CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214460Orig1s000
214461Orig1s000

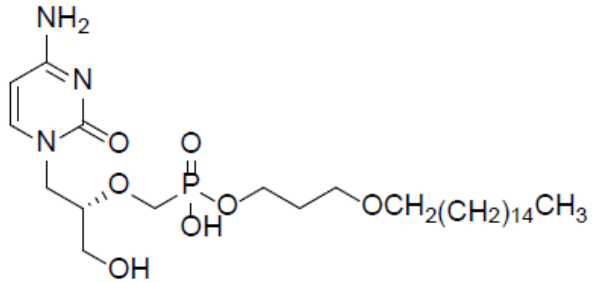
CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRALS / VIROLOGY REVIEW

NDA: 214461 (tablets), 214460 (suspension) Original NDA **REVIEW COMPLETED:** 05/06/2021

Virology Reviewer: Eric F. Donaldson, Ph.D.

Reviewer's Name(s): Eric F. Donaldson, Ph.D.
NDA#: 214461 (tablets), 214460 (suspension, cross-references to NDA 214461)
Applicant: Chimerix, Inc.
2505 Meridian Parkway, Suite 100
Durham, NC 27713
A. Heather Knight, Pharm D, VP Regulatory Affairs
Complete Submission Dates:
Correspondence Date: 10/7/2020
CDER Receipt Date: 10/7/2020
PDUFA Date: 4/7/2021 (7/7/2021 major amendment goal date)

Proprietary Name	**TEMBEXA®**
Drug Names	brincidofovir, CMX001
IND #	67681
Chemical Name	Phosphonic acid, [[(S)-2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl) ethoxy]methyl]mono[3-(hexadecyloxy)propyl] ester
Structure	
Molecular Formula	$C_{27}H_{52}N_3O_7P$
Molecular Weight	561.69 Daltons

Dosage Form and Route of Administration: tablet and suspension / oral
Dispensed: Rx x OTC__
Proposed Indication: Treatment of human smallpox disease in adult and pediatric patients

DIVISION OF ANTIVIRALS / VIROLOGY REVIEW

NDA: 214461 (tablets), 214460 (suspension) Original NDA **REVIEW COMPLETED:** 05/06/2021

Virology Reviewer: Eric F. Donaldson, Ph.D.

Assigned/Reviewed SDNs:

NDA	SDN	eCTD#	Rec'd Date	Content
214460	002	0002	10/30/2020	Response to Clinical Virology request for OPXV DNA sequences.
214460	005	0005	12/1/2020	Response to Clinical Virology request for additional supporting information for NGS data.
214460	035	0035	3/26/2021	Response to Clinical Virology request for additional supporting information for NGS data.
214460	041	0041	4/19/2021	Response to virology request regarding timelines and analyses for a virology PMC concerning development of viral resistance and acknowledgement of intended redaction of resistance information.
214461	005	0005	10/30/2020	Final part of rolling original NDA for tablet formulation; included NGS files on a hard drive.
214461	006	0006	10/30/2020	Response to Clinical Virology request for OPXV DNA sequences.
214461	009	0009	12/1/2020	Response to Clinical Virology request for additional supporting information for NGS data.
214461	021	0021	2/3/2021	Synopsis for post-marketing field study and response to Clin. Pharm. request.
214461	036	0036	3/26/2021	Response to Clinical Virology request for additional supporting information for NGS data.
214461	044	0044	4/19/2021	Response to virology request regarding timelines and analyses for a virology PMC concerning development of viral resistance and acknowledgement of intended redaction of resistance information.

Abbreviations: ATCC, American Type Culture Collection; BARDA, Biomedical Advanced Research and Development Authority; (b) (4) (b) (4) BCV, brincidofovir; CC_{50}, 50% cytotoxic concentration; CDC, Centers for Disease Control and Prevention; CDV, cidofovir; CDV-PP, cidofovir diphosphate; CMLV, camelpox virus; CMV, cytomegalovirus; EC_{50}, 50% effective concentration; ECTV, ectromelia virus; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GLP, good laboratory practice; IV, intravenous(ly); LD_{50}, 50% lethal dose; LLOQ, lower limit of quantification; MOI, multiplicity of infection; MPXV, monkeypox virus; MVA, modified vaccinia Ankara; NGS, next generation sequencing; NHP, nonhuman primate; NZW, New Zealand white (rabbits); PCR, polymerase chain reaction; PFU, plaque-forming unit(s); PK, pharmacokinetics; PRNT, plaque reduction neutralization test; RPXV, rabbitpox virus; SI, selectivity index; USAMRIID, U.S. Army Research Institute of Infectious Diseases; VACV, vaccinia virus; VARV, variola virus; VIGIV, vaccinia immune globulin intravenous

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I. EXECUTIVE SUMMARY

This review focused on an independent analysis of next generation nucleotide sequencing (NGS) data to assess for the development of brincidofovir (BCV) resistance in pivotal animal model studies performed in two animal models: the rabbitpox virus (RPXV) model in rabbits and the ectromelia virus (ECTV) model in mice. For a comprehensive review of all of the virology data for this NDA, please see the review of Clinical Virology Reviewer, Dr. Patrick Harrington (Dr. Harrington's review). Based on the results of the independent assessment of NGS resistance data, DAV agrees that the Applicant made a reasonable effort to collect RPXV and ECTV NGS data for assessment of BCV genotypic resistance and based on the reported amino acid coding changes, there was no clear evidence of BCV resistance selection in the RPXV and ECTV challenge models. (b) (3) (A), (b) (3) (B)

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

This Original NDA for TEMBEXA® (brincidofovir [BCV], CMX001), orthopoxvirus deoxynucleotide analog DNA polymerase inhibitor, is approvable from the Clinical Virology perspective for the treatment of human smallpox disease caused by variola virus.

1.2 Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

Clinical Virology recommends one post-marketing commitment (PMC) for the Applicant to assess the phenotypes of amino acid substitutions that arose in (b) (3) (A), (b) (3) (B) ECTV in animals treated with BCV.

(b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

For a detailed review of virology assessments, please see the review of Clinical Virology Reviewer, Dr. Patrick Harrington (Dr. Harrington's review).

2.1 Review of Next Generation Sequencing Data

Resistance assessments conducted for the pivotal animal model studies included NGS analyses to identify BCV treatment-emergent amino acid substitutions in the viral (b) (3) (A), (b) (3) (B) genes (b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B) as well as cell culture phenotypic resistance analyses of virus isolates from treated animals. Raw NGS fastq files were independently analyzed and the results were compared to those reported by the Applicant in this review. The NGS analyses revealed no clear genetic evidence of BCV resistance selection in the RPXV and ECTV studies. There was no confirmed detection of any treatment-emergent amino acid substitutions at known BCV or CDV resistance-associated positions. (b) (3) (A), (b) (3) (B)

Based on the results of the independent assessment of NGS resistance data, DAV agrees that the Applicant made a reasonable effort to collect RPXV and ECTV NGS data for assessment of BCV genotypic resistance and based on the reported amino acid coding changes, there was no clear evidence of BCV resistance selection in the RPXV and ECTV challenge models using primary DAV resistance criteria: 1) treatment-emergent substitutions detected at a frequency ≥15%; 2) substitutions detected in two or more animals but not in control animals; and 3) sequence read coverage of ≥500 at the amino acid position. (b) (3) (A), (b) (3) (B)

3. ADMINISTRATIVE

3.1 Reviewer's Signatures

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer, Division of Antivirals

Patrick Harrington, Ph.D.
Senior Clinical Virology Reviewer, Division of Antivirals

3.2 Concurrence

Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antivirals

II. REVIEW OF NEXT GENERATION SEQUENCING DATA

1. INTRODUCTION

BCV efficacy was evaluated under the Animal Rule (21 CFR part 314, subpart I). In theory, the ideal animal model for variola virus (VARV) would assess infection with VARV itself, but natural VARV infection and disease is specific to humans. Two animal models of smallpox were primarily used to evaluate BCV efficacy: RPXV-infected rabbits and ECTV-infected mice. Use of these models to establish BCV efficacy is in alignment with conclusions and recommendations from a 2011 Antiviral Drugs Advisory Committee Meeting on the development of antiviral drugs for smallpox under the Animal Rule. For a more thorough review of this NDA and the Applicant's resistance analyses, please see the review of Clinical Virology Reviewer, Dr. Patrick Harrington (Dr. Harrington's review).

Rabbit/RPXV model. The rabbit/RPXV model employs intradermal inoculation of RPXV, resulting in a disease course with characteristics similar to that in human smallpox, including an incubation/viral replication phase, fever, lesions, and a high mortality rate. RPXV natural history studies identified the onset of fever, which occurs around ~3-4 days post-challenge, as a potential trigger for treatment for the purpose of modeling treatment of symptomatic smallpox. The pivotal rabbit/RPXV study, CMX001-VIR-106, was a blinded, placebo-controlled study. The primary objective was to compare the survival benefit of BCV over placebo. BCV-treated animals received a 20 mg/kg loading dose with treatment in different arms initiated on Day 3, 4, 5, or 6 post-challenge, followed by two additional 5 mg/kg doses every other day. Survival rates through Day 42 were 69-100% in BCV-treated groups compared to 29% in the placebo control group, with survival rates trending higher for those that started BCV on Day 3 (100%) or Day 4 (90%). Fever was detected in 50% of animals by Day 3, and in 98% of animals by Day 4. The Applicant's analyses of body weight, body temperature, and respiration rates did not show clear or consistent differences in disease signs between treatment groups. Quantitative viral PCR results for whole blood samples showed a modest trend of higher peak viral DNA levels in the control group and lower peak viral DNA levels in the group that started BCV on Day 3. Peak viral DNA levels within each group were consistently higher among animals that died compared to those that survived.

Mouse/ECTV model. In the mouse/ECTV model, BALB/c mice are inoculated intranasally with the ECTV-Moscow strain, resulting in high rates of mortality with low challenge doses. In this model there are no clear, objective disease triggers for treatment as animals generally do not show overt disease signs until late in the infection when they become moribund. The pivotal mouse/ECTV study, CMX001-VIR-044, was a randomized, blinded, placebo-controlled study. For the efficacy evaluation, mice were randomized across 8 groups in which animals received BCV or placebo by oral gavage, with BCV 10/5/5 or 20/5/5 mg/kg administered every other day starting on Study Day 5, 6, 7, or 8 (20/5/5 mg/kg dose only), corresponding to 4 to 7 days post-challenge (i.e., challenge occurred on Study Day 1). The primary efficacy endpoint was the survival rate at Day 43. Survival rates were 34-84% in the BCV-treatment groups compared to 13% in the placebo control group. Survival rates were higher for groups that started BCV at earlier times post-challenge and trended slightly higher for the 20/5/5 mg/kg dose relative to the 10/5/5 mg/kg dose when administered at the same timepoints. Clinical disease signs were not substantially different between groups. In untreated mice, viral DNA was detected in blood by Day 5 (4 days post-challenge) and continued to increase through Day 7 (6 days post-challenge). Limited virologic data from exploratory mouse groups showed viral DNA and PFU levels in liver and spleen tissues trended modestly lower in BCV-treated versus control mice.

Resistance assessments by NGS. Resistance assessments conducted for the pivotal animal model studies included NGS analyses to identify BCV treatment-emergent amino acid substitutions in the viral (b) (3) (A), (b) (3) (B) genes (b) (3) (A), (b) (3) (B) as well as cell culture phenotypic resistance analyses of virus isolates from treated animals. Raw NGS fastq files were independently analyzed and the results were compared to those reported by the Applicant.

NGS analysis performed by the Applicant for RPXV. RPXV samples planned for NGS analysis included: 1) blood samples from all animals at Post-Challenge Day 8, 2) blood samples from all animals at 24 or 48 hours after the last dose of BCV (dependent upon the cohort, either a 24 or a 48 hour sample was available), and 3) terminal samples (blood, if available and liver) from all animals that did not survive to the scheduled collection timepoints; animals that survived beyond 48 hours after the last BCV dose were not planned for analysis. Samples analyzed from control-treated animals were obtained at timepoints close to the BCV-treated animal time points. (b) (3) (A), (b) (3) (B) NGS analyses were performed at (b) (4) using the Thermo-Fisher Ion Torrent system. DNA extractions and concentration were performed by (b) (4). Library preparation included four major steps: DNA target amplification, partial amplicon digestion, and library amplification with barcodes. A concentration of 1,000 to 3,000 DNA copies per microliter of purified and concentrated viral DNA sample were used for target amplification. The primers included unique molecular tags to identify each amplicon originating from the viral DNA template. Positive controls were included in selected runs to verify assay performance and consisted of mixtures of wild-type and mutant DNA (1% and 5% concentrations) encoding resistance-associated substitutions (b) (3) (A), (b) (3) (B) The RPXV-Utrecht strain was used as a reference sequence (Genbank AY484669). A total of 285 samples were analyzed, of which 191 samples had available/successful NGS data (including 10 samples that were repeated for a total of 201 sequences), defined as having sequence results derived from >10 unique viral DNA templates. All nucleotide variants identified in any sequencing reaction, including sequences of low quality according to the Applicant's NGS analysis pipeline, were reported in a summary frequency table. The frequency table included results for 153 samples from 102 animals, which included 15 animals in Group 5 (controls) and 18-26 animals/group from the BCV treatment groups. Analyzed timepoints included Post-Challenge Days 6-12 and terminal samples.

NGS analysis performed by the Applicant for ECTV. NGS analyses for ECTV study CMX001-VIR-044 were conducted targeting the viral (b) (3) (A), (b) (3) (B) These regions correspond to the same regions analyzed for RPXV. These analyses were conducted for liver samples from Groups 1-8 mice that died or were euthanized due to disease prior to the end of the study (n=117; except for one animal without an available liver DNA sample), as well as liver samples from serially sacrificed mice in Groups 10-12 (n=42). This work was conducted under two other protocols: CMX001-VIR-119 (conducted at (b) (4)) for preparation of DNA samples and phenotypic resistance analyses, and CMX001-VIR-108 (conducted at (b) (4)) for performance of NGS analyses. Positive controls were included in selected assay runs and consisted of wild-type, mouse liver-extracted ECTV DNA spiked at a 1% frequency with synthetic DNA containing DNA polymerase gene (EVM049) nucleotide substitutions corresponding to resistance-associated substitutions (b) (3) (A), (b) (3) (B) Negative controls included a no template control sample (NTC) which consisted of nuclease-free water in place of tissue DNA sample. Additional negative controls included DNA extracted from uninfected purchased mouse liver tissue (n=3, from BioIVT) that was analyzed in selected NGS runs.

Reviewer's *Note:* *The Applicant found the negative control liver samples were contaminated with ECTV DNA (discussed below).*

NGS analyses were performed at (b) (4) using the Thermo-Fisher Ion Torrent system. DNA extractions and concentration were performed by (b) (4) Library preparation involved target genome amplification using the Thermo-Fisher Ion AmpliSeq™ HD Library Kit. The viral DNA sample input for each reaction was as follows: 1 µL of 10,000 copies/µL sample when starting concentrations were ≥10,000 copies/µL, 1 µL for samples with starting concentrations <10,000 copies/µL but ≥5,000 copies/µL, or 3 µL for samples with <5,000 copies/µL and negative control samples. The library preparation protocol included initial target amplification, partial amplicon digestion, barcoding amplification, and purification. The amplification primers included unique molecular tags to identify each amplicon originating from the viral DNA template. Data were analyzed in reference to ECTV strain Moscow (Genbank AF012825). Nucleotide variants were identified using a Torrent Variant Caller with analysis settings to detect variants at a ≥5% frequency.

2. OBJECTIVES OF INDEPENDENT ASSESSMENT OF NGS RESISTANCE DATA

The objectives of the independent assessment of the NGS resistance data were as follows:

- Perform independent alignments of the IonTorrent sequence reads from animal studies designed to assess for the development of resistance and generate a frequency table of variations for each study for comparison to the results provided by the Applicant.
- Verify the Applicant's resistance findings by comparing their results to those generated independently.
- Determine any additional resistance-associated substitutions using DAV virology criteria (treatment-emergent at a frequency ≥15% in two or more animals).
- Assess for any substitutions at known resistance sites that were detected at any frequency.
- Look for resistance signals in low frequency variants that were detected in treated animals only.
- Look at specific reads to see if multiple substitutions were linked and to assess the types of mutations at specific codon positions that were driving the amino acid substitutions.

3. (b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

4. DAV NGS ANALYSIS METHODOLOGY

CLC Genomics Workbench (CLC Genomics) and the High-Performance Integrated Virtual Environment (HIVE) (Simonyan and Mazumder, 2014), were used in conjunction to perform the independent assessment of the NGS data for this NDA. The HIVE tool contains specific tools that allow the reviewer to batch rename files to meet nomenclature rules for the analysis pipeline, to assess quality control on all sequence files, to convert variant call files (VCF) to frequency tables at the amino acid level, and to generate tables comparing results submitted by the Applicant and those generated by HIVE or CLC Genomics (Figure 2).

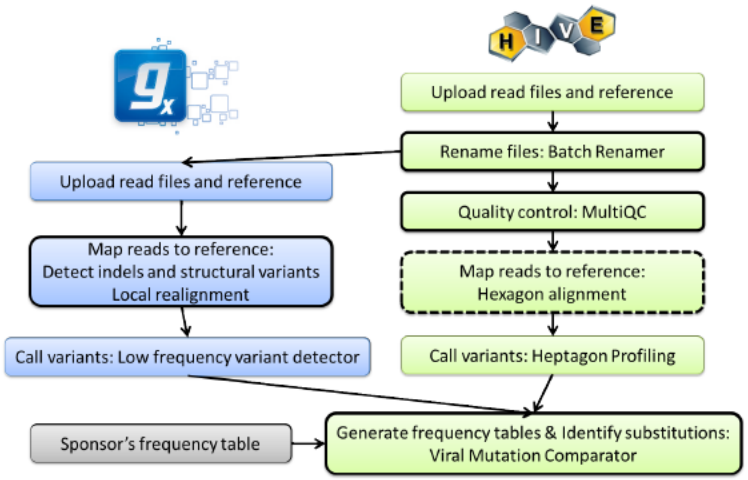

Figure 2. Overview of the NGS analysis pipeline using HIVE and/or CLC Genomics Workbench (DAV Analysis).

The initial analysis of NGS data in this review was performed using the optimized CLC Genomics Workbench pipeline. The optimized HIVE analysis pipeline was used to assess variants for which there was a major disagreement (novel variants or major disagreements in frequency of 10% or greater) between the Applicant's results and the HIVE pipeline (Figure 2).

The optimized CLC Genomics Workbench analysis pipeline. The workflow in CLC Genomics workbench was as follows: 1) trimmed reads were mapped to the reference sequence, 2) structural variants were detected based on the information derived from unaligned ends and locally realigned to the reference sequence, 3) consensus sequences were generated, 4) variants were called at the amino acid level, exported in Excel, and formatted manually to generate frequency tables.

The parameters used in CLC Genomics were the following:

A. Mapping using the CLC aligner
    a. Match score: 1
    b. Mismatch cost: 2
    c. Insertion cost: 3
    d. Deletion cost: 3
    e. Length fraction of read required for mapping: 0.5
    f. Similarity fraction: 0.8
    g. Local alignment
B. InDels and Structural Variants
    a. p-value threshold of unaligned end breakpoints: 0.0001
    b. maximum number of mismatches: 3
    c. minimum number of reads to filter variants: 2
C. Local realignment
    a. multi-pass realignment: 2
    b. maximum guidance-variant length (defined by InDels and Structural Variants): 100
D. Low frequency variant detection
    a. Minimum frequency: 1%
    b. Minimum coverage was set to 100
    c. Frequency was set to 1%
    d. Significance: 1%

The optimized HIVE analysis pipeline. The workflow in HIVE was as follows: 1) sequence reads were mapped to the reference sequence using Hexagon aligner tool, 2) variants were called at the amino acid level using the Heptagon profiling tool, and 3) frequency tables were generated using the Viral Mutation Comparator tool.

The parameters used in HIVE were the following:

A. Mapping using Hexagon aligner
    a. Match benefit: 5

   b. Mismatch penalty: -4
   c. Mismatch continuation penalty: -6
   d. Gap continuation cost: -4
   e. Gap opening cost: -12
   f. Local alignment

B. Heptagon profiling
   a. Minimum coverage was set to 100

Overview of analysis process. Each step of the analysis process is briefly described below.

A. Processing fastq files. Data files were submitted to the FDA on a portable hard drive, which included fastq files for each sample that was sequenced using the Ion Torrent system. The nucleotide sequences were uploaded into CLC Genomics Workbench or via the Regulatory HIVE interface and assessed for quality control by looking at the following parameters: position statistics, where the mean phred score is calculated; read length, the relative base population of A, C, G, T for each sample; and average base quality for each file. Outlier files were flagged and evaluated more closely.

B. Preparing sequence reads and reference sequences prior to mapping. The fastq files were imported into HIVE or CLC Genomics and the RPXV-Utrecht strain (Genbank AY484669) was used as a reference sequence for the RPXV resistance analysis and the ECTV Moscow strain (Genbank AF012825) was used as a reference sequence for the ECTV resistance analysis. (b) (3) (A), (b) (3) (B) [redacted]

C. Mapping reads to the appropriate reference sequence. The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each sample. The mapping contained the target of interest (b) (3) (A), (b) (3) (B) [redacted] and was used to identify variants that differed from the reference sequence. In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias.

D. Generating frequency tables of amino acid substitutions. From the read mappings, variants were called and variant tables were generated for each nucleotide sequence run using the low frequency variant caller in CLC Genomics workbench and the built in variant caller in HIVE (Simonyan et al., 2017) (only used in cases of disagreement between CLC Genomics and the Applicant). The variant call tables were converted into amino acid frequency tables manually from CLC Genomics or using the HIVE Viral Mutation Comparator tool. Below are descriptions for the variant callers and the frequency table:

   a. **Low Frequency Variant Detection (LFVD)** – according to the CLC Genomics Workbench Manual: *"a statistical test is performed at each site to determine if the nucleotides observed in the reads at that site could be due simply to sequencing errors, or if they are significantly better explained by there being one (or more) alleles than the reference present in the sample at some unknown frequency. If the latter is the case, a variant corresponding to the significant allele will be called, with estimated frequency".*

b. **HIVE Variant Detector (HVD)** – calls variants from a read mapping using the Heptagon Sequence Profiler tool (Simonyan et al., 2017). Following alignment, the reference-based variant profile is computed by mapping nucleotides of short read sequences and counting occurrences of distinct bases at every genomic position on the reference. This variant-calling procedure produces consensus, per-base, forward/reverse, and total coverage maps for all reference segments of the specified reference genomes/sets. The frequencies of each variant call are then computed relative to the reference genome or relative to the accumulated consensus genome, depending on user specifications. Amino acids (AA) are called based on the contribution of every read and its alignment in relation to an annotated open reading frame (ORF). AA substitution calls are made from codon translations of individual reads, based on mapping to annotated open reading frames. This is to contrast the variant calling from a consensus-based AA calling procedure.

c. **Frequency tables** – Tables were generated manually from variant call files (VCFs) produced by CLC Genomics or using the HIVE Viral Mutation Comparator tool. The frequency table contains information for each position of each viral gene and each animal for which variation from the reference occurs. The frequency table contains the following columns: unique subject or animal identifier (USUBJID), treatment regimen (ARM), visit (VISIT), the amino acid position within the gene of interest (AAPOS), total coverage at the nucleotide position (TCOV), the amino acid found in the reference sequence (AAREF), the amino acid substitution (AASUB), the coverage at the nucleotide level for the variant (VCOV), and the frequency by which the variant was detected (AAFREQ). Frequency tables are generated by:
    i. The variant tables were combined by arm (BCV or placebo)
    ii. The variant tables were filtered to remove synonymous substitutions
    iii. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the Applicant
    iv. Any amino acid substitution ≥1% was presented in the table, although the Applicant's analysis was performed with a ≥5% cutoff.

E. Generating resistance analysis tables. Excel macros and the HIVE Viral Comparator tool were used to convert the frequency tables into resistance analysis tables, allowing the resistance tables to be populated using different frequency thresholds. For example, the frequency tables generated from CLC Genomics Workbench output or submitted by the Applicant contained all variants with a frequency greater than or equal to 1%, and this tool allowed resistance analysis tables to be generated showing variants at different levels of sensitivity (5%, 15%, 25%, etc.) as defined by the user.

F. Conducting independent resistance analysis. The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above various defined frequency thresholds using the following criteria:

b. **Resistance criteria** – resistance-associated substitutions were defined by several criteria listed below. In general, substitutions that occurred at highly conserved amino acid positions in the virus of animals in a BCV treatment group, but not the placebo group, were of interest, particularly if these occurred at known resistance positions, occurred in the virus from more than one animal, or occurred while the animal was still on BCV treatment.
    i. Amino acid positions conserved at ≥96% identity (across all studies)
    ii. Substitution occurs in <5% of animals in the placebo arm
    iii. Amino acid substitution frequency ≥5% in an animal in the BCV arm; looking for patterns above 2%
    iv. Substitutions occurred in n≥2 animals
    v. Detected at known positions at any frequency

vi. Novel, unreported substitutions at a polymorphic position
vii. Identify amino acid changes that occurred as the result of two or more mutations in a codon

(b) (3) (A), (b) (3) (B)

[redacted]

G. **Comparing results to those submitted by the Applicant.** The remainder of this review provides details on how the NGS data submitted by the Applicant were independently evaluated using the above described NGS analysis pipeline. In general, the NGS data analysis was performed using data generated in this pipeline and provided by the Applicant, and the results were compared as follows:
   a. Frequency and resistance analysis tables were compared directly, and major differences were noted.
   b. Amino acid substitutions were identified by at least two and up to three algorithms, including the Applicant's algorithm and HVD and LFVD (used by DAV) and major differences between algorithms were reported.
   c. Novel resistance-associated amino acid substitutions reported by different NGS analysis approaches were compared and major differences were reported.
   d. Novel resistance-associated substitutions identified by the independent analysis were noted and discussed with the review team for potential labeling/post-marketing actions.

5. THRESHOLDS

The following thresholds and cutoffs were employed for the independent assessment of the NGS resistance data:

- Substitutions detected at frequencies <1% were ignored due to potential sequence error thresholds. Based on structural variant (insertions and deletions) frequencies, it appeared that a frequency of <3% would more accurately reflected the error rate in this dataset.
- Insertions and deletions were ignored for the most part due to the impact such large changes would likely have on the protein sequence (i.e., frameshifts, nonsense mutations, etc.). Synonymous substitutions were also ignored.
- Total coverage minimum of 500
- Substitutions that were also detected in the control animals were eliminated from resistance consideration

6. CROSS CONTAMINATION OF SAMPLES

Uninfected mouse liver samples (n=3) were included as controls to assess potential cross contamination in study CMX001-VIR-044; however, these samples yielded sequence results indicating they were contaminated with ECTV DNA. To investigate, the no template control (NTC; only water used for sample) sample results were compared with the naïve liver sample results. The NTC samples produced short reads of poor coverage that aligned across the entire reference sequence and the Applicant determined these reads to be primer-dimers based on the mean read length of 36 base pairs and a median coverage of 0. The naïve liver samples generated mean read lengths of 111 to 219 with median coverages of 21 to 839 across the reference region, which was similar to samples producing quality ECTV sequences that generally had median read lengths of 200-230 base pairs and coverage of >500. The

Applicant followed up with the sequencing vendor to determine that the liver samples were cross contaminated at or before the time of homogenization.

