CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208627Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Antiviral Products
Center for Drug Evaluation and Research

Date:	May 11, 2018
Reviewer:	Jules O'Rear, Ph.D. Supervisory Microbiologist
NDA #/SDN #/date:	(b) (4)/000
Sponsor:	SIGA Technologies Inc.
Drug Product:	tecovirimat (ST-246)
Indication:	Treatment of variola virus infection (smallpox)
Recommended Action:	Approval

This NDA application for the use of tecovirimat (TPOXX) in the treatment of variola virus infection (smallpox) is approvable from a Clinical Virology perspective. The applicant, SIGA Technologies, Inc., has demonstrated antiviral activity against variola virus and other orthopoxviruses in cell culture and against multiple orthopoxviruses in multiple animal hosts. Four key studies in the monkeypox virus/cynomolgus macaque model and two key studies in the rabbitpox virus/New Zealand white rabbit model were submitted in support of approval and these are thoroughly described in the review of Dr. Pat Harrington, Clinical Virology Reviewer.

Some have proposed destroying the variola virus stocks once two antiviral drugs for smallpox are approved. This reviewer strongly disagrees with this proposal as other drugs may be needed in the future and isolates of variola virus are essential for drug development. Resistance has been found for virtually all direct-acting antiviral drugs and there are many examples of transmitted resistant virus. The genetic barrier to resistance of tecovirimat is low so development of a transmissible, resistant variant is a possibility, especially given that variola virus is naturally highly transmissible. Additionally, variola virus may replicate in humans in an essential organ where tecovirimat levels are inadequate and thereby compromises efficacy. The efficacy of antiviral drugs for smallpox will not be clearly established until these are used against variola virus in humans during an outbreak.

The threat of an accidental/intentional release of variola virus is a real possibility. Long sense forgotten frozen virus isolates have been found, some countries may have

retained stocks illegally, and it is possible that the virus could be synthesized (Noyce et al., 2018). In a release, the government is expected to initiate widespread vaccination of healthy individuals. Individuals known to have compromised immune systems, of which there are thought to be millions in the US, would not be vaccinated due to life-threatening complications arising from replication of the vaccine virus. However, it's likely that some individuals unknowingly having a compromised immune system will be vaccinated. The sponsor has conducted several studies to evaluate the activity of tecovirimat in such populations and these studies indicate that individuals with partially compromised immune systems, i.e. lacking T or B cell immunity, may be successfully treated (Grosenbach et al., 2009). However, individuals with severe combined immunodeficiency may need a combination of antiviral drugs (Grosenbach et al., 2009; Lederman et al., 2012; Vora et al., 2008). These same studies may also provide a false sense of security with respect to variola virus infection in individuals with partially compromised immune systems. The high mortality of smallpox and molecular characterization of variola virus and other orthopoxviruses indicate that variola virus encodes functions capable of compromising host immune function. Therefore, the response to tecovirimat in these variola virus infected individuals may be more like mice with severe combined immunodeficiency in which tecovirimat was not effective.

The uncertainty about the durability of drugs approved under the animal rule within an individual and during an outbreak, and, the needs of special populations argue for retention of the variola virus stocks for future drug development.

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/s/

JULIAN J O REAR
05/10/2018

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208627 **SDN** 004 **DATE REVIEWED:** 03/13/2018

NDA#: 208627 **Serial #: 004**

Reviewer's Name: Eric F. Donaldson, Ph.D.

Sponsor: SIGA Technologies, Inc.
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Annie Frimm, VP Regulatory, Clinical and Quality
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Submission Dates:

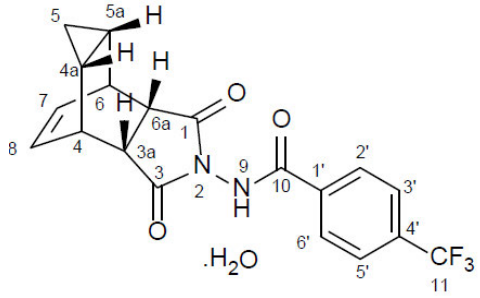
Correspondence Date: 12/8/2017

CDER Receipt Date: 12/8/2017

Assigned Date: 12/21/2017 (receipt of NGS data)

