded for each animal twice during acclimation, once 2-4 hours post-dose) during Week 25 (Day 169), and once during Week 38 or 39. There were no drug-related findings.

Hematology/Coagulation

Blood samples were collected pretreatment an on Days 79, 82, 170, 264, 359 and 450. There were no drug-related changes.

Clinical Chemistry

Blood samples were collected pretreatment an on Days 79, 82, 170, 264, 359 and 450. Increased alanine transaminase (ALT; about 2- to 4-fold compared to baseline) was observed in all BCV-treatment Groups on Days 79, 170 and 264. The change was dose-related in females but not males and values were comparable on Days 79, 170, and 264. ALT values decreased but remained high in at least one female in each treated group during Week 52 (the first post-treatment recovery sampling point); however, values in all BCV-treatment groups were comparable to control values during Week 65. The differences in ALT in middle and high dose females were statistically significant ($p<0.05$) relative to the female control group values on Days 79, 170, and 264. There was no histopathologic correlation to this change.

Menstrual Cycle Observations

Evaluated once daily. At the 6-month interim sacrifice, possible BCV-related changes in length and number of menstrual cycles were observed in high dose (25 mg/kg) females. The average cycle length was 33 days for all groups. The total number of cycles calculated was 55, 62, 58, and 47 in control, low, middle, and high dose, respectively. The extended cycle length and decreased number of cycles in high dose females relative to control females may be a direct effect of BCV.

At the terminal (9-month) sacrifice, the average cycle length was 34 days (range: 19 to 76), 31 days (range:13 to 67), 30 days (range: 14 to 96) and 41 days (range: 18 to 177) in control, low, middle, and high dose, respectively. The total number of cycles calculated was 74, 87, 84, and 64 in control, low, middle, and high dose, respectively. The extended cycle length and decreased number of cycles in high dose relative to control females may be a direct effect of BCV or related to changes observed in the body weight of several high dose females.

Extended cycle length and/or a decreased number of cycles was observed in 4 of 10 high dose animals, all of them had pronounced (≥10% of baseline) body weight decreases at one or multiple intervals during the dosing or post-treatment recovery periods of the study. In the 6 animals without menstrual cycle changes, only 2 had pronounced body weight decreases at any time while on study.

Testicular Volume Measurements

Animals were sedated with ketamine and the testicular volume (mL) of both testes was derived from linear measurements (length x width x height) made using a caliper. Measurements are presented in **Table 57**. A dose-dependent and progressive decrease in testicular volume was observed in all treated groups beginning during Week 8. By interim necropsy, mean group values were decreased 22% (low dose), 51% (middle dose), and 52% (high dose), as compared to the respective baseline values in those groups. During the post-treatment recovery period, mean testicular volume (TV) increased in low and high dose as early as Week 47. Middle dose TV remained low compared to baseline. Full recovery, as compared to average baseline TV, was observed during Week 52 for low dose males and during Week 59 for high dose males.

Table 57: Testicular volume changes from the 26/39-week monkey tox study

	Average Baseline Volume (mL)	Change from Average Baseline (%)								
		Week 2	Week 8	Week 13	Week 24	Week 38/39	Week 47	Week 52	Week 59	Week 65
Group 1	37.99	+9.9	+13.9	+17.3	+21.6	+25.3	+17.3	+19.0	+26.8	+10.7
Group 2	45.10	+4.6	-10.3	-14.2	-21.6	-29.0	-1.7	+10.6	+37.7	+40.1
Group 3	50.16	+1.3	-33.2	-44.2	-51.1	-53.7	-67.8	-65.8	-59.0	-58.4
Group 4	43.20	+4.3	-36.6	-40.0	-51.8	-41.4	-3.1	-6.3	+10.7	+17.7

Group 1, control; Group 2, low dose; Group 3, middle dose; Group 4, high dose

Semen Analysis

A dose dependent and progressive decrease, both relative to baseline and to control, in semen characteristics (sperm motility, concentration, and sperm count) was observed in treated males beginning Week 12/13. These data are presented in **Tables 58-60**. By the interim necropsy at Week 26, sperm motility was decreased 33 to 85%, and sperm count was decreased 49 to 96%, as compared to average baseline. A decrease in ejaculate volume (43%) was observed only in the high dose group by interim necropsy, as compared to average baseline. During the post-treatment recovery period, increased sperm motility, count and concentration were observed as compared to the treatment period of the study, indicating partial or full recovery in all BCV-treatment groups following cessation of treatment.

Table 58: Sperm motility changes from the 26/39-week monkey tox study

	Average Baseline (%)	Sperm Motility – % Change from Baseline (Total Number of Samples Collected and Analyzed)				
		Week 12/13 (n=10)	Week 24/25/26 (n=10)	Week 37/38/39 (n=6)	Week 51/52 (n=2)	Week 64/65 (n=2)
Group 1	79	-10 (10)	-12 (8[a])	-14 (6)	-10 (2)	-57 (2)
Group 2	74	+1 (7[a])	-34 (10)	-32 (4[a])	+16 (1[a])	-6 (2)
Group 3	73	-59 (9[b])	-72 (9[c])	-90 (6)	-80 (2)	-39 (2)
Group 4	71	-70 (10)	-86 (9[d])	-79 (6)	+21 (2)	-26 (2)

[a] A maximum of three attempts to collect semen were made per time point, except as noted in the Deviations Summary (Appendix 2). Samples missing or with insufficient volume collected for analysis was seen for select males in control and CMX001 treatment groups.
[b] Sample for SSAN 42 collected but not analyzed.
[c] Sample for SSAN 48 excluded due to possible urine contamination.
[d] Sample for SSAN 62 collected but not analyzed.

Table 59: Sperm count changes from the 26/39-week monkey tox study

	Average Baseline (M/ejaculation)	Total Sperm Count – % Change from Baseline (Total Number of Samples Collected and Analyzed)				
		Week 12/13 (n=10)	Week 24/25/26 (n=10)	Week 37/38/39 (n=6)	Week 51/52 (n=2)	Week 64/65 (n=2)
Group 1	174.2	-3.5 (10)	+40.2 (8[a])	+2.7 (6)	+56.3 (2)	+218.8 (2)
Group 2	214.5	-3.9 (7[a])	-49.7 (10)	-44.5 (4[a])	+10.9 (1[a])	+44.4 (2)
Group 3	258.2	-80.2 (9[b])	-96.4 (9[c])	-99.2 (6)	-96.4 (2)	-91.9 (2)
Group 4	172.8	-78.2 (9[a])	-90.9 (9[d])	-88.0 (6)	-37.6 (2)	+31.2 (2)

[a] A maximum of three attempts to collect semen were made per time point, except as noted in the Deviations Summary. Samples missing or with insufficient volume collected for analysis was seen for select males in control and CMX001 treatment groups.
[b] Sample for SSAN 42 collected but not analyzed.
[c] Sample for SSAN 48 excluded due to possible urine contamination.
[d] Sample for SSAN 62 collected but not analyzed.

Table 60: Sperm morphology changes from the 26/39-week monkey tox study

	Percent of Males with 20% or Greater Abnormal Sperm or No Cells for Evaluation (Total Number of Samples Collected and Analyzed)						
	Acclimation 1 (n=10)	Acclimation 2 (n=10)	Week 12/13 (n=10)	Week 24/25 (n=10)	Week 37/38/39 (n=6)	Week 51/52 (n=2)	Week 64/65 (n=2)
Group 1	0 (10)	10 (10)	10 (10)	20 (10)	17 (6)	0 (2)	0 (2)
Group 2	10 (10)	0 (10)	40 (10)	20 (10)	33 (6)	50 (2)	0 (2)
Group 3	0 (10)	0 (10)	56 (9[a])	67 (9[b])	83 (6)	100 (2)	50 (2)
Group 4	10 (10)	0 (10)	70 (10)	89 (9[c])	83 (6)	50 (2)	0 (2)

[a] Sample for SSAN 42 not analyzed.
[b] Sample for SSAN 48 excluded due to possible urine contamination.
[c] Sample for SSAN 62 not analyzed.

Gross Pathology

Evaluated at necropsy. Changes (decreased testes size) were observed in middle and high dose males. This change correlated with decreased testes, epididymides, and prostate/seminal vesicles weights. Mild pale discoloration of the kidneys was observed in 1 of 4 high dose males at the terminal necropsy and in the surviving high dose female at the recovery necropsy.

Organ Weights

Measured at necropsy. BCV-related decreases were observed in the weights (absolute and percent relative to brain and body weight) of the testes and epididymides in middle dose (15 mg/kg) and high dose (25 mg/kg) males at the interim necropsy. Increased kidney weight was noted in 1 of 4 high dose males. Edema in the kidney of that animal was considered to be secondary to accidental dosing into the lung of this animal. Mildly decreased sizes of the testes were noted in 2 of 4 low dose males, 4 of 4 middle dose males and 4 of 4 high dose males at terminal necropsy, and in 1 of 2 middle dose males at recovery necropsy.

Histopathology

Adequate Battery: yes
Peer Review: yes

Testes: Atrophy of seminiferous tubules was observed in 2 of 4 low dose males (mild or moderate), 4 of 4 middle dose males (moderate or marked) and 4 of 4 high dose males (marked) at the terminal necropsy. Mild vacuolation of Sertoli cells in the testes was observed in one animal each at the middle and high dose at the terminal necropsy. At the recovery necropsy, atrophy of seminiferous tubules was observed in 1 of 2 middle dose males (moderate) and 1 of 2 high dose males (moderate). Minimal vacuolation of Sertoli cells in the testes was observed in one middle dose male, and absence of Sertoli cells in atrophied seminiferous tubules was observed in one high dose male. These changes were considered to be related to treatment with BCV because they were proportional to dose and duration of treatment. According to the Pathologist's report, there was evidence of recovery during the post-treatment recovery period. Decreased sperm was observed in 1 of 4 low dose males (minimal) and 2 of 4 middle dose males (mild or moderate), and absence of sperm was observed in 1 of 4 low dose males, 2 of 4 middle dose males and 4 of 4 Group 4 males at the terminal necropsy. Mild atrophy of ducts was also observed in 1 of 4 high dose males at the terminal necropsy. At the recovery necropsy, decreased sperm was observed in 1 of 2 middle dose males (marked) and 1 of 2 high dose males (moderate). These changes were considered to be related to treatment with BCV because they were proportional to dose and duration of treatment. As with the testicular changes, there was evidence of reversal during the post-treatment recovery period.

Pituitary gland: Observed pituitary gland changes (minimal vacuolation in pars intermedia) in one male and one female from the high dose groups may be secondary to the effects on sex organs.

Kidney: Minimal or mild karyomegaly of tubular epithelial cells was observed in 2 of 4 high dose males and 2 of 4 high dose females at the terminal necropsy. At the recovery necropsy, minimal or mild karyomegaly of tubular epithelial cells was present

in 1 of 2 middle dose males, 1 of 2 high dose males, and the surviving high dose female. The middle dose male also had mild dilatation of renal tubules with protein casts and the high dose female had mild atrophy of tubular epithelium, minimal dilatation of renal tubules with protein casts, and minimal edema in the interstitium.