7. (b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

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(b) (3) (A), (b) (3) (B)

8. CONCLUSIONS

This review focused on an independent analysis of NGS data to assess for the development of BCV resistance in pivotal animal model studies performed in two animal models: the rabbitpox virus (RPXV) model in rabbits and the ectromelia virus (ECTV) model in mice. Based on the results of the independent assessment of NGS resistance data, DAV agrees that the Applicant made a reasonable effort to collect RPXV and ECTV NGS data for assessment of BCV genotypic resistance and based on the reported amino acid coding changes, there was no clear evidence of BCV resistance selection in the RPXV and ECTV challenge models using primary DAV resistance criteria: 1) treatment-emergent substitutions detected at a frequency ≥15%; 2) substitutions detected in two or more animals but not in control animals; and 3) sequence read coverage of ≥500 at the amino acid position.

(b) (3) (A), (b) (3) (B)

9.

(b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

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(b) (3) (A), (b) (3) (B)

[redacted]

III. REFERENCES

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/s/

ERIC F DONALDSON
05/07/2021 10:39:02 AM

PATRICK R HARRINGTON
05/07/2021 11:01:27 AM

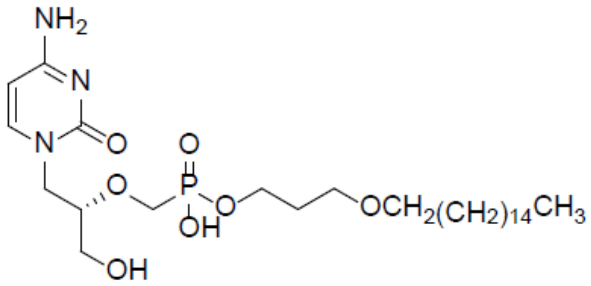
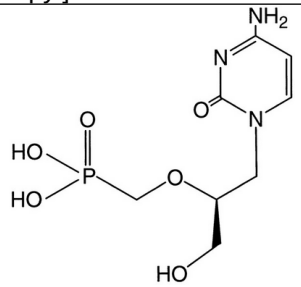
JULIAN J O REAR
05/07/2021 11:29:28 AM

DIVISION OF ANTIVIRALS / VIROLOGY REVIEW
NDA: 214461(tablets), 214460(suspension) **SDN**: Original NDA **REVIEW COMPLETED:** 4/23/2021
Virology Reviewer: Patrick R. Harrington, Ph.D.

Reviewer's Name(s): Patrick R. Harrington, Ph.D.
NDA#: 214461 (tablets), 214460 (suspension, cross-references to NDA 214461)

Sponsor: Chimerix, Inc.
2505 Meridian Parkway, Suite 100
Durham, NC 27713
A. Heather Knight, Pharm D, VP Regulatory Affairs

Complete Submission Dates:
Correspondence Date: 10/7/2020
CDER Receipt Date: 10/7/2020
PDUFA Date: 4/7/2021 (7/7/2021 major amendment goal date)

Proprietary Name	**TEMBEXA®**	
Drug Names	brincidofovir, CMX001	
IND #	67681	
Chemical Name	Phosphonic acid, [[(S)-2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl) ethoxy]methyl]mono[3-(hexadecyloxy)propyl] ester	
Structure	 **brincidofovir**	 **cidofovir** **(parent nucleotide analogue)**
Molecular Formula	$C_{27}H_{52}N_3O_7P$	
Molecular Weight	561.69 Daltons	

Dosage Form and Route of Administration: tablet and suspension / oral
Dispensed: Rx x OTC__
Proposed Indication: Treatment of human smallpox disease in adult and pediatric patients

Assigned/Reviewed SDNs (submitted thru 5/6/21):

NDA	SDN	eCTD#	Rec'd Date	Content
214460	001	0001	10/7/2020	Original NDA for oral suspension.
214460	002	0002	10/30/2020	Response to Clinical Virology request for OPXV DNA sequences.
214460	004	0004	11/17/2020	Response to Clin. Pharm. request related to datasets.
214460	005	0005	12/1/2020	Response to Clinical Virology request for additional supporting information for NGS data.
214460	012	0012	1/7/2021	Response to Clinical request related to distribution plans.
214460	019	0019	1/29/2021	Synopsis for post-marketing field study and response to Clin. Pharm. request.
214460	021	0021	2/3/2021	Updated labeling.
214460	029	0029	3/2/2021	Updated labeling.
214460	032	0032	3/12/2021	Response to Clinical Virology requests re-clinical cowpox case and variola virus isolate
214460	035	0035	3/26/2021	Response to Clinical Virology request related to ECTV resistance analyses.
214460	036	0036	3/30/2021	PMR field study correspondence.
214460	040	0040	4/16/2021	PMR field study correspondence.

214460	041	0041	4/19/2021	Virology PMC correspondence and acknowledgement of redaction of resistance data.
214460	042	0042	4/20/2021	Updated labeling.
214460	043	0043	5/6/2021	Updated labeling.
214461	001	0001	5/29/2020	Rolling NDA submission #1.
214461	002	0002	6/30/2020	Rolling NDA submission #2.
214461	003	0003	8/28/2020	Rolling NDA submission #3.
214461	005	0005	10/7/2020	Final part of rolling Original NDA for tablet formulation.
214461	006	0006	10/30/2020	Response to Clinical Virology request for OPXV DNA sequences.
214461	008	0008	11/17/2020	Response to Clin. Pharm. request related to datasets.
214461	009	0009	12/1/2020	Response to Clinical Virology request for additional supporting information for NGS data.
214461	015	0015	1/7/2021	Response to Clinical request related to distribution plans.
214461	021	0021	1/29/2021	Synopsis for post-marketing field study and response to Clin. Pharm. request.
214461	023	0023	2/3/2021	Updated labeling.
214461	031	0031	3/2/2021	Updated labeling.
214461	034	0034	3/12/2021	Response to Clinical Virology requests re-clinical cowpox case and variola virus isolate
214461	036	0036	3/26/2021	Response to Clinical Virology request related to ECTV resistance analyses.
214461	038	0038	3/30/2021	PMR field study correspondence.
214461	043	0043	4/16/2021	PMR field study correspondence.
214461	044	0044	4/19/2021	Virology PMC correspondence and acknowledgement of redaction of resistance data.
214461	045	0045	4/20/2021	Updated labeling.
214461	047	0047	5/6/2021	Updated labeling.

Abbreviations: ATCC, American Type Culture Collection; BARDA, Biomedical Advanced Research and Development Authority; (b) (4) BCV, brincidofovir; CC_{50}, 50% cytotoxic concentration; CDC, Centers for Disease Control and Prevention; CDV, cidofovir; CDV-PP, cidofovir diphosphate; CMLV, camelpox virus; CMV, cytomegalovirus; EC_{50}, 50% effective concentration; ECTV, ectromelia virus; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GLP, good laboratory practice; IL, interleukin; IV, intravenous(ly); LD_{50}, 50% lethal dose; LLOQ, lower limit of quantification; MOI, multiplicity of infection; MPXV, monkeypox virus; MVA, modified vaccinia Ankara; NGS, next generation sequencing; NHP, nonhuman primate; NZW, New Zealand white (rabbits); PCR, polymerase chain reaction; PFU, plaque-forming unit(s); PK, pharmacokinetics; PRNT, plaque reduction neutralization test; RPXV, rabbitpox virus; SI, selectivity index; USAMRIID, U.S. Army Research Institute of Infectious Diseases; VACV, vaccinia virus; VARV, variola virus; VIGIV, vaccinia immune globulin intravenous

TABLE OF CONTENTS

EXECUTIVE SUMMARY

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

These Original NDAs for tablet and suspension oral formulations of TEMBEXA® (brincidofovir [BCV], CMX001), an orthopoxvirus nucleotide analog DNA polymerase inhibitor, are approvable from a Virology perspective for the treatment of human smallpox disease caused by variola virus.

1.2 Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

We recommend the following post-marketing commitment (PMC):

Conduct cell culture studies to characterize brincidofovir antiviral activity against recombinant vaccinia viruses encoding specific amino acid substitutions that emerged in ectromelia virus in brincidofovir-treated animals in mouse study CMX001-VIR-044.

PMC Schedule Milestones (in agreement with sponsor as of 4/19/2021):

Draft Protocol Submission:	01/2022
Final Protocol Submission:	07/2022
Study/Trial Completion:	07/2023
Final Report Submission:	12/2023

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

Brincidofovir (BCV) is a lipid conjugate of cidofovir (CDV), which is an acyclic nucleotide analog of (deoxy)cytidine monophosphate. Once inside cells, the lipid ester linkage of BCV is cleaved to liberate CDV, which is then phosphorylated to produce the active triphosphate, referred to as CDV diphosphate (CDV-PP). CDV-PP is an inhibitor of orthopoxvirus replication by inhibiting viral DNA synthesis mediated by the E9L (vaccinia virus [VACV] nomenclature) viral DNA polymerase. Results from biochemical (b) (3) (A), (b) (3) (B) studies support the mechanism of action of CDV-PP targeting the viral DNA polymerase.

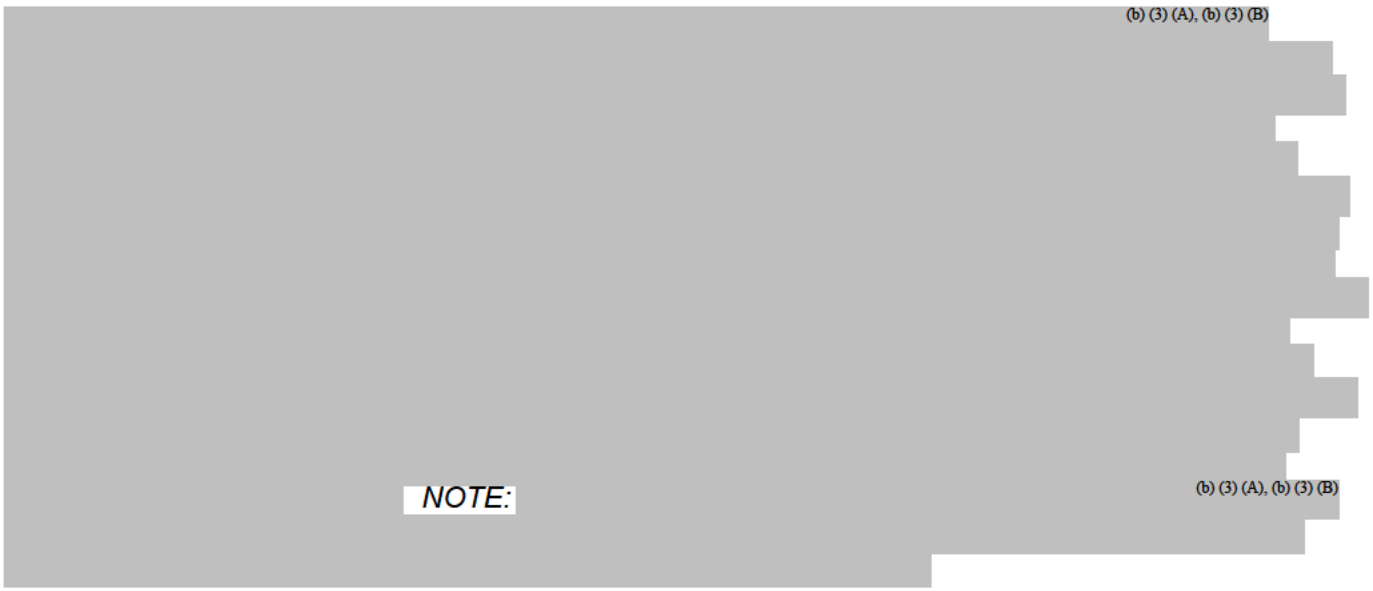
Brincidofovir had broad and consistent antiviral activity in cell culture assays against a variety of orthopoxviruses, including variola virus (VARV), rabbitpox virus (RPXV), and ectromelia virus (ECTV). Under analogous assay conditions, BCV EC_{50} values were 1.15 µM, 0.33 µM, and 0.11 for RPXV, ECTV, and VARV (median for 5 isolates), respectively. BCV is highly protein bound (>98%) in plasma from human and various animal species, which reduces BCV antiviral activity in cell culture. BCV has a distinct mechanism of action and non-antagonistic antiviral activity with tecovirimat, which is the only currently approved antiviral drug for smallpox.

(b) (3) (A), (b) (3) (B)

BCV efficacy was evaluated under the Animal Rule (21 CFR part 314, subpart I). In theory, the ideal animal model for VARV would involve infection with VARV itself, but natural VARV infection and disease is specific to humans. Two animal models of smallpox were primarily used to evaluate BCV efficacy: RPXV-infected rabbits and ECTV-infected mice. Use of these models to establish BCV efficacy is in alignment with conclusions and recommendations from a 2011 Antiviral Drugs Advisory Committee Meeting on the development of antiviral drugs for smallpox under the Animal Rule.

The pivotal rabbit/RPXV study, CMX001-VIR-106, was a blinded, placebo-controlled study. The primary objective was to compare the survival benefit of BCV over placebo. BCV-treated animals received an initial 20 mg/kg loading dose on Day 3, 4, 5 or 6 post-challenge (challenge occurred on Study Day 0), followed by two additional 5 mg/kg doses every other day. Survival rates through Day 42 were 69-100% in BCV-treated groups compared to 29% in the placebo control group, with survival rates trending higher for those that started BCV on Day 3 (100%) or Day 4 (90%). Fever was detected in 50% of animals by Day 3, and in 98% of animals by Day 4. The sponsor's analyses of body weight, body temperature and respiration rates did not show clear or consistent differences in disease signs between treatment and control groups. Quantitative viral PCR results for whole blood samples showed a modest trend of higher peak viral DNA levels in the control group and lower peak viral DNA levels in the group that started BCV on Day 3. Peak viral DNA levels within each group were consistently higher among animals that died compared to those that survived.

The pivotal mouse/ECTV study, CMX001-VIR-044, was a randomized, blinded, placebo-controlled study. For the efficacy evaluation, mice were randomized across 8 groups in which animals received BCV or placebo by oral gavage, with BCV 10/5/5 or 20/5/5 mg/kg administered every other day starting on Study Day 5, 6, 7 or 8 (20/5/5 mg/kg dose only), corresponding to 4 to 7 days post-challenge (i.e., challenge occurred on Study Day 1). The primary efficacy endpoint was the survival rate at Day 43. Survival rates were 34-84% in the BCV-treatment groups compared to 13% in the placebo control group. Survival rates were higher for groups that started BCV at earlier times post-challenge and trended slightly higher for the 20/5/5 mg/kg dose relative to the 10/5/5 mg/kg dose when administered at the same timepoints. Clinical disease signs were not substantially different between groups. In untreated mice, viral DNA was detected in blood by Day 5 (4 days post-challenge) and continued to increase through Day 7 (6 days post-challenge). Limited virologic data from exploratory mouse groups showed viral DNA and PFU levels in liver and spleen tissues trended modestly lower in BCV-treated versus control mice.



Results from other exploratory and published orthopoxvirus animal model studies indicate that the immune response likely contributes to viral clearance during BCV treatment, and BCV efficacy may be reduced in the setting of severe immune deficiency. In a post-exposure prophylaxis dosing strategy, a combination of BCV and tecovirimat provided significant protection of mice challenged with a highly virulent recombinant ECTV expressing murine interleukin-4 (IL-4), whereas neither compound alone protected mice from lethal infection, providing proof-of-concept that the combination of BCV plus tecovirimat provides greater antiviral activity than either drug alone in a highly rigorous challenge model.

As a result of its antiviral activity against VACV, BCV has the potential to interfere with VACV-based smallpox vaccines when it is administered around the time of vaccination, and studies conducted in animal models indicate BCV can have a modest impact on smallpox vaccine-induced immune responses. The clinical relevance of this interaction is unclear, and likely varies depending on the specific circumstance in which vaccine and BCV are used concomitantly.

2.2 Clinical Virology

No clinical trials evaluating BCV efficacy for orthopoxvirus infection have been conducted. BCV has been administered in emergency use cases for the treatment of human orthopoxvirus infections. In general, because of the anecdotal nature of the cases, limited laboratory analyses, and confounding treatments and clinical care, it is unknown if BCV provided a treatment benefit in any of these cases.

Approval of a drug under the Animal Rule requires the conduct of a post-marketing clinical/field trial if or when such studies are feasible and ethical. The review team is recommending that the sponsor develop a factorial clinical trial design to compare the safety and efficacy of BCV vs. tecovirimat vs. BCV + tecovirimat for the treatment of smallpox. Negotiations on this required post-marketing study were ongoing at the time of finalization of this review.

3. OVERALL VIROLOGY SUMMARY

BCV has a well-characterized mechanism of action as an orthopoxvirus nucleotide analog DNA polymerase inhibitor, has broad and consistent activity against orthopoxviruses, and appears to have a favorable resistance barrier. BCV efficacy has been demonstrated in pivotal efficacy studies using the rabbit/RPXV and mouse/ECTV models, and activity was also demonstrated in several additional exploratory animal studies. Collectively, results from the sponsor's nonclinical development program indicate BCV is likely to be an effective treatment for human smallpox, particularly if administered early in the course of disease. Based on its mechanism of action and lack of cross-resistance with tecovirimat, BCV could in theory be administered in combination with tecovirimat to provide a greater antiviral effect and higher resistance barrier than either drug alone.

4. ADMINISTRATIVE

4.1 Reviewer's Signature

_____
Patrick R. Harrington, Ph.D.
Senior Clinical Virology Reviewer, Division of Antivirals, FDA/CDER/OND/OID

4.2 Concurrence

_____
Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antivirals, FDA/CDER/OND/OID

OND CLINICAL VIROLOGY REVIEW

1. INTRODUCTION AND BACKGROUND

1.1 Important Milestones in Product Development and Prior FDA Reviews

These are the Original NDA submissions for brincidofovir (BCV, TEMBEXA®) tablets and suspension for the proposed indication of "Treatment of human smallpox disease in adult and pediatric patients."

BCV was developed under IND 67681. The IND was first opened in 2005. Besides smallpox/variola virus, BCV was investigated for the treatment of a variety of other infections of viruses with a DNA genome, most notably cytomegalovirus (CMV). These NDA submissions are the first for any BCV indication.

Numerous meetings were held between the Sponsor and DAVP throughout development. A 2011 Antiviral Drugs Advisory Committee Meeting was held to discuss smallpox antiviral drug development, and key conclusions from this meeting helped guide the development of BCV under the Animal Rule (21 CFR part 314, subpart I). Pre-NDA discussions were held with the sponsor in 2019 and 2020. The NDA was submitted on a rolling submission basis, with the first submission received on 5/29/2020, and the final complete NDA package was received on 10/7/2020.

Virology reviews throughout product development for the smallpox indication under IND 67681 were conducted primarily by Dr. Jules O'Rear, Ph.D., DAVP Clinical Virology Team Leader/Supervisor.

1.2 State of Antivirals Used for the Indication(s) Sought

The sponsor's proposed indication for BCV is the treatment of human smallpox disease in adult and pediatric patients. Variola virus (VARV), the cause of smallpox, is a DNA genome virus in the orthopoxvirus genus. Smallpox was one of the most devastating diseases in human history. The virus is transmitted via aerosolized droplets from an infected individual, by direct contact with contaminated surfaces, or by airborne spread in enclosed areas or ventilation systems. Variola major, the most serious and prevalent form of the disease, was associated with an approximately 30% case fatality rate (CDC). As a result of an intense global vaccination campaign, no cases of human smallpox have occurred since 1978, and the disease was declared eradicated from the world in 1980. Despite the eradication of naturally acquired smallpox, the disease remains a threat as VARV could be developed as a bioterrorism agent. Although currently there are only two locations in the world where VARV is allowed to be stored, one in the U.S. and one in Russia, long forgotten isolates have been found in freezers, some countries may have illegally kept VARV stocks, and it may also be possible to synthesize the virus in a laboratory (Noyce et al., 2018). Routine vaccination in the U.S. ended in the 1970s, so most of the population is immunologically susceptible to smallpox.

Tecovirimat (TPOXX™) is currently the only approved antiviral drug for smallpox. Like BCV, it was developed following the FDA animal rule, and was ultimately approved in 2018. Tecovirimat has a distinct mechanism of action from BCV; it is an inhibitor of the orthopoxvirus VP37 envelope wrapping protein. Although it has potent antiviral activity in cell culture against orthopoxviruses, and demonstrated efficacy in multiple orthopoxvirus animal models, tecovirimat has a low resistance barrier, with certain single amino acid substitutions in the drug target conferring high level resistance. Therefore, the public would potentially benefit with the availability of additional antiviral drugs for smallpox. With its distinct mechanism of action and potentially higher resistance barrier, BCV could be used in combination with tecovirimat to enhance the antiviral activity and overall resistance barrier of the regimen, or as an

alternative to tecovirimat in the event that a tecovirimat-resistant VARV bioweapon is engineered or tecovirimat-resistant virus emerges in the population.

1.3 Methodology

BCV was developed under the Animal Rule (21 CFR part 314, subpart I) to support an indication for the treatment of smallpox. Cell culture and biochemical studies were used to characterize the antiviral activity and mechanism of action of BCV or its active metabolite, cidofovir diphosphate. Numerous animal studies were conducted to assess the effect of BCV treatment on orthopoxvirus disease outcomes. These animal models primarily involved rabbitpox virus (RPXV) infection of rabbits, ectromelia virus (ECTV) infection of mice, and vaccinia virus (VACV) infection in mice. During development, BCV was also used under emergency IND (E-IND) in a small number of patients with complications related to VACV-based vaccination or cowpox virus (CPXV) infection, and limited clinical and laboratory data were collected from these cases.

The two pivotal animal model studies were CMX001-VIR-106, conducted using the rabbit/RPXV model, and CMX001-VIR-044, conducted using the mouse/ECTV model. These orthopoxvirus animal models and their relevance to human smallpox disease are described in greater detail in Section 3.1. These studies were conducted in a placebo-controlled, laboratory technician-blinded manner. Primary efficacy endpoints were based on survival versus mortality (including euthanasia due to moribund disease).

Virology analyses for the animal efficacy studies included quantification of viral DNA levels in whole blood samples collected at various times post-challenge before and after initiation of BCV treatment. Viral DNA levels were measured using a quantitative PCR assay targeting the conserved orthopoxvirus hemagglutinin gene. The lower limit of quantification (LLOQ) for the quantitative PCR assays for whole blood samples were 496 copies/mL for the rabbit/RPXV study, and 1,007 copies/mL for the mouse/ECTV study. Additional virologic analyses included quantitative PCR for viral DNA in tissue samples collected at necropsy, plaque assays using whole blood and tissue samples, and analyses of neutralizing antibody levels. Additional details for virology studies are included with the results described in Section 3.

Blood and tissue samples were also collected and processed to investigate the emergence of BCV-resistant virus in the pivotal animal studies. (b) (3) (A), (b) (3) (B) [redacted] Additional details are described along with the resistance results in Section 3.4. FDA/DAVP Clinical Virology Reviewer Dr. Eric Donaldson, Ph.D., also independently analyzed raw NGS fastq files used by the sponsor to produce amino acid substitution data; see Dr. Donaldson's review for more details (link to Dr. Donaldson's review).

2. MECHANISM OF ACTION, CELL CULTURE ACTIVITY, AND DRUG RESISTANCE STUDIES

2.1 Mechanism of Action

Brincidofovir (BCV) is a lipid conjugate of cidofovir (CDV), which is an acyclic nucleotide analog of (deoxy)cytidine monophosphate. The lipid conjugate is designed to mimic a natural lipid, lysophosphatidylcholine, and thereby use endogenous lipid uptake pathways to facilitate cellular uptake of the drug. Once inside cells, the lipid ester linkage of brincidofovir is cleaved to liberate CDV, which is then phosphorylated to produce the active triphosphate, referred to as CDV diphosphate (CDV-PP).

CDV (Vistide®) is an FDA-approved intravenously (IV)-infused antiviral drug indicated for the treatment of cytomegalovirus (CMV) retinitis in HIV-infected patients with acquired immunodeficiency syndrome (AIDS). The lipid conjugate in BCV allows for oral administration, changes the drug's tissue distribution, and results in >100-fold higher intracellular concentrations of CDV-PP, leading to enhanced BCV antiviral activity relative to CDV against a variety of DNA viruses (Pharmacokinetics Written Summary; Aldern et al., 2003). The terminal half-life of CDV-PP in peripheral blood mononuclear cells is 3.9-6.8 days (Pharmacokinetics Written Summary).

CDV-PP is an inhibitor of orthopoxvirus replication by inhibiting viral DNA synthesis mediated by the E9L (vaccinia virus [VACV] nomenclature) viral DNA polymerase. In a biochemical assay, CDV-PP was incorporated into a growing DNA chain opposite of template guanosine (G) nucleotides by the VACV E9L DNA polymerase, resulting in non-obligate chain termination generally at the CDV +1 position (Magee et al., 2005). DNA chain elongation is also inhibited when CDV is incorporated into the template strand (Magee et al., 2008).

The E9L protein encodes both a DNA polymerase domain and a 3'-5' exonuclease domain that serves a proofreading function to reduce replication errors and also plays a role in genetic recombination (Gammon and Evans, 2009). Once CDV is incorporated into DNA, and beyond its chain terminating activity, it also inhibits the 3'-5' exonuclease activity of E6L (Magee et al., 2005).

The viral DNA polymerases of orthopoxviruses are highly conserved. The DNA polymerases of both RPXV and ECTV have approximately 98% amino acid identity with the consensus of 48 VARV isolates (confirmed in an independent analysis by Dr. Eric Donaldson, Ph.D.).

2.2 Cell Culture Antiviral Activity, Cytotoxicity and Effect of Human Serum Proteins

Antiviral Activity in Cell Culture

Brincidofovir has broad antiviral activity against a variety of orthopoxviruses, including VARV, RPXV, ECTV, VACV, CPXV, camelpox virus (CMLV) and monkeypox virus (MPXV) (Table 1; compiled from Summary of Virology, pg. 12, and references therein).

Table 1. BCV EC_{50} values and selectivity indices against different orthopoxviruses.