Review Complete Date: 04/19/2018

PDUFA Date: 08/08/2018

Proprietary Name	**TPOXX™**
Drug Names	tecovirimat, ST-246
IND #	69019
Chemical Name	Benzamide, N-[(3aR,4R,4aR,5aS,6S,6aS)-3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl]-4-(trifluoromethyl), rel-(monohydrate)
Structure	 **TECOVIRIMAT**
Molecular Formula	$C_{19}H_{15}F_3N_2O_3 \cdot H_2O$
Molecular Weight	376.33 Daltons (monohydrate=394.35 Daltons)

Amendments: none

Related/Supporting Documents: IND 69019; NDA 208627 SDN 000

Dosage Form and Route of Administration: Oral

Dispensed: Rx **x** OTC _

Proposed Indication(s): Treatment of human smallpox disease caused by variola virus in adults and pediatric patients

Abbreviations: CEV, cell-associated enveloped virus; CLC, CLC Genomics Workbench; EEV, extracellular enveloped virus; GT, genotype; HIVE, High-Performance Integrative Virtual Environment; ID, intradermal; IEV, intracellular enveloped viruses; IMV, intracellular mature viruses; IV, intravenous; LE, late endosomal; LLOQ, lower limit of quantification; LOD, limit of detection; MPXV, monkeypox virus; NGS, Next Generation Sequencing; NZW, New Zealand white (rabbits); ORF, open reading frame; PFU, plaque-forming unit; PK, pharmacokinetic; PRNT, plaque reduction neutralization assay; RPXV, rabbitpox virus; RPXV-UTR, RPXV Utrecht; SIV, simian immunodeficiency virus; SOP, standard operating procedure; SRV, simian retroviruses; STLV, simian T-cell leukemia virus; USAMRIID, U.S. Army Research Institute of Infectious Diseases; VACV, vaccinia virus; VARV, variola virus;

Table of Contents

EXECUTIVE SUMMARY

The sponsor submitted this original New Drug Application (NDA) for tecovirimat (ST-246; TPOXX™), with the proposed indication for treatment of human smallpox disease caused by variola virus (VARV) in adults and pediatric patients. VARV is a DNA virus of the *Orthopoxvirus* genus. Smallpox disease was one of the most devastating infectious diseases in human history, and as a result of an intense global vaccination campaign, no naturally acquired cases of human smallpox disease have occurred since 1978. Smallpox disease was declared eradicated from the world in 1980; however, despite the eradication of naturally acquired smallpox, the disease remains a threat over concerns that VARV could be developed as a bioterrorism agent. Routine vaccination in the U.S. ended in the 1970s, so most of the population is immunologically susceptible to smallpox. No antiviral drugs are approved for the treatment of VARV infection.

The primary Clinical Virology reviewer for this NDA is Dr. Patrick Harington, Ph.D and his review can be found under NDA 208627 SDN 000. This review was written to document an independent assessment of the Next Generation Sequencing (NGS) data submitted in support of the NDA from nucleotide sequencing data generated while assessing for the development of resistance against tecovirimat in challenge studies performed in rabbits and monkeys challenged with rabbitpox virus (RPXV) and monkeypox virus (MPXV), respectively.

NGS data were generated using the Illumina MiSeq™ platform to sequence blood samples collected at a timepoint near the time of treatment failure for animals who failed tecovirimat treatment to assess for amino acid substitutions in the viral target protein VP37 that could confer resistance, detected as amino acid substitutions, which correlate with treatment failure. The goals of the independent assessment of NGS data were to confirm the results reported by the sponsor, to determine which known tecovirimat resistance-associated substitutions were present in the virus of animals that failed treatment, and to determine if additional substitutions occurring in two or more animals could be associated with treatment failure.

Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few minor variations in sequencing depth and frequency. No additional substitutions were determined to be associated with resistance to tecovirimat.