Liver: In the liver, minimal karyomegaly of hepatocytes was observed in 1 of 2 middle dose males at the recovery necropsy.

Toxicokinetics

Blood samples were collected on Day 1 and Weeks 13, 26 and 39 pre-dose (Weeks 13, 26 and 39 only) and 0.5, 1, 2, 4, 8, 12, 24, 36, 48, and 72 hours post-dose. Toxicokinetic parameters are presented in **Table 61**.

BCV: C_{max} increased slightly greater than in proportion to dose with mean values of 4.04, 17.37, and 33.21 ng/mL in the 5, 15, and 25 mg/kg groups, respectively, during Week 39. $AUC_{0\text{-}tau}$ increased approximately in proportion to dose with mean values of 21.57, 63.74, and 112.50 ng.hr/mL at doses of 5, 15, and 25 mg/kg, respectively, during Week 39. $AUC_{0\text{-}tau}$ during Weeks 13, 26, and 39 was very similar to $AUC_{0\text{-}inf}$ on Day 1 indicating there was no meaningful change in enzymes involved in the elimination of BCV with repeated administration. As on Day 1, BCV concentrations in plasma declined rapidly after reaching T_{max} and were below the limit of quantitation within 24 hours after administration in all dose groups. The estimated $t_{1/2elim}$ values during Weeks 13, 26 and 39 were similar to Day 1 values, ranging from 1.87 to 3.08 hours.

CMX021: After twice weekly administration for 13, 26, and 39 weeks, the mean T_{max} for CMX021 ranged from 14.0 to 22.5 hours after oral administration of BCV. CMX021 C_{max} increased approximately in proportion to dose with mean values of 14.40, 63.60, and 66.55 ng/mL at BCV doses of 5, 15, and 25 mg/kg, respectively, for 39 weeks. $AUC_{0\text{-}tau}$ increased approximately in proportion to dose with mean values of 592.06, 1715.57, and 2306.63 ng.hr/mL at BCV doses of 5, 15, and 25 mg/kg, respectively, for 39 weeks. After steady-state was achieved, there was no evidence of accumulation of CMX021 and concentrations in plasma declined slowly after reaching T_{max}, with quantifiable concentrations at the last sampling time point (72 hours) in most monkeys receiving 25 mg/kg BCV. The mean $t_{1/2elim}$ for CMX021 after twice weekly administration of BCV ranged from 16.36 to 28.57 hours.

Table 61: BCV TK data from the 26/39-week monkey tox study

Dose (mg/kg) given twice weekly for 26 or 39 Weeks:	0 (Control)		5		15		25	
Gender: Number of Animals:	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics, CMX001	Combined Sexes							
C_{max} (ng/mL)								
Day 1	NA		3.04		11.04		26.99	
Week 13	NA		3.49		13.64		21.76	
Week 26	NA		3.65		21.32		21.31	
Week 39	NA		4.04		17.37		33.21	
$AUC_{0\text{-}inf}$ (ng•hr/mL)								
Day 1	NA		14.20		60.27		117.67	
Week 13	NA		13.81		59.28		117.11	
Week 26	NA		19.82		66.67		117.68	
Week 39	NA		21.57		63.77		112.54	
Toxicokinetics, CMX021	Combined Sexes							
C_{max} (ng/mL)								
Day 1	NA		10.31		21.66		50.53	
Week 13	NA		11.39		28.55		50.50	
Week 26	NA		17.40		49.79		67.49	
Week 39	NA		14.40		63.60		66.55	
$AUC_{0\text{-}inf}$ (ng•hr/mL)								
Day 1	NA		497.66		968.22		1726.56	
Week 13	NA		808.88		1380.06		2238.39	
Week 26	NA		939.35		1354.91		2741.24	
Week 39	NA		704.68		1794.70		2400.14	

7. Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

7.1.1 Study Title: Bacterial Reverse Mutation Assay of CMX001

Study no.: CMX001-TX-004
Study report location: 4.2.3.3.1
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: CMX001 (lot #CMX001-9, purity = 94.5%)

Key Findings

Under the conditions of this study, BCV was not mutagenic in the Ames reverse mutation assay.

Methods

Strains: TA98, TA100, TA1535, TA1537 and WP2 *uvrA*
Concentrations in definitive study: 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
Basis of concentration selection: A non-GLP exploratory study
Negative control: Sterile dH_2O
Positive control:

Control (µg/plate)	S9	Strain
2-nitrofluorene (1)	-	TA98
Sodium azide (1)	-	TA100 & TA1535
9-aminoacridine (75)	-	TA1537
Methyl methanesulfonate (1000)	-	WP2 *uvrA*

2-aminoanthracene (10)	+	All strains

Formulation/Vehicle: Sterile dH_2O
Incubation & sampling time: Plates were incubated at 37°C for 48 hours with and without S9-induced metabolic activation
Comment on Study Validity: Valid

Results

BCV was assessed for mutagenic potential in the Ames assay at concentrations up to 5000 µg/plate, both with and without S-9 metabolic activation. The metabolic activation system was the S9 fraction of a rat liver homogenate from animals treated previously with Aroclor 1254 (500 mg/kg). No cytotoxicity was observed in each of the S. typhimurium strains at the highest concentrations tested with and without S9 activation (5000 µg/plate). No cytotoxicity was observed with E. coli strains. The histamine+ and tryptophan+ revertant frequencies in each of the treated cultures were essentially at the concurrent negative controls. As expected, there were significant increases in the histidine+/tryptophan+ revertant counts in the cultures treated with the positive control articles. Under the conditions of this study, BCV was not mutagenic in the Ames reverse mutation assay.

7.2 *In Vitro* Assays in Mammalian Cells

7.2.1 Study Title: In Vitro Mammalian Chromosome Aberration Test

Study no.: CMX001-TX-003
Study report location: 4.2.3.3.1
Conducting laboratory and location: (b) (4) [redacted]
GLP compliance: Yes
Drug, lot #, and % purity: CMX001 (lot #CMX001-9, purity = 94.5%)

Key Findings

Under the conditions of this study, BCV was weakly positive for the induction of structural chromosome aberrations in the in vitro mammalian chromosome aberration test using human peripheral lymphocytes in the non-activated test system. Thus, it is concluded that BCV is clastogenic to cultured human lymphocytes.

Methods

Cell line: Peripheral blood lymphocytes from a healthy non-smoking 27-year-old adult female

Concentrations in definitive study:

Time (hr)	S9	Concentration (µg/mL)
4	-	3.13, 6.25, 12.5, 25, 50, 75 & 100
20	-	0.39, 0.78, 1.56, 3.13, 6.25, 12.5 & 25
4	+	3.13, 6.25, 12.5, 25, 50, 75 & 100

Basis of concentration selection: A non-GLP exploratory study
Negative control: Sterile dH_2O
Positive control: Mitomycin C (0.3 & 0.6 µg/mL) in the absence of S9. Cyclophosphamide (20 & 40 µg/mL) in the presence of S9.

Formulation/Vehicle:	Sterile dH_2O
Incubation & sampling time:	Cells were incubated at 37°C for 4 and 20 hours without S9-induced metabolic activation, and for 4 hours with S9-induced metabolic activation.
Comment on Study Validity:	Valid

Results

In this study, hemolysis was observed at concentrations >50 µg/mL in the S9 activated 4 hr exposure group. In the non-activated 4 hr exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations (75 µg/mL), mitotic inhibition was 51%, relative to the solvent control.

The dose level selected for analysis of chromosome aberrations were 25, 50 and 75 µg/mL. The percentage of cells with structural aberrations in the non-activated 4 hr exposure group at 25, 50 and 75 µg/mL were statistically significantly increased at 3.5%, 3% and 2.5%, respectively, above that of the solvent control ($p<0.01$ to <0.05). The percentage of cells with numerical aberrations in the treated group was not significantly increased above that of the solvent control at any dose level. The percentage of structurally damaged cells in the positive control (mitomycin) group, significantly increased to 24%. In the non-activated 20 hr exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations (25 µg/mL), mitotic inhibition was 58%, relative to the solvent control. The dose level selected for analysis of chromosome aberrations were 6.25, 12.5 and 25 µg/mL. The percentage of cells with structural aberrations at the high dose (25 µg/mL) was not significantly increased (3.5%, $p<0.01$) above that of the solvent control. The percentage of aberrant cells at the low and mid-dose was within the historical control range. The percentage of cells with numerical aberrations was not significantly increased at any dose level. The percentage of structurally damaged cells in the positive control (mitomycin) group, significantly increased to 26%.

In the S9 activated 4 hr exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations (25 µg/mL), mitotic inhibition was 54%, relative to the solvent control. The dose level selected for analysis of chromosome aberrations were 6.25, 12.5 and 25 µg/mL.

The percentage of cells with structural aberrations in the non-activated 4 hr exposure group at 25, 50 and 75 µg/mL were statistically significantly increased 3.5%, 3% and 2.5%, respectively, above that of the solvent control ($p<0.01$ to <0.05). The percentage of cells with numerical or structural aberrations in the treated group was not significantly increased above that of the solvent control at any dose level. The percentage of structurally damaged cells in the positive control (cyclophosphamide) group, significantly increased to 16%.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

7.3.1 Study Title: Mammalian Erythrocyte Micronucleus Test in Mice Dosed by Oral Gavage with CMX001

Study no:	CMX001-TX-005
Study report location:	4.2.3.3.2
Conducting laboratory and location:	(b) (4) [redacted]
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001 (lot #CMX001-9, purity = 94.5%)

Key Findings

Under the conditions of the assay, BCV was negative for micronucleus formation in the in vivo rat micronucleus assay.

Methods

Doses in definitive study:	0, 500, 1000 & 2000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Sterile dH_2O
Species/Strain:	ICR mice
Number/Sex/Group:	5/sex/group
Basis of dose selection:	A non-GLP exploratory study
Negative control:	Sterile dH_2O
Positive control:	Cyclophosphamide (50 mg/kg)
Comment on Study Validity:	Valid

Results

Clinical Signs and mortality: There were no deaths in this study. Clinical signs included piloerection in males (low, mid and high) and females (high). In addition, lethargy was seen in males (mid) and in females (high).

Micronucleus evaluation: Reductions in the ratio of polychromatic erythrocyte to total erythrocytes were 6-8% (low), 6-16% (mid) and 10-15% (high) was observed in the treated mice relative to the respective vehicle controls. The number of micronucleated polychromatic erythrocytes per 10,000 polychromatic erythrocytes (PCEs) in drug treated groups relative to the controls was not statistically increased in male and female mice at 24 or 48 hr post-dose. Cyclophosphamide (positive control) induced a significant increase ($p>0.05$) in micronucleated polychromatic erythrocytes in both male and female mice, regardless of dose level or bone marrow collection time.