Virus	Isolate/Strain	Cells	EC_{50} value (µM)	CC_{50} value (µM)	SI	Reference
Variola virus	BSH74	BSC-40	0.21[1]	15	71	Olson et al., 2014
	SOM77	BSC-40	0.077[1]	15	195	Olson et al., 2014
	JAP51	BSC-40	0.11[1]	15	136	Olson et al., 2014
	UNK52	BSC-40	0.05[1]	15	300	Olson et al., 2014
	BRZ66	BSC-40	0.11[1]	15	136	Olson et al., 2014
	BSH	Vero 76	0.1	ND	ND	Huggins et al., 2002[2]
	BSH	MK2	0.04	ND	ND	Huggins et al., 2002[2]
Variola virus range			**0.04-0.21**			
Rabbitpox virus	Utrecht	BSC-40	1.15[1]	>10	>9	CMX001-VIR-107
	Utrecht	Vero 76	1.05	ND	ND	CMX001-VIR-069
Rabbitpox virus range			**0.5-1.89[3]**			
Ectromelia virus	Moscow	BSC-40	0.33[1]	>10	>30	CMX001-VIR-107
	Moscow	CV-1	0.5	ND	ND	Buller et al., 2004
	Moscow	BSC-1	0.125	25.3	202	Ruiz et al., 2011
Ectromelia virus range			**0.125-0.5**			
Vaccinia virus	WR	CV-1	0.7	ND	ND	Buller et al., 2004
	WR	HEL	0.007	≥2.1	≥300	Duraffour et al., 2013
	WR	HEL	0.013	≥2.4	≥180	(b) (3) (A), (b) (3) (B)
	WR	HFF	0.13	42	323	Quenelle et al., 2007
	WR	Vero	0.4	ND	ND	(b) (3) (A), (b) (3) (B)
	WR	Vero	0.24	ND	ND	
	WR	C127I	0.31	ND	ND	
	WR	HFF	1.1	31	28	Kern et al., 2002
	Copenhagen	HEL	0.004	≥2.1	≥525	Duraffour et al., 2013
	Copenhagen	HEL	0.005	≥2.4	≥467	(b) (3) (A), (b) (3) (B)
	Copenhagen	HFF	0.08	25	313	Ruiz et al., 2011
	Copenhagen	HFF	0.6	29	48	Keith et al., 2004
	Copenhagen	HFF	0.8	31	37	Kern et al., 2002
	Copenhagen	HFF	0.14	42	300	Quenelle et al., 2007
	Lister	HEL	0.094	≥2.1	≥22	Duraffour et al., 2013
	Lister	HEL	0.023	≥2.4	≥106	(b) (3) (A), (b) (3) (B)
	Elstree	HFF	1.2	31	26	Kern et al., 2002
	IHD	HFF	0.2	31	155	Kern et al., 2002
	NYC Pres. NYCDOHL)	HFF	0.4	31	78	Kern et al., 2002
	Lederle-Ch.	HEL	0.022	0.6	28	Lebeau et al., 2006
	Lederle-Ch.	PHK	0.8	0.3	0.4	Lebeau et al., 2006
Vaccinia virus range			**0.004-1.2**			
Cowpox virus	Brighton	HFF	0.2	25	125	Ruiz et al., 2011
	Brighton	HEL	0.021	≥2.4	≥114	(b) (3) (A), (b) (3) (B)
	Brighton	HFF	0.5	29	58	Keith et al., 2004
	Brighton	HFF	0.6	31	50	Kern et al., 2002
	Brighton	HFF	0.24	42	175	Quenelle et al., 2007
	Brighton	HEL	0.06	0.6	11	Lebeau et al., 2006
	Brighton	PHK	0.5	0.3	0.6	Lebeau et al., 2006
	5 different isolates	HEL	0.007-0.03	≥2.1	≥71	Duraffour et al., 2013
	ND	ND	ND	ND	ND	Huggins et al., 2002[2]
Cowpox virus range			**0.007-0.6**			
Camelpox virus	Iran (CML1)	HEL	0.021	≥2.1	≥100	Duraffour et al., 2013
	Iran (CML1)	HEL	0.024	≥2.4	≥100	(b) (3) (A), (b) (3) (B)
Camelpox virus range			**0.021-0.024**			
Monkeypox virus	ND (n=2)	ND	0.023-0.12	ND	ND	Huggins et al., 2002[2]
VACV/RPXV chimera	VACV-WR/RPXV E9L	BSC-40	1.75	>10	>6	CMX001-VIR-107

[1]RPXV and ECTV studies followed methods by Olson et al., 2014 to facilitate direct comparisons of EC_{50} values.
[2]Meeting abstract, limited details available.
[3]Range includes data from a M. Prichard communication, and from slightly different assay conditions.
ND, no data or not reported; SI, selectivity index: CC_{50}/EC_{50}, showing results reported in reference, calculated if not in reference.

(b) (3) (A), (b) (3) (B)

[redacted]

For optimal comparison of BCV antiviral activity against RPXV, ECTV and VARV, the sponsor evaluated BCV against RPXV and ECTV following the same assay conditions reported by Olson et al., 2014 (conducted by David Evans, Ph.D.; report CMX001-VIR-107). Under these consistent assay conditions, BCV EC_{50} values were 1.15 µM, 0.33 µM, and 0.11 for RPXV, ECTV, and VARV (median) respectively.

CDV had ~100-fold reduced activity against VARV compared to BCV (Table 2). The published study by Baker et al., 2003 evaluated CDV activity against a panel of 35 geographically and temporally diverse VARV isolates, and the results confirm that CDV, and presumably BCV since it shares the same active metabolite, has consistent activity against known VARV isolates with a ~5.6-fold range in reported EC_{50} values.

(b) (3) (A), (b) (3) (B)

[redacted]

Table 2. Cidofovir EC_{50} values against variola virus.

Virus	Isolate/Strain	Cells	EC_{50} value (µM)	Reference
Variola virus	BSH74	BSC-40	6.07	Olson et al., 2014
	SOM77	BSC-40	1.37	Olson et al., 2014
	JAP51	BSC-40	10.81	Olson et al., 2014
	UNK52	BSC-40	7.08	Olson et al., 2014
	BRZ66	BSC-40	28.45	Olson et al., 2014
	35 different isolates	Vero	38 (mean), range: 16-89	Baker et al., 2003

BCV has reported cell culture antiviral activity against a variety of other DNA viruses beyond orthopoxviruses, with variable selectivity indices (SIs) (Table 3; adapted from Summary of Virology, pgs. 16-17). BCV activity against these viruses is generally attributed to activity against viral DNA polymerases, although this cannot always be the case as polyomaviruses and papillomaviruses do not encode their own DNA polymerases. The sponsor speculates that antiviral activity against these viruses may be due to specific inhibition of viral DNA synthesis arising from effects on the viral T antigen, selective anti-proliferative effects on infected cells, or a combination of these or other mechanisms. In addition, modest antiviral activity has been reported against certain RNA viruses and retroviruses (Table 3), again consistent with a nonspecific or off-target effect, or an alternative mechanism of action. In the case of Ebola virus, it has been reported that the lipid moiety of BCV, and not the active triphosphate metabolite, contributes to its activity against Ebola virus in cell culture (McMullan et al., 2016). The sponsor noted that no BCV activity (SI <10) has been demonstrated against most RNA virus families, including influenza A virus, respiratory syncytial virus, Rift Valley fever virus, Tacaribe virus, Venezuelan equine encephalitis virus, dengue virus, West Nile virus, yellow fever virus, enterovirus 71, poliovirus, and SARS-Coronavirus. None of these data were independently reviewed as this review is focused on the proposed smallpox indication.

Table 3. BCV antiviral activity against other viruses outside of the orthopoxvirus genus.

Viral Family	Virus/Genome Type	BCV EC_{50} value (μM)				SI (Approx.)
		Mean	Median	Range	n	
Adenoviridae	AdV/dsDNA	0.03	0.008	0.004-0.28	23	1,200
Herpesviridae	CMV/dsDNA	0.0011	0.00045	0.000002-0.030	102	40,000
	EBV/dsDNA	0.03	0.03	0.020-0.040	3	660
	HHV-6/dsDNA	0.005	0.005	0.003-0.007	2	40
	HHV-8/dsDNA	0.02	0.02	0.02-0.02	1	24
	HSV-1/dsDNA	0.016	0.01	0.008-0.060	8	3,400
	HSV-2/dsDNA	0.03	0.023	0.009-0.080	9	1,000
	VZV/dsDNA	0.0052	0.0052	0.0004-0.01	2	870
Papillomaviridae	HPV-11/dsDNA	17	17	17-17	1	5
Polyomaviridae	BKV/dsDNA	0.034	0.018	0.001-0.27	26	22
	JCV/dsDNA	0.04	0.028	0.0055-0.10	4	1,000
Poxviridae	ORF/dsDNA	0.044	0.0098	0.0004-0.18	10	600
Hepadnaviridae	HBV/(+)ssRNA cccDNA provirus	1.84	1.66	0.30-3.6	3	10
Caliciviridae	HNV/(+)ssRNA	12.4	4.8	4.8-20	2	>20
Rhabdoviridae	VSV/(-)ssRNA	4.34	4.34	2.56-6.12	2	>20
Filoviridae	EBOV/(-)ssRNA	1.48	0.96	0.17-8.05	16	1 to 133
Flaviviridae	HCV/(+)ssRNA	0.64	0.64	0.4-0.89	2	20 to 35
Retroviridae	HIV/(+)ssRNA DNA provirus	0.03	0.03	0.03-0.03	1	10

Abbreviations: AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; dsDNA, double-stranded DNA; EBOV, Ebola virus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus genotype 1b; HHV-6 (or -8), human herpesvirus 6 (or 8); HIV, human immunodeficiency virus; HNV, human norovirus; HPV-11, human papilloma virus 11; HSV-1 (or -2), herpes simplex virus 1 (or 2); JCV, JC virus; ORF, Orf parapoxvirus; rt, reverse transcribed; SI, selectivity index; ssRNA, single-stranded RNA; VSV, vesicular stomatitis virus; varicella-zoster virus

Cytotoxicity

In the conditions used for orthopoxvirus antiviral activity assessments, BCV was generally cytotoxic at low micromolar levels across a variety of different cell types (Table 1). In the study by Olson et al., 2014, the CC_{50} value of BCV for BSC-40 (African green monkey kidney) cells after 3 days of exposure was approximately 15 μM, reflecting an average SI of ~136. The SIs for RPXV and ECTV under similar conditions were lower at >9 and >30, respectively, due to the relatively higher EC_{50} values against these viruses compared to VARV.

The reported K_i values of CDV-PP toward human DNA polymerases α, β, and γ are 51, 520, and 299 μM, respectively (Ho et al., 1992; Cherrington et al., 1994). The sponsor noted that K_i values are not available for CDV-PP against orthopoxvirus DNA polymerases, precluding an assessment of specificity, but sub- to low micromolar K_i values have been reported for herpesvirus DNA polymerases.

Neither BCV nor CDV appeared to have selective mitochondrial toxicity in HepG2 cells based on a MitoBiogenesis In-Cell ELISA assay (Report CMX001-VIR-113). This assay simultaneously measures the levels of two different mitochondrial proteins, one encoded by mitochondrial DNA (subunit I of Complex IV, cytochrome c oxidase I, COX-I), and the other encoded by nuclear DNA (70 kDa subunit of Complex II, succinate dehydrogenase complex, subunit A, SDH-A). Across a range of drug concentrations, the ratio of COX-I:SDH-A is assessed to determine if the drug has a specific impact on COX-I levels, which would indicate specific mitochondrial toxicity. The sponsor found the COX-I:SDH-A ratio remained comparable to that of untreated cells across all BCV and CDV concentrations evaluated, up to 10 μM and 100 μM, respectively, after 7 days of exposure. In contrast, dideoxycytidine caused a ≥50% reduction in COX-I:SDH-A ratio at a concentration of ≥0.21 μM. However, general cytotoxicity was observed with both BCV and CDV, with CC_{50} values of 0.74 μM and 22.9 μM, respectively. Inclusion of 40 mg/mL human serum albumin in cell culture media reduced BCV cytotoxicity (CC_{50} value >10 μM).

Effect of Human Serum Proteins on Antiviral Activity

Based on equilibrium dialysis methods, BCV is >98% protein bound in Sprague Dawley rat, New Zealand white rabbit, beagle dog, mini pig, cynomolgus monkey, and human plasma (reports CMX001-NCA-006, CMX-NCA-108).

Consistent with its serum protein binding, BCV cell culture antiviral activity was reduced substantially in the presence of increasing amounts of serum or serum proteins (Table 4 and Table 5; Summary of Virology, pg. 15, details in report CMX001-CBI-003). Human serum appeared to have a greater impact on BCV antiviral activity compared to fetal bovine serum (FBS), although the results are challenging to interpret given the different magnitude of effects for VACV versus CPXV. A precise fold-change impact of human serum protein binding on BCV antiviral activity cannot be determined from these data, but it appears that protein binding likely increases the cell culture EC_{50} value by at least 50-fold. CDV antiviral activity was not clearly impacted by serum protein except at the highest human serum concentration tested.

Table 4. Effect of human serum and fetal bovine serum on cell culture antiviral activity of BCV and CDV against vaccinia virus and cowpox virus.

	EC_{50}, µM (fold change from 2% FBS)			
	BCV		CDV	
Serum	Vaccinia Virus	Cowpox Virus	Vaccinia Virus	Cowpox Virus
2% FBS	0.012	0.05	2.3	7.5
2% Human serum	0.04 (3.3)	0.19 (3.8)	0.8 (0.3)	5.7 (0.8)
10% Human serum	0.19 (15.8)	0.39 (7.8)	2.1 (0.9)	2.8 (0.4)
25% Human serum	>2 (>167)	1.6 (32)	>317 (>138)	75.2 (10)

EC_{50} = half maximal effective (drug) concentration; BCV = brincidofovir; CDV = cidofovir

Table 5. Effect of human α-1-acid glycoprotein and fetal bovine serum on cell culture antiviral activity of BCV and CDV against vaccinia virus and cowpox virus.

	EC_{50}, µM (fold change from 2% FBS)			
	BCV		CDV	
FBS Concentration	No AGP	1 mg/mL AGP	No AGP	1 mg/mL AGP
Vaccinia Virus				
2% FBS	0.005	0.04 (8)	4.8	4.8 (1)
10% FBS	0.027 (5.4)	0.038 (7.6)	5.7 (1.2)	6.7 (1.4)
25% FBS	0.3 (60)	0.25 (50)	3.5 (0.7)	2.9 (0.6)
Cowpox Virus				
2% FBS	0.01	0.04 (4)	5.7	5.4 (0.9)
10% FBS	0.18 (18)	0.28 (28)	ND	ND
25% FBS	0.4 (40)	0.81 (81)	5.1 (0.9)	6.3 (1.1)

ND = Not determined, EC_{50} = half maximal effective (drug) concentration; FBS = fetal bovine serum; AGP = α-1-acid glycoprotein

2.3 (b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

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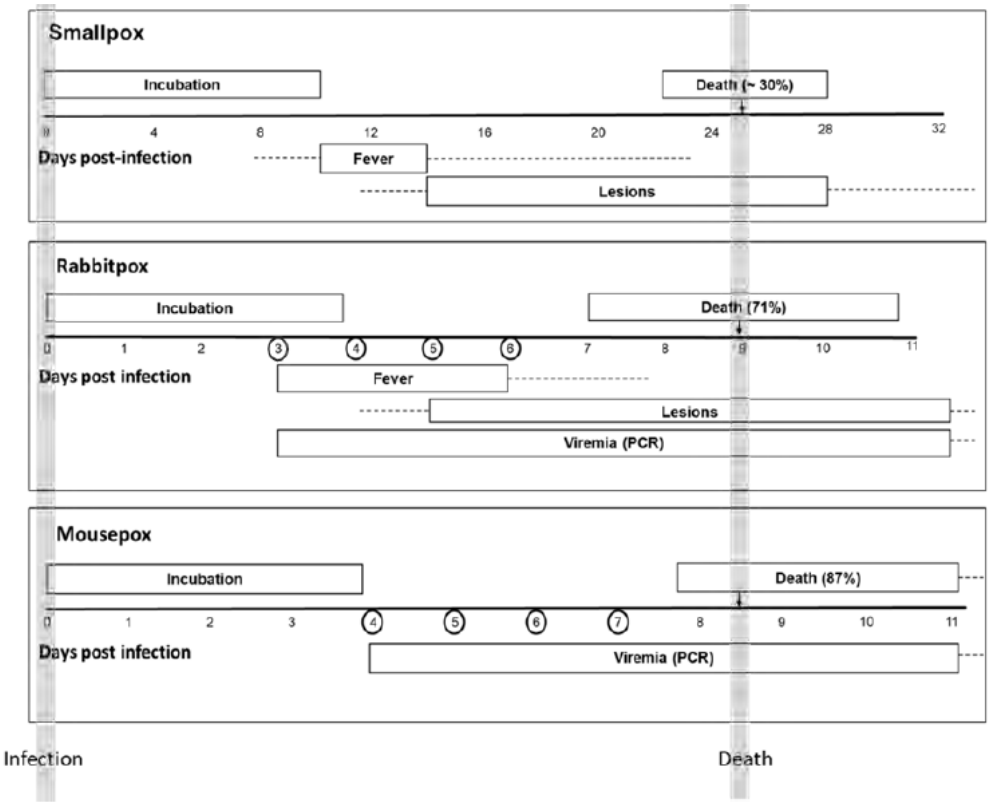
3. "REGISTRATIONAL" ANIMAL MODEL STUDIES

3.1 Orthopoxvirus Animal Models Used to Establish Brincidofovir Efficacy

As smallpox has been eradicated and VARV does not replicate in other animals, related orthopoxviruses were used as surrogates to evaluate BCV efficacy in animals to support BCV approval under the Animal Rule. The NDA package includes a white paper document titled, "Brincidofovir for the Treatment of Smallpox: Animal Model Justification," which details the sponsor's use and justification of the

rabbit/RPXV and mouse/ECTV animal models. Key elements from this document are summarized briefly in this review section. In general, we agree with the sponsor's perspectives on the use of the rabbit/RPXV and mouse/ECTV animal models for the purpose of evaluating BCV efficacy.

Figure 1 (Report pg. 49) illustrates the time course of progression of disease in the rabbit/RPXV and mouse/ECTV animal models relative to human smallpox, and Table 8 (adapted from Report pgs. 51-53) summarizes the key features of these models. In general, the disease course is compressed in these animal models relative to human smallpox. Note that poor pharmacokinetics of BCV in cynomolgus macaques did not allow for BCV to be evaluated using a macaque/monkeypox virus model. Additional specific features for each of the animal models, including details on the viral challenge strains, are summarized below.



Circled days indicate the day post-inoculation (PID) that BCV treatment was initiated in the pivotal rabbit and mouse studies.
↓ = median day of death (for animals that died) for each species
Plots are aligned for day of infection and day of death within each species.
Smallpox: fever indicates the day of initial occurrence of fever (typical range 10-14 days, median Day 12) with dotted line indicating continued variably elevated temperature. Death indicates a typical range of 22-28 days post-infection, with an arrow at the midpoint. Lesions indicate the typical timeframe of lesion development; following Day 28, the dotted line indicates scab formation and eventual resolution.
Rabbitpox: fever indicates the typical range of first observation (from Study CMX001-VIR-106). Death indicates the timeframe of death (and percentage) in pivotal study CMX001-VIR-106, along with the median day of deat of the animals that died indicated by an arrow. Lesions indicate the timeframe of typical lesion development, at first pinprick lesions, subsequently becoming more prominent and eventually resolving. Viremia indicates the detection of viral DNA by PCR in Study CMX001-VIR-106 with peak at Day 6 and dotted line indicates continued detection of viral DNA.
Mousepox: death indicates the timeframe of death (and percentage) in pivotal study CMX001-VIR-044, along with the median day of death of the animals that died indicated by an arrow. One mouse survived to Day 21 an is indicated by the dotted line. Viremia indicates the detection of viral DNA by PCR in Study CMX001-VIR-044 (100% positive on Day 4) with dotted line indicating continued detection of viral DNA. Lesions are typically not observed in sensitive mouse strains such as BALB/c.

Figure 1. Disease progression in rabbit/RPXV and mouse/ECTV models and human smallpox.

Table 8. Summary of rabbit/RPXV and mouse/ECTV animal models relative to human smallpox.

	NZW Rabbit	BALB/c Mouse	Human (Historical Information)
Characteristics of the Etiologic or Challenge Agent			
Agent	Rabbitpox virus (RPXV) (Utrecht)	Ectromelia virus (ETCV) (Moscow)	Variola virus
BCV EC_{50} value	1.15 ± 0.11 μM	0.33 ± 0.05 μM	0.11 ± 0.06 μM
Patho-physiological mechanisms of toxicity or virulence	RPXV replicates at the inoculation site and spreads to the local lymph nodes. An asymptomatic viremia then spreads the infection to the lymphoid organs. Upon replication in the lymphoid organs, particularly the spleen and liver, a secondary viremia is thought to further disseminate the infection. The respiratory tract is a key site of replication.	ECTV spreads from the site of infection to the regional lymph nodes. The primary viremia spreads via infected macrophages, especially to the phagocytic cells of the spleen and liver. The virus then replicates extensively in the parenchymal cells of the liver and spleen, resulting in a secondary cell-associated viremia that further disseminates the infection.	VARV spreads from the site of infection (mucosal epithelium of the respiratory tract) to the regional lymph nodes. Viral replication produces a primary viremia, resulting in infection of cells of the reticuloendothelial system including those in the lymph nodes, spleen, liver, and bone marrow. Viral replication in these tissues produces a secondary cell-associated viremia that further disseminates the infection.
Route of exposure	Intradermal challenge in all studies. Animal to animal transmission via inhalation of aerosols from infected rabbits in nature (Adams 2007).	Intranasal challenge in all studies. Animal to animal transmission via skin abrasions in nature (Buller 2004a; Buller 2004b).	Inhalation (via droplets of viral particles onto the mucosal surfaces of the respiratory tract (Fenner 1988).
Dose and quantification of exposure	Inoculums administered: 3-1000 PFU Mortality: 52% to 100% (VIR-033, VIR-038, VIR-039, VIR-041); 71% at 600 PFU (VIR-106)	Inoculums administered: 5-1000 PFU Mortality: 87.5% to 100% at 200 PFU (VIR-044, VIR-102, VIR-109)	Humans cannot be experimentally challenged to determine a lethal dose. Natural exposure is not quantifiable, thus only estimates are available. The infective dose is believed to be very small (e.g., 10 to 100 PFU). Mortality rate of variola major often cited as approximately 30% (WHO).
Host susceptibility and response	New Zealand white (NZW) rabbits are naturally susceptible to lethal infection when exposed to RPXV.	BALB/c mice are naturally susceptible to lethal infection when exposed to ECTV.	Humans are naturally susceptible to infection (with risk of mortality) when exposed to variola virus.
Natural History of the Disease or Condition – Pathophysiological Comparability			
Time to onset and time course of progression	Viral DNA detectable in the blood of most animals by ~3 days post-challenge (VIR-106). First sign of systemic disease (fever) appears ~3-4 days post-challenge. Lesions appear on mucocutaneous sites ~4-5 days post-infection. Respiratory distress is evident after 6 to 8 days. Mean time to death in natural history and efficacy studies was 6.9 to 9.8 days post-challenge.	Viral DNA is detectable in the blood, spleen, or liver in most animals ~3 days post-challenge, and 100% of mice at 4 days post-challenge (VIR-111, VIR-044). First sign of systemic disease ~5.5-6.5 days post-challenge. Clinical signs of disease, if present, may begin to appear ~5.5-6.5 days post-challenge. Median time to death in natural history studies was 8.2 to 9 days post-challenge.	First signs of systemic disease (fever, head and body aches, malaise, and prostration) appear ~10-14 days after exposure, with a median incubation time of 12 days. Initial symptoms last ~2-4 days. Rash first appears 1-2 days later, then progresses to papular, vesicular, and pustular lesions over several days; fever may recur. Pustules scab over and gradually desquamate after 14-21 days. Death in fatal cases generally occurs ~10-16 days after onset of fever, which is about 22-28 days after exposure (CDC).
Disease manifestations	Fever, weight loss, and necrosis of the site of infection. The appearance of secondary lesions is followed by respiratory distress (profuse mucopurulent discharge from the nostrils and slow, labored breathing). Death in natural history studies was generally euthanasia due to respiratory distress.	Weight loss (or failure to gain weight/thrive in young mice), lethargy, ruffled fur, and hunched posture. Lesions may or may not be present. Major body systems involved include liver, spleen, skin, respiratory, and lymphoid. Death attributed to liver and spleen necrosis.	Fever, headache, malaise, backache, and prostration. Lesions, appearing as minute red dots, appear on the tongue and palate, then a macular rash begins to develop on the skin, which evolves into papules, vesicles, and then pustules before scabbing over. Deep pitted scars (pockmarks) remain for 65% to 85% of survivors. Death results from massive inflammatory response causing shock and multiple organ failure.
Trigger for intervention	A clinical trigger for intervention may be onset of fever (>40°C) (VIR-041). A set time period post-challenge was used to initiate treatment in the primary rabbit study (VIR-106): Treatment began on Post-Challenge Day 3, 4, 5, or 6 to cover full range of conditions under which treatment may be used in the clinical setting of a smallpox outbreak. Viremia was detectable prior to treatment start.	Clinical triggers are not reliable and occur late in the disease course, making them unsuitable as a trigger for intervention. A set time period post-challenge was used to initiate treatment in the primary mouse study (VIR-044): Treatment began on Post-Challenge Day 4, 5, 6, or 7 to cover full range of conditions under which treatment may be used in the clinical setting of a smallpox outbreak. Viremia was detectable prior to treatment start.	Medical and epidemiologic history of exposure; onset of clinical signs and symptoms (e.g., fever, lesions). In a future outbreak, initial diagnosis may be made by detection of virus in blood or respiratory secretions via PCR.

Rabbit/Rabbitpox Virus Model
The rabbit/RPXV model employs intradermal inoculation of RPXV, resulting in a disease course with some characteristics similar to human smallpox, including an incubation/viral replication phase, fever, lesions, and a high mortality rate.

For the sponsor's key RPXV studies, including pivotal efficacy study CMX001-VIR-106, the sponsor used the RPXV Utrecht challenge strain, Lot 050310-ALS. This virus was originally isolated in the 1940s and was obtained in 1980 by Dr. Richard Moyer at the University of Florida (b) (4) Dr. Moyer's laboratory plaque purified this virus (b) (4) this stock is referred to as Master stock Lot RPV93B. To produce the Lot 050310-ALS stock suitable for use in GLP studies, Dr. Moyer provided the RPV39B stock to (b) (4) which produced a subsequent working stock under GLP conditions (b) (4) (detailed in Report CMX001-SRI-001). The resulting working stock of virus was evaluated by testing for titer, DNA sequence, sterility, pH, mycoplasma, and endotoxin, and met all of the release criteria. RPXV Lot 050310-ALS was subsequently transferred to (b) (4) and used directly in Chimerix studies.

There has been some variability of mortality results across several RPXV studies (Table 9; Report pg. 26). The 50% lethal dose (LD_{50}) of RPXV was estimated to be <10 plaque-forming units (PFU) in 9-week-old NZW rabbits (Studies 2817-2818, summarized in Appendix A). The infection tended to be less severe in 6-month-old animals although they still developed disease with a high mortality rate when challenged with 1,000 PFU (Adams et al., 2007). Rabbits of ~9 weeks of age were used in some early studies, but during development the supplier of the 9-week-old rabbits (Myrtle's Rabbitry) was purchased by (b) (4) and the Myrtle's lineage was later discontinued. According to the sponsor, the change in rabbit lineage, and associated changes in animal husbandry, weaning, and shipping practices between vendors, resulted in rabbits that had much lower body weights at the time of receipt and appeared highly susceptible to the stress of shipping (evidenced by diarrhea and dehydration, continued poor health after receipt, and unexpected mortality). In response, Chimerix (in consultation with BARDA and (b) (4)) added weight specifications for (b) (4) rabbits in future studies to align with mean body weights of Myrtle's rabbits that were used in previous studies. Adding weight specifications to ensure consistency and comparability of data generated in the RPXV model also necessitated the use of slightly older rabbits (13 to 16 weeks versus 9 weeks) in the later studies. Across BARDA studies 2819 and 2820 (Appendix A), similar mortality was observed in 9-week-old and 6-month-old rabbits infected with target inocula of 300 PFU (back titer of 230 PFU) and 1000 PFU (back titer of 734 PFU), respectively.