BACKGROUND AND SUMMARY

Smallpox disease is caused by infection with variola virus (VARV), which is most commonly transmitted via the aerosol route with a low number of infectious units thought to be required for successful transmission. Once transmission has occurred there is an incubation period of 7-19 days before disease onset. During the incubation period, primary viremia occurs as the virus replicates in lymph nodes and begins to spread to other tissues. A secondary viremia occurs as the virus replicates in newly infected tissues generating high levels of viremia leading to the “prodrome phase” of disease, during which symptoms, such as high fever, headaches, and joint pain occur. The virus then spreads to the skin and oropharyngeal mucous membranes leading to the “eruptive” phase of disease, during which the characteristic smallpox rash begins to form. Viral shedding typically occurs with the onset of rash, and a person is thought to be most contagious during the first week of the rash when the skin legions are still mostly intact. If the infected individual does not succumb to disease, pocks on the skin are usually fully resolved 21 days after their initial appearance.

The virus that causes smallpox disease, VARV, was declared to be officially eradicated from the world on May 8, 1980 at the 33rd World Health Assembly (CDC Smallpox History). Despite the eradication of naturally acquired smallpox, the disease remains a threat because there are concerns that VARV could

be developed as a bioterrorism agent. Routine vaccination in the U.S. ended in the 1970s, so most of the population is immunologically susceptible to smallpox. No antiviral drugs are approved for the treatment of variola virus infection.

Tecovirimat is a small molecule antiviral drug that targets the orthopoxvirus VP37 protein within an infected cell, ultimately resulting in inhibition of viral spread to uninfected cells (Figure 1). The VP37 protein is encoded by the VARV C17L gene, as well as homologous genes in other orthopoxviruses, including vaccina virus (VACV), F13L; monkeypox virus (MPXV), C19L; rabbitpox virus (RPXV), RPXV041; and cowpox virus (CPXV), V061.

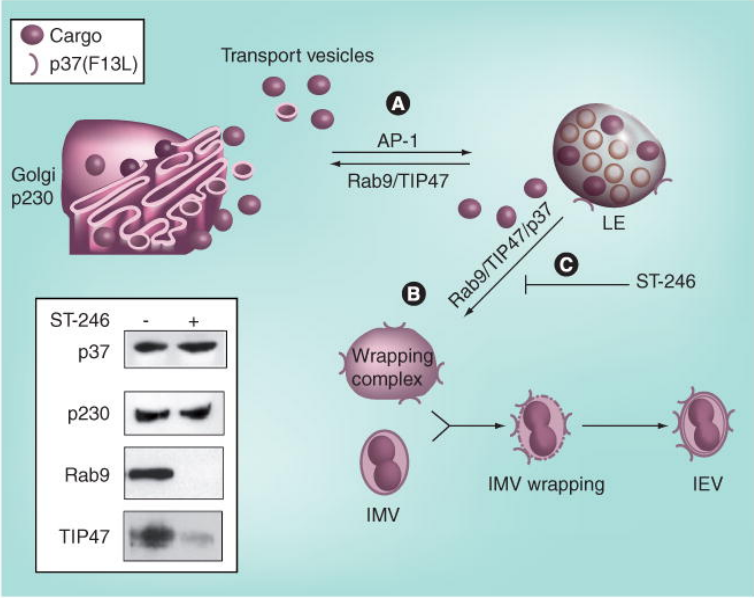


Figure 1. Tecovirimat mechanism of action (Figure 4, copied from Grosenbach et al., 2011). Vaccinia VP37 nucleates formation of a wrapping complex derived from LE membranes that catalyzes the envelopment of IMV particles to produce IEV. **(A)** Normally, LE-derived transport vesicles shuttle cellular 'cargo' between the LE and Golgi compartments. LE-derived transport vesicles assemble around cargo proteins through specific interactions with the Rab9 GTPase, and TIP47, a Rab9-specific effector protein. **(B)** The wrapping complex is formed through interactions of VP37 with components of LE-derived transport vesicles. Vaccinia virus VP37 acts like cellular cargo and interacts with Rab9 and TIP47 to nucleate a virus-specific wrapping complex required for assembly of IEV. **(C)** ST-246 blocks interaction of VP37 with Rab9 and TIP47 (but not p230, a Golgi-specific marker protein), and prevents wrapping complex formation. The inset shows a western blot analysis of proteins that coimmunoprecipitated with VP37 in the presence and absence of ST-246. IEV: Intracellular enveloped virus; IMV: Intracellular mature virus; LE: Late endosome. IEV fuses with the cellular membrane forming cell-associated enveloped viruses (CEV) and extracellular enveloped viruses (EEV).