8. Carcinogenicity

8.1 Study Title+: A 26-Week Oral Toxicity and Carcinogenicity Study of CMX001 in Sprague Dawley Rats, with a 26-Week Post-Treatment Period

Study no.:	CMX001-TX-029
Study report location:	4.2.3.2
Study initiation date:	October 14, 2014
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001-097; 99.8%
Prior Exec CAC Dose Concurrence:	N
Basis for Dose Selection:	13-week study

Reviewer Carcinogenicity Conclusion (negative/ positive): Positive

Tumor Findings:

Neoplastic findings considered to be related to CMX001 were present in both sexes. These included:

- **Zymbal's gland tumors** in males and females.
  - For **adenoma**, the incidence in the control, low-, mid-, and high-dose groups was 0/19, 0/20, 2/20, and 7/17, respectively, in males and 0/20, 2/20, 0/20, and 4/19, respectively, in females.
  - For **carcinoma**, the incidence in the control, low-, mid-, and high-dose groups was 0/19, 2/20, 1/20, and 4/17, respectively, in males and 0/20, 0/20, 0/20, and 1/19, respectively, in females.
- **Hemangiosarcomas** in the mesenteric lymph node of males.
  - The incidence was 0/19, 0/20, 0/20, and 3/17 in the control, low-, mid-, and high-dose groups, respectively.
- A hemangiosarcoma also was present in the mesenteric lymph node of 1/19 high-dose females.
- **Mammary gland tumors** in females.
  - The incidence was 3/20, 0/20, 6/20, and 6/19 in the control, low-, mid-, and high-dose groups, respectively.
- **Skin tumors** in males.
  - The incidence was 0/19, 0/20, 0/20, and 2/17 in the control, low-, mid-, and high-dose groups, respectively. In the high-dose group, one male had a **basal cell adenoma** and **hair follicle tumor**, and another male had a **squamous cell carcinoma**.

Methods

Doses:	0, 0.5, 1 & 5 mg/kg
Frequency of dosing:	Twice weekly
Number/Sex/Group:	20/sex/main group, 20/sex/recovery group

Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) in dH_2O
Route of administration:	ORAL GAVAGE
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age:	Approximately 7 weeks
Comment on Study Design and Conduct:	Animals were euthanized on either Week 26 (main group) or 52 (recovery).
Dosing Comments (Dose Adjustments or Early Termination):	The high dose groups were sacrificed before completing the 6 month recovery period upon reaching 5 survivors.
Dosing Solution Analysis:	Dosing solution did not always meet acceptance criteria; however, based on plasma concentrations of test article the discrepancies did not invalidate the results.

Observations and Results

Mortality

At 0.5 mg/kg there were two deaths related to CMX001. Morbidity/mortality was attributed to skin tumor in one male and to Zymbal's gland tumor in one female.

At 1 mg/kg there were 12 deaths related to CMX001. Morbidity/mortality was attributed to Zymbal's gland tumors in six males and one female, to gastrointestinal tumor in one male, to hemangiosarcoma in one male, and to a skin tumor in one male, and to mammary gland tumors in two females.

At 5 mg/kg there were 32 deaths related to CMX001. Morbidity/mortality was attributed to mammary gland tumors in 11 females, to Zymbal's gland tumors in eight males and three females, to hemangiosarcoma in six males, and to skin tumors in three males and one female.

Clinical Signs

There were no test article-related clinical signs during the treatment phase.

Body Weights

Mean body weight was lower in both sexes at 5 mg/kg beginning in Week 3 through the end of the treatment period (-16 to -19%).

Food Consumption

Mean food consumption tended to be lower in both sexes at 5 mg/kg.

Gross Pathology

In males at 5 mg/kg, macroscopic findings included small epididymides and testes, enlarged mesenteric lymph node (correlated to hemangiosarcoma), abrasion/scab (correlated to basal cell adenoma) and subcutaneous mass (correlated to hair follicle tumor or squamous cell carcinoma).

In females at >1 mg/kg macroscopic findings included subcutaneous mass (correlated to mammary gland adenocarcinoma).

At the post dosing interval necropsy, test article-related macroscopic observations were noted in both sexes in all treated groups. In males, these were noted in the liver, skin, skin subcutis, jejunum, Zymbal's gland, spleen, abdominal cavity, mediastinal and mesenteric lymph nodes, testes, and/or epididymides.

Most macroscopic observations were associated with either primary or metastatic tumors. Exceptions were the testes and epididymides (small; correlated with seminiferous tubule degeneration), and the spleen (enlarged, correlated with increased extramedullary hematopoiesis, except in one animal with lymphoma).

Histopathology
Peer Review Conducted: Yes
Historical Control Provided for Tumor Incidence: No

Neoplastic findings considered to be related to CMX001 were present in both sexes. These included:

- **Zymbal's gland tumors** in males and females.
  - For **adenoma**, the incidence in the control, low-, mid-, and high-dose groups was 0/19, 0/20, 2/20, and 7/17, respectively, in males and 0/20, 2/20, 0/20, and 4/19, respectively, in females.
  - For **carcinoma**, the incidence in the control, low-, mid-, and high-dose groups was 0/19, 2/20, 1/20, and 4/17, respectively, in males and 0/20, 0/20, 0/20, and 1/19, respectively, in females.
- **Hemangiosarcomas** in the mesenteric lymph node of males.
  - The incidence was 0/19, 0/20, 0/20, and 3/17 in the control, low-, mid-, and high-dose groups, respectively.
- A hemangiosarcoma also was present in the mesenteric lymph node of 1/19 high-dose females.
- **Mammary gland tumors** in females.
  - The incidence was 3/20, 0/20, 6/20, and 6/19 in the control, low-, mid-, and high-dose groups, respectively.
- **Skin tumors** in males.
  - The incidence was 0/19, 0/20, 0/20, and 2/17 in the control, low-, mid-, and high-dose groups, respectively. In the high-dose group, one male had a **basal cell adenoma** and **hair follicle tumor**, and another male had a **squamous cell carcinoma**.

Non-neoplastic findings considered to be related to CMX001 were:

- Mild to severe degeneration/atrophy of the seminiferous tubules of the testes in all males at 5 mg/kg, with a severe lesion present in 12 of them. This was associated with mild to severe oligospermia/germ cell debris in the epididymides of 16 of the 17 males, with a severe lesion in 12 of them.
- Minimal to mild cystic glandular hyperplasia in the uterus of eight of 19 females at 5 mg/kg, with six of the eight animals affected only minimally.
- Focal hyperplasia of the Zymbal's gland in one male at 1 mg/kg and in two males and two females at 5 mg/kg.

9. Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development+

9.1.1 Study Title: An Oral (Gavage) Fertility and General Reproduction Toxicity Study of CMX001 in Rats

Study no.:	CMX001-TX-023
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001 (lot #CMX001-042 and CMX001-047, purity = 95.8% and 99.7%, respectively)

Key Findings

The NOAEL for general toxicity in paternal rats was 1 mg/kg due to gross lesions in male rats dosed with 4 and 15 mg/kg and reduced body weights and food consumption in male rats dosed with 15 mg/kg. The NOAEL for male reproductive parameters is also 1 mg/kg due to findings in male reproductive organ weights, with microscopic correlates, and adverse effects on sperm parameters at 4 and 15 mg/kg. Male fertility was impacted at 15 mg/kg.

The NOAEL for maternal toxicity was defined as 0.25 mg/kg after daily dosing due to reduced weight gain at doses ≥1 mg/kg. The maternal reproductive NOAEL could not be defined, as a dose-dependent effect on embryo viability was noted at all dose levels.

Methods

Doses:	Females – 0, 0.25, 1 & 4 mg/kg Males – 0, 1, 4 & 15 mg/kg
Frequency of dosing:	Females – Once daily Males – Twice weekly
Number/Sex/Group:	25/sex/group
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) in dH_2O
Route of administration:	ORAL GAVAGE
Species:	Rats
Strain:	Crl:CD(SD)
Comment on Study Design and Conduct:	Female rats were dosed once daily beginning 15 days before cohabitation, during cohabitation (maximum 21 days) and continuing through Gestation Day (GD) 7 (total of 23-30 doses over 23-30 days). Male rats were dosed twice weekly beginning 70 days before the initial cohabitation period and continuing until the day before

	scheduled sacrifice (total duration of treatment was 25-39 doses over 85-134 days).
Dosing Solution Analysis:	Day 1, Week 8 and Extended Study formulations were within acceptable criteria. Results of the End of Study analyses indicated that the 1 and 4 mg/kg dose were slightly above 25% of the nominal concentration (actual range was 122.6-126.9% of nominal), but the 15 mg/kg dose level (0.8 mg/mL) was within acceptable limits. All formulations were considered to be acceptable for use. The above deviations were not considered to have negatively affected the overall integrity or outcome of the study.

Observations and Results

Mortality

Assessed twice daily. Early deaths in one male from the 1 mg/kg dose group (Day 85; perforated lung) and one male from the 15 mg/kg dose group (Day 88; no lung perforations) were thought to be due to gavage error, although an exact cause of death was not determined in the high dose animal. All females survived to terminal sacrifice.

Clinical Signs

Assessed at least once daily. There were no drug-related clinical observations.

Body Weight

Measured at least once weekly. In males, body weight gain in 15 mg/kg dose group was decreased throughout the study. Body weight gains in the 1, 4, 15 mg/kg dose groups were 91%, 94% and 68% of the vehicle control group value, respectively, over the Day 1 to 134 period.

In females, body weight gains in the 0.25, 1 and 4 mg/kg/day dosage groups were 104%, 43% and -20% of the vehicle control group value, respectively, for the entire period prior to cohabitation (Days 1 to 15). Body weight gains in the 0.25, 1 and 4 mg/kg/day dosage groups were 96%, 81% and 87% of the vehicle control group value, respectively, on GDs 0 to 13.

Food Consumption

Measured at least twice weekly. In males, decreased food consumption in males from the 15 mg/kg dose group correlated with decreased body weight gain noted above. Absolute food consumption values for the entire dosage period (Days 1 to 134) were 96%, 97% and 87% of the vehicle treated group values in the 1, 4 and 15 mg/kg dosage groups, respectively.

In females, absolute food consumption values in the 0.25, 1 and 4 mg/kg/day dosage groups were 101%, 99% and 84% of the vehicle control group value, respectively, on Days 1 to 15. Absolute food consumption values in the 0.25, 1 and 4 mg/kg/day dosage groups were 99%, 95% and 92% of the vehicle control group value, respectively, on GDs 8 to 13.

Fertility and Pregnancy Parameters

Males treated with 15 mg/kg BCV had a fertility index of 66.7%, compared to a fertility index of 87.5% in the vehicle control group. The number of pregnancies per cohabitated pair at 15 mg/kg was 64.0% compared to 82.0% in the vehicle control group. To follow up on the observations of reduced fertility index, reduced pregnancies, and an increase in nonviable embryos, the treated male rats were re-mated with untreated female rats. At the time of re-mating, male rats had been dosed for approximately 18 weeks. Treated male rats in the 15 mg/kg dose group were infertile in the second cohabitation period.

In females, the number of pregnancies per number of rats that mated (i.e., fertility index) and the number of pregnancies per number of rats in cohabitation in the 4 mg/kg/day dosage group were lower than the corresponding vehicle control group values following mating of treated female rats with treated male rats. The fertility index at 4 mg/kg/day was 66.7% in comparison to 87.5% in the vehicle control group. The number of pregnancies per number of rats in cohabitation at 4 mg/kg/day was 64.0% compared to 84.0% in the vehicle control group. Caesarean section and litter observations are summarized in **Table 62**.