Key study design issues (including animal age, virus related issues [including, but not limited to, strain, stock, challenge dose]) were discussed by the Applicant and the Agency and consensus was reached before the pivotal efficacy study, CMX001-VIR-106, was conducted. Animals were challenged with a target RPXV dose of 600 PFU.

Table 9. RPXV lethality across studies.

Parameter or Assessment	RPXV Model Studies					Efficacy Studies (Placebo Group)		
	2819	2820	2923	CMX001-VIR-033	CMX001-VIR-038	CMX001-VIR-039	CMX001-VIR-041	CMX001-VIR-106
Viral Stock	RPXV-(b) (4)-01	RPXV-(b) (4)-01	RPXV-(b) (4)01	050310-ALS	050310-ALS	050310-ALS	050310-ALS	050310-ALS
Rabbit lineage	(b) (4)							
Rabbit age	9 weeks	6 months	9 weeks	9 weeks	9 weeks	9 weeks	13-16 wks	13-16 wks
Inoculum Target PFU	300	1000	300	300	300	300	300	600
Mortality – n/N	32/32 (100%)	12/12 (100%)	8/8 (100%)	8/8 (100%)	8/8 (100%)	12/16 (75%)	15/29 (52%)	20/28 (71%)
MTD – days PI	7.8 days	7.7 days	6.9 days	9.7 days	8.3 days	7.9 days	9.8 days	9.4 days
First qPCR detected in blood – hours PI	48 h	36 h	48 h	ND	48 h	ND	24 h	ND

Abbreviations: h=hours; MTD=median time to death; ND=not done; PI=post infection/inoculation; qPCR=quantitative polymerase chain reaction

RPXV natural history studies identified the onset of fever and visualization of the earliest detectable secondary lesions as potential triggers for treatment for the purpose of modeling treatment of symptomatic smallpox. The initial efficacy studies used the first appearance of secondary lesions as the trigger to begin treatment (CMX001-VIR-039) or used onset of fever to begin treatment immediately or to delay treatment for 24, 48, or 72 hours following fever onset (CMX001-VIR-041). The pivotal efficacy study (CMX001-VIR-106) was designed with treatment initiation beginning at set time points after inoculation (Post-challenge Day 3, 4, 5, or 6, depending on treatment group) to model different conditions under which the drug may be used in the clinical setting of a smallpox outbreak. Initiation of treatment at fixed intervals relative to viral inoculation also decreased study complexity for lab technicians. Note that in the pivotal RPXV study for tecovirimat (SR14-008F), animals were treated starting on Day 4 post-challenge, based on the consistent development of fever by this timepoint.

Mouse/Ectromelia Virus

In the mouse/ECTV model, BALB/c mice are inoculated intranasally with the ECTV-Moscow strain resulting in high rates of mortality with low challenge doses. ECTV spreads from the site of infection to regional lymph nodes where it produces a primary viremia, and then spreads to the spleen and liver. In this model there are no clear, objective disease triggers for treatment as animals generally do not show overt disease signs until late in the infection when they become moribund. Viral DNA is consistently detected in blood, liver and spleen by Day 3.5, which the sponsor uses as a reference for treatment initiation. ECTV pathogenesis in mice is affected by mouse strain and inoculation route. According to the sponsor, the BALB/c strain is more sensitive to ECTV than C57BL/6, with published intranasal LD_{50} values ranging from 1 to 5 PFU for BALB/c mice (Paran et al., 2013; Xiao et al., 2007).

Early studies conducted by (b) (4) (studies 2955, 2956, 2957) showed a ~200 PFU ECTV challenge in 6- to 8-week-old mice resulted in 98% (41/42) lethality. To provide an ECTV viral stock suitable for use in GLP studies, a 3x plaque purified early passage sample of ECTV-Moscow was provided by (b) (4) (b) (4) and used directly to prepare a working stock of virus under GLP guidelines (study CMX001-VIR-050). Briefly, the virus was propagated in L929 mouse cells with a low MOI of 0.01, infected cells were collected and homogenized, and the cell lysates were sonicated and purified through a sucrose cushion. This ECTV stock, designated CMRX-3571 (also referred to as 032516-ECTV), was used in subsequent

GLP studies. Natural history and LD_{50} studies confirmed the high lethality of this stock at low challenge doses, as well as the late development of disease signs (Appendix A).

Table 10 (Report pg. 38) summarizes mortality and virology results across several studies of the mouse/ECTV model. In all of these studies, ECTV-Moscow was administered to BALB/c mice (aged 6-8 weeks across studies) by intranasal inoculation. These studies show relatively consistent, high lethality in the model with a target challenge dose of ~200 PFU. Study VIR-111 is the key GLP natural history study (summarized in Appendix A). Study VIR-044 is the pivotal efficacy study and key results are summarized below.

Table 10. ECTV lethality across different studies.

Parameter or Assessment	Mousepox Natural History Studies				Efficacy Studies (Placebo Groups)	
	2956	2957	CMX000-VIR-109	CMX000-VIR-111	CMX001-VIR-102	CMX001-VIR-044
Viral Stock	BARDA 2954	BARDA 2954	CMRX 3571	CMRX 3571	(b) (4) MOS-3	CMRX 3571
Inoculum Target PFU	200	200	200	200	270	200
Laboratory	(b) (4)					
Mortality – n/N (%)	8/8 (100%)	18/18 (100%)	18/18 (100%)	13/16 (81%)	10/10 (100%)	28/32 (87.5%)
MTD – hours PI	215 h (8.9 d)	197 h (8.2 d)	203 h (8.5 d)	206 h (8.6 d)	264 h (11 d)	206 h (8.6 d)
Peak Mean Blood Viral Load – hour PI	180 h	216 h	N/A	216 h	N/A	216 h[a]
Time of First qPCR Detection[b] – hours (day) post-infection						
Spleen	60 h (PID 2.5)	72 h (PID 3)	N/A	48 h[c] (PID 2)	N/A	48 h[d] (PID 2)
Liver	60 h (PID 2.5)	72 h (PID 3)	N/A	60 h[c] (PID 2.5)	N/A	48 h[d] (PID 2)
Blood	84 h (PID 3.5)	96 h (PID 4)	N/A	60 h[c] (PID 2.5)	N/A	48 h[d] (PID 2)

Abbreviations: h=hours; MTD=median time to death; N/A=not available; PFU=plaque-forming units; PI=post-infection/inoculation; PID=post-infection/inoculation day; qPCR=quantitative polymerase chain reaction; (b) (4)

[a] Across all groups.
[b] Studies CMX000-VIR-111 and CMX001-VIR-044 used a different qPCR assay than the earlier studies (2956 and 2957).
[c] 100% of mice tested positive at 84 hours (PID 3.5) and later.
[d] 100% of mice in the untreated group (viral load assessment Group 9) tested positive on PID 4 and later.

3.2 Study CMX001-VIR-106 (Rabbits/Rabbitpox Virus)

Title
CMX001-VIR-106, "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"

Summary of Design

Study CMX001-VIR-106 was a randomized, blinded, placebo-controlled study of orally administered BCV in the rabbit/RPXV model, conducted at (b) (4)
Treatment assignment was blinded for the Sponsor (with the exception of a member of the Sponsors' Quality Assurance Unit), Study Director, and staff who evaluated animals and their responses to make decisions about animal care and euthanasia. In addition, treatment assignment was blinded to virologists.

The primary objective of this study was to compare the survival benefit of BCV over placebo. The study treatment groups are summarized in Table 11 (Report pg. 17). New Zealand White rabbits from (b) (4) were shipped to the laboratory in five separate cohorts of 30 animals each (15 males/15 females in each shipment) with each cohort making up a different challenge day (Challenge Days A, B, C, D, and E).

Table 11. CMX001-VIR-106 (rabbit/RPXV) study design.

			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
Group	Planned Number of Animals	Actual Number of Animals	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

On the day of RPXV challenge, animals must have weighed 1.7-2.6 kg, been between 13 and 16 weeks (± 4 days) of age, and been free from obvious clinical signs of ill health or malformation, or any other condition that would, in the judgment of a veterinarian or the Study Director, interfere with the conduct of the study. Animals were quarantined for 7 days prior to study and were housed individually. A total of 6 animals were excluded from the study for reasons of "not being suitable for study per inclusion criteria" (n=3), died prior to challenge immediately after anesthesia (n=1), euthanized due to broken leg (n=1), or found dead (n=1).

On study Day 0, animals were challenged intradermally with a target of 600 PFU of RPXV strain Utrecht (lot 050310-ALS), diluted in calcium- and magnesium-free Dulbecco's Phosphate Buffered saline. Following preparation, challenge material was maintained on wet ice until use. The challenge dose (~200 µL) was divided in half and all animals were inoculated by bilateral intradermal injections in each thigh region (~100 µL in each thigh). The challenge dose was confirmed by back titration of remaining sample, which was initiated no more than 4 hours after the last challenge for each challenge day.

Starting on Day 3 (i.e., 3 days post-challenge), each animal received a total of 8 single consecutive daily oral administrations of a blinded dose regimen per assigned dosing kit. BCV-treated animals received an initial 20 mg/kg loading dose on Day 3, 4, 5 or 6, followed by two additional 5 mg/kg doses every other day. Weights collected on Study Day 2 were used for the dose calculations for treatments that occurred on Day 3 through Day 6, and weights collected on Study Day 6 were used for the dose calculations for treatments that occurred on Day 7 through Day 10.

Animals were observed clinically following the schedule in Table 12 (Report pg. 19). During the pre-challenge period, rabbits were implanted with temperature transponder chips for monitoring body temperature. Challenge sites were observed for erythema (redness) and edema (swelling) prior to challenge (Day 0) and once per day from Study Day 1 through Study Day 42. Secondary lesions were assessed on the head (ears, eyes, nose, mouth), genitals, back, and pads of paws; only the temperature chip implantation sites and the challenge sites were clipped of fur prior to implant and/or challenge.

Table 12. In-life clinical monitoring activities for study CMX001-VIR-106 (rabbit/RPXV).

<table>
<tr><th>Time-point (Study Days)</th><th>Observations</th><th>Body Temps</th><th>^Respiration Rates</th><th>Morbidity &Mortality Checks</th><th>Body Weight</th><th>Lesion Assessments / Challenge Site Monitoring</th></tr>
<tr><td>-7 through -3</td><td>2 X Day</td><td>NA</td><td>NA</td><td rowspan="4">NA</td><td>1 X Day on Days -7, -3</td><td rowspan="2">NA</td></tr>
<tr><td>-2</td><td>2 X Day</td><td>2 X Day</td><td>2 X Day</td><td>NA</td></tr>
<tr><td>-1</td><td>2 X Day</td><td>2 X Day</td><td>2 X Day</td><td rowspan="4">1 X Day</td></tr>
<tr><td>0 (Challenge)</td><td>2 X Day</td><td>1 X Day</td><td>1 X Day</td><td rowspan="3">1 X Day</td></tr>
<tr><td>1 through 3</td><td>2 X Day</td><td>1 X Day</td><td>1 X Day</td></tr>
<tr><td>4 through 11</td><td>0800 ±1 hr
1600 ±1 hr
0000 ±1 hr</td><td>0800 ±1 hr
1600 ±1 hr
0000 ±1 hr</td><td>0800 ±1 hr
1600 ±1 hr
0000 ±1 hr</td><td>0400 ±1 hr
1200 ±1 hr
2000 ±1 hr</td></tr>
<tr><td>12 through 42*</td><td>2 X Day</td><td>1 X Day</td><td>1 X Day</td><td>NA</td><td colspan="2">1 X Day</td></tr>
</table>

^ Respiration Rates were determined prior to conduct of other activities (see Appendix B, DR-B05007-(b) (4)0009, and DR-B05007-(b) (4)0031).
NA = Not Applicable
*Clinical observations were only performed once on Day 42.

Euthanasia criteria were based on meeting any of the following:

1. Severe respiratory distress as assessed by clinical observations or morbidity and moribundity checks including open mouth breathing and/or forced abdominal respirations.
2. Moribund, persistent prostration, seizures, and/or unresponsive to external stimuli (e.g., gentle prodding by hand).
3. Any animal(s) meeting at least two (2) of the following criteria:
    a. 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).
    b. Body temperature less than 37.2ºC from either chip (confirmed by a second temperature measurement 1 hr ± 10 min later).
    c. 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.

Animals requiring euthanasia were anesthetized and blood samples were obtained. All animals found dead or euthanized (with the exception of animals which were euthanized for not meeting the inclusion criteria) had a full gross necropsy examination. Sections of liver, spleen, and lung tissues were collected from all animals, split into three approximately equal sections (each ~1 cm cubed), flash-frozen in liquid nitrogen and stored at ≤ -70ºC.

Blood collections for virology analyses were collected on staggered schedules according to Challenge Day Cohort (Table 13; report pgs. 22 and 23). Blood samples were taken from a marginal ear vein or auricular artery, or cardiac puncture for terminal collections. In the event there were insufficient volumes obtained, the order of priority for the blood was as follows: treatment confirmation > qPCR > plaque assay > plaque reduction neutralization test (PRNT). Whole blood samples collected in K_3EDTA tubes

were processed for plaque assay and quantitative PCR assay targeting the RPXV hemagglutinin gene. PRNT assessments were conducted on serum samples. Plaque assays, qPCR and PRNT assessments were not conducted until after the completion of the in-life portion of the study.

Table 13. Blood collection schedule for CMX001-VIR-106 (rabbit/RPXV) Challenge Day Cohorts A, C and E (top) or B and D (bottom).

Time-point (Days)	Blood Collection Volume/Tube	PRNT Assay	qPCR	Plaque Assay	Treatment Confirmation Analysis (shipped offsite)
Day -1	~0.4 mL SST ~1.25 mL K_3EDTA	X	X	X	
Day 3	^ ~1.25 mL K_3EDTA # ~0.5 mL K_2EDTA		X	X	X
Day 4	^ ~1.25 mL K_3EDTA # ~0.5 mL K_2EDTA		X	X	X
Day 5	# ~0.5 mL K_2EDTA				X
Day 6	^ ~1.25 mL K_3EDTA # ~0.5 mL K_2EDTA		X	X	X
Day 8	^ ~1.25 mL K_3EDTA		X	X	
Day 10	^ ~1.25 mL K_3EDTA		X	X	
Day 12	~1.25 mL K_3EDTA		X	X	
Day 14	~1.25 mL K_3EDTA		X	X	
Day 21	~1.25 mL K_3EDTA		X	X	
Day 28	~1.25 mL K_3EDTA		X	X	
Day 35	~1.25 mL K_3EDTA		X	X	
Day 42	~0.4 mL SST ~4.0 mL K_3EDTA	X	X	X	
* Terminal (if possible)	~0.4 mL SST ~2.0 mL K_3EDTA	X	X	X	

Time-point (Days)	Blood Collection Volume/Tube	PRNT Assay	qPCR	Plaque Assay	Treatment Confirmation Analysis (shipped offsite)
Day -1	~0.4 mL SST ~1.25 mL K_3EDTA	X	X	X	
Day 3	^ ~1.25 mL K_3EDTA # ~0.5 mL K_2EDTA		X	X	X
Day 4	# ~0.5 mL K_2EDTA				X
Day 5	# ~0.5 mL K_2EDTA				X
Day 6	^ ~1.25 mL K_3EDTA # ~0.5 mL K_2EDTA		X	X	X
Day 8	^ ~1.25 mL K_3EDTA		X	X	
Day 9	^ ~1.25 mL K_3EDTA		X	X	
Day 11	~1.25 mL K_3EDTA		X	X	
Day 13	~1.25 mL K_3EDTA		X	X	
Day 15	~1.25 mL K_3EDTA		X	X	
Day 21	~1.25 mL K_3EDTA		X	X	
Day 28	~1.25 mL K_3EDTA		X	X	
Day 35	~1.25 mL K_3EDTA		X	X	
Day 42	~0.4 mL SST ~4.0 mL K_3EDTA	X	X	X	
* Terminal (if possible)	~0.4 mL SST ~2.0 mL K_3EDTA	X	X	X	

NOTE: The day of RPXV challenge in the report and protocol is Study Day 0. The day of challenge in the SEND datasets is Day 1 in compliance with SEND specifications. Accordingly, all study days starting with the day of challenge in the SEND dataset are 1 day later than the corresponding day in the protocol and study report. This reviewer's analyses followed the sponsor's convention in the study report/protocol, i.e., RPXV challenge was on Day 0.

Pre-treatment Disease Characteristics and Clinical Efficacy Results

The overall median RPXV challenge dose was 534 PFU/animal. The challenge dose in Cohort C (272 PFU/animal) was approximately half of the challenge dose of all other Cohorts. For each Challenge Cohort, animals were appropriately balanced across different treatment groups (Table 14; FDA analysis). The median age in each treatment group was 14 weeks.

Table 14. Challenge Cohorts and Treatment Groups in CMX001-VIR-106 (rabbit/RPXV).

Challenge Cohort	Challenge Date (Day 0)	Challenge Dose (PFU)	Number of Animals/Group				
			Group 1 (Day 3 Tx Start)	Group 2 (Day 4 Tx Start)	Group 3 (Day 5 Tx Start)	Group 4 (Day 6 Tx Start)	Group 5 (Control)
A	2018-10-15	562	6	6	6	5	6
B	2018-10-30	546	6	5	6	6	6
C	2018-11-09	272	6	6	5	6	6
D	2018-11-28	528	6	6	6	6	5
E	2018-12-07	534	5	6	6	6	5
All Cohorts		534 (median)	29	29	29	29	28

Survival results are summarized in Table 15 (FDA analysis) and Figure 2 (report pg. 33), and show a clear benefit with BCV treatment, with survival rates higher for groups that started BCV at earlier times post-challenge. Note that for one of the Group 5 control animals (B05007-52), there were discrepant results between two different datasets indicating whether the animal was euthanized or found dead. Nevertheless, this inconsistency does not impact the overall conclusions, and these results confirm (a) that the model is lethal in the absence of euthanasia, and (b) there is no major imbalance in the proportions of animals euthanized versus found dead across treatment groups.

Table 15. Overall survival results in study CMX001-VIR-106 (rabbit/RPXV).

Treatment Group	% Survival (n/N)	N Euthanized/Found Dead
Group 1 (Day 3 Tx Start)	100% (29/29)	0/0
Group 2 (Day 4 Tx Start)	90% (26/29)	3/0
Group 3 (Day 5 Tx Start)	69% (20/29)	6/3
Group 4 (Day 6 Tx Start)	69% (20/29)	6/3
Group 5 (Control)	29% (8/28)	12/8*

*One animal in Group 5 (B05007-52) noted as euthanized in CL dataset, but 'found dead' in DS dataset. In the table the animal was considered found dead, which matches the sponsor's numbers.

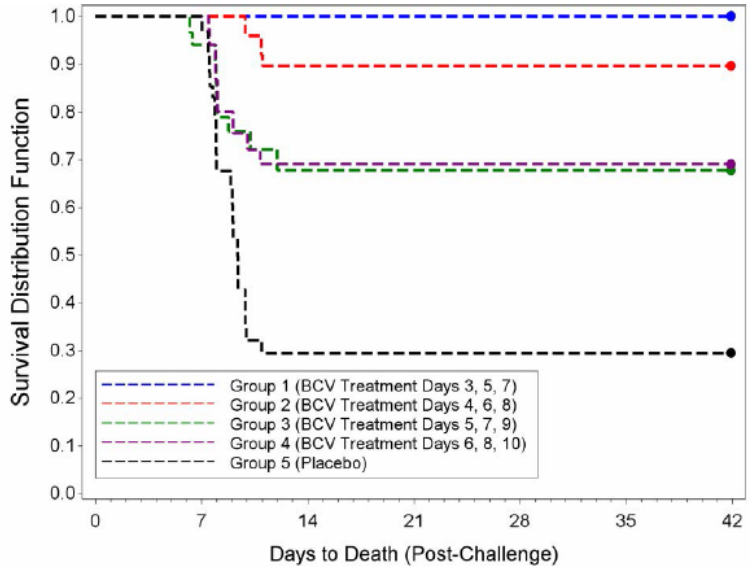


Figure 2. Kaplan-Meier plot of survival results in study CMX001-VIR-106 (rabbit/RPXV).

Survival results were also compared between Challenge Cohort C versus Cohorts A/B/D/E to assess the impact of the ~50% lower RPXV challenge dose in Cohort C. The numbers of animals for this comparison were small, and the available data did not show a consistent difference in survival rates between Challenge Cohort C versus Cohorts A/B/D/E across different treatment groups (FDA analysis, data not shown).

According to the sponsor, abnormal clinical observations were generally noted as early as Day 3 through Day 7 post-challenge and included stool abnormalities, nasal discharge, reduced food consumption, lacrimation, and lethargy. These clinical observations progressively worsened in most animals until they were either found dead/euthanized or recovered and survived to the end of the study. Overall, the sponsor concluded there did not appear to be a difference in the clinical observations noted between placebo (Group 5) and BCV treated groups (1, 2, 3, and 4). In animals that succumbed to RPXV infection, clinical observations progressed to include severe respiratory abnormalities prior to death.

The sponsor's analyses of body weight, body temperature and respiration rates are summarized in Figure 3 (Report pgs. 39-41), and generally did not show clear or consistent differences between treatment groups. The sponsor concluded there were no significant overall group effects for body weight. Significant increases in body temperatures were observed on Study Days 3 through 6 for all groups (sponsor's analyses), without a consistent pattern of changes throughout the study between different groups. Respiration rates trended lower in Group 5 (placebo) compared to most other groups around the peak of illness (Days ~7-14), although again there was not a consistent relationship between body weight and BCV treatment start day. The sponsor commented that RPXV lesions were noted as early as 2 days following challenge and resolved by Day 33 (generally most lesions noted between Study Days 5 and 20), and overall there did not appear to be a difference in the duration lesions were noted between groups.

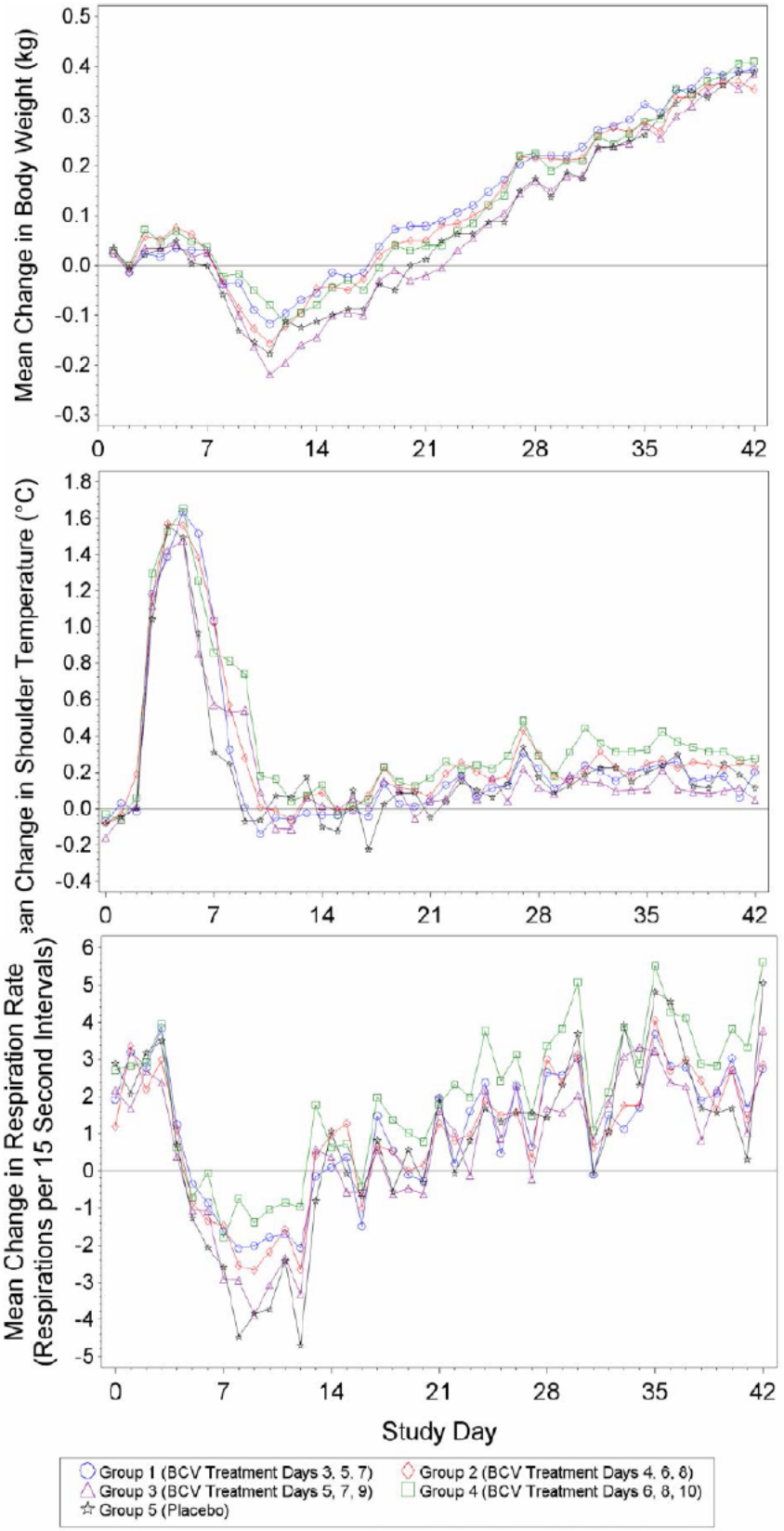


Figure 3. Sponsor's analyses of body weight, body temperature and respiration rate in study CMX001-VIR-106 (rabbit/RPXV).

An independent analysis of body temperature data was conducted to confirm the timing of development of fever in animals, defined as a ≥1.5˚C increase in temperature from baseline measured either in the rump/hip or shoulder/back regions (Table 16; FDA analysis). By Study Day 3, 50% (72/144) of all animals had a fever detected, while by Day 4, 98% (141/144) of animals had a fever detected.

Table 16. Timing of first detection of fever in study CMX001-VIR-106.

	Number of Animals with First Fever Detected (≥1.5˚C increase in temperature from baseline in rump/hip or shoulder/back)			
Arm	**Study Day 2**	**Study Day 3**	**Study Day 4**	**Study Day 5**
Group 1 (Day 3 Tx Start)	1	16	11	1
Group 2 (Day 4 Tx Start)	0	14	15	0
Group 3 (Day 5 Tx Start)	0	18	10	1
Group 4 (Day 6 Tx Start)	0	13	16	0
Group 5 (Control)	0	10	17	1
All Groups	**1% (1/144)**	**49% (71/144)**	**48% (69/144)**	**2% (3/144)**

Virology Laboratory Results

Quantitative viral PCR results for whole blood samples are summarized in Figure 4 (FDA analysis), and showed a modest trend of highest viral DNA levels in Group 5 (placebo) and lowest in Group 1 (Day 3 Tx Start) around the time of peak viral DNA levels at Day ~6 post-challenge. Medians and ranges in viral DNA levels in blood for all timepoints are summarized in Table 17 (FDA analysis). On Day 3, 97% (139/144) of animals had quantifiable viral DNA levels, and the other 5 animals had viral DNA <LLOQ/Detected. All 86 animals with data on Day 4 had quantifiable viral DNA levels.