Animal Models, Challenge Strains, and Reference Sequences

Because VARV is a tier 1 select agent and the virus has been eradicated, the sponsor is seeking approval of tecovirimat under the animal rule, whereby all efficacy studies were performed in animal models. The ideal animal model to test for the efficacy of tecovirimat would be one wherein the human VARV was used as the challenge virus, and while there is evidence of tecovirimat antiviral activity in the NHP/VARV model (Huggins *et al.*, 2009; Mucker *et al.*, 2013), there are several limitations to this approach, including 1) natural VARV infection and disease is specific to humans; 2) for nonhuman primates (NHPs), VARV challenge doses as high as 10^9 plaque-forming units (PFU) have been evaluated and do not cause uniform lethality or disease that resembles typical human smallpox (Jarhling et al., 2004); and 3) experiments with live VARV have significant feasibility issues due to the

requirement to use a World Health Organization (WHO)-approved biosafety level-4 laboratory, a resource that is limited. Therefore, the sponsor used other animal models of surrogate orthopoxviruses for pivotal efficacy studies, specifically the NHP/MPXV intravenous (IV) challenge and rabbit/RPXV intradermal (ID) challenge models.

The animal studies from which resistance data were generated by Next Generation Sequencing are summarized below. For a complete description and analysis of these and other animal studies, please refer to primary virology review performed by Dr. Patrick Harrington, Ph.D. (NDA 208627 SDN 000).

NHP/MPXV Lethal Challenge Model

The sponsor used an NHP/MPXV model in which cynomolgus macaques were challenged by IV with a high viral challenge dose of 5 x 10^7 PFU MPXV. The MPXV challenge strain used in key efficacy studies was referred to as the "Zaire '79" strain" (also referred to as V79-I-005, ZAI-1979-005, or Zaire-79-I-05). Viral challenge stocks were derived from scab material originally collected on 1/11/1979 from a severely ill 1-year-old boy living in Zaire (now Democratic Republic of Congo) during a 1978-1979 monkeypox outbreak (Zaire case 1134, monkeypox case #38; Breman et al., 1980; NDA 208627 SDN 23). According to the sponsor, the infected boy who was the source of the virus did not die from the infection, but experienced severe disease characterized by >100 lesions, severe incapacity, and required medical care. Other cases of severe or fatal infection occurred during the same outbreak.

Although the MPXV-infected boy in Zaire was the original source of challenge virus used in the key tecovirimat NHP/MPXV efficacy studies, the final challenge stocks used in each study were not uniformly produced (Animal Models White Paper, pg. 14).

Study SR10-037F (NHPs/Monkeypox Virus)

Title: SR10-037F, "Double-Blind Placebo Controlled Study to Evaluate Effect of Delayed ST-246 Treatment on Efficacy Following Lethal Monkeypox Virus Challenge in Cynomolgus Macaques"

Summary of Design: Study SR10-037F was a randomized, double-blind, placebo-controlled study of tecovirimat administered orally to cynomolgus monkeys infected with MPXV, conducted at (b) (4) The primary purpose of this study was to determine the effect of a delay in initiation of treatment on the efficacy of tecovirimat in MPXV-infected cynomolgus monkeys. A total of 21 cynomolgus monkeys that were negative for pre-existing antibodies against orthopoxviruses were randomly assigned to four groups to receive either placebo or tecovirimat 10 mg/kg for 14 days starting either on Day 4, Day 5 or Day 6 post-MPXV-challenge. All monkeys were challenged on Day 0 with 5.0 x 10^7 PFU of the MPXV Zaire '79 strain (NR-523 stock).