Table 62: Caesarean section/litter observations from the rat oral fertility study

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.25	1.00	4.00
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	21(84.0)	23(92.0)	20(80.0)	16(64.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	21	23b	20	16
CORPORA LUTEA	MEAN±S.D.	15.1 ± 2.2	15.6 ± 2.2	14.6 ± 2.1	10.0 ± 2.2**
IMPLANTATIONS	MEAN±S.D.	14.5 ± 2.9	15.1 ± 2.2	13.8 ± 3.0	5.9 ± 3.4**
% PREIMPLANTATION LOSS	MEAN±S.D.	4.9 ± 13.9	3.5 ± 5.5	6.0 ± 13.2	41.9 ± 28.7**
LITTER SIZES	MEAN±S.D.	13.8 ± 3.0	10.9 ± 3.3**	1.5 ± 4.6**	0.2 ± 0.8**
VIABLE EMBRYOS	N	289	250	30	3
	MEAN±S.D.	13.8 ± 3.0	10.9 ± 3.3**	1.5 ± 4.6**	0.2 ± 0.8**
NONVIABLE EMBRYOS	N	16	97	245	91
	MEAN±S.D.	0.8 ± 0.8	4.2 ± 2.3**	12.2 ± 5.1**	5.7 ± 3.6**
% POSTIMPLANTATION LOSS	MEAN±S.D.	6.2 ± 8.2	28.6 ± 15.2**	90.0 ± 30.8**	95.3 ± 18.8**
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	12(57.1)	22(95.6)**	18(90.0)**	16(100.0)**
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	18(90.0)**	15(93.8)**

a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Includes values for dam 6250, which did not have a confirmed mating date.
% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE EMBRYOS) / NUMBER OF IMPLANTATIONS] x 100
** Significantly different from the vehicle control group value (p≤0.01).

Necropsy

There were no clear pathology findings in females, though histopathology was not performed in females beyond Caesarean sectioning. Caesarean section and litter observations are summarized below (see “Fertility and Pregnancy Parameters”). In male rats treated with 4 and 15 mg/kg BCV, reduction in the size of one or both testes, flaccid and/or purple appearance to one or both testes, and decreased size of one or

both epididymides (including the left cauda) was noted. The macroscopic changes were associated with microscopic changes that included an increased incidence and severity of minimal to moderate multifocal, or marked diffuse degeneration in the testes of all rats in these groups, characterized by partial or complete loss of spermatogenic epithelium in the affected seminiferous tubules. Associated changes in the epididymides consisted of mild to marked bilateral hypospermia. Also, in the epididymal tubules of most rats in these groups, there was an increased amount of spermatogenic cells that had undergone degeneration. These data are presented in **Table 63**.

Table 63: Male necropsy findings from the rat oral fertility study

Dose Group:	I	II	III	IV
Sex:	M	M	M	M
Number of Animals/Group:	25	24	25	24
TESTES:				
NO. EXAMINED	25	24	25	24
NO. NORMAL	24	22	0	0
-degeneration, diffuse, bilateral				
marked	0	0	7	24
Total Incidence, All Grades	0	0	7	24
-degeneration, multifocal, bilateral				
minimal	1	2	3	0
mild	0	0	5	0
moderate	0	0	10	0
Total Incidence, All Grades	1	2	18	0
EPIDIDYMIDES:				
NO. EXAMINED	25	24	25	24
NO. NORMAL	16	19	0	0
-exfoliated spermatogenic cells				
minimal	2	1	1	4
mild	0	0	6	11
moderate	0	0	16	0
Total Incidence, All Grades	2	1	23	15
-hyperplasia, focal				
minimal	0	0	1	0
mild	0	0	0	3
Total Incidence, All Grades	0	0	1	3
-hypospermia, bilateral				
mild	0	0	3	0
moderate	0	0	11	0
marked	0	0	8	24
Total Incidence, All Grades	0	0	22	24
-infiltration, mononuclear-cell, focal/multifocal				
minimal	9	4	4	7
mild	0	0	0	1
Total Incidence, All Grades	9	4	4	8

Sperm Evaluation

Sperm concentration and motility were evaluated in males at necropsy. At doses of 4 and 15 mg/kg, the number of motile sperm were decreased. At 15 mg/kg, reduced motility was in addition to a decrease in total sperm count, and the combined effect resulted in reduced fertility during the first cohabitation period and infertility during the second cohabitation period.

9.2 Embryonic and Fetal Development+

9.2.1 Study Title: A Study of the Effect of Orally-Administered CMX001 on Embryo/Fetal Development in Rats

Study no.:	CMX001-TX-019
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001 (lot #CMX001-029, purity = 93.3%)

Key Findings

The NOAEL for maternal toxicity was defined as 0.5 mg/kg/day due to significantly decreased mean maternal body weight and body weight gain at doses of 1.5 and 4.5 mg/kg/day and significantly reduced food consumption at 4.5 mg/kg/day. The NOAEL for embryofetal development was defined as 1.5 mg/kg/day due to a reduction in mean fetal weight at 4.5 mg/kg/day.

Methods

Doses:	0, 0.5, 1.5 & 4.5 mg/kg
Frequency of dosing:	Once daily
Number/Sex/Group:	22 females/main group, 8 females/satellite group
Dose volume:	5 mL/kg
Formulation/Vehicle:	dH_2O
Route of administration:	ORAL GAVAGE
Species:	Rats
Strain:	Crl:CD(SD)
Comment on Study Design and Conduct:	Main study animals were dosed once daily from GD 6 through 17. Satellite animals were treated once daily from GD 6 through 20. In a dose range-finding study, all 5 females in the 15 m/kg/day dose group were sacrificed moribund. Findings included decreased body weight, decreased body weight gain and decreased food consumption. Dose-related decreases in maternal body weight, body weight gain, food consumption, gravid uterine weight and fetal body weight were also observed in the 2.5 and 6 mg/kg/day groups.

Dosing Solution Analysis: Initial concentration analysis of the BCV dosing formulations demonstrated mean potency results between 94.4% and 100.8% of target for Groups 2-4, although analysis of one of the Group 2 sample revealed no peak at the expected retention time, with no explanation given. Due to concerns with appearance (cloudiness), samples for homogeneity determination were collected on 7 June 2007. The Group 2 and 3 dosing formulations did not meet (b) (4) SOP requirements for concentration acceptability (90%-100% of target). The reported TK parameters suggest that there were not significant issues with respect to formulation concentrations during the dosing phase, so the apparent discrepancy may be due to analytical deficiencies.

Observations and Results (F_0 Dams)

Mortality

Assessed twice daily. There were no mortalities among dosed females.

Clinical Signs

Assessed at least once daily. There were no drug-related clinical observations.

Body Weight

Measured on GD 0 and GD 6-20. Mean body weight gain was intermittently decreased compared to controls in rats dosed with 0.5 mg/kg/day, and consistently in females dosed with 1.5 and 4.5 mg/kg/day when data from GD 6-20 were assessed.

Food Consumption

Measured on GD 0 and GD 6-20. Mean food consumption was decreased compared to controls in females dosed with 4.5 mg/kg/day, but not other dose levels.

Caesarean Section Data

At terminal necropsy, one female in the high dose (4.5 mg/kg) TK group was not gravid. All remaining mated females were pregnant. Parameters evaluated included post-implantation loss, live litter size, mean fetal body weights and fetal sex ratios. No test article-related effects were noted on fetal sex ratio or fetal survival. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of preimplantation loss were similar across all groups.

Necropsy

Gross pathology was evaluated at necropsy on GD 20. There were no macroscopic findings in the treated female rats.

Toxicokinetics

Blood samples were collected on GD 6 and 17 at 1, 2, 4, 8, 12 and 24 hours post-dose. Toxicokinetic parameters are provided in **Table 64**. Results indicate that steady-state exposures were achieved after the first dose. On GD 17, 80-90% of test article-derived material detected in blood was CMX021.

Table 64: F_0 TK parameters from the rat embryofetal development study

Dose (mg/kg/day)	Day	CMX001					CMX021					CMX064				
		C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)
0.5	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	BQL	ND	ND	ND	ND
	17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	BQL	ND	ND	ND	ND
1.5	6	3.95	2	10.9	37.6	54.3	8.17	12	ND	101.6	ND	BQL	ND	ND	ND	ND
	17	7.24	4	5.2	72.6	76.1	17.7	12	ND	308.3	ND	BQL	ND	ND	ND	ND
4.5	6	24.0	2	9.3	181.7	209.4	18.53	12	ND	348.4	ND	6.59	4	ND	29.4	ND
	17	47.6	2	5.1	314.9	328.5	47.28	8	17.5	802.1	1422.9	8.96	4	ND	63.7	ND

Abbreviations: BQL, below quantifiable limit (1.0 ng/mL for CMX001, 5.0 ng/mL for CMX021 & CMX064); ND, not determined due to insufficient data

Observations and Results (F_1 Offspring)

Terminal Observations

A statistically significant decrease in mean fetal weight was noted in the 4.5 mg/kg/day group (3.0 g) compared with the concurrent control group (3.5 g). As noted above, no test article-related effects were noted on fetal sex ratio or fetal survival.

Fetal Malformations/Variations (external, visceral, skeletal)

External: A single external malformation, umbilical herniation of the intestine, was noted for 1 fetus in the 1.5 mg/kg/day group and was considered spontaneous in origin. There were no drug-related external variations.

Visceral: There were no drug-related visceral malformations. Renal papillae not fully developed (Woo and Hoar Grade 1) was observed for 1 fetus each in the control and 1.5 mg/kg/day groups. The mean litter proportion of "renal papillae not developed" (Woo and Hoar Grade 0) finding in the 4.5 mg/kg/day group (1.2% per litter) was equal to the maximum mean value in the (b) (4) historical control data range. These findings were not considered to be test article-related.

In the 0.5 mg/kg/day group, but not at higher doses, a major blood vessel variation was noted in 2 fetuses and a pale spleen was observed in 1 fetus. In the absence of a dose-related response, these findings were not considered to be test article-related. In addition, 1 fetus in the 1.5 mg/kg/day group was noted with a dark red area in the kidney. These findings were not classified as either malformations or developmental variations.

Skeletal: There were no drug-related skeletal malformations. Findings in the control and treatment groups consisted primarily of ossified cervical centrum no. 1, unossified sternebrae (nos. 5 and/or 6), 14th rudimentary ribs, malaligned sternebrae (slight or moderate), reduced ossification of the vertebral arches and 7th cervical rib(s). The mean incidences were not statistically significant when compared to the concurrent control group and were within the mean (b) (4) developmental historical data.

9.2.2 Study Title: A Study of the Effect of Orally-Administered CMX001 on Embryo/Fetal Development in Rabbits

Study no.:	CMX001-TX-020
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4) [redacted]
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001 (lot #CMX001-029, purity = 93.3%)

Key Findings

The NOAEL for maternal toxicity was defined as 1.5 mg/kg due to decreased mean body weight and food consumption in dams treated with 4.5 mg/kg/day Group. The NOAEL for embryofetal development was defined as 1.5 mg/kg due to BCV-related effects on intrauterine growth (lower mean fetal weights), survival (higher late resorptions) and morphology (external malformations and visceral and skeletal developmental variations) at 4.5 mg/kg/day.