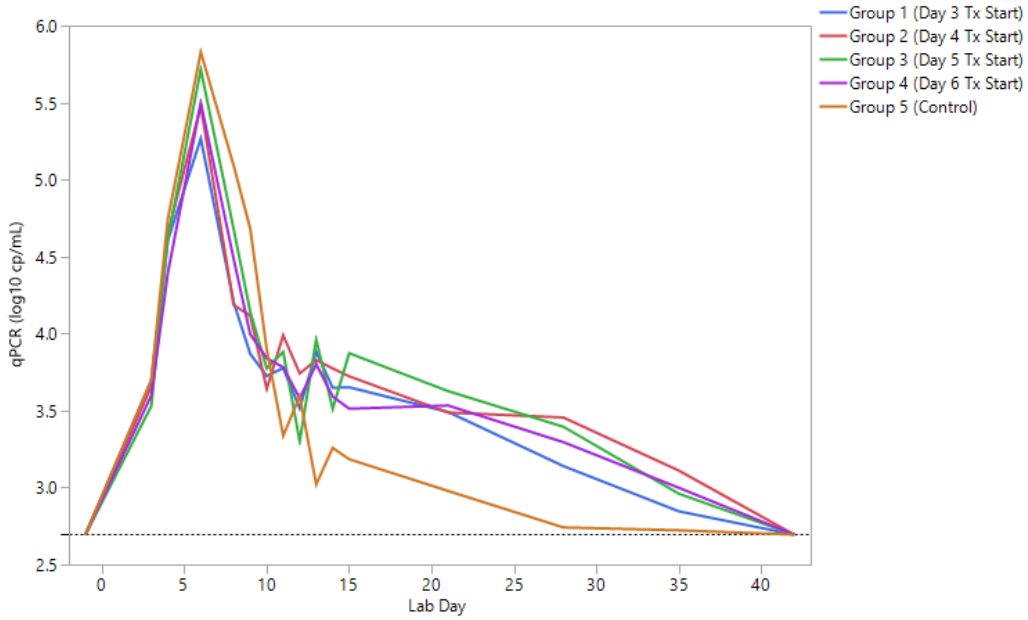
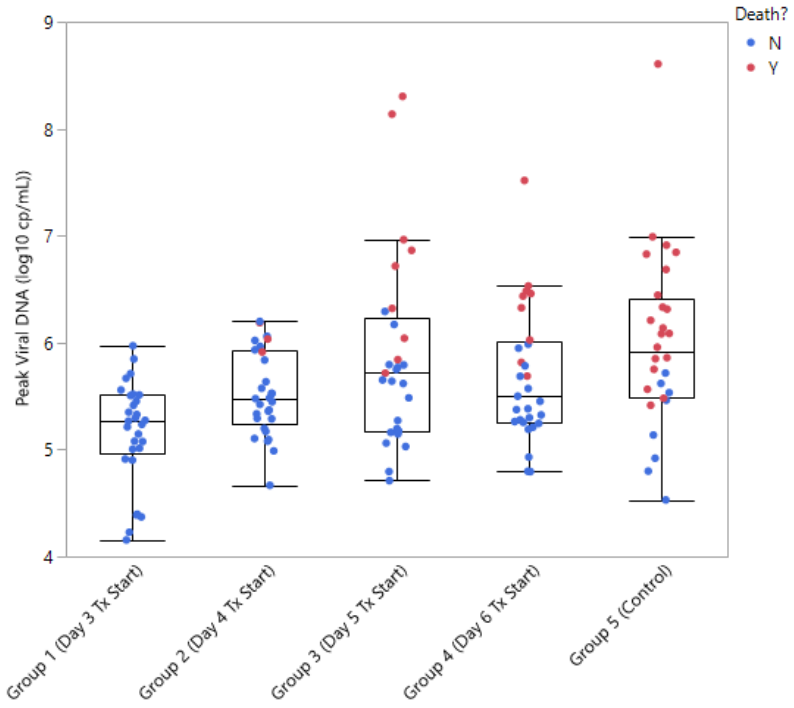


Figure 4. Median viral DNA levels in whole blood samples in study CMX001-VIR-106 (rabbit/RPXV). Results do not include unscheduled timepoints. Dashed line indicates the assay LLOQ. All results <LLOQ, target detected or not detected, were assigned LLOQ minus 1 (495 copies/mL) for illustrative purposes. Results are shown only for animals that had results available at that timepoint. Median time to death in controls was Day 10.

Table 17. Summary of viral DNA levels in whole blood samples at scheduled timepoints in study CMX001-VIR-106 (rabbit/RPXV).

	Group 1 (Day 3 Tx Start)				Group 2 (Day 4 Tx Start)				Group 3 (Day 5 Tx Start)				Group 4 (Day 6 Tx Start)				Group 5 (Control)			
Day	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max
-1	29	2.7	2.7	2.7	29	2.7	2.7	2.7	29	2.7	2.7	2.7	29	2.7	2.7	2.7	28	2.7	2.7	2.7
3	29	3.7	2.7	4.3	29	3.7	3.0	4.3	29	3.5	2.7	4.2	29	3.6	2.7	4.3	28	3.7	2.7	4.3
4	17	4.6	3.2	5.0	18	4.7	4.0	5.4	17	4.6	3.8	5.6	17	4.4	3.2	5.4	17	4.7	4.1	5.2
6	29	5.3	4.2	6.0	29	5.5	4.7	6.2	29	5.7	4.7	8.3	29	5.5	4.8	7.5	28	5.8	4.5	7.1
8	29	4.2	3.1	4.7	29	4.2	3.6	5.4	24	4.7	3.5	6.0	25	4.5	3.5	6.5	20	5.1	3.1	5.8
9	12	3.9	3.0	4.4	11	4.1	3.2	4.6	8	4.1	3.5	5.0	9	4.0	3.1	4.7	7	4.7	3.0	5.4
10	17	3.7	2.9	4.1	17	3.6	2.9	4.6	14	3.8	3.0	4.4	14	3.8	3.5	5.2	5	3.9	2.7	4.3
11	12	3.8	2.7	4.4	10	4.0	3.4	4.3	8	3.9	2.7	4.5	8	3.8	3.3	3.9	3	3.3	2.8	4.3
12	17	3.5	2.7	4.1	16	3.7	3.3	4.4	13	3.3	2.7	4.2	12	3.6	3.1	4.1	5	3.6	2.7	3.7
13	12	3.9	2.7	4.2	10	3.8	3.0	4.4	7	4.0	3.0	4.2	8	3.8	3.2	4.0	3	3.0	2.7	4.1
14	17	3.6	2.7	4.0	16	3.8	3.1	4.4	13	3.5	2.7	4.2	12	3.6	2.7	4.1	5	3.3	2.7	3.4
15	12	3.7	2.7	4.3	10	3.7	3.5	4.2	7	3.9	3.1	4.6	8	3.5	2.9	4.1	3	3.2	2.9	3.9
21	29	3.5	2.7	4.2	26	3.5	2.7	4.2	20	3.6	2.7	4.3	20	3.5	2.9	4.2	8	3.0	2.7	3.9
28	29	3.1	2.7	3.9	26	3.5	2.7	3.9	20	3.4	2.7	4.4	20	3.3	2.7	3.8	8	2.7	2.7	3.6
35	29	2.8	2.7	3.9	25	3.1	2.7	3.6	20	3.0	2.7	4.1	20	3.0	2.7	3.6	8	2.7	2.7	3.4
42	29	2.7	2.7	3.7	26	2.7	2.7	3.7	20	2.7	2.7	4.1	20	2.7	2.7	3.6	8	2.7	2.7	2.9

Peak viral DNA levels in whole blood were similar between groups but modestly higher (median ~0.5 log_{10} copies/mL) in Group 5 (Control) (Figure 5; FDA analysis). Peak viral DNA levels within each group were consistently higher among animals that died compared to those that survived.



Tx Group	Median Peak Viral DNA (log_{10} cp/mL)
Group 1 (Day 3 Tx Start)	5.3
Group 2 (Day 4 Tx Start)	5.5
Group 3 (Day 5 Tx Start)	5.7
Group 4 (Day 6 Tx Start)	5.5
Group 5 (Control)	5.9

Figure 5. Peak viral DNA levels in whole blood in study CMX001-VIR-106 (rabbit/RPXV). These analyses did not exclude unscheduled assessments (e.g., terminal samples).

Whole blood samples were also analyzed by plaque assay, although there was a low frequency of detected and quantifiable plaques across all treatment groups (Table 18; FDA analysis). Only 0.7% of plaque assays yielded a quantitative result, all from Groups 3, 4 or 5 on Days 6 or 7 post-challenge. No samples from Group 1 (Day 3 Tx Start) or Group 2 (Day 4 Tx Start) had quantifiable plaques. Note that

the plaque assay was not highly sensitive, with an LLOQ of 6,360 PFU/mL. The sponsor, but not this reviewer, analyzed results that were <LLOQ despite not being in the quantitative range of the assay. Nevertheless, there was a trend of detected or quantifiable plaques more likely observed in the Groups 3-5 compared to the earlier BCV treatment groups (Groups 1 and 2).

Table 18. Plaque assay results for whole blood samples in study CMX001-VIR-106 (rabbit/RPXV).

Treatment Group	<LLOQ/Det.	>LLOQ
Group 1 (Day 3 Tx Start)	0.6% (2/348)	0% (0/348)
Group 2 (Day 4 Tx Start)	1.2% (4/331)	0% (0/331)
Group 3 (Day 5 Tx Start)	3.8% (11/286)	1.4% (4/286)
Group 4 (Day 6 Tx Start)	3.1% (9/286)	0.7% (2/286)
Group 5 (Control)	4.1% (8/197)	2.0% (4/197)
All Animals	2% (34/1448)	(0.7%) 10/1448

Individual Results **≥LLOQ**

USUBJID	Treatment Group	Day	PFU/mL	Death Day
B05007-114	Group 3 (Day 5 Tx Start)	6	2150000	6
B05007-16	Group 3 (Day 5 Tx Start)	6	2050000	6
B05007-16	Group 3 (Day 5 Tx Start)	6	1700000	6
B05007-43	Group 3 (Day 5 Tx Start)	6	13900	8
B05007-124	Group 4 (Day 6 Tx Start)	6	7260	7
B05007-60	Group 4 (Day 6 Tx Start)	6	41500	7
B05007-41	Group 5 (Control)	6	95400	7
B05007-41	Group 5 (Control)	7	5560000	7
B05007-67	Group 5 (Control)	6	13100	7
B05007-94	Group 5 (Control)	6	9880	7

Viral DNA levels in terminally collected tissue samples were analyzed by the sponsor, but to this reviewer's knowledge the data were not provided in an independently analyzable format. Nevertheless, the results are not highly informative as they are confounded by the variable timing of sample collection. Samples from non-surviving animals were collected on Study Day 6-12, which includes the time of peak viremia, while samples from survivors were collected at the time of planned euthanasia on Study Day 42. Therefore, it is not surprising that tissue viral DNA levels were substantially higher in non-survivors, which were also most common in Group 5 (Control/PBO) (Figure 6; Summary of Virology, pgs. 32-33).

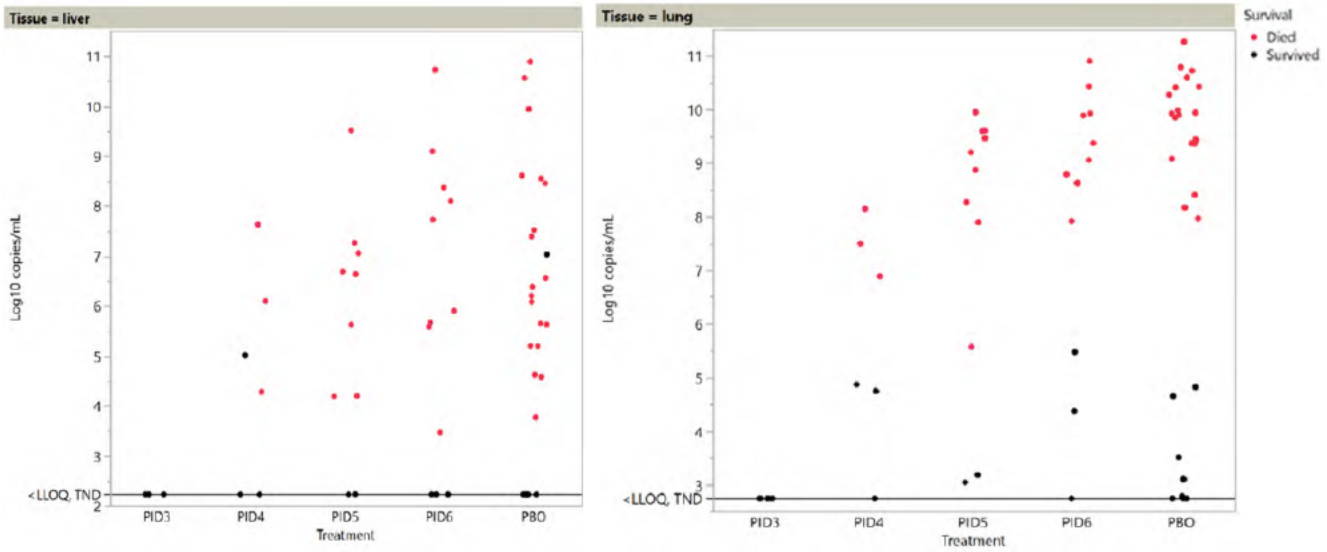


Figure 6. Sponsor's analyses of viral DNA levels in selected terminal liver (left) and lung (right) tissues in study CMX001-VIR-106 (rabbit/RPXV).

Neutralizing antibody titers by $PRNT_{50}$ assay for terminal samples collected from non-survivors (pre-Day 42) and survivors (Day 42) are summarized in Figure 7 (FDA analysis). Not surprisingly, $PRNT_{50}$ titers were higher at later study timepoints, although data from non-survivors were not sufficient to compare results between different treatment groups.

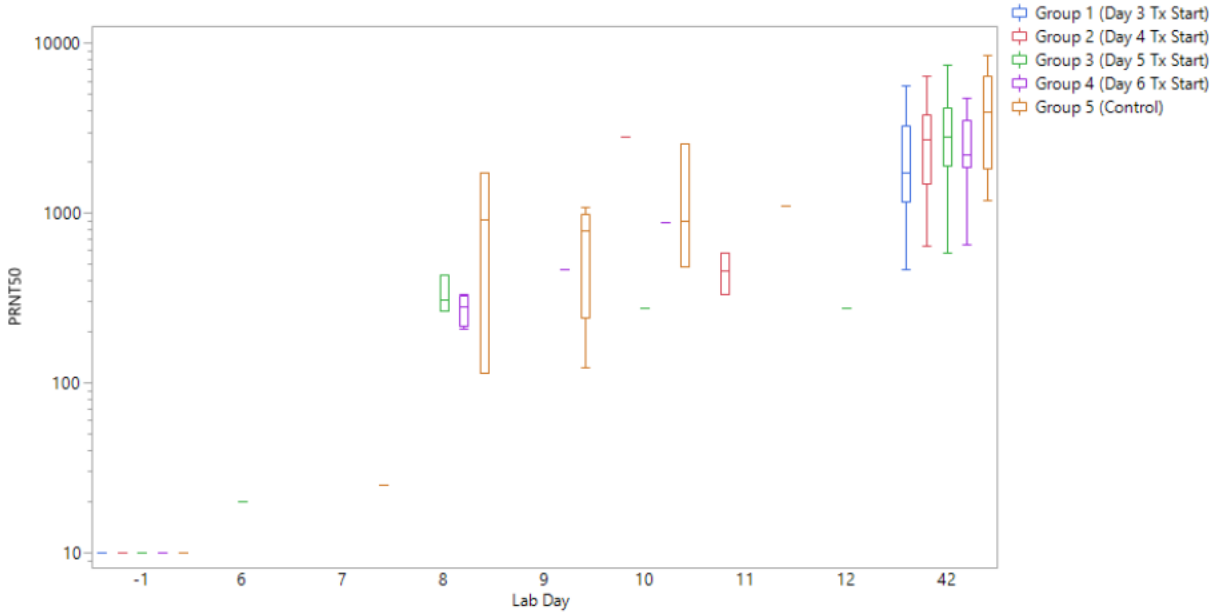


Figure 7. Serum RPXV neutralizing antibody titers ($PRNT_{50}$) from non-survivors (pre-Day 42) and survivors (Day 42) in study CMX001-VIR-106 (rabbit/RPXV).

Among survivors, there was a numerical trend of lower $PRNT_{50}$ titers at Day 42 in BCV-treated animals, consistent with an antiviral effect of BCV, although the titers in BCV-treated animals were still reasonably high and not significantly different from control animals (P=0.177 Wilcoxon Rank Sum Test) (Figure 8; FDA analysis).

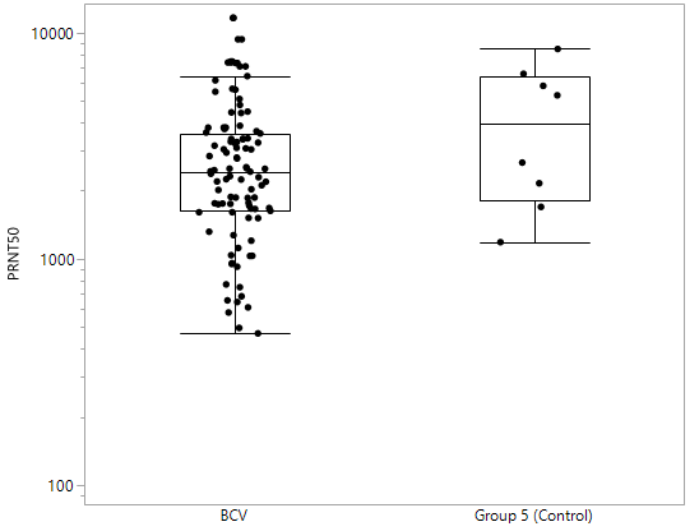


Figure 8. Serum RPXV neutralizing antibody titers ($PRNT_{50}$) from survivors (Day 42) in study CMX001-VIR-106 (rabbit/RPXV), pooling data from all BCV treatment groups versus placebo-treated control animals.

3.3 Study CMX001-VIR-044 (Mice/Ectromelia Virus)

Title
CMX001-VIR-044, "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"

Summary of Design
Study CMX001-VIR-044 was conducted at (b) (4) This was a randomized, blinded, placebo-controlled study. Study groups are summarized in Table 19 (report pg. 15). A total of 358 BALB/c mice, 15-30 grams each, which included 338 mice designated for study and 20 extra animals, were purchased from (b) (4) and arrived in three shipments to be used in three different challenge cohorts (A, B, C). Animals were quarantined for 5 days upon arrival at the facility.

For the efficacy evaluation, mice were randomized to Groups 1-8, in which animals were to receive blinded BCV or placebo by oral gavage, with BCV 10/5/5 or 20/5/5 mg/kg administered every other day starting on Study Day 5, 6, 7 or 8 (20/5/5 mg/kg dose only). The primary efficacy endpoint was the survival rate at Day 43 (42 days post-infection) in Groups 1 through 8. Note that the Clinical Pharmacology review team considered the 10/5/5 mg/kg dose as the fully effective dose in the mouse/ECTV model for the purpose of extrapolating efficacy to humans.

Group 9 was a "Viral Load" arm comprised of unblinded, untreated mice that were sacrificed at different times for analysis of ECTV viral levels in blood, liver and spleen samples. Groups 10 through 12 were the "Resistance" groups comprised of unblinded, BCV-treated mice sacrificed at various times for collection of samples to be analyzed for BCV resistance emergence.

Mice were challenged intranasally with a target dose of 200 PFU per mouse of ECTV strain Moscow (lot 032516- ECTV). Note that ECTV challenge occurred on Study Day 1 (in contrast to RPXV study CMX001-VIR-106 in which challenge occurred on Study Day 0). Therefore, the start of BCV dosing on Study Day 5, 6, 7 or 8 corresponds to post-challenge Day 4, 5, 6 or 7, respectively. This reviewer's

analyses below used the sponsor's Study Day designations (i.e., challenge on Day 1). Groups 1 through 9 were conducted concurrently and split across all three challenge day cohorts, while Groups 10 through 12 were all conducted in the challenge day C cohort.

Table 19. Study groups in mouse/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.

On each day of challenge (Day 1), the virus was diluted to a target concentration of 8,000 PFU/mL in 1X Dulbecco's phosphate buffered saline without calcium and magnesium, and maintained on wet ice following preparation. Mice were anesthetized with ketamine and xylazine, and 12.5 µL of diluted virus was administered into each nare using a micropipette with a rest period in-between each side. Confirmation of dose was demonstrated by plaque assay of the challenge dilution (i.e., back-titer) in VeroE6 cells within 1 hour of completing the mouse inoculations on each challenge day. Mice were ~8 weeks of age at the time of challenge.

Clinical observations were conducted as summarized in Table 20 (report pg. 20). Body temperature was assessed using temperature transponder chips implanted in the area between the scapulae on Day -8. The weight obtained during quarantine was used to randomize the animals into groups, while the weight obtained on Day 1 served as the individual animal's baseline weight. The treatment dose for each animal was based on the individual animal's weight collected on Day 4 for treatments occurring on Day 5 through Day 8 and the individual animal's weight collected on Day 8 for treatments occurring on Day 9 through Day 12.

Table 20. Summary of in-life clinical monitoring activities in mouse/ECTV study CMX001-VIR-044.

Study Day[a]	Naïve/Clinical Observation	Morbidity & Mortality	Body Weights	Body Temperatures
Quarantine	2X Daily	N/A	Once for Randomization	N/A
Acclimation (-7 to -1)	2X Daily	N/A	1X Daily on -7, -3, and -1	1X Daily
1	2X Daily	N/A	1X prior to challenge	1X prior to challenge
2 to 4	2X Daily	N/A	1X Daily	1X Daily
5 to 12	0800 ± 1 hr 1600 ± 1 hr 0000 ± 1 hr	0400 ± 1 hr 1200 ± 1 hr 2000 ± 1 hr	1X Daily	1X Daily
13 to 43	2X Daily	N/A	1X Daily	1X Daily

[a] Monitoring activity continued until the end of the study for each animal.

At the time of euthanasia, whole blood samples were collected for possible virology (qPCR and plaque assays) and resistance analyses. Blood samples were not to be collected from animals found dead, although samples were collected soon after death from 3 animals. Liver and spleen samples were also collected for hematoxylin and eosin (H&E) staining, as well as virology and resistance analyses.

Euthanasia criteria included the following:

- 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.

All animals that succumbed during the study demonstrated clinical signs consistent with ECTV infection as determined by the Study Director and Study Veterinarian. These clinical signs included lethargy, rough coat/not grooming, hunched posture, lacrimation, respiratory abnormalities, and ocular abnormalities.

Results

Survival results are summarized in Table 21 (FDA analysis) and Figure 9 (report pgs. 31-33), and show a clear benefit with BCV treatment in all groups, with survival rates higher for groups that started BCV at earlier times post-challenge. Survival rates trended slightly higher for the 20/5/5 mg/kg dose relative to the 10/5/5 mg/kg dose when administered at the same timepoints. Most animals that died were found dead rather than euthanized due to moribund disease, particularly driven by the high proportion of animals found dead in the controls and Day ≥7 treatment start groups.

Table 21. Overall survival results in study CMX001-VIR-044 (mice/ECTV). Challenge on Study Day 1.

Treatment Group	% Survival (n/N)	N Euthanized/Found Dead
Group 1 (Day 5 Tx Start 10/5/5)	78% (25/32)	4/3
Group 2 (Day 6 Tx Start 10/5/5)	66% (21/32)	5/6*
Group 3 (Day 7 Tx Start 10/5/5)	34% (11/32)	3/18
Group 4 (Day 5 Tx Start 20/5/5)	84% (27/32)	3/2
Group 5 (Day 6 Tx Start 20/5/5)	75% (24/32)	3/5
Group 6 (Day 7 Tx Start 20/5/5)	47% (15/32)	2/15
Group 7 (Day 8 Tx Start 20/5/5)	38% (12/32)	3/17
Group 8 (Control)	13% (4/32)	1/27

*Result from DS dataset differs by 1 animal in each category as reported by sponsor (6/5). Inconsistency does not affect conclusions.

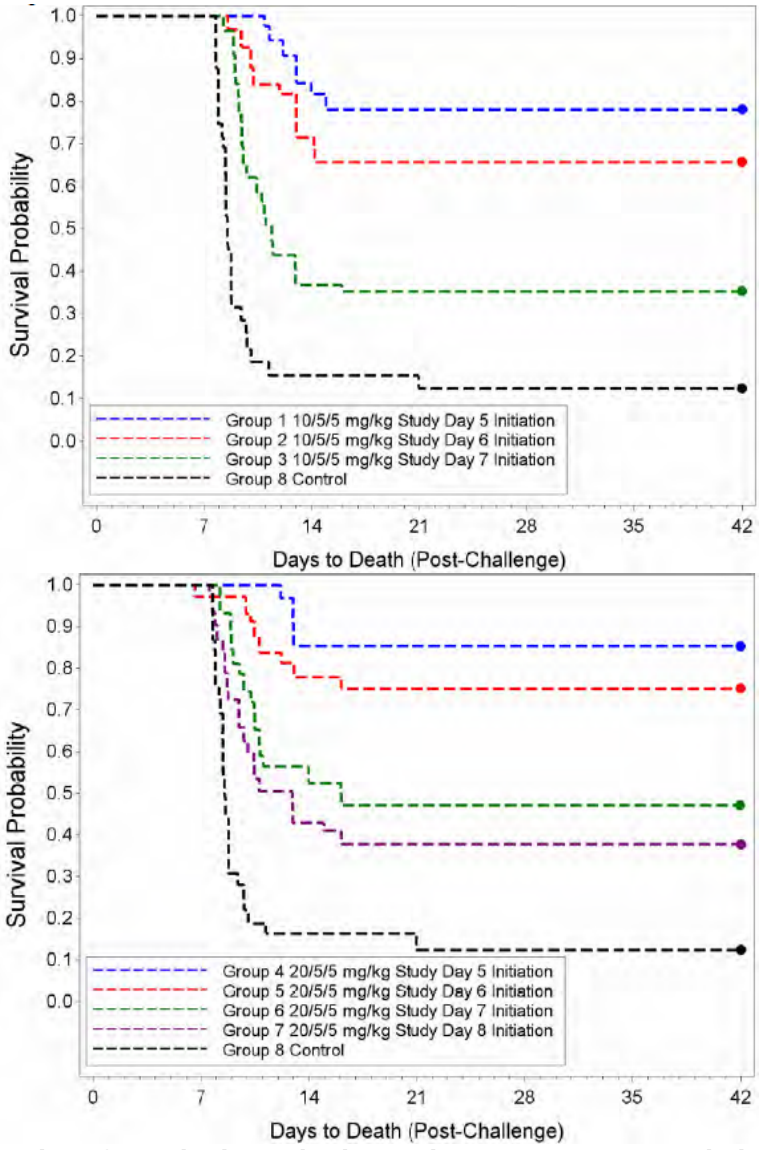
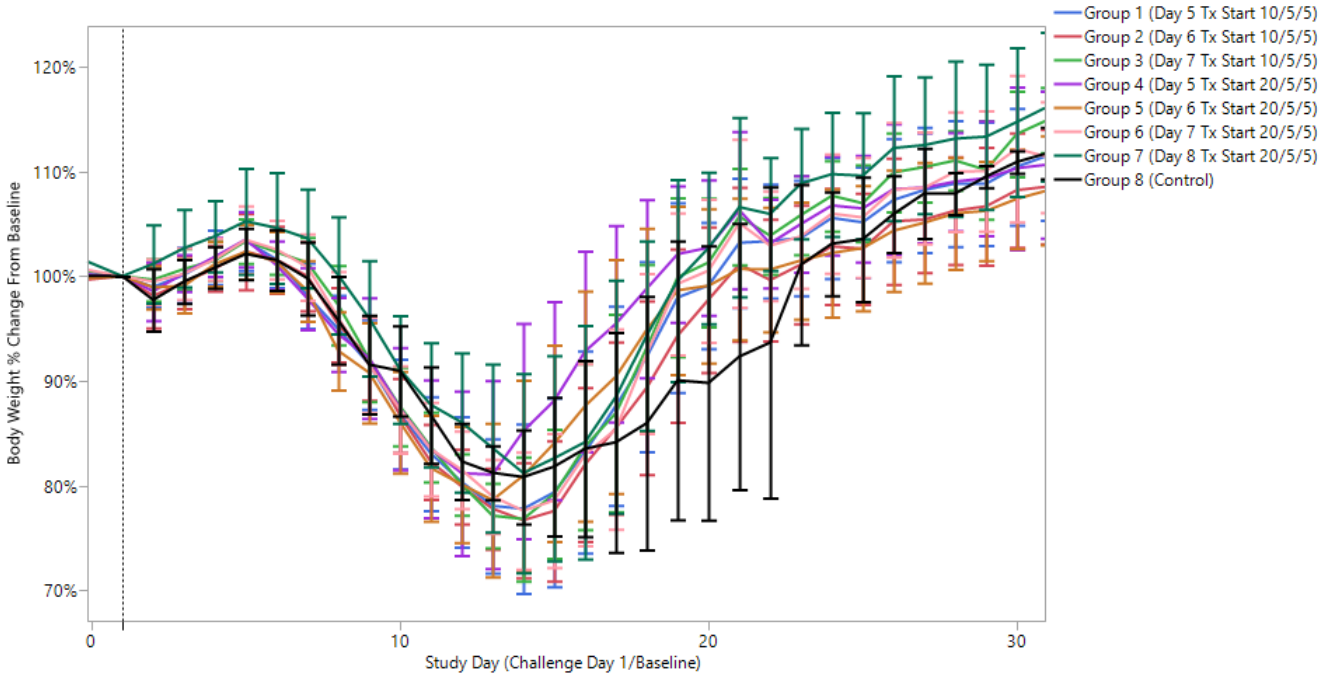


Figure 9. Kaplan-Meier plot of survival results in study CMX001-VIR-044 (mice/ECTV).