Summary of Results: All 3 animals in the Placebo group died by post-challenge Day 10, while survival rates in the tecovirimat Day 4, 5, and 6 groups were 83% (5/6), 83% (5/6), and 50% (3/6), respectively. According to the sponsor's analyses, survival rates were significantly greater (p=0.0476) in the tecovirimat Day 4 and tecovirimat Day 5 groups relative to the placebo group. In general, viral DNA levels were consistent with clinical efficacy observations, with reduced viral DNA levels (e.g., lower peak and more rapid decline) observed in animals that initiated tecovirimat treatment at Post-Challenge Day 4 or Day 5 relative to those that started tecovirimat at Post-Challenge Day 6.

Resistance Assessments: Genotypic resistance results generated by NGS were obtained from blood samples from 4 NHPs in Study SR10-037F. Of these 4 NHPs, 3 received tecovirimat and 1 received placebo:

- SR10-037F NHP blood samples w/NGS resistance data (Treatment Group):

- Animal 4762-Day 7 (Placebo); euthanized/died on Day 7
- Animal 4768-Day 12 (Tecovirimat 10 mg/kg Day 4-17); euthanized/died on Day 12
- Animal 4770-Day 11 (Tecovirimat 10 mg/kg Day 6-19); euthanized/died on Day 11
- Animal 4773-Day 8 (Tecovirimat 10 mg/kg Day 5-18); euthanized/died on Day 8

Emergence of tecovirimat VP37 resistance-associated substitutions was demonstrated in 2 of the 3 tecovirimat-treated NHPs and included the following amino acid positions: H238, N267, R268, A295, L297, and I372. For an in depth analysis of these resistance-associated substitutions, please see the Deep Sequencing Analysis of Animal Studies section below.

Study SR10-038F (NHPs/Monkeypox Virus)

Title: SR10-038F, "Double-Blind, Placebo-Controlled Study to Evaluate Effect of Duration of ST-246 Treatment on Efficacy Following Lethal Monkeypox Virus Challenge in Cynomolgus Macaques"

Summary of Design: Study SR10-038F was a double-blind, randomized, placebo-controlled study of oral tecovirimat in cynomolgus monkeys infected with MPXV, conducted at (b) (4) . The primary purpose of this study was to determine the effect of varying the duration of treatment on the efficacy of tecovirimat in MPXV-infected cynomolgus monkeys.

A total of 25 cynomolgus macaques that were negative for pre-existing antibodies against orthopoxviruses were randomly assigned to five groups to receive either placebo or 3, 5, 7, or 10 days of 10 mg/kg tecovirimat starting on post-challenge Day 4. All monkeys were challenged on Day 0 with 5.0×10^7 PFU of the MPXV Zaire '79 strain (NR-2324 stock). The virus was provided by BEI Research Resources Repository at a titer of 4.1×10^8 PFU/mL, and was thawed and diluted to a concentration of 5×10^7 PFU/mL for inoculation.

Summary of Results: Survival rates were 25% (1/4) in the Placebo group and 86% (18/21) in the pooled tecovirimat treatment groups. There was a trend of reduced efficacy in the tecovirimat 3-dose group relative to other tecovirimat groups, although sizes of the treatment groups for comparison were small.

Resistance Assessments: Genotypic resistance results generated by NGS were obtained from blood samples from 2 NHPs in Study SR10-038F. Of these 2 NHPs, 1 received tecovirimat and 1 received placebo:

- SR10-038F NHP blood samples w/NGS resistance data (Treatment Group):
  - Animal 4803-Day 12 (Placebo); euthanized/died on Day 13
  - Animal 4819-Days 9 and 11 (tecovirimat 10 mg/kg Day 4-13); euthanized/died on Day 11

Emergence of tecovirimat VP37 resistance-associated substitutions was demonstrated in the tecovirimat-treated NHP and included the following amino acid positions: N267 and A295. For an in depth analysis of these resistance-associated substitutions, please see the Deep Sequencing Analysis of Animal Studies section below.