Methods

Doses:	0, 0.5, 1.5 and 4.5 mg/kg
Frequency of dosing:	Once daily
Number/Sex/Group:	22 females/main group, 3 females/satellite group
Dose volume:	5 mL/kg
Formulation/Vehicle:	dH_2O
Route of administration:	ORAL GAVAGE
Species:	Rabbits
Strain:	New Zealand White
Comment on Study Design and Conduct:	In the present study, time-mated females were dosed once daily from GD 7 through 20. In a dose range-finding study, time-mated females were administered 2.5, 6 or 15 mg/kg/day. Severe body weight loss and reduced food consumption led to abortions in 4 of 5 rabbits at 15 mg/kg/day and 1 of 5 rabbits at 6 mg/kg/day. Post-implantation loss was significantly increased and mean fetal weight was significantly decreased in at 6 mg/kg/day. Based on these findings, dose levels of 0, 0.5, 1.5 and 4.5 mg/kg/day were selected for a definitive embryo/fetal development study in rabbits.
Dosing Solution Analysis:	Low potency results were obtained for dosing solutions from Groups 3 and 4 as presented in **Table 64**. The solutions submitted for homogeneity evaluation demonstrated a substantially higher result for the Middle aliquot than the Top and Bottom aliquots.

Table 65: Dosing solution analysis results from the rabbit embryofetal development study

Metrics ID	(b) (4) Study Number	Group	Dose Concentration (mg/mL)	CMX001 Found (% Label Claim)
12870-1	(b) (4)-623004	1	0	0.0[2]
12870-2		2	0.5	97.7
12870-2A		2 (Top)	0.5	86.0
12870-2B		2 (Middle)	0.5	117.5
12870-2C		2 (Bottom)	0.5	87.0
12870-3		3	1.5	48.1
12870-4		4	4.5	64.4

Observations and Results (F_0 Dams)

Mortality

Assessed twice daily. One female in the 4.5 mg/kg/day group aborted on GD 27; at necropsy, dark red discoloration of the trachea and white areas of the liver were noted. The abortion was likely related to late fetal death as 6 late fetal resorptions were noted at necropsy. One dam in the 0.5 mg/kg/day group was euthanized in extremis on GD 23 due to clinical findings of rales and labored respiration. The cause of moribundity was not determined.

Clinical Signs

Assessed once daily. There were no drug-related clinical signs.

Body Weight

Measured once daily. Mean body weight gain in the 1.5 mg/kg and 4.5 mg/kg dose groups was decreased 20% and 21% when compared with the control group. BCV-related reduced body weight gain and reductions in food consumption were noted in individual animals in the 4.5 mg/kg/day group and were particularly notable immediately following initiation of dose administration on GDs 7-9, followed by increased body weight gains on GDs 9-10.

Food Consumption

Measured once daily. The only statistically significant difference in food consumption between control and treated groups was an increase in the group mean for animals treated with 4.5 mg/kg (139 g/animal/day compared to 109 g/animal/day) over the non-dosing period of GDs 21-29.

Necropsy

Gross pathology was evaluated at necropsy on Day 29. Histopathology was performed in the GI tracts of all treated animals only. Three of 21 rabbits from the 4.5 mg/kg/day group had dark red contents in the cervix, and an additional 3 of 21 rabbits from the 4.5 mg/kg/day group had dark red contents of the vagina. These tissues with gross lesions were not examined microscopically. There were no histopathological

findings in GI tissues from treated animals, possibly due to recovery during the non-dosing period (9 days from GD 21 to 29).

Caesarean Section Data

Evaluated at necropsy on Day 29. Findings and historical control (HC) data are presented in the following table. **Table 66** presents the test article-related effects noted in the 4.5 mg/kg/day group with statistical significance and comparison to the testing facility's historical control data. The mean proportion of post-implantation loss in dams treated with 4.5 mg/kg BCV exceeded the current control value as well as the testing facility's historical control value. As might be expected, more post-implantation loss correlated with decreases in parameters associated with viable fetuses. Mean fetal weight of fetuses from dams dosed with 4.5 mg/kg BCV was 23% lower than the value from the concurrent control group and 15% lower than the value from the testing facility's historical control database. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups.

Table 66: Caesarean section data from the rabbit embryofetal development study

Group:	Control	0.5	1.5	4.5	HC[a]
Number of Litters Evaluated	22[b]	19	20	19[b]	Not Applicable
Postimplantation Loss (% Per Litter)	10.1	2.6	2.5	30.5	4.2 (1.1-7.3)
Early Resorptions (% Per Litter)	8.2	1.6	0.8	10.6	2.6 (0.5-5.7)
Late Resorptions (% Per Litter)	1.9	1.0	1.7	19.9*	1.5 (0.5-2.8)
Viable Fetuses (% Per Litter)	89.9	97.4	97.5	69.5	95.8 (92.7-98.9)
Number Viable Fetuses	8.3	9.5	8.4	6.2*	8.8 (8.0-9.6)
Male Fetal Weight (g)	42.1	40.7	41.5	33.5**	41.2 (38.8-43.6)
Female Fetal Weight (g)	40.6	38.9	40.6	30.9**	40.1 (37.4-42.7)
Combined Fetal Weights (g)	41.9	39.8	41.3	32.1**	40.6 (38.0-43.0)

[a] = (b) (4) historical control data mean (minimum-maximum) for rabbits from the (b) (4) facility.
[b] = With the exception of fetal weight data for 1 litter (due to 100% resorption of litter).
* = Statistically significant; $p<0.05$ ** = Statistically significant; $p<0.01$

Toxicokinetics

Blood samples were collected on GD 7 and 19 at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose, and 1 hour post-dose on GD 20. Toxicokinetic parameters are presented in **Table 67**. Fetal blood was also collected at the time of necropsy on Day 29. Fetal plasma concentration of CMX021 (CDV) ranged from 39.3% to 43.5% of maternal plasma concentrations 1 hour post-dose. CMX064 (a major metabolite) appeared soon after maternal administration of BCV with a T_{max} of 2.7 to 5.3 hours after dosing. Maternal C_{max} and AUC for CMX064 increased approximately dose-proportionately. Fetal exposure to CMX064 was minimal.

Table 67: BCV and metabolite TK data from the rabbit embryofetal development study

Dose (mg/kg/day)	Gestation Day	CMX001					CMX021					CMX064				
		C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)
0.5	7	6.3	4.0	ND	27.7	ND	BQL	ND	ND	ND	ND	40.8	4.0	2.7	198.0	245.0
	19	7.3	1.8	6.0	38.7	63.1	6.2	6.7	ND	71.9	ND	40.0	2.7	3.8	246.4	307.8
1.5	7	25.4	3.0	3.1	150.1	156.9	5.15	8.0	45.8	90.63	330.9	94.3	4.0	3.6	719.8	781.5
	19	20.4	1.0	7.2	100.0	133.7	14.5	4.2	45.2	284.0	910.1	132.0	2.0	3.5	957.2	1142.7
4.5	7	91.2	3.0	4.5	486.3	497.5	15.8	14.7	ND	294.7	ND	343.3	5.3	4.5	2597.0	2697.2
	19	112.1	2.3	4.8	474.4	484.8	45.7	6.7	33.0	871.2	2318.4	487.3	2.7	4.4	3012.4	3068.1

Abbreviations: BQL, below quantifiable limit (1.0 ng/mL for CMX001, 5.0 ng/mL for CMX021 & CMX064); ND, not determined due to insufficient data

Observations and Results (F_1 Offspring)

Fetal Malformations/Variations

Malformations were observed in 4(3), 4(3), 1(1) and 6(6) fetuses (litters) in control, 0.5, 1.5 and 4.5 mg/kg/day groups, respectively.

External: Four fetuses in the 4.5 mg/kg/day group had external malformations, compared with none in the control, 0.5 and 1.5 mg/kg/day groups. Findings included: meningocele (1 fetus), exencephaly, without open eyelid (1 fetus), microphthalmia (1 fetus), exencephaly without open eyelid, microphthalmia, and bent tail (no apparent skeletal origin) (1 fetus). The mean litter proportions of meningocele, exencephaly without open eyelid and microphthalmia exceeded the concurrent control group value and the maximum mean values in the testing facility's historical control database and were considered to be related to BCV administration. There were no drug-related external variations.

Visceral: No visceral malformations were observed for fetuses in the test article-treated groups. Drug-related variations included absent or small gallbladder in 24% of fetuses per litter, and pale spleen in 8% of fetuses per litter in the 4.5 mg/kg group.

Skeletal: There were no drug-related skeletal malformations. Drug-related skeletal variations included bent hyoid arches (affecting all 18 litters) and unossified hyoid bodies and/or arches in the 4.5 mg/kg group. The mean litter proportions of bent hyoid arches and unossified hyoid bodies and/or arches were 72.4% and 5.3% per litter, respectively.

9.3 Prenatal and Postnatal Development (PPND)+

9.3.1 Study Title: An Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of CMX001 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	CMX001-TX-024
Study report location:	4.2.3.5.3
Conducting laboratory and location:	(b) (4) [redacted]
GLP compliance:	Yes
Drug, lot #, and % purity:	CMX001 (lot #CMX001-042, purity = 95.8%)
Enhanced PPND Study:	N

Key Findings

In F_0 dams, the NOAEL for maternal toxicity was defined as 1.00 mg/kg based on decreased food consumption and corresponding decreases in maternal body weight and body weight gain. The reproductive NOAEL in the dams is 1.00 mg/kg based on a failure to thrive noted at 4.00 and 15.00 mg/kg.

The NOAEL for viability and growth in the offspring preweaning was defined as 1.00 mg/kg based on decreased pup body weight per litter and the increased incidence of dehydration and abdominal distention in the 4.00 and 15.00 mg/kg dosage groups.

The NOAEL for growth and development of the F_1 generation postweaning was 1.00 mg/kg. Maternal treatment with 4.00 and 15.00 mg/kg BCV reduced fetal survivability shortly after weaning (between days 23 and 26 postpartum), slowed sexual maturation, and caused reductions in male reproductive organ weights. F_1 male and female rats at 4.00 mg/kg also had notable reductions in mating and/or fertility. Finally, Caesarean sectioning parameters (corpora lutea, implantation sites, litter size and live fetuses) evaluated in F_1 female rats were also affected.

Methods

Doses:	0, 0.25, 1, 4 & 15 mg/kg (Groups 1 through 5, respectively)
Frequency of dosing:	Groups 1-4 – Once daily Group 5 – GDs 7, 10, 13, 16 and 19 and on LDs 1, 3, 6, 9, 12, 15, 18 and 20 (total of 13 doses over 34-35 days
Number/Sex/Group:	25/sex/main groups, 4/sex/satellite groups (4 and 15 mg/kg F_0 animals only)
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) [redacted] in dH_2O
Route of administration:	ORAL GAVAGE
Species:	Rat
Strain:	Crl:CD(SD)
Comment on Study Design and Conduct:	Pregnant rats in Groups 1-4 received vehicle, 0.25, 1 or 4 mg/kg once daily from GD 7 through

either lactation day (LD) 20 or GD 24 for those that did not deliver a litter (total of 18-36 doses over 18-36 days). Group 5 rats (15 mg/kg) were treated on GDs 7, 10, 13, 16 and 19 and LD 1, 3, 6, 9, 12, 15, 18 and 20 (total of 13 doses over 34-35 days).