Viral challenge doses were similar for each challenge cohort, calculated at 188, 196 and 198 PFU/mouse for Challenge Day Cohort A, B and C, respectively.

Body weight changes over time are summarized in Figure 10 (FDA analysis). All groups had declining weights starting around Study Day 7-8. Body weights in the control group animals that survived beyond Day ~15 tended to rebound more slowly compared to BCV-treated animals, while body weights in Group 4 (Day 5 Tx Start 20/5/5 mg), which had the highest survival rate, tended to rebound slightly more quickly compared to all other groups.



Arm	Number of Animals with Body Weight Data @ Study Day																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	43
Group 1 (Day 5 Tx Start 10/5/5)	32	32	32	32	32	32	32	32	32	32	31	30	29	27	26	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Group 2 (Day 6 Tx Start 10/5/5)	32	32	32	32	32	32	32	32	32	31	30	27	27	26	23	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Group 3 (Day 7 Tx Start 10/5/5)	32	32	32	32	32	32	32	32	32	29	20	16	14	13	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11
Group 4 (Day 5 Tx Start 20/5/5)	32	32	32	32	32	32	32	32	32	32	32	32	32	29	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
Group 5 (Day 6 Tx Start 20/5/5)	32	32	32	32	32	32	32	31	31	31	30	27	27	26	25	25	25	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Group 6 (Day 7 Tx Start 20/5/5)	32	32	32	32	32	32	32	32	32	28	24	19	18	18	17	17	17	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Group 7 (Day 8 Tx Start 20/5/5)	32	32	32	32	32	32	32	32	29	23	20	16	16	15	14	14	13	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Group 8 (Control)	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**25**	**10**	**7**	**6**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**

Figure 10. Body weight changes from Baseline in study CMX001-VIR-044 (mice/ECTV). Shown in chart are group mean ± standard deviation.

All animals in Groups 1-8 had reported clinical signs. The first reported post-challenge clinical signs most frequently occurred on Study Day 7 for all groups (Figure 11; FDA analysis), consistent with clinical signs not being a reliable trigger to treat early in this model. The most commonly first reported clinical sign was “Rough Coat/Not Grooming” (>99%, 255/256).

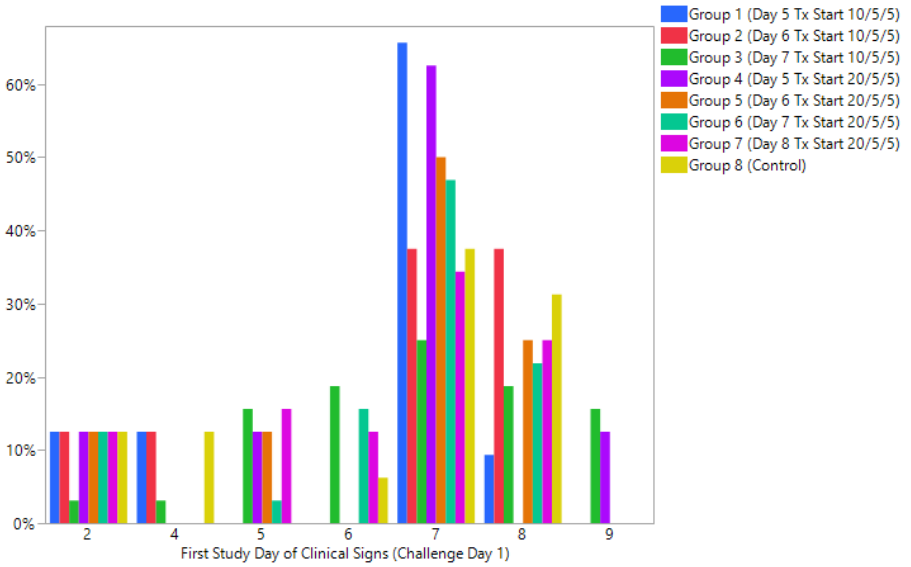


Figure 11. First Study Day of clinical signs in study CMX001-VIR-044 (mice/ECTV).

Changes in body temperature in Groups 1-8 generally were not remarkable until Study Day ~9 when all groups started showing a decline in mean body temperature (Figure 12; report pg. 779). Therefore, like clinical signs and body weight, body temperature would not have served as a reliable early trigger to treat in this study.

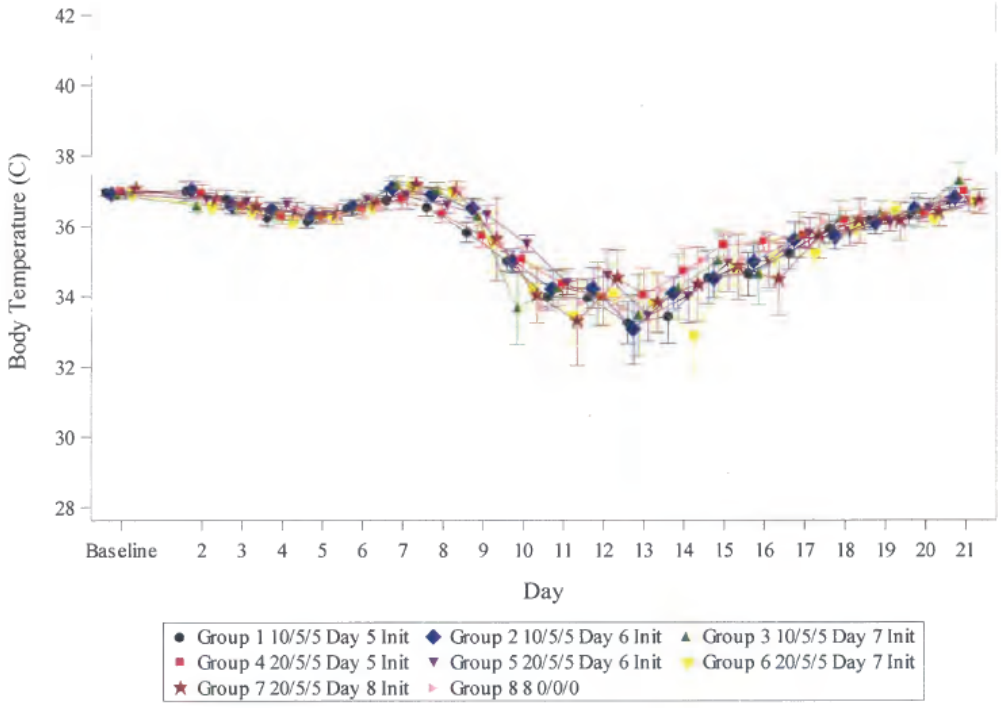


Figure 12. Body temperatures in study CMX001-VIR-044 (mice/ECTV). ECTV challenge on Study Day 1.

Quantitative viral DNA analyses from the untreated Group 9 mice are summarized in Figure 13 (FDA analysis). Viral DNA was detected in blood of all analyzed mice by Day 5 (7/8 >LLOQ), but clearly the levels continued in to increase through Day 7, the last timepoint evaluated. Viral DNA was more readily

detected in liver and spleen samples at earlier times post-challenge, but again levels continued to increase through Day 7. Thus, under the conditions used in this study, the peak in viral burden for untreated animals would not have occurred until Day 7, at the earliest.

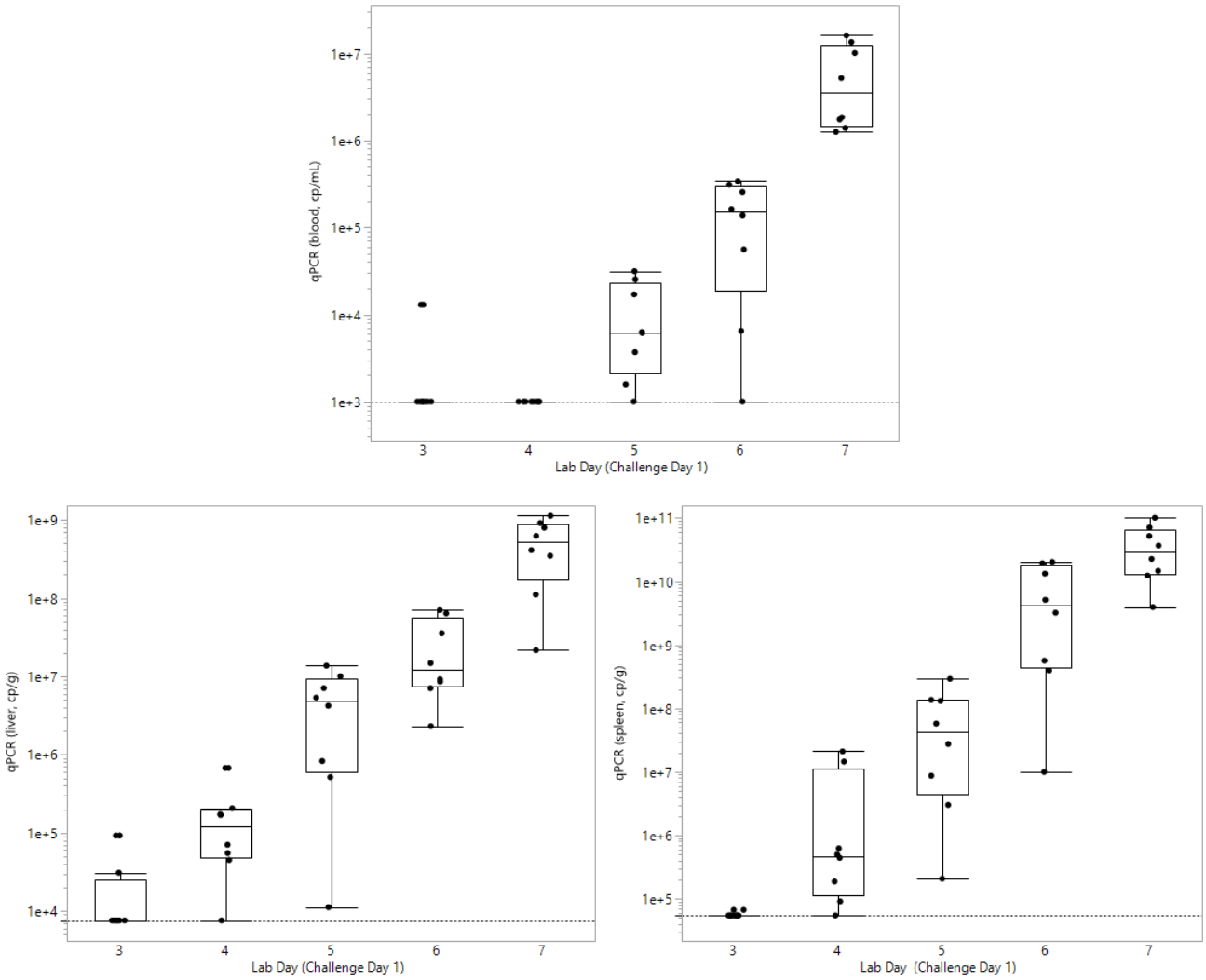


Figure 13. Viral quantititative PCR results in Group 9. Dashed lines indicate assay LLOQ for the sample type.

Plaque assay results showed similar patterns, in which plaque forming units were more readily detected in liver and spleen tissues compared to blood at earlier timepoints, but again levels continued to increase through Day 7 (Figure 14; FDA analysis).

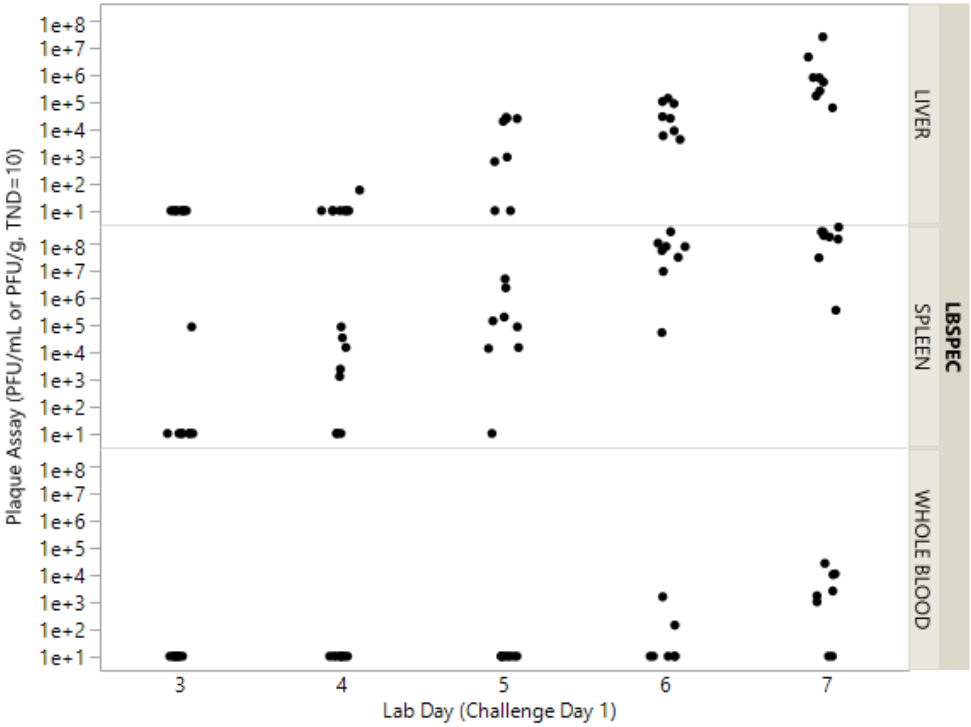


Figure 14. Plaque assay results in Group 9. Assay LLOQ for whole blood was 1,671 PFU/mL, but assay was not formally qualified for liver and spleen samples. For graphing purposes, all reported quantitative results are shown, and results reported as target not detected (TND) were arbitrarily assigned 10 PFU/mL or 10 PFU/g.

Virologic data in Groups 1-8 were not sufficient to assess the impact of BCV treatment on viral replication since only terminal samples were collected from these mice. However, limited data from the resistance Groups (10-12) for samples collected on Days ≥8 provide some indication of BCV antiviral activity. As shown in Table 19, 2 animals/timepoint were sacrificed at specific times for collection of blood and tissue samples. Blood samples were not collected from animals found dead, and ultimately only one blood sample was analyzed for viral DNA levels, so these analyses are restricted to liver and spleen tissues.

As shown in Figure 15 (FDA analysis), although the numbers of animals for analysis are small for each timepoint, viral DNA and PFU levels in liver and spleen tissues trended somewhat higher in the control group (Group 12) compared to the BCV treatment groups (Groups 10, 11), particularly for timepoints where more complete data were available.

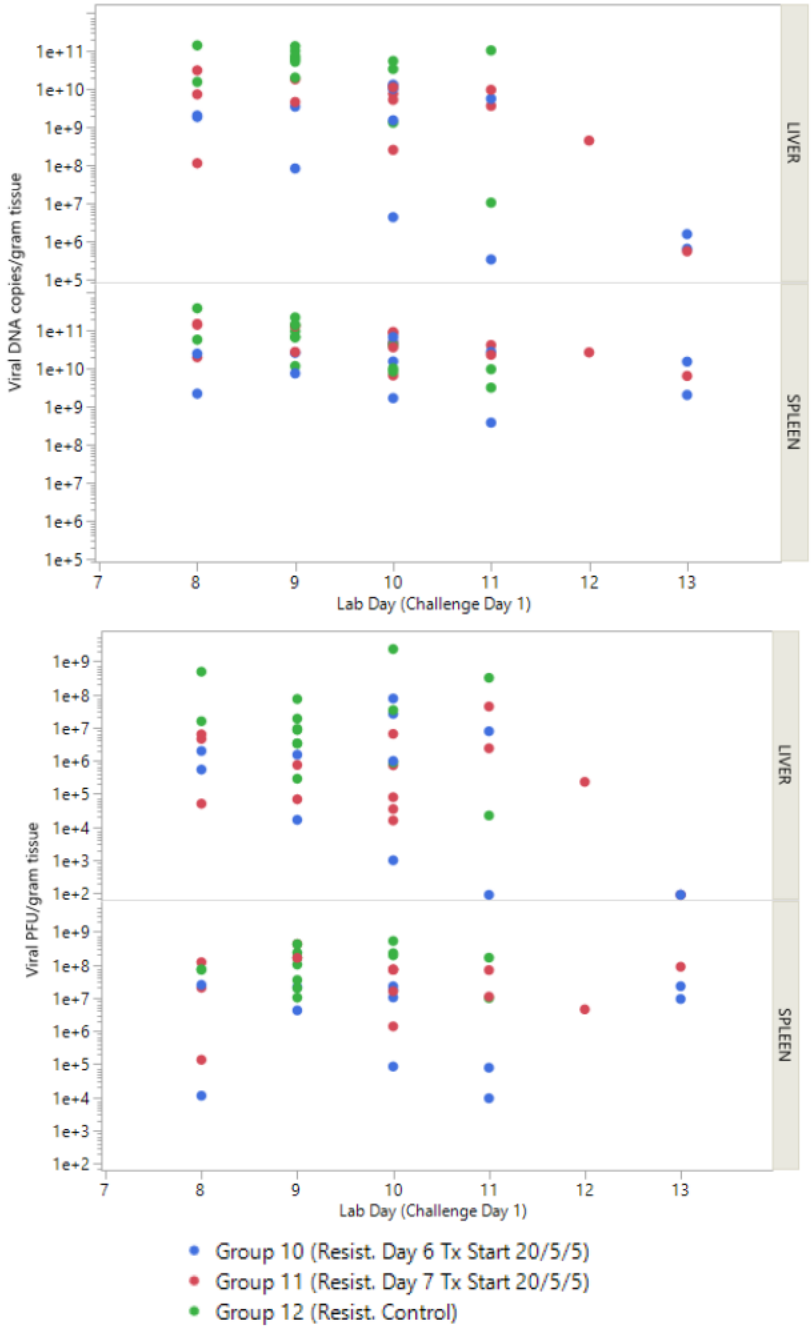


Figure 15. Viral qPCR and plaque assay results for liver and spleen samples collected from Groups 10-12. Each point represents one animal. Results shown are from animals that were euthanized according to the schedule in Table 19, as well as animals found dead at the specified timepoints. Plaque assay results for 3 liver samples were target not detected; for graphical purposes these results were arbitrarily assigned a value of 90 PFU/mL, based on lowest quantitative value (91) obtained minus 1.

3.4 (b) (3) (A), (b) (3) (B)

(b) (3) (A), (b) (3) (B)

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4. VARIOLA VIRUS ANIMAL MODEL STUDIES

BCV has not been evaluated in a VARV animal model, which as noted above is generally not suitable for efficacy evaluation of antiviral drugs. Furthermore, the evaluation of BCV in currently available VARV NHP models would not be informative due to the poor pharmacokinetics of BCV in NHPs.

The sponsor briefly summarized 3 studies of intravenous CDV in cynomolgus monkeys infected with VARV, which were conducted by investigators at CDC and USAMRIID and have only been published in abstract form (Huggins et al., 2003; Huggins et al., 2004; Huggins et al., 2006), so study details are somewhat limited. A copy of the slide presentation by Huggins et al., 2004 was available to the sponsor. Collectively, these studies reported CDV activity in the cynomolgus monkey VARV model, which provide some indirect evidence that BCV (through the delivery of the CDV-PP active moiety) may have anti-VARV activity *in vivo*.

5. BRINCIDOFOVIR ACTIVITY IN IMMUNE DEFICIENT MICE

Results from multiple published studies indicate that the immune response likely contributes to viral clearance during BCV treatment.

BCV was evaluated in a BALB/c mouse model of VACV infection under varying conditions of immune deficiency in a study published by Zaitseva et al., 2015. BCV treatment starting at Day 1 or Day 2 post-challenge provided dose-related protection from lethal i.n. VACV challenge in immunocompetent BALB/c mice, and also in immunodeficient BALB/c nude mice with partially reconstituted T cells from normal mice. Furthermore, BCV dosing in these mice did not prevent the development of an immune response that protected against lethal re-challenge. However, in BALB/c nude mice lacking T cells, BCV delayed but ultimately did not clear the infection, resulting in post-treatment lethality.

Other published studies have similarly demonstrated reduced activity of CDV in severely immune deficient mice infected with either CPXV or VACV (Neyts and De Clercq, 1993; Smee et al., 2002; Smee et al., 2014). These studies showed CDV could delay but ultimately not prevent death.

Tecovirimat was similarly shown to have reduced activity in the setting of severe immune deficiency in orthopoxvirus-infected animals, resulting in a limitation of use statement in the TPOXX® prescribing information: *"TPOXX efficacy may be reduced in immunocompromised patients based on studies demonstrating reduced efficacy in immunocompromised animal models."* Data are not available to determine if one drug is more effective than the other in the setting of severe immune deficiency. However, there is evidence from a mouse model study (see Section 6) and from a human case of progressive vaccinia (see Section 9) that the combination of both BCV and tecovirimat may improve overall antiviral efficacy in the setting of severe immune deficiency or other conditions when the efficacy of either individual drug is suboptimal.

6. COMBINATION ANTIVIRAL ACTIVITY WITH TECOVIRIMAT

BCV is predicted to have non-antagonistic antiviral activity when combined with other anti-orthopoxvirus agents with different mechanisms of action. Tecovirimat, an inhibitor of the orthopoxvirus VP37 envelope wrapping protein, is the only FDA-approved small molecule antiviral drug for smallpox, and studies conducted in cell culture and in mice indicate the potential for enhanced antiviral activity when BCV and tecovirimat are dosed in combination.

In a study by Quenelle et al., 2007, BCV and tecovirimat had non-antagonistic antiviral activity in cell culture against both VACV and CPXV. Furthermore, in CPXV-infected mice, enhanced activity of the combination was evident when antiviral treatment was administered for 5 days starting relatively late in the course of infection, particularly starting on Day 6 post-challenge. Under these stringent conditions, treatment with BCV or tecovirimat alone provided no significant survival benefit, while treatment with certain combinations of BCV plus tecovirimat resulted in significantly enhanced survival.

(b) (3) (A), (b) (3) (B)

Although this models a post-exposure prophylaxis approach and not a symptomatic treatment, it provides proof-of-concept that the combination of BCV plus tecovirimat provides greater antiviral activity than either drug alone in a highly rigorous challenge scenario.

Virus[a]	ST-246[b]	CMX001[c]	ST-246® Veh[d]	CMX001® Veh[e]	Mean time to death	Mortality (%)
-11KL-IL-4	–	–	–	–	6 ± 0	8/8 (100)
	–	–	+	+	6 ± 0	8/8 (100)[f]
	+	–	–	+	14.1 ± 1.6[g]	8/8 (100)[f]
	+	+	–	–	100.5 ± 16.5[g]	2/8 (25)[g]
	–	+	+	–	7.9 ± 0.1[g]	8/8 (100)[f]
Mock	+	+	–	–	NA[h]	0/8 (0)[g]
	–	–	+	+	NA	0/8 (0)[g]
	–	–	–	–	NA	0/8 (0)[g]

[a] Mice were infected IN with 200 PFU of ECTV-11KL-IL-4 or were mock-infected.
[b] Mice were dosed with 100 mg/kg of ST-246 for 14 days.
[c] Mice were dosed with 4 mg/kg of CMX001 for 14 days.
[d] ST-246® vehicle controls received 0.75% methylcellulose and 1% tween.
[e] CMX001® vehicle controls received saline.
[f] $P = 1.0$ compared to infected non-treated controls.
[g] $P < 0.05$ compared to infected non-treated controls.
[h] Not applicable.

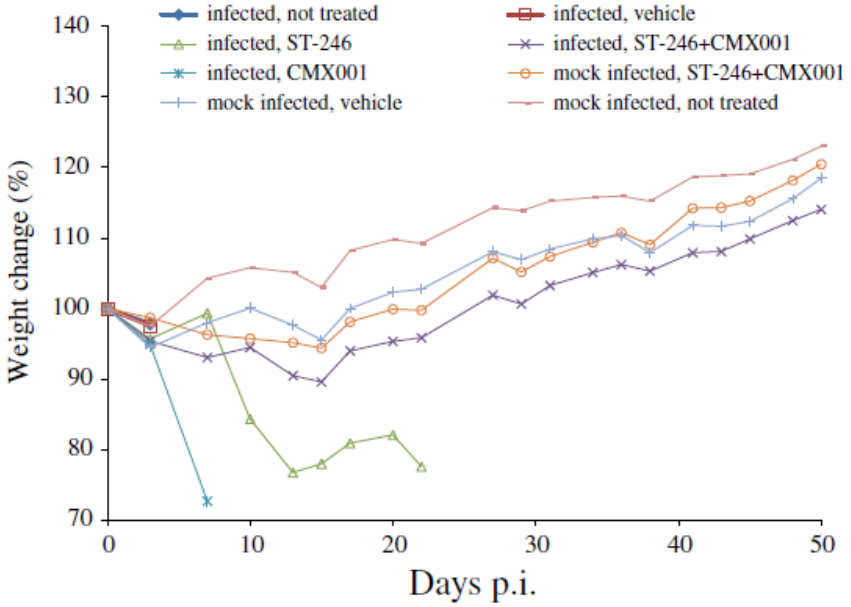

Figure 16. Effect of BCV (CMX001) and tecovirimat (ST-246), alone or in combination, in mice challenged with ECTV or ECTV-IL-4.