Dosing Solution Analysis: Analysis of the end-of-dosage-period samples showed a lack of homogeneity for the 3 mg//mL suspension. The average replicate bottom samples were 124% of nominal concentration (see **Table 68**). The 0.05 and 0.8 mg/mL end of study concentration samples were also out of specification (high). Most significantly, the 0.8 mg/mL concentration was prepared as 8.0 mg/mL and one animal was dosed with the incorrect formulation (see **Table 69**). Data from that animal were excluded where appropriate.

Table 68: Dosing solution analysis results from the second dose preparation samples from the rat PPND study

(b) (4) Container i.d.	Sample Stratum	Nominal Conc (mg/mL)	Measured Conc [a] (mg/mL)	% Nominal [b]
1 of 12T	Top	3	3.22 [c]	107.23
2 of 12T	Top	3	3.62 [c]	120.65 [d]
5 of 12M	Middle	3	3.44 [c]	114.57
6 of 12M	Middle	3	3.28 [c]	109.40
9 of 12B	Bottom	3	3.88 [c]	129.30 [d]
10 of 12B	Bottom	3	3.54 [c]	118.05 [d]

a. Assumes a density of 1 g/mL for all samples.
b. % Nominal values are calculated using unrounded concentrations.
c. The relative standard deviation (RSD) of the six top, middle, and bottom sample concentrations is 5.28 % (5.27 % for reported values rounded to two decimal places).
d. This result does not meet the acceptance criteria for the formulation ((b) (4) % of nominal). Investigation at (b) (4) found no evidence of laboratory error or assignable cause contributing to the OOS result. Therefore, the result is considered to be a valid measure of sample concentration.

Table 69: Dosing solution analysis results from the end-of-dosage-period samples from the rat PPND study

End of Study Samples				
Analyte: CMX001				
[redacted] (b) (4) Container i.d.	Sample Stratum	Nominal Conc (mg/mL)	Measured Conc [a] (mg/mL)	% Nominal [b]
1 of 4M	Middle	0	None Detected	N/A
2 of 4M	Middle	0	None Detected	N/A
1 of 4M	Middle	0.05	0.068	136.97 [c]
2 of 4M	Middle	0.05	0.071	142.17 [c]
1 of 4M	Middle	0.8	8.02	1002.29 [c]
2 of 4M	Middle	0.8	8.02	1002.27 [c]

a. Assumes a density of 1 g/mL for all samples.
b. % Nominal values are calculated using unrounded concentrations.
c. This result does not meet the acceptance criteria for the formulation ([redacted] (b) (4) % of nominal). Investigation at [redacted] (b) (4) found no evidence of laboratory error or assignable cause contributing to the OOS result. Therefore, the result is considered to be a valid measure of sample concentration.

Observations and Results (F_0 Dams)

Parameters evaluated in the main study rats included viability, clinical observations, body weight, food consumption, mating performance, maternal behavior, natural delivery observations, necropsy observations, milk assessments (LD 14) and maternal toxicokinetics (LD 15). As presented in **Table 70**, notable findings in F_0 were related to decreased food consumption and corresponding decreases in body weight and body weight gain during gestation and lactation.

Table 70: Notable F_0 findings from the rat PPND study

Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_0 Females:	No. Pregnant	24	23	24	25	25
	No. Died or Sacrificed Moribund	0	0	0	0	0
	No. Aborted or with Total Res. Of Litter	0	0	0	0	0
	Clinical Observations	-	-	-	-	-
	Necropsy Observations	-	-	-	-	-
	Gestation Body Weight (g) (DG 20) (%)[a]	388.8	-1.49	-2.49	-9.44**	-8.95**
	Lactation Body Weight (g) (DL 21) (%)[a]	343.7	-0.90	-1.31	-2.82	-5.50*
	Gestation Food Consumption (g) (DGs 7 to 20) (%)[a]	26.0	-4.23	-4.23	-14.23**	-13.31**
	Lactation Food Consumption (g) (DLs 1 to 14) (%)[a]	56.6	-6.54	-6.71*	-19.08**	-17.31**
	Mean Duration of Gestation (days)	22.5	22.4	22.4	22.3	22.2

- No noteworthy findings. DG = Gestation day DL = Lactation day
* = $p \leq 0.05$ ** = $p \leq 0.01$ Statistical significance is based off of actual data (not on the percent differences)
a - At end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

Observations and Results (F_1 Generation)

Parameters evaluated in the F_1 generation rats included viability, clinical observations, body weights, food consumption values, sexual maturation, pup toxicokinetics (LD 15), learning and memory, mating performance, necropsy observations, Caesarean sections/litter parameters, and F_2 fetal gross external alterations. As noted in **Table 71**, test article-related findings in F_1 litters included a decrease in the mean number of implantations, a decrease in four-day post-partum survival (failure to thrive), and lower pup weight per litter. Clinical observations related to test article administration included dehydration and abdominal distention in pups from dams dosed with 15 mg/kg. Administration of BCV to F_0 dams did not affect learning or memory of F_1 generation rats as evaluated by passive avoidance or water maze tests.

Table 71: Drug-related F_1 litter findings from the rat PPND study

Maternal Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_1 Litters: (Preweaning)	No. Litters Evaluated	24	23	24	25	25
	Mean No. of Implantations	17.0	16.0	15.7	9.2**	13.8**
	Mean No. Pups/Litter	13.8	14.1	13.6	13.2	13.8
	Mean No. Liveborn Pups/Litter	13.7	14.0	13.5	13.0	13.8
	Mean No. of Stillborn Pups/Litter	0.0	0.0	0.0	0.2	0.0
	Postnatal Survival to Day 4 Postpartum (%)[a]	99.7	98.4	99.1	94.8**	96.0**
	Postnatal Survival to Weaning (%)[b]	99.7	99.7	99.7	99.0	96.6**
	No. of Total Litters Losses	0	0	0	0	0
	Pup Wight/Litter (g) (Day 21 postpartum) (%)[c]	43.5	-8.96	-5.98	-20.23**	-26.21**
	Pup Sex Ratios (% males) (Day 1 postpartum)[d]	51.4	46.9	48.0	52.9	57.4
	No. of Litters with Pup Clinical Observations:					
	Dehydration (mild or slight)	0	0	1	4*	7**
	Abdominal Distension	0	0	0	0	3**
	Pup Necropsy Observations[e]					
	Appeared Normal (% Pup Incidence)	100	100	100	100	96.9**
	Intestines Filled with Digestive Material (% Pup Incidence)	0	0	0	0	3.1**

- No noteworthy findings.
* = p≤0.05 ** = p≤0.01 Statistical significance is based off of actual data (not on the percent differences)
a - Number of live pups on day 4 postpartum / number of liveborn pups on day 1 postpartum
b - Number of live pups on day 21 postpartum / number of liveborn pups on day 4 postpartum
c - For controls, group means are shown. For treated groups, percent differences from controls are shown.
d - Includes liveborn pups and pups that died before weighing on day 1 postpartum.
e - Pups sacrificed on day 15 or 21 of lactation

Table 72 presents test article-related findings in F_1 males. Clinical observations include dehydration and distended abdomens. At necropsy, findings were related to decreases in the size of testes and epididymis. Also, sexual maturation was delayed, the number of F_1 males that mated was decreased, and male offspring from dams treated with 4 or 15 mg/kg BCV were less likely to be fertile when compared with male offspring of control dams.

Table 72: Drug-related F_1 male findings from the rat PPND study

Maternal Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_1 Males: (Postweaning)	No. Evaluated Postweaning	25	25	25	25	25
	No. Died or Sacrificed Moribund	1	0	1	3	3
	No. of Rats with Clinical Observations:					
	Dehydration	0	3	1	5	4
	Abdominal Distension	0	0	0	1	2
	Necropsy Observations:					
	Testes:					
	Left and/or Right, Small	0	1	0	12**	7**
	Left and/or Right, Flaccid	0	0	1	0	1
	Epididymides:					
	Left and/or Right, Small	0	1	0	2	3
	Organ Weights (g) (%)[a]:					
	Testes Paired	3.67	-0.82	-5.18	-41.1**	-33.5**
	Epididymides Paired	1.51	-3.97	-3.97	-30.5**	-23.8**
	Body Weights (g) (Day 92 postpartum) (%)[b]	515.3	-7.02	-4.48	-8.09*	-10.89**
	Food Consumption (g/day) (Days 22 to 92 postpartum) (%)[b]	27.6	-5.07	-2.90	-9.42**	-8.33**
	Mean Age of Preputial Separation (Days)	47.5	48.4	47.4	50.3**	51.5**
	Mean No. Days Prior to Mating	2.4	4.5	3.8	5.6	4.2
	No. of Males that Mated	24	23	23	16**	19*
	No. of Fertile Males	22	22	21	13	16

- No noteworthy findings.
* = $p \leq 0.05$ ** = $p \leq 0.01$ Statistical significance is based off of actual data (not on the percent differences)
a - For controls, group means are shown. For treated groups, percent differences from controls are shown.
b - For controls, group means are shown. For treated groups, percent differences from controls are shown. Because body weight values were recorded at weekly intervals, based on each rat's day postpartum, day 92 postpartum was the last day in which the youngest rats had a body weight value recorded before cohabitation.

Table 73 presents test article-related findings in F_1 females. Premating body weight and gestation body weight were lower in female offspring of treated dams, and the difference from controls was statistically significant at 4 and 15 mg/kg BCV. Sexual maturation was delayed in the female offspring of dams treated with 4 and 15 mg/kg BCV, and mating (number of days prior to mating, number of females sperm positive) appear to have been impacted by treatment of the dams. In pregnant F_1 females, the mean number of corpora lutea and implantations was decreased by treatment of dams with 4 and 15 mg/kg BCV, and preimplantation loss appeared to be higher at all doses.

Table 73: Drug-related F_1 female findings from the rat PPND study

Maternal Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_1 Females: (Postweaning)	No. Evaluated Postweaning	25	25	25	25	25
	No. Died or Sacrificed Moribund	0	1	0	1	4
	No. of Rats with Clinical Observations:					
	Dehydration	0	0	0	2	3
	Abdominal Distension	0	0	0	1	2
	Necropsy Observations	-	-	-	-	-
	Premating Body Weight (g) (Day 92 postpartum) (%)[a]	287.1	-4.46	-4.32	-7.10	-8.08
	Gestation Body Weight (g) (DG 21) (%)[a]	492.4	-4.94	-7.23	-16.82**	-12.22**
	Premating Food Consumption (g/day) (Days 22 to 92 postpartum) (%)[a]	20.0	0.50	-3.5	-3.0	-3.0
	Gestation Food Consumption (g/day) (DGs 0 to 21) (%)[a]	26.8	-3.73	-4.85	-7.84	-5.22
	Mean Age of Vaginal Patency (days)	32.3	33.2	32.9	34.5	34.6*
	Mean No. Days Prior to Mating	2.5	4.6	3.8	7.7	4.5
	No. of Females Sperm Positive	25	24	25	18**	21
	No. of Pregnant Females	22	23	23	14**	18
	Mean No. Corpora Lutea	17.5	17.4	16.4	10.4**	13.8**
	Mean No. Implantations	17.0	16.2	15.7	9.2**	13.1**
	Mean % Preimplantation Loss	2.9	7.3	4.5	16.0*	4.6

- No noteworthy findings.
* = p≤0.05 ** = p≤0.01 Statistical significance is based off of actual data (not on the percent differences)
a - For controls, group means are shown. For treated groups, percent differences from controls are shown. Because body weight values were recorded at weekly intervals, based on each rat's day postpartum, day 92 postpartum was the last day in which the youngest rats had a body weight value recorded before cohabitation.

Observations and Results (F_2 Generation)

As presented in **Table 74**, there is an apparent decrease in the mean number of live pups per litter and fetal body weights, and increase in post-implantation loss as a result of treatment of dams with BCV.

Table 74: Drug-related F_2 litter findings from the rat PPND study

F_0 Maternal Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F2 Litters:	Mean No. Live Conceptuses/Litter	16.1	15.3	14.7	8.4**	13.4**
	Mean No. Resorptions	0.8	0.8	1.6	0.8	0.5
	No. of Litters with Dead Conceptuses	0	1	0	0	0
	No. Dead Conceptuses	0	1	0	0	0
	Mean % Postimplantation Loss	4.6	5.3	10.8	7.2	8.9
	Fetal Body Weights (g) (%)[a]	5.34	-1.87	-2.06	0.19	-0.94
	Fetal Sex Ratios (% males)	50.8	52.5	44.3	51.6	51.9
	Fetal Anomalies	-	-	-	-	-

Toxicokinetics and Lactation (exposures)

F_0 Blood samples were collected on GD 7 and 19 pre-dose and 2 hours post-dose. F_0 milk samples were collected on LD 14 2 hours post-dose. Toxicokinetic and lactation data from the F_0 dams and F_1 fetal drug plasma levels are presented in **Tables 75 & 76**. The tables below present findings from the bioanalytical assessment of plasma and milk from treated dams, and plasma from pooled fetal blood samples. Apparent discrepancies in data from dams treated with 15 mg/kg (i.e., a lack of detected or quantifiable parent drug, or lower than expected values) is likely due to the twice per week dosing in that group, compared with daily dosing at 0.25, 1 and 4 mg/kg. Notable

findings include the significant levels of CMX021 (CDV) in the milk of dams treated with 4 and 15 mg/kg.

Table 75: F_0 serum and milk concentrations of BCV and metabolites from the rat PPND study

Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_0 Females:	Mean Milk and Pre-dose Plasma Concentrations					
	CMX001					
	Milk, DL 14 (2 hr postdose)	NA	BQL	BQL	0.68 ± 1.51	BQL
	Plasma, DL 15 (predose)	NA	BQL	0.23 ± 0.52	3.54 ± 1.42	BQL
	CMX021					
	Milk, DL 14 (2 hr postdose)	NA	BQL	BQL	29.72 ± 2.44	26.65 ± 3.72
	Plasma, DL 15 (predose)	NA	BQL	4.86 ± 2.74	24.58 ± 2.06	1.02 ± 2.29
	CMX064					
	Milk, DL 14 (2 hr postdose)	NA	BQL	BQL	BQL	BQL
	Plasma, DL 15 (predose)	NA	BQL	BQL	BQL	BQL
	Mean Plasma Concentrations					
	CMX001					
	DG 7 (2 hr postdose)	NA	NA	NA	8.35 ± 9.60	57.15 ± 13.64
	DG 19 (predose)	NA	NA	NA	3.15 ± 1.14	BQL
	DG 19 (2 hr postdose)	NA	NA	NA	20.70 ± 6.65	172.25 ± 49.68
	CMX021					
	DG 7 (2 hr postdose)	NA	NA	NA	BQL	13.28 ± 2.39
	DG 19 (predose)	NA	NA	NA	19.65 ± 5.56	3.19 ± 3.68
	DG 19 (2 hr postdose)	NA	NA	NA	48.00 ± 8.32	47.33 ± 12.48
	CMX064					
	DG 7 (2 hr postdose)	NA	NA	NA	BQL	5.73 ± 4.18
	DG 19 (predose)	NA	NA	NA	BQL	BQL
	DG 19 (2 hr postdose)	NA	NA	NA	BQL	24.63 ± 13.58

NA = not applicable (no sample collected); hr = hour; BQL = below the limit of quantitation
CMX001 = parent compound; CMX021 = cidofovir; CMX064 = major metabolite

Table 76: F_1 fetal blood concentrations of BCV and metabolites from the rat PPND study

Maternal Dose (mg/kg)		0 (Vehicle)	0.25	1.00	4.00	15.00
F_1 Litters: (Preweaning)	Mean Fetal Plasma Concentrations[a]					
	CMX001					
	DG 19 (2 hr postdose)	NA	NA	NA	BQL	1.63 ± 0.34
	CMX021					
	DG 19 (2 hr postdose)	NA	NA	NA	22.27 ± 2.83	12.93 ± 1.71
	CMX064					
	DG 19 (2 hr postdose)	NA	NA	NA	BQL	BQL
	Mean Pup Plasma Concentrations[b]					
	CMX001					
	Plasma, DL 15 (predose)	NA	BQL	BQL	BQL	BQL
	CMX021					
	Plasma, DL 15 (predose)	NA	BQL	BQL	BQL	BQL
	CMX064					
	Plasma, DL 15 (predose)	NA	BQL	BQL	BQL	BQL

NA = not applicable (no sample collected); hr = hour; BQL = below the limit of quantitation
CMX001 = parent compound; CMX021 = cidofovir; CMX064 = major metabolite
a. Pooled per litter
b. Pooled per sex per dosage group

11. Integrated Nonclinical Summary and Safety Evaluation

Efficacy/Primary Pharmacology Summary

Multiple primary pharmacology studies were submitted in support of approval of this NDA via the Animal Rule pathway. Due to the difficulties in modeling and evaluating variola virus infection and disease in nonclinical species, it was established via the Antiviral Drugs Advisory Committee Meeting in December 2011 that confirmation of drug efficacy in two surrogate animal models, with surrogate viruses closely related to variola, would meet the Animal Rule requirements for approval. Due to relatively low intracellular concentrations of BCV and its metabolites in monkeys, the monkeypox model was considered an inadequate measure of efficacy and was not pursued. The rabbit/RPXV and mouse/ECTV models were therefore considered the pivotal models for evaluation of BCV efficacy.

The RPXV model was established in several studies, particularly natural history study #2819-100017958 in which 9-week old rabbits were challenged with 300 pfu RPXV, strain Utrecht (actual titer 230 pfu), by intradermal injection. The objectives of this study were to ensure that RPXV challenge results in a robust, reproducible viremia and disease course consistent with prior studies in this model, and to identify potential early markers of RPXV infection that could be used as “triggers to treat” in follow-up efficacy studies. To qualify as triggers to treat, these parameters must have been specific for RPXV infection, unambiguous, reproducible and objective, and early enough in the disease course to allow intervention corresponding to the therapeutic window in human smallpox. All animals challenged with RPXV in this study succumbed to illness within 6-10 days post-challenge. RPXV-infected animals also exhibited clinical signs (decreased food consumption, lethargy, ataxia, eye closure, labored/rapid/open-mouth breathing, nasal flaring, and discharge from the nose and/or mouth), decreased body weight gain, changes in body temperature and respiratory rate, challenge site reactions, RPXV lesions, fluctuations in various clinical pathology parameters, and gross pathology findings (necrosis, inflammation, hemorrhage and/or edema in the skin, tongue, lungs, spleen, testes/ovaries, liver, thymus and brain). All findings were consistent with RPXV infection in this species, and occurred roughly 1-5 days prior to death. RPXV viremia was also confirmed in the blood and nose starting roughly 3 days post-challenge. Of these endpoints, viremia and lesion count were considered the most promising triggers to treat. Additional RPXV studies also identified body temperature and viremia to be reliable treatment triggers. Due to inconsistencies in timing and occurrence, however, these markers were eventually deemed unreliable. The pivotal efficacy study CMX001-VIR-106 was therefore designed to evaluate BCV administration starting on either Day 3, 4, 5 or 6 post-challenge, which covers the period of symptom onset.

BCV efficacy was evaluated in two GLP RPXV studies, CMX001-VIR-041 and CMX001-VIR-106. In the first of these, 13- to 18-week-old rabbits were challenged intradermally on Day 0 with purified RPXV, strain Utrecht (target titer = 300 pfu, actual titer = 316-350 pfu). BCV was administered in 3 doses spaced 48 hours apart by oral gavage starting 0, 1, 2 or 3 days after symptom onset (Groups 1-4, respectively; placebo = Group 5). Survival was 28/28 (100.0%), 27/29 (93.1%), 27/29 (93.1%), 20/29 (68.9%), and 14/29 (48.3%) in Groups 1-5, respectively, with death occurring 8-10 days

post-challenge. Treatment with BCV therefore resulted in significantly greater survival relative to placebo (Group 5), and survival increased the sooner treatment was initiated. BCV also prevented RPXV-induced weight loss when administered up to 24 hours post-onset of symptoms, particularly in Group 1. RPXV-induced changes in body temperature, respiratory rates, lesion counts, and viremia were also all alleviated in the BCV groups relative to placebo. These results suggest that BCV treatment protected against RPXV-induced mortality and disease progression. However, significant data quality and integrity concerns were raised during an audit of this study. Specifically, BCV was detected in blood samples of several placebo animals as well as several animals on days when placebo should have been administered. The root cause could not be identified or remedied, and the reliability of this study was therefore questioned. As a result, this study was considered supportive only, and efficacy in the RPXV model was definitively established in the follow-up study CMX001-VIR-106.

As previously mentioned, the design of CMX001-VIR-106 was simplified to address the unreliability of previously identified treatment triggers. In this study, 13- to 16-week old rabbits were challenged intradermally on Day 0 with purified RPXV, strain Utrecht (target titer = 600 pfu, actual titer = 488 pfu). BCV was administered in 3 doses spaced 48 hours apart (20, 5 and 5 mg/kg/dose) by oral gavage starting 3, 4, 5 or 6 days post-challenge (Groups 1-4, respectively; placebo = Group 5). No clear, drug-related differences in RPXV-induced clinical signs, body weights, lesion counts or challenge site erythema/edema were observed. However, survival was 100%, 90%, 69%, 69%, and 29% in Groups 1-5, respectively, indicating that BCV was protective against RPXV-induced mortality, particularly in Groups 1 and 2. Viral loads also tended to be lower on Days 6 and 8 in Groups 1 and 2 relative to Group 5 (control). Body temperatures were also significantly above concurrent controls in Groups 1, 2 and 4 on Days 6 and 7, in Group 4 on Days 8 and 9, and in Group 3 on Day 9. Overall, BCV was shown to be effective in the treatment of RPXV-induced infection and disease, particularly in the reduction of RPXV-induced mortality, under the conditions of this study.