7. INTERACTIONS BETWEEN BRINCIDOFOVIR AND SMALLPOX VACCINES

Although outside the treatment indication being considered for this NDA, it is possible that BCV will be considered for use in combination with VACV-based vaccines in some clinical circumstances. As a result of its anti-VACV activity, BCV has the potential to interfere with VACV-based smallpox vaccines when it is administered around the time of vaccination, and studies conducted in animal models indicate BCV can have a modest impact on smallpox vaccine-induced immune responses. The clinical relevance of this interaction is unclear, and likely varies depending on the specific circumstance in which vaccine and BCV are used concomitantly. A similar vaccine interaction was noted for tecovirimat, and as a result the following text is included in the TPOXX® prescribing information Section 7.4 Vaccine Interactions: *"Some animal studies have indicated that co-administration of TPOXX at the same time as live smallpox vaccine (vaccinia virus) may reduce the immune response to the vaccine. The clinical impact of this interaction on vaccine efficacy is unknown."*

The most relevant investigations into the potential interaction of BCV with smallpox vaccines come from a published study by Parker et al., 2014, in which the authors evaluated the impact of BCV on the protective efficacy of VACV-based smallpox vaccines in the mouse/ECTV model. Co-administration of BCV with Dryvax™ live VACV smallpox vaccine improved vaccine-related lesion healing without significantly altering the development of short-term protective immunity following lethal ECTV challenge. However, antibody responses were measurably lower and ECTV-associated weight loss was greater for mice that had received vaccine with BCV compared to those that received vaccine alone (Figure 17; adapted from Parker et al., 2014). Similarly, co-administration of BCV with ACAM2000 vaccine (live VACV derived from Dryvax™ clone) resulted in lower antibody titers as well as modestly greater weight loss following lethal ECTV challenge, although without affecting $CD8^+$ T cells responses or overall survival.

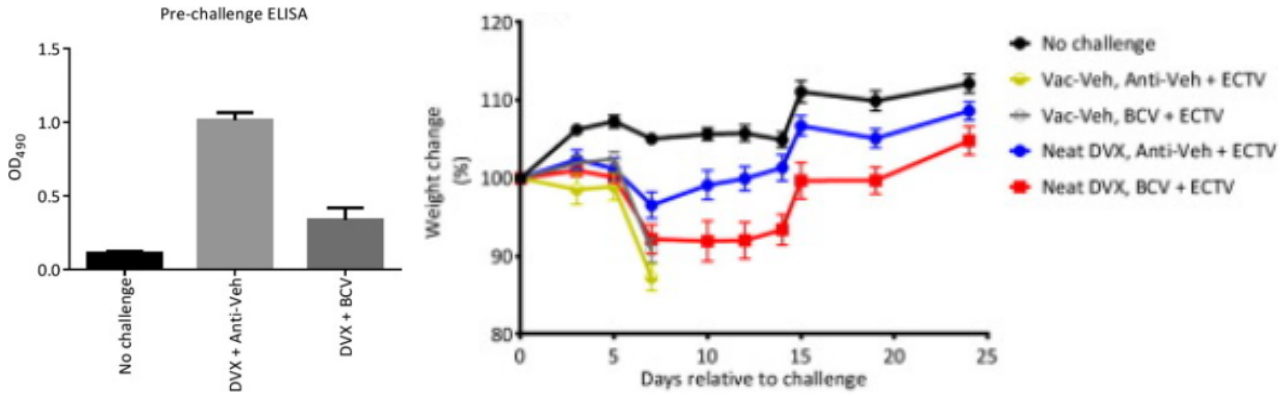

Figure 17. Impact of BCV on VACV-based smallpox vaccine immunogenicity. Left panel, virus-specific antibody titers in mice at Day 50 following Dryvax™ vaccine (DVX) administered (neat) with or without BCV prophylaxis. Right panel, weight loss following ECTV challenge on Day 50 in animals administered Dryvax™ vaccine (DVX) administered with or without BCV prophylaxis. Vaccine was administered at Day 0 and mice were treated with BCV (or vehicle) by oral gavage at a dose of 10 mg/kg on Day 0 followed by 2.5 mg/kg on Days 2, 4, 6, 8, 10, 12, and 14. Other mouse groups received various dilutions of Dryvax™ vaccine with or without BCV and similarly had greater weight loss following ECTV challenge if vaccines were administered with BCV (details included in Parker et al., 2014).

The impact of BCV co-administration with a non-replicating smallpox vaccine (ACAM3000, modified vaccinia Ankara [MVA]) was also evaluated in Parker et al., 2014 and again showed a possible interaction based on slightly greater weight loss following ECTV challenge in the ACAM3000 + BCV groups, although this vaccine was overall less active in the model. Although MVA does not undergo a full replication cycle in mammalian cells, viral DNA replication and gene expression still occur (Volz and Sutter, 2017), and thus BCV interference with MVA immunogenicity is theoretically plausible. In contrast, interference of MVA immunogenicity by tecovirimat is not anticipated, as tecovirimat inhibits viral spread, for which MVA is already defective in mammalian cells.

The Parker et al., 2014 study also evaluated the combination of BCV and Dryvax™ as a pre- or post-exposure prophylaxis in the ECTV model. Administration of Dryvax™ or BCV, or the combination, starting on Day -4 (i.e., 4 days pre-ECTV challenge) protected mice from ECTV-related mortality. However, Dryvax™ alone administered on Day 0, 2 or 4 did not protect mice from lethal infection, while BCV administration starting on Day 0 or Day 2 post-challenge protected mice, indicating there is a window of time following initial virus exposure during which BCV ± vaccine, but not vaccine alone, may be effective as a post-exposure prophylaxis.

Other studies indicate BCV treatment is unlikely to have a significant impact on immune responses when treatment is administered in the setting of a lethal viral exposure or established infection. In fact, the study by Zaitseva et al., 2015, noted above, showed that in VACV-infected mice, the immune response that develops during BCV treatment plays an important role in helping to clear the virus infection and also provides at least short term protection from lethal re-challenge. Similarly, in a study in the mouse/ECTV model, mice infected with ECTV and treated with BCV were protected from a subsequent lethal re-challenge (report CMX001-(b) (4)-005-RPT-01; summarized in Appendix A). Consistent with these results, in the pivotal rabbit/RPXV study CMX001-VIR-106, BCV-treated animals that survived RPXV challenge developed high titers of RPXV neutralizing antibody (see Section 3.2).

8. OTHER SUPPORTING ANIMAL MODEL STUDIES

In addition to the studies summarized above, the sponsor or collaborators conducted numerous other pilot or exploratory studies of BCV antiviral activity in the rabbit/RPXV and mouse/ECTV models. These additional studies are summarized briefly in Appendix A. Although many of these studies were small and exploratory in scope, in general they further confirm the activity of BCV these orthopoxvirus animal models.

9. CLINICAL VIROLOGY

No clinical trials evaluating BCV efficacy for orthopoxvirus infection have been conducted. BCV has been studied in clinical trials with other viruses, such as HCMV and adenovirus; clinical virology data from these trials were not reviewed to support this NDA.

BCV has been administered in single emergency use cases for the treatment of human orthopoxvirus infections. Because of the anecdotal nature of the cases, limited laboratory analyses, and confounding treatments and clinical care, it is unknown if BCV provided an antiviral treatment benefit in any of these cases, summarized below.

In March 2009, a 20-year-old male in the military who recently received the ACAM2000™ smallpox vaccine smallpox vaccine developed progressive vaccinia. Following receipt of the vaccine, the individual was diagnosed with acute myelogenous leukemia (AML) and had to start aggressive chemotherapy (cytarabine and idarubicin) for his malignancy. As a result, the patient had a persistent and enlarged vaccine lesion and additional symptoms of progressive vaccinia. The patient initially received antiviral treatment with vaccinia immune globulin intravenous (VIGIV) and both oral and topical tecovirimat. The vaccination site began to respond, but then satellite lesions developed and VACV was detected in the blood, and the patient subsequently received granulocyte colony-stimulating factor, tapered ongoing corticosteroid therapy, and started oral BCV weekly (200 mg initially, followed by 100 mg for 6 doses). Higher doses of VIGIV and tecovirimat were also administered around this time. The vaccine site gradually improved, although a bacterial superinfection developed and the patient had below-the-knee amputation on both legs due to gangrene as a consequence of pseudomonal sepsis before being discharged in stable condition. The individual contribution of the antiviral drugs towards eventual VACV clearance and healing of the vaccine and satellite lesions was unclear. Viral DNA and culturable virus were detected and present at relatively high and stable levels in vaccine and/or satellite lesion swabs 28-29 days after first starting VIGIV and tecovirimat, and 7 days after starting BCV. Immune cell counts also improved during this period. Of particular interest, there was strong evidence of emergence of tecovirimat-resistant VACV in the patient, but no BCV phenotypic resistance was observed following BCV treatment. Results from this case indicate that tecovirimat alone or in combination with polyclonal VIGIV may not have sufficient antiviral activity and durability to treat a highly pathogenic orthopoxvirus infection in the setting of severe immune deficiency, particularly if the virus is exposed to sub-therapeutic tecovirimat levels during the initial days of treatment. In retrospect, it is possible that

initiating combination treatment at the same time might have reduced the risk of tecovirimat resistance selection. Additional details from this case are published in the article by Lederman et al., 2012.

The sponsor described three additional anecdotal cases of BCV use for the treatment of CPXV infection, summarized as follows:

- In (b) (6), BCV was administered at a dose of 100 mg twice weekly to a 45-year-old-female with CPXV infection. The patient lived in a rural area of Germany and was believed to have contracted the virus from a cat. The virus had been refractory to all other treatments (not described), and the patient had severe pustules on her extremities and was in danger of losing fingers from one hand. Within 3 weeks of BCV administration, the patient was completely free from detectable virus and no amputations were necessary. Data for this case are unpublished and are anecdotal based on physician feedback.
- In (b) (6) a severely immunocompromised, 17-year-old male in the UK developed progressive swelling in his neck, followed by the development of skin lesions. Six days after initial presentation the patient required intubation and emergency tonsillectomy and tracheostomy for upper airway obstruction. The patient did not respond to broad spectrum antibiotic therapy, and eventually CPXV was identified in the skin lesions, tonsillar tissue, and in blood, confirming disseminated cowpox. Despite empiric antibiotics, a reduction in immunosuppressive therapy, initiation of VIGIV and CDV, and subsequently a 100 mg twice weekly regimen of BCV, the patient developed fusobacterium infection and new cowpox skin lesions and died of septic shock and multi-organ failure. Additional details are published in the article by Gazzani et al., 2017, although the article does not include significant additional virology data.
- In (b) (6) an immunosuppressed female lung transplant patient in Sweden was diagnosed with CPXV infection and started BCV at 200 mg once weekly. Tecovirimat was added about a week later. VIGIV was also used for a period of about 3 weeks starting in late August. According to the sponsor, the patient's viral load generally trended downward and eventually reached undetected in November, but the virus was not completely cleared, and the patient continued to have intermittent viremia. (b) (3) (A), (b) (3) (B) The patient succumbed in March 2020 due to pneumonia and progression of renal failure. Data for this case are unpublished and are anecdotal based on physician feedback, and the sponsor noted additional information will be provided when available (the sponsor confirmed no updated data as of 3/12/2021).

10. PROPOSED POST-MARKETING FIELD TRIAL

Approval of a drug under the Animal Rule requires the conduct of a post-marketing clinical/field trial if or when such studies are feasible and ethical.

The review team is recommending that the sponsor (or collaborator, e.g., NIH or BARDA) develop a factorial clinical trial design to compare the safety and efficacy of BCV vs. tecovirimat vs. BCV + tecovirimat. Negotiations on this required post-marketing study were ongoing at the time of completion of this review. This reviewer agrees with the overall intention of a factorial trial design, as neither BCV nor tecovirimat have been proven to be effective for the treatment of smallpox or any other orthopoxvirus disease in a clinical trial. Furthermore, the combination of BCV plus tecovirimat may reduce the possibility of emergence of drug resistant virus.

11. CONCLUSIONS

These Original NDAs are approvable from a Virology perspective for the treatment of human smallpox disease caused by variola virus.

12. PACKAGE INSERT

Section 12.4 Microbiology of the proposed prescribing information is shown below, with our cumulative proposed edits and minor sponsor updates shown in track changes. The sponsor accepted this labeling text as of 5/6/2021, with the exception of the wording of the pharmaceutical class ("orthopoxvirus nucleotide analog DNA polymerase inhibitor") which was still under negotiation at the time of finalization of this review.

12.4 Microbiology

Mechanism of Action
Brincidofovir is a lipid conjugate of cidofovir, an acyclic nucleotide analog of deoxycytidine monophosphate. The lipid conjugate is designed to mimic a natural lipid, lysophosphatidylcholine, and thereby use endogenous lipid uptake pathways. Once inside cells, the lipid ester linkage of brincidofovir is cleaved to liberate cidofovir, which is then phosphorylated to produce the active antiviral, cidofovir diphosphate. Based on biochemical and mechanistic studies using recombinant vaccinia virus E9L DNA polymerase, c(b)(4)idofovir diphosphate selectively inhibits orthopoxvirus ~~replication by inhibiting viral~~ DNA polymerase-mediated viral DNA synthesis. (b) (4) Incorporation of cidofovir into the growing viral DNA chain results in reductions in the rate of viral DNA synthesis.

Activity in Cell Culture
The median 50% effective concentration (EC_{50}) of brincidofovir against variola virus (b) (4) was 0.11 µM (range 0.05 to 0.21 µM) across 5 variola virus strains chosen to represent (b) (4) 5 distinct variola virus DNA polymerase genotypes. (b) (4) The median EC_{50} values of brincidofovir against rabbitpox, ectromelia, vaccinia, and monkeypox viruses were 1.10 µM (n=4, 0.5-1.89 µM), 0.33 µM (n=5, 0.12-0.51 µM), 0.17 µM (n=22, 0.004-1.2 µM), and 0.074 µM (n=2, 0.023-0.12 µM), respectively.

Resistance
There are no known instances of naturally occurring brincidofovir resistant orthopoxviruses, although (b) (4) brincidofovir resistance may develop under drug selection. (b) (4) Cell culture studies have shown that certain amino acid substitutions in the target viral DNA polymerase protein can confer reductions in brincidofovir antiviral activity. The possibility of resistance to brincidofovir should be considered in patients who either fail to respond to therapy or who develop recrudescence of disease after an initial period of responsiveness. (b) (4)

Cross-resistance
Cross-resistance between brincidofovir and tecovirimat is not expected based on their distinct mechanisms of action. Where tested, orthopoxvirus isolates resistant to tecovirimat have not been

resistant to brincidofovir and/or cidofovir and vice versa [redacted] (b) (4) Non-antagonistic activity of brincidofovir and tecovirimat has been demonstrated in cell culture and animal models.

In addition, we recommended the following changes to other text outside of Section 12.4.

- Change description (Highlights and Section 11) and pharmaceutical class from [redacted] (b) (4) to "orthopoxvirus nucleotide analog DNA polymerase inhibitor."
- Section 7.3 Vaccine Interactions (now Section 7.2): "No vaccine-drug interaction studies have been performed in human subjects. Animal studies have indicated that co-administration of TEMBEXA at the same time as live smallpox vaccine (vaccinia virus) may reduce the immune response to the vaccine. It is also possible that TEMBEXA may reduce the immune response to replication-defective smallpox vaccine (modified vaccinia virus Ankara). The clinical impacts of [redacted] (b) (4) these potential interactions on vaccine efficacy ~~is~~ are unknown."
- Section 12.1 Mechanism of Action: "Brincidofovir is an antiviral drug [redacted] (b) (4) against variola (smallpox) virus [see [redacted] (b) (4) Microbiology (12.4)]."

13. RECOMMENDATIONS

We recommend the following post-marketing commitment (PMC):

> Conduct cell culture studies to characterize brincidofovir antiviral activity against recombinant vaccinia viruses encoding specific amino acid substitutions that emerged in ectromelia virus in brincidofovir-treated animals in mouse study CMX001-VIR-044.

[redacted] (b) (3) (A), (b) (3) (B)

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15. APPENDICES

Appendix A. Additional supporting animal model studies of brincidofovir

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
RPXV Natural History Studies						
(b) (4) Studies 2817-2818-100017958	Determination of the Potency and LD_{50} of Rabbitpox virus Utrecht Strain in the Rabbit Model	RPXV-Utrecht Propagated under Study 2814 (from ATCC VR-1591 stock, which was derived from VR-157); 4.66-220 PFU (actual), i.d.	NZW Rabbits ((b) (4) lineage) (9 wk at challenge)	40	BARDA-sponsored RPXV potency studies w/220 PFU (2817) and LD_{50}/LD_{90} determination (2818).	All 8 animals in study 2817 (220 PFU) succumbed (7/8 found dead), median time to death 6.64 days. In 2818, no differences in survival rates by challenge dose, 2/8 survivors with 6.66 PFU, all others including lowest dose (4.66 PFU) succumbed (mix of euthanized and found dead), unexpected low weight loss possibly due to food enrichment, all animals with clinical disease signs, estimated LD_{50} ≤4.66 PFU, LD_{90} ≤220 PFU.
(b) (4) Study 2819-100017958	Natural History Study of Intradermal Rabbitpox Virus (Utrecht Strain) Infection in the Nine Week Old Rabbit Model	RPXV-Utrecht (b) (4)-01 (from study 2814), 230 PFU actual (300 PFU target), i.d.	NZW Rabbits ((b) (4) lineage) (9 wk at challenge)	54	BARDA-sponsored natural history study of RPXV (b) (4)-01 stock in 9-wk rabbits, included control group.	All RPXV-infected animals succumbed, median time to death 7.8 days. Clinical disease signs, fever (first by median of 2.8 days post-challenge), lesions, no weight loss but less gain relative to sham controls, viral DNA detected in blood in all animals by 60 hours post-challenge, buccal swab by 108 hours. Viral DNA in tissues post-mortem. Increased temperature and genome copy number observed before lesions and provide the earliest and most reliable biomarker for a trigger to treat; however, none of the parameters showed a significant correlation between time of onset and death.
(b) (4) Study 2820-100017958	Characterization of the Intradermal Rabbitpox Model/Natural History in the Six Month New Zealand White Rabbit	RPXV-Utrecht (b) (4)-01 (from study 2814), 734 PFU actual (1000 PFU target), i.d.	NZW Rabbits ((b) (4) lineage) (6 months + 6 days at challenge)	18	BARDA-sponsored natural history study of RPXV (b) (4)-01 stock in 6-mo rabbits, included control group.	All RPXV-infected animals succumbed, median time to death 7.7 days. First clinical sigs at 120 hours. Decreases on body weight. Fever by 60 hours. Skin lesions beginning 96 hours (4/12) and all animals on Day 6. Viral DNA detected in whole blood of all animals by 96 hours, 9/12 buccal swabs by 120 hours. Fever and positive qPCR first signs (2.5 and 2.25 days, respectively). Respiration rates abnormal for infected and controls. Hematology and clinical chemistry parameters not consistently abnormal in infected rabbits. In summary, a combination of temperature and qPCR of whole blood offer earliest and most reliable trigger to treat; however, none of the parameters showed a significant correlation between time of onset and death.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
(b) (4) Study 2923-100017958	Blinded Study to Assess Ability to Identify Triggers to Treat in the ID Rabbitpox Model	RPXV-Utrecht (b) (4) -01 (from study 2814), 173 PFU actual (300 PFU target), i.d.	NZW Rabbits (b) (4) (9 wk at challenge)	22	BARDA-sponsored blinded natural history study to evaluate fever and viremia (qPCR+) as indicator of disease. Fever defined as >= 2 Std dev above baseline, significant increase above baseline temperature (SIBT) defined as three consecutive fever readings. qPCR conducted at determination of SIBT. Included control group.	All infected animals succumbed, median time to death of 6.9 days. Clinical signs, fever beginning at 60 hours. Viral DNA detected in blood of all animals by 48 hours. SIBT observed for 5/8 animals at 60 hours (4 w/qPCR >LLOQ, 1 detected/<LLOQ). Remaining 3 animals had SIBT at 72-96 hours (qPCR >LLOQ). Lesions in all animals at some time between 4 and 9 days. No weight loss, but weight gain lower in infected animals. Acute inflammation at 72 hours (CRP), some clinical chemistry changes. Concluded combination of fever and qPCR of whole blood for viral nucleic acid provides the best (earliest and most reliable) indicator of disease onset.
CMX001-VIR-033	A Randomized, Blinded Study of the Potency of Rabbitpox Virus in New Zealand White Rabbits Infected Via the Intradermal Route	RPXV-Utrecht (050310-ALS), 15-630 actual PFU, i.d.	Female and Male NZW Rabbits (b) (4) (9weeks)	40	Challenge on Day 0. Natural history study. Blinded. Actual challenge doses (target) of 15 PFU (30 PFU), 43 PFU (100 PFU), 117 PFU (300 PFU) and 630 PFU (900 PFU); control group 31 PFU (100 PFU, "Batch 5-7-2008" from R. Moyer). Conducted a (b) (4)	Mortality results w/050310-ALS: 75% (6/8) with 15 PFU, 63% (5/8) with 43 PFU, 100% (8/8) with 117 PFU, 100% (8/8) with 630 PFU. 50% (4/8) mortality in control group. LD_{50} and LD_{90} values estimated at 8 PFU and 83 PFU, respectively. Median time to death 8-10 days, 13 days in control group. No consistent different in body weight changes, generally started declining around Day 8. Body temperatures started to increase between Day 0 and 2. Respiration rates started to decrease around Day 4. Appearance of secondary lesions between 5-10 days, earlier with higher challenge doses. Viral load measured at euthanasia, higher in moribund/non-survivors.
CMX001-VIR-038	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	RPXV-Utrecht (050310-ALS), 0.666-102 actual PFU, i.d.	Female and Male NZW Rabbits (b) (4) (9weeks)	36	Challenge on Day 0. Natural history study. Blinded. Actual challenge doses (target) of 0.666 PFU (3 PFU), 8.66 PFU (30 PFU), 96.8 PFU (300 PFU) and 102.2 PFU (300 PFU). Four animals at highest dose euthanized on Days 0 and 2 for ear punch biopsy. Conducted at (b) (4)	All animals (n=24) that received challenge doses of >=8.66 PFU succumbed to infection. 50% (4/8) challenged with 0.666 PFU succumbed to infection. Median times to death were ~8-10 days. Combining survival data with CMX001-VIR-033, LD_{50} and LD_{90} values estimated at 0.6 PFU and 46 PFU, respectively. Body temperatures increased in all groups from Days 1-6. Weight loss occurred just prior to death. Characterized ear lesions.
ECTV Natural History Studies						
(b) (4) Study 2955-100029460	Determination of the Potency and LD_{50} of Ectromelia virus-Moscow in the Mouse Model	ECTV-Mos from study 2954, 2.44-700 PFU actual, i.n.	BALB/c Mice (6-8 wks at arrival)	80	BARDA-sponsored potency and LD_{50}/LD_{90} study in BALB/c mouse model.	Mean time to death 8 to 10.84 days post-challenge, significantly longer in lower challenge groups. Weight loss with challenge doses >= 27.5 PFU. Estimated LD_{50} <2.44PFU, LD_{90} = 32.1 PFU. Overt clinical signs of disease observed in mice challenged with 700 PFU as early as 4 days post-challenge. The typical progression of clinical signs was lethargy, ruffled fur, hunched posture (Day 7 post-challenge), lacrimation, and lesions in some mice on the ears and tails on Day 9 post-challenge. Hunched posture, lacrimation and lesions all occurred around the mean time to death. Most deaths "found dead."