Minor discrepancies were noted in the designs of efficacy studies #CMX001-VIR-041 and CMX001-VIR-106 versus natural history study #2819-100017958. In particular, a higher RPXV titer was used in CMX001-VIR-106 (488 pfu) than the natural history study (230 pfu), but RPXV-induced mortality was lower in the placebo group in CMX001-VIR-106 (71% vs. 100% in the natural history study). This difference may be attributed to the age of the animals (13-16 weeks in CMX001-VIR-106 vs. 9 weeks in the natural history study) or differences in body weight. Regardless, these differences did not affect study interpretation or preclude establishment of BCV efficacy in this model.

As with the RPXV model, the ECTV model was first established in several studies, particularly natural history study #CMX000-VIR-111. Eight-week-old mice were challenged with 200 pfu ECTV, strain Moscow (actual titer = 194 pfu), by intranasal aspiration in this study, and as with the RPXV natural history study, the objectives were to ensure a robust, reproducible disease course consistent with previous ECTV studies was established and potential triggers to treat were identified. Survival in the ECTV-infected group was 15.7% by Day 21, with a median time of death of 8.6 days post-challenge. Clinical signs in the ECTV-challenged animals included rough hair coat,

breathing abnormalities, lethargy, lacrimation, hunched posture, and moribundity. Decreased body weights and temperature and histopathologic lesions in the spleen and liver were observed in the ECTV-infected groups, though no gross, pustular rashes or lesions and no meaningful changes in clinical pathology parameters were observed in these animals. All findings were consistent with ECTV infection in this species. ECTV viremia was also confirmed in the blood, liver and spleen starting roughly 60 hours post-challenge. Of the endpoints evaluated, ECTV viremia was considered the most promising trigger to treat, being elevated starting roughly 2-3 days post-challenge. As with the RPXV model, however, these triggers were considered too unreliable to be used as treatment triggers.

Similar to study #CMX001-VIR-106, the pivotal ECTV study #CMX001-VIR-044 was designed to cover the period of symptom onset. Six- to eight-week-old mice were challenged with 200 pfu purified ECTV, strain Moscow (actual titer = 188-196 pfu), by intranasal aspiration on Day 1. BCV was administered in 3 doses spaced 48 hours apart (either 10, 5 and 5 mg/kg/dose or 20, 5 and 5 mg/kg/dose) by oral gavage starting on Days 5, 6, 7 or 8 post-challenge. Both BCV regimens (10/5/5 and 20/5/5) were protective against ECTV-induced mortality, with survival highest in mice treated with 20/5/5 starting on Day 4 (84%) and Day 5 (75%). The lower regimen of 10/5/5 was also protective, with survival of 78% and 66% when started on Days 4 and 5, respectively. By comparison, survival in the placebo group was 12.5%. No clear, drug-related differences in ECTV-induced secondary endpoints of clinical signs, body weights, viral loads were observed. BCV was therefore shown to be effective in the treatment of ECTV-induced infection and disease, particularly in the reduction of ECTV-induced mortality, under the conditions of this study.

Toxicology Summary

The nonclinical toxicology of BCV has been evaluated in both *in vitro* and *in vivo* genotoxicity assays and safety pharmacology studies, and in single- and repeat-dose general toxicology studies up to 2 weeks in mice, 26 weeks in rats, and 39 weeks in cynomolgus monkeys. Carcinogenicity was noted in 13-week studies in rats, prompting a 6-month, rather than 2-year, carcinogenicity study in rats. Reproductive and developmental toxicity studies included fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a PPND study in rats.

In animal species assessed as part of the nonclinical program, absolute oral bioavailability of BCV was reported to be as low as 0.3% in cynomolgus monkeys when administered as a capsule and 8% in rats and rabbits when administered via oral gavage. Clinically, bioavailability was determined to be 16.8% when trial participants were administered an oral suspension. Following oral and intravenous administration, systemic exposures to BCV, its active metabolite CDV (CMX021), and inactive metabolites CMX064 and CMX103 were approximately proportional to, or less than proportional to dose. Systemic accumulation (>2-fold) of BCV-related compounds in plasma was limited to CDV, consistent with its longer terminal phase half-life. No significant sex differences were noted with respect to systemic exposures or metabolite profiles.

Toxicity findings occurred in laboratory animals at exposures to BCV less than exposures in humans following the clinical dose (200 mg). In all orally-dosed nonclinical

toxicology studies, the defined NOAEL resulted in a safety margin of <1 with respect to human exposures. The mean systemic exposure in clinical trial subjects (AUC_{last}) was 4492 ng.hr/mL following a single 200 mg oral dose (data from clinical trial CMX001-127). In nonclinical studies, systemic exposures (AUC) equal to or greater than human exposures were only achieved at the highest dose following IV administration in rats (see **Table 77**). In each of those IV dose studies, the NOAEL was identified as the low dose.

The following organs or organ systems were identified as targets of BCV toxicity in nonclinical species:

Gastrointestinal (GI) Tract:

In rats dosed orally, BCV caused enteropathy, including loss of mucosal architecture that led to secondary bacterial infection. The jejunum and ileum were the most severely affected portions of the GI tract. In orally dosed monkeys, gross findings at necropsy included dark areas on the mucosa and/or fluid in the lumen of the stomach and large intestine, and at the ileocecal junction. These findings correlated histologically with dose-related minimal enteropathy localized in the posterior portion of the small intestine and in the large intestine. In both species, GI tract findings were reversible following a treatment-free period. Gastric-related adverse events were observed in clinical trials.

Reproductive Organs:

In rats, germ cell depletion of testicular seminiferous tubules and secondary epididymal intraluminal cellular debris, correlated with decreased testicular weights, decreased spermatogenesis and hypospermia, macroscopic findings of soft testes. In monkeys, changes in menstrual cycle (extended cycles, decreased number of cycles) were noted in females. Dose-related testicular, epididymal, and seminal vesicle/prostate changes, including decreased size and weight, as well as atrophy, in males resulted in decreases in sperm motility and sperm count.

Liver:

In monkeys dosed with BCV orally for ≥13-weeks, minor, but statistically significant, elevations in ALT values occurred in monkeys. The increases were not associated with any macroscopic or microscopic changes and were fully reversible after cessation of dosing. In rats, minor increases of ALP were noted following 13-weeks of IV administration. Elevated liver enzymes were noted in clinical trials.

Kidney:

In monkeys dosed with BCV orally for 39 weeks, minimal or mild karyomegaly (enlarged nuclei) of renal tubular epithelial cells was noted at the high dose in both sexes. Following a 26-week treatment-free period, karyomegaly, dilatation of renal tubules, and mild atrophy of the tubular epithelium was noted in mid- and high dose animals. CDV is known to cause proximal tubular cell injury and acute renal failure in humans.

Bone marrow:

In rats dosed for with BCV for 4 weeks via IV administration, a slight decrease of bone marrow cellularity was noted at the end of a 2-week treatment-free period, but not at the end of the dosing period. No effects on hematologic parameters were noted. CDV is known to cause decreased red and white cell parameters, most commonly neutropenia.

Carcinogenicity:

In genotoxicity assays, BCV was negative for mutagenicity in the Ames assay, negative for micronucleus formation in an *in vivo* assay, and positive for increased structural chromosomal aberrations in the absence of metabolic activation in an *in vitro* assay. In rats, treatment with either CDV or BCV has been shown to cause malignant mammary tumors and squamous cell carcinomas. Previously, palpable tumors were noted in rats as early as six weeks from the start of weekly oral dosing with CDV at a dose (15 mg/kg) that resulted in systemic exposures of approximately 40 µg.hr/mL. Here, in nonclinical toxicology studies with the prodrug BCV, tumors were detected in rats as early as 17 weeks following the first IV dose (i.e., at the end of a four week recovery period following a 13-week dosing period) and as early as 21 weeks following the first oral dose (i.e., 8 weeks following the last weekly dose in a 13-week study).

Whereas tumors were noted later than in the study with CDV, in the oral BCV study, tumors were noted in rats at much lower CDV exposures. For example, in studies with CDV malignant mammary tumors were noted in rats at systemic exposures ≥12,660 ng.hr/mL. In studies with BCV, malignant mammary tumors were noted in 3 of 5 female rats at systemic exposures (CDV) as low as 193 ng.hr/mL.

BCV, the prodrug of CDV, should be considered a carcinogen in rats as well as a potential carcinogen in humans.

Reproductive Toxicology:

Treatment with BCV caused notable reductions in mating and/or fertility, and resulted in reduced maternal and fetal body weights, and decreased embryo viability in rats. In rabbits, decreased mean body weight and food consumption in dams, and BCV-related effects on intrauterine growth (lower mean fetal weights), survival (higher late resorptions) and morphology (external malformations and visceral and skeletal developmental variations) were noted. Findings in reproductive toxicology studies were associated with systemic exposures in rats and rabbits less than the expected human exposure.

Table 77: Exposure Margins Based on High Dose from Pivotal Toxicology Studies[#]

Nonclinical				Clinical
Species/ Duration	Route/ Frequency	High Dose from Study (mg/kg)	AUC at end of Dosing (ng.hr/mL)	Exposure Margins (Based on 200 mg Clinical Dose)*
Mouse/14 days	Oral gavage (daily)	10	1444	0.3
Rat /3 doses	IV infusion (once weekly for 3 total doses)	15	4082	0.9
Rat /28 days	IV infusion (once or twice weekly)	15	9893	2
Rat /13 weeks	IV infusion (once or twice weekly)	15	9298	2
Rat /13 weeks	Oral gavage (twice weekly)	15	973	0.2
Monkey/13 weeks	Oral gavage (twice weekly)	15	81	<0.1
Pregnant Rats/ Dosing gestation days [GD] 6-17	Oral gavage (daily)	4.5	329	0.1
Pregnant Rabbits/ Dosing gestation days [GD] 7-19;	Oral gavage (daily)	4.5	474	0.1
Monkey/39 Weeks	Oral Gavage (twice weekly)	25	109	<0.1

AUC = area under the concentration-time curve
*Human AUC_{last} = 4492 ng.hr/mL following a single 200 mg oral dose (Study CMX001-127).
[#]Data taken from Table 3: Comparison of Animal vs. Human TK Data from Longest Duration Study in Each Nonclinical Species, in the Applicant's submission 2.6.7 Toxicology Tabulated Summary, p. 11-12.

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/

DAVID H MCMILLAN
05/03/2021 05:10:01 PM

MARK J SEATON
05/03/2021 05:32:27 PM

LAINE P MYERS
05/10/2021 08:35:36 AM
I concur with Dr. Seaton and Dr. McMillan on the recommendation that BCV be approved as a treatment of human smallpox disease caused by variola virus in adult and pediatric patients.

HANAN N GHANTOUS
05/10/2021 08:41:25 AM
I concur on the recommendation that BCV be approved as a treatment of human smallpox disease caused by variola virus in adult and pediatric patients.”