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
(b) (4) Study 2956-100029460	Natural History Study of Intranasal Ectromelia virus Infection in the BALB/c Mouse Model	ECTV-Mos from study 2954, 125 PFU actual (200 PFU target), i.n.	BALB/c Mice (6-8 wks at arrival)	180	BARDA-sponsored natural history study of ECTV in BALB/c mouse model. Included control group. Serial sacrifices to obtain blood/tissues for analysis.	All infected animals succumbed (not incl. serially euthanized animals), median time to death of 8.94 days. No difference in time to death by sex. Most experienced weight loss, primarily occurring around Day 8. No consistent changes in body temperature. Overt clinical signs included lethargy, ruffled fur, hunched posture, ocular abnormalities, lacrimation, and lesions, observed as early as Day 3. Median time-to-abnormal (TTA) for animals displaying mild clinical signs was 4 days, and 7+ days for moderate and severe clinical signs. Viremia (qPCR+) first identified at 84 hours, with most being viremic at 132 hours. Viral titers in spleen and liver first detected at 60 hours. Some measures of hematology and clinical chemistry abnormal between Day 4 and 6 post-challenge. Gross pathological findings rare, incl. discoloration in liver and spleen, pocks on tail. Ocular abnormalities, such as discharge from the eyes and partial closures of one or both eyes, were the clinical parameters found to provide the earliest indication of disease. No correlation among any of the clinical parameters and the time-to-death or survival.
(b) (4) Study 2957-100029460	Assessment of the Ability to Identify Triggers to Treat in the ECTV Mouse Model	ECTV-Mos from study 2954, 98 PFU actual (200 PFU target), i.n.	BALB/c Mice (6-8 wks at arrival)	132	BARDA-sponsored, blinded natural history study of ECTV BALB/c mouse model to identify triggers to treat. Serial sacrifices to obtain blood/tissues for analysis.	All infected animals succumbed to disease (not incl. serially euthanized animals), median time to death of 8.2 days. Females shorter time to death. Body weight relative to pre-challenge baseline and the sham-infected group significantly decreased on Days 1, 2, 3, 7, 8, 9, 10, and 11, but subtle until Day 7. Body temperature changes sporadic and subtle. Clinical signs did not appear until Days 7-8. Viremia by qPCR first measured at 96 hours in 3/6 animals, 120 hours in 3/4 animals. Viral titers in spleen and liver first observed at 72 hours, all spleens qPCR+ at 96 hours. Some levels of liver and kidney abnormalities. Gross pathological findings rare. Minority of infected animals experienced mild, moderate and severe clinical signs/conditions of disease (6, 24 and 17 percent, respectively) with the median time to onset of 8.0 and 8.33 days for moderate and severe signs, i.e., too late and close to the average time animals succumbed (8.2 days). No correlation among any of the clinical parameters and the time-to-death or survival.
CMX000-VIR-107	Determination of Lethality for Ectromelia Virus (ECTV) Strain Moscow in the BALB/c Mouse Model	ECTV-Mos Lot 032516-ECTV/3571, 192 or 948 PFU actual (200 or 1000 PFU target), i.n.	BALB/c Mice (8 wks at arrival)	36	Challenge on Day 0. Lethality/natural history study conducted at (b) (4)	All animals (18/18) in Group 1 (1000 PFU target dose) succumbed to infection, most (15/18) found dead. Most animals (14/18, 78%) in Group 2 (200 PFU target dose) succumbed to infection, all (14/14) found dead. Disease signs in survivors. Median times to death in Groups 1 and 2 were 193.3 hours and 237.4 hours, respectively (p-value = 0.0012). Weight loss starting around Day 5-6.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX000-VIR-109	Determination of LD_{50}/LD_{90} of Ectromelia Virus, CMRX 3571, in the Intranasal BALB/c Mouse Model	ECTV-Mos stocks: CMRX 3571/032516, (b) (4) ECTV-Mos-3-P5 and BARDA 2954, 5-1000 target PFU range, i.n.	BALB/c mice (8 weeks)	252	Challenge on Day 0. Blinded study to compare lethality of 3 different ECTV-Mos stocks. Actual PFU challenge doses back calculated. Conducted at (b) (4)	LD50 and LD90 doses, respectively, for each stock: CMRX 3571/032516-ECTV: 1.73 and 14.17; (b) (4) ECTV-Mos-3-P5: 0.76 and 6.25; BARDA 2954: 2.26 and 35.07. all animals succumbed to infection at the target 200, 500, and 1000 PFU doses. All animals experienced a decrease in mean body weight following challenge, with the greatest overall change in body weight occurring from Days 6 to 19. Some differences in clinical observations between stocks at lower challenge doses. Shorter median time to death with higher challenge doses.
CMX000-VIR-111	Natural History Study of Intranasal Ectromelia Virus (CMRX 3571) Infection in the BALB/c Mouse Model	ECTV-Mos Lot 032516-ECTV/3571, 194 PFU actual, i.n.	BALB/c mice, female and male (8 weeks)	340	Challenge on Day 0. Detailed natural history study, not blinded, infected and sham infected groups. Serial euthanasia to obtain blood and tissue samples for analyses of viral load, hematology, clinical chemistry, etc. Conducted at (b) (4)	Unscheduled deaths first occurred at 188 hours post-challenge. Median time to death 8.6 days. Estimated survival of 15.7% through Day 21. 100% of infected animals with clinical disease signs by Day 7. Time to onset of clinical signs earlier compared to blinded natural history study 2957. Sharp initial body weight drop in first 24 hours, then more body weight loss starting around Day 6. Body temp decline starting around Day 8. Various hematology assessments did not show a remarkable or consistent difference between ECTV-infected and sham-infected animals, except that ECTV-infected animals starting as early as Day 4 had a spike in neutrophil and monocyte percentages concurrent with a drop in lymphocyte percentage. Viral DNA first consistently detected in whole blood by Day 3.5. Viral PFU consistently detected by Day 6. IL-4 levels highest on Day 5 in liver samples.
Rabbit/RPXV BCV Studies						
CMX001-(b) (4)-001	Pre-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with 10 mg/kg BID or 20 mg/kg QD Initiated 1 Day Prior to Infection	RPXV-Utrecht (ATCC), 1000 PFU (>=100x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (8wks, ~1.8-2.2 kg)	18	Challenge on Day 0. BCV 20 mg/kg on Day -1 followed by 10 mg/kg BID to Day 3, or 20 mg/kg QD Day -1 to Day 3. (b) (4) lab.	Controls developed fever starting on Day 3 and all 6 died by Day 7, all 12 BCV-treated animals survived. Only followed to Day 10. Some mild respiratory symptoms, decreased weight gain, some fever in QD group on Day 9-10, plus some late primary/secondary lesions, indicating poss ble delayed disease post-dosing in QD group. At death/euthanasia or end of study, all animals developed nAb, including controls, with variable titers. Little or no virus detected in tissues of BCV-treated animals.
CMX001 (b) (4) -002	Pre-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with 1 or 5 mg/kg BID Initiated 1 Day Prior to Infection	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}) or 500 PFU, i.d.	Female NZW Rabbits (b) (4) (8wks, ~1.6-1.9 kg)	26	Challenge on Day 0. BCV oral 1 mg/kg BID or 5 mg/kg BID Day -1 to Day 3 (b) (4) lab.	All controls died by Day 7, 2/4 BCV-treated animals survived with 500 PFU challenge and 1 mg/kg BCV dose, all others survived. More severe disease and lesions with 1 mg/kg vs. 5 mg/kg dose. All groups developed fever. Little virus detected in tissues other than primary lesion area in BCV-treated animals. At death/euthanasia or end of study, nAb titers in all but 2 animals (both in control group)

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-(b) (4)-003	Pre- and Post-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment Initiation at Days -1, 0, 1, or 2	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (8wks, ~1.9-2.2 kg)	18	Challenge on Day 0. BCV oral 5 mg/kg BID for 5 days starting on Day -1, 0, 1 or 2. (b) (4) lab.	2/4 controls died, all BCV-treated animals survived. All BCV groups with mild weight loss/reduced weight gain, and fever. Little virus detected in tissues other than primary lesion area in BCV-treated animals. At death/euthanasia or end of study, all but 1 animal developed nAb titers, w/lower titers in controls.
CMX001-(b) (4)-004	Post-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with 5 mg/kg BID Initiated at Days 3, 4, 5, or 6	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (8wks, ~1.7-2.1 kg)	18	Challenge on Day 0. BCV oral 5 mg/kg BID for 5 days starting on Day 3, 4, 5 or 6. (b) (4) lab.	Controls (n=2) died, 100% survival with tx start on Day 3 (4/4) or Day 4 (4/4), 75% (3/4) with tx start on Day 5, 25% (1/4) with tx start on Day 6. All groups with fever starting Day 3, and weight loss. More clinical disease signs with later tx start. Some virus detected in lung, but not in other tissues other than primary lesion area in BCV-treated animals. At death/euthanasia or end of study, all animals had nAb titers, w/lower titers in controls.
CMX001-(b) (4)-005	Post-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Evaluation of Dose with Treatment Initiated at Day 1 Post Infection	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (8wks, ~1.7-2.1 kg)	18	Challenge on Day 0. BCV oral 2-20 mg/kg QD for 5 days starting on Day 1. (b) (4) lab.	Controls (n=2) died, 100% survival in all BCV treatment groups. 20 mg/kg double-dosed on Day 1. More disease signs, lesions and weight loss with lower doses. All groups developed fever. No virus detected in tissues other than primary lesion area in BCV-treated animals. At death/euthanasia or end of study, all animals had nAb titers.
CMX001-(b) (4)-006	Post-Exposure Prophylaxis Activity of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Comparison of 1, 2, or 3 doses of 20 mg/kg CMX001 starting on Day 3 or 4 Post Infection	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (8wks, ~2.1-2.6 kg)	18	Challenge on Day 0. BCV oral 20 mg/kg, 1, 2 or 3 doses starting on Day 3 or 4. (b) (4) lab.	Controls (n=2) died. Survival rates: Day 3 (3/3), Day 4 (2/3), Day 3+5 (3/3), Day 4+6 (3/3), Day 3+5+7 (3/3), Day 4+6+8 (2/3). Thus, 1-3 doses at 20 mg/kg associated with survival when started on Day 3 or 4. All groups lost weight, least in 3-dose groups. All groups developed fever. Lowest disease signs in Day 3+5+7 group. Secondary lesions not statistically different between groups.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-(b) (4)-007	Efficacy of CMX001 in the Treatment of New Zealand White Rabbits Infected with Rabbitpox Virus by Animal to Animal Spread: Treatment at the Onset of Fever	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d. in index animals; transmitted RPXV to co-housed sentinel animals	Female NZW Rabbits (b) (4) (9wks, ~2.2-2.5 kg)	18 (?)	Index animals challenged w/RPXV on Day 0 and not treated. Two index animals housed with 4 sentinel animals per cage representing each of the 4 different treatment groups. Sentinel animals treated orally with BCV 20 mg/kg at onset of fever for 1, 2 or 3 qod doses, or vehicle. (b) (4) (b) (4) lab.	Among sentinel animals, BCV groups had 100% (9/9) survival, but 2/3 (67%) in vehicle control group also survived, so unable to demonstrate efficacy. All groups had weight loss and fever, without major differences between groups. Inoculum transferred from index to sentinel rabbits may have been insufficient to achieve a high mortality rate.
CMX001-(b) (4)-008	Efficacy of CMX001 in the Treatment of New Zealand White Rabbits Infected with Rabbitpox Virus by Animal to Animal Spread: Treatment at the Onset of Lesions I	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d. in index animals; transmitted RPXV to co-housed sentinel animals	Female NZW Rabbits (b) (4) (9wks, ~2.2-2.4 kg)	12 (?)	Index animals challenged w/RPXV on Day 0 and not treated. Two index animals housed with 4 sentinel animals per cage representing each of the 4 different treatment groups. Sentinel animals treated orally with BCV 20 mg/kg at onset of secondary lesions for 1, 2 or 3 qod doses, or vehicle. (b) (4) lab.	Among sentinel animals, 100% survival for all animals, including vehicle controls, so unable to demonstrate efficacy. Minimal and similar weight loss in all groups. Modest fever in controls group but not treated groups.
CMX001-(b) (4)-009	Efficacy of CMX001 in the Treatment of New Zealand White Rabbits Infected with Rabbitpox Virus by Animal to Animal Spread: Treatment at the Onset of Lesions II	RPXV-Utrecht (ATCC), 1000 PFU (>=100x LD_{50}), i.d. in index animals; transmitted RPXV to co-housed sentinel animals	Female NZW Rabbits (b) (4) (9wks, ~1.7-2.1 kg)	18	Index animals challenged w/RPXV on Day 0 and not treated. Two index animals housed with 4 sentinel animals per cage representing each of the 4 different treatment groups. Sentinel animals treated orally with BCV 20 mg/kg at onset of secondary lesions for 1, 2 or 3 qod doses, or vehicle (b) (4) lab.	Identical design to CMX001-(b) (4)-008 study except 10-fold higher RPXV challenge dose, and rabbits were smaller. 6/9 (67%) survival in treated groups with 1 death in each dosing group, 0/3 (0%) survival in vehicle controls (p=0.09). All groups with weight loss but more loss in vehicle group. All groups with fever but higher peak fever in vehicle group.
CMX001-(b) (4)-010	Efficacy of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with 20 mg/kg CMX001 Every Other Day for Three Doses Beginning at the Appearance of Secondary Lesions	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female NZW Rabbits (b) (4) (9wks, ~1.9-2.3 kg)	24 (+6 for PK in PBMCs)	Challenge on Day 0. Blinded study. BCV oral 20 mg/kg or vehicle qod for 3 doses, starting at time of appearance of secondary lesions. (b) (4) lab.	Treatments started around Day 3-4. Survival of 11/12 (92%) in BCV group, 2/12 (17%) in vehicle control group. Weight loss in both groups but more in vehicle group. Fever in both groups but lasted longer in vehicle group. Primary lesion size smaller on Day 7 in BCV group. Fewer secondary lesions at Day 7 in BCV group. Correlation between #lesions at Day 7 and survival. Few animals with detected virus in blood at onset of secondary lesions, no clear difference in titers later b/w groups or relationship with survival. Female and male animals exhibited similar course of disease and survival rates.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-(b) (4)-011	Efficacy of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with one dose of 20 mg/kg CMX001 at the Appearance of Secondary Lesions	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female and Male NZW rabbits (b) (4)(9.5wks, ~2.3-2.6 kg)	24 (+6 for PK in PBMCs)	Challenge on Day 0. Blinded study. BCV oral 20 mg/kg or vehicle single dose starting at time of appearance of secondary lesions. (b) (4) lab.	Treatments started around Day 3-4. Survival of 7/12 (58%) in BCV group, 1/12 (9%) in vehicle control group. Weight loss in both groups but more in vehicle group. Fever similar in both groups. Trend of fewer secondary lesions in BCV group. No significant difference in viral PFU/mL in blood between groups, most with non-detected virus at Day 4. PK of CDV, CDV-P and CDV-PP in PMBCs at 24 hours post-dose all <LLOQ.
CMX001-(b) (4)-012	Efficacy of CMX001 in New Zealand White Rabbits Intradermally Challenged with Rabbitpox Virus: Treatment with two doses of 20 mg/kg CMX001 at the Appearance of Secondary Lesions	RPXV-Utrecht (ATCC), 100 PFU (>=10x LD_{50}), i.d.	Female and Male NZW rabbits (b) (4)(9wks, ~2.0-2.3 kg)	30	Challenge on Day 0. Blinded study. BCV oral 20 mg/kg or vehicle, two doses 2 days apart, starting at time of appearance of secondary lesions. (b) (4) lab.	Treatments started around Day 3-5. Survival of 8/12 (67%) in BCV group, 1/12 (9%) in vehicle control group. Weight loss in both groups but more in vehicle group. Fever similar in both groups. Trend of slightly fewer secondary lesions in BCV group. PK of CDV-P and CDV-PP in PMBCs at 24 hours post-second-dose variable but not clearly different between infected and uninfected animals.
CMX001-VIR-039	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	RPXV-Utrecht (050310-ALS), 187 PFU (300 PFU target), i.d.	Female and Male NZW Rabbits (b) (4)(9weeks)	61	Challenge on Day 0. Blinded study. Included an unblinded PK group. Animals randomized and dosed relative to timing of appearance of secondary pox lesions. CMX001 5/5/5, 20/5/5, 20/20/20 mg/kg or vehicle on randomization days (RD) 0/2/4. Conducted at (b) (4)	Randomization start: 24 on Day 3 post-challenge, and 19 on both Days 4 and 5 post-challenge (one animal mis-dosed and removed from analysis). Survival 47%, 73%, 80% and 25% in CMX001 5/5/5, 20/5/5, 20/20/20 mg/kg or vehicle groups, respectively. Body temperatures elevated starting on Day ~3, respiration rates decreased starting by Day ~4. Peak lesion counts on Day 7-8 for CMX001 groups and Day 11 for vehicle group. All animals viremic by qPCR on day of first lesion observation, no significant differences in median DNA/mL levels between groups over time, except terminal/end-of-study samples. No differences between groups by plaque assay, most were negative results. Consensus RPXV polymerase gene sequences 100% identical to reference. No significant differences between mean PRNT values of the groups at any of the study days examined. Saliva and blood samples in PK group analyzed for viral DNA, first detected around Day 3-4 in blood, Day 4-5 in saliva.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-VIR-041	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	RPXV-Utrecht (050310-ALS), 316 or 350 actual PFU (300 target PFU), i.d.	Female and Male NZW Rabbits (b) (4) (11 weeks)	144	Challenge on Day 0. Blinded, randomized study. Animals monitored at 8 hour intervals, and randomization occurred on first day of confirmed fever. BCV 20/5/5 mg/kg qod starting on Randomization Day 0, 1, 2 or 3. Two challenge cohorts. Conducted at (b) (4)	Previously intended as pivotal RPXV study. Study quality problems, including dosing errors. Randomization trigger 14-71 hours post-challenge (median 62 hours). Survival rates: Tx start on Randomization Day (RD) 0=100%, RD1=93%, RD2=93%, RD3=69%, PBO=48%. Less weight loss, fewer secondary lesions with earlier BCV tx start. Lower peak viral DNA levels in blood and buccal swabs with BCV vs. PBO, lower with earlier BCV tx. PFU rarely >LLOQ in blood, more often in PBO and BCV RD3 animals. Surviving animals development neutralizing antibody, with slightly lower titers in the BCV RD0 group.
Mouse/ECTV BCV Studies						
CMX001-(b) (4)-005-RPT-01	Efficacy of a Single Therapeutic Dose of CMX001 in Protection of Intranasally Challenged A/NCR Mice from Severe Mousepox and the Generation of Immunologic Memory	ECTV-Mos 20 PFU (67x LD_{50}), i.n.	Female A/NCR mice (6-8 wks)	150	Challenge on Day 0. BCV 100 uL oral 20-30 mg/kg, single dose on Day 4, 5, 6 or 7 post-challenge. Surviving mice at Day 63 re-challenged w/280 PFU (b) (4) lab.	All untreated controls died by Day 10. All 3 doses associated with >=90% survival when started at Day 4. Reduced survival for later treatment times, low survival for Day 7 treated mice. All mouse groups lost weight but generally less for Day 4 treated mice. Day 4 treated mice had most reduced viral load, reduced ALT, reduced IFNγ at Day 8. Day 4/5 treated mice w/CD4+ and CD8+ T cell responses. Some weight loss after re-challenge but all survived.
CMX001-(b) (4)-006-RPT-01	Efficacy of a Loading Dose of CMX001 Administered at Days 4, 5 and 6 Post-Infection in Protection of A/Ncr Mice from Severe Mousepox Disease	ECTV-Mos 4 PFU (10x LD_{50}), i.n.	Male A/NCR mice	150	Challenge on Day 0. BCV 100 uL oral 10-30 mg/kg on Day 4, 5 or 6, or 20 mg/kg on Day 4, 5 or 6 followed by 2.5 mg/kg qod for 14 days total tx. (b) (4) lab.	All untreated controls died by Day 9. >=80% survival for mice that started tx on Day 4. Lower survival with later start times, except 100% survival w/ Day 5 20 mg/kg dose. Less weight loss for mice that started tx on Day 4. More weight loss w/ Day 4 10 mg/kg dose (lowest dose). No obvious benefit of additional maintenance dosing. Viral load reduced in Day 4 20-30 mg/kg tx mice. ALT, IFNγ, CD4/CD8 T cells evaluated. Seroconversion in all infected mice except Day 4 30 mg/kg and Day 5 20 mg/kg tx groups.
CMX001-(b) (4)-007-RPT-01	Optimization of the CMX001 Maintenance Dose for Therapeutic Intervention Following Intranasal Ectromelia Virus Infections in A/Ncr Mice	ECTV-Mos 15 PFU (10x LD_{50}), i.n.	Female A/NCR mice (5-7 wks)	80	Challenge on Day 0. BCV 100 uL oral loading dose 20 mg/kg, followed by maintenance dose 0.31-2.5 mg/kg for 14 days, starting on Day 0 (one tx group) or Day 5 (all others) (b) (4) lab.	All controls died by Day 11. All treated groups had 100% survival except for 1 death in Day 5/0.63 maintenance group (90% survival). Weight changes similar between maintenance dose groups. Maintenance dose of limited value.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-(b) (4)-008-RPT-01	Delayed Therapeutic Intervention with CMX001 to Treat A/Ncr Mice Infected Intranasally with a Lethal Dose of Ectromelia Virus	ECTV-Mos 0.6 PFU (2x LD_{50}), i.n.	Male A/NCR mice (5-7 wks)	130	Challenge on Day 0. BCV 100 uL oral loading dose 10 mg/kg on Day 0,1,2,3,4,5,or 6, followed by 2.5 mg/kg maintenance doses qod for 14 days total; 100 mg/kg IP CDV on Day 0 or 3. (b) (4) lab.	All controls died by Day 10. BCV dosing started by Day 3 or earlier associated with >=90% survival and less weight loss. Lower survival at later BCV tx start times. CDV tx at Day 0 resulted in 90% survival, CDV tx at Day 3 resulted in 20% survival.
CMX001-(b) (4)-009-RPT-01	Delayed Therapeutic Intervention with CMX001 to Treat A/Ncr Mice Infected Intranasally with a Lethal Dose of Ectromelia Virus	ECTV-Mos 4.5 PFU (12x LD_{50}), i.n.	Male A/NCR mice (5-7 wks)	130	Challenge on Day 0. BCV 100 uL oral loading dose 10 mg/kg on Day 0,1,2,3,4,5,or 6, followed by 2.5 mg/kg maintenance dose qod for 14 days total; 100 mg/kg IP CDV on Day 0 or 3 (b) (4) lab.	All controls died by Day 9. BCV dosing starting on Day 4 or earlier associated with >=90% survival and less weight loss. No survival when dosing started on Day 5 or 6. Both CDV tx regimens associated w/>=90% survival.
CMX001-(b) (4)-010	Efficacy of HDP-CDV Treatment in Preventing Mortality of A/Ncr Mice Following Inoculation with Different Doses of Ectromelia Virus	ECTV-Mos, 27-2700 PFU (90-9000x LD_{50}), i.n.	Female A/NCR mice (5-7 wks)	52	Challenge on Day 0. BCV 100 uL oral 1, 2 or 4 mg/kg for 5 days starting 4 hours post-challenge. Small sample size (n=4/group) (b) (4) lab.	All controls died. 4 mg/kg dose associated with best survival and least weight loss: 50% survival w/2700 PFU, 100% survival w/27 or 270 PFU. More rapid death with higher PFU challenge.
CMX001-(b) (4)-011-RPT-01	Efficacy of CMX001 in Protecting SKH-1 Mice from Mortality Following an Intranasal Challenge with Various Doses of Ectromelia Virus I	ECTV-Mos 160, 320 or 630 PFU, i.n.	Female SKH-1 mice (7-9 wks)	65	Challenge on Day 0. BCV oral 25 mg/kg loading dose at Day 3, 6 or 9, followed by 2.5 mg/kg maintenance dose qod for 14 days. Surviving mice re-challenged w/8800 PFU at Day 68. Hairless mice to visualize pox lesions (b) (4) lab.	Across all 3 PFU challenges, 14/15 of the mice treated with BCV beginning at Day 3 survived the infection (1 died at highest challenge dose), compared to 5/12 vehicle treated mice (inconsistent). Low survival for animals that started treatment on Day 6 or 9. Weight loss in all groups. Papules first detected around Days 7-10 (inconsistent-not a good trigger). 100% protection from lethality w/re-challenge.
CMX001-(b) (4)U-012	Efficacy of CMX001 in Protecting SKH-1 Mice from Mortality Following an Intranasal Challenge with Various Doses of Ectromelia Virus II	ECTV-Mos 750, 1500 or 3000 PFU, i.n.	Female SKH-1 mice (6-8 wks)	64	Challenge on Day 0. BCV oral 25 mg/kg loading dose on Day 3, 6 or 9, followed by 2.5 mg/kg maintenance dose qod for 14 days. Surviving mice re-challenged with 19,000 PFU. Hairless mice to visualize pox lesions (b) (4) lab.	80-100% of vehicle treated controls died, median 11-12 days. 100% of mice treated beginning Day 3 survived. Lower survival for later treatment start times (0-60%). All groups lost weight relative to mock infected, Day 3 groups w/reduced weight loss. Papules first detected around Day 9-10 (inconsistent-not a good trigger). 100% protection from lethality w/re-challenge, although 50% of previously mock infected mice also survived.

Study w/link to study report	Title	Challenge Virus	Animals (age*)	Total N	Design#/Treatments	Results and Comments
CMX001-(b) (4)-013-RPT-01	Efficacy of CMX001 in Protecting SKH-1 Mice from Mortality Following an Intranasal Challenge with Various Doses of Ectromelia Virus III	ECTV-Mos 920, 1800, or 3700 PFU, i.n.	Female SKH-1 mice (6-8 wks)	65	Challenge on Day 0. BCV oral 25 mg/kg loading dose on Day 3, 6 or 9, followed by 2.5 mg/kg maintenance dose qod for 14 days. Surviving mice re-challenged with 20,000 PFU. Hairless mice to visualize pox lesions. (b) (4) (b) (4) lab.	60% survival in controls w/920 PFU challenge, so cannot measure survival benefit with this challenge. 0-20% survival of controls with higher challenge doses. 80-100% survival for mice treated beginning on Day 3. Lower survival for later treatment start times (0-40% with 1800 and 3700 PFU challenge, 60-80% w/920 PFU challenge). All groups lost weight relative to mock infected, Day 3 groups w/reduced weight loss (for 1800/3700 PFU challenges). Papules first detected around Day 8 (inconsistent-not a good trigger). 100% protection from lethality w/re-challenge, although 40% of previously mock infected mice also survived.
CMX001-(b) (4)-014-RPT-01	Efficacy of CMX001 in Protecting C57BL/6 Mice from Mortality Following an Intranasal Challenge with Ectromelia Virus	ECTV-Mos 800 PFU, i.n.	Female C57BL/6/Ncr (NCI) or C57BL/6 (Harlan) mice (6-8 wks)	170	Challenge on Day 0. M. (b) (4) lab. BCV oral 20 mg/kg every third day for 5 doses, initiated on Day 0, 4, 5, 6, 7, 8 or 9. Or single dose 10 mg/kg on Day 4 or 6. (b) (4) (b) (4) lab.	13% survival in controls, mean Day 11 death. Single or multiple doses of BCV starting on Day 6 associated with significant survival. 100%, 73%, 80%, 53% survival for multiple-dose groups starting tx on Day 0, 4, 5 or 6, respectively. 90% and 50% survival for Day 4 and Day 6 single dose groups, respectively. Weight loss generally in all infected groups except low weight loss in Day 0 treated mice.
CMX001-VIR-102	Determination of the Lethal Dose of Ectromelia Virus and Efficacy of Delayed Brincidofovir Treatment in Infected BALB/c Mice	ECTV-Mos-(b) (4) stock, target challenge doses of 0.27-2700 PFU, or 270 PFU, i.n.	BALB/c mice, female (8 or 11 weeks)	90	LD_{50}/LD_{90} study of ECTV-Mos (b) (4) stock, and assessment of BCV 10/5/5 mg/kg oral qod starting 2-6 days post-challenge. Actual PFU not back calculated. Unblinded study. (b) (4) lab.	In LD_{50}/LD_{90} study, 100% mortality with target doses of 270 or 2700 PFU. LD_{50}=1.6-1.8 PFU, LD_{90}=83.5 PFU. Shorter median time to death with higher challenge doses. Survival results for BCV start days: Day 2 (10/10, 100%), Day 3 (9/10, 90%), Day 4 (3/10, 30%), Day 5 (5/10, 50%), Day 6 (3/10, 30%); controls (0/5, 0%). Less weight loss with earlier BCV start.
NHP/MPXV BCV Studies						
CMX001-VIR-003	Evaluation of Intramuscular CMX001 for Treatment in the Lesional Monkeypox Cynomolgus Monkey Model	MPXV-Zaire'79 5 x 10^7 PFU i.v.	Male Cynomolgus monkeys, 5.3-8 kg	7	Challenge on Day 0. CMX001 4 mg/kg IM starting on Day 2 (n=3) or Day 3 (n=3) post-challenge, or control (n=1), for up to 16 days. Conducted at USAMRIID.	Placebo-treated monkey died on Day 17. CMX001 starting on Day 2 resulted in deaths in 2 of 3 animals (Days 8, 13). CMX001 starting on Day 3 resulted in deaths of all 3 animals (Days 12, 14 and 15). No difference in viral loads or lesion counts between different groups. Single surviving monkey had lower viral load and lesion counts.
CMX001-VIR-001	Evaluation of Three Oral Dosing Regimens of CMX001 (HDP-cidofovir) in Cynomolgus Monkeys Infected Intravenously with Monkeypox Virus	MPXV-Zaire'79 5 x 10^7 PFU i.v.	Male Cynomolgus monkeys, 7-9 kg	15	Challenge on Day 0. Randomized, blinded, PBO-controlled. CMX001 20-35 mg/kg (total doses, i.e., 2.5-10 mg/kg per dose, 3-5 doses of tx or PBO) oral on Days 1, 3, 6, 9 and 12. Conducted at USAMRIID.	All animals died by Day 28. CMX001 dosed groups had longer time to death compared to PBO (15 vs. 12 days), trends of slightly lower or delayed viral loads in blood, lower in tissues, slightly lower lesion counts.

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/s/

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