Rabbit/RPXV Lethal Challenge Model

The RPXV Utrecht (RPXV-UTR) strain, first isolated in 1941, causes a rapid and consistently fatal disease in rabbits with a relatively low viral challenge dose. The sponsor noted that the RPXV-UTR

isolate is much more pathogenic than a "Rockefeller" strain that was isolated earlier in 1932, and therefore RPXV-UTR has been the primary RPXV isolate used in published studies over the last 40+ years. The early passage and transfer history of RPXV-UTR is not well-documented. The virus was transferred to multiple international academic laboratories before it was obtained by (b) (4) in 1963 and subsequently transferred to the American Type Culture Collection (ATCC) (Animal Models White Paper, pg. 19).

Study SR13-007F (Rabbits/Rabbitpox Virus)

Title: SR13-007F, "Exploratory Pilot Efficacy Study to Evaluate the Impact of Time of Tecovirimat Treatment Initiation on Mortality Following Lethal Intradermal Rabbitpox Virus Challenge of NZW Rabbits"

Summary of Design: Study SR13-007F was a double-blind, randomized, placebo-controlled study of oral tecovirimat in New Zealand White (NZW) rabbits infected with RPXV. The primary purpose of this study was to evaluate the impact of time of tecovirimat treatment initiation on mortality in RPXV-infected NZW rabbits. A total of 48 NZW rabbits that were negative for pre-existing antibodies against orthopoxviruses were randomly assigned to six groups to receive tecovirimat 80 mg/kg QD oral, or placebo, for 14 days starting on Day 2-6 post-challenge. All rabbits were challenged on Day 0 with 300 PFU ID of the Rabbitpox Virus-Utrech.

Summary of Results: Tecovirimat starting on Day 2, 3, or 4 post-challenge protected all animals from lethal disease, with lower viral DNA levels in blood compared to placebo-treated animals. Reduced protection occurred when treatment started on Day 5 and no protection was observed when treatment started on Day 6.

Resistance Assessments: Genotypic resistance results were obtained from blood samples from 4 rabbits in Study SR13-007F. The 4 blood samples are as follows (SR13-007F Treatment Group):

- Animal 714-Day 7 (Tecovirimat 80 mg/kg Day 6-19); euthanized/died on Day 7
- Animal 719-Day 7 (Tecovirimat 80 mg/kg Day 6-19); euthanized/died on Day 7
- Animal 741-Day 7 (Tecovirimat 80 mg/kg Day 6-19); euthanized/died on Day 7
- Animal 751-Day 7 (Tecovirimat 80 mg/kg Day 6-19); euthanized/died on Day 7

Emergence of tecovirimat VP37 resistance-associated substitutions was demonstrated in 1 out of 4 tecovirimat-treated rabbits and included only one amino acid position: N179. For an in depth analysis of these resistance-associated substitutions, please see the Deep Sequencing Analysis of Animal Studies section below.

Reference Sequences for Assembly of NGS Reads

A multiple alignment of the VP37 reference sequences was generated by the Division of Antiviral Products (DAVP) to confirm that the tecovirimat VP37 protein targets are highly conserved between MPXV, RPXV, and VARV. The VP37 amino acid sequences of the specific MPXV Zaire '79 and RPXV-UTR strains used in the pivotal animal studies were approximately 98% identical to a prototypical VARV Bangladesh '74 strain (Figure 2A and B). Furthermore, while tecovirimat efficacy was primarily established from studies using these models, tecovirimat has also been shown to have broad anti-orthopoxvirus activity in cell culture and also in numerous other orthopoxvirus animal models (see NDA 208627 SDN 000 for detailed information), providing further evidence that tecovirimat is reasonably likely to be effective for the treatment of VARV infection.

Based on these analyses, the VP37 reference sequences selected and used by DAVP for the NGS analyses were: rabbitpox virus strain Utrecht (RPXVUTR41; AY484669.1) and monkeypox virus strain Zaire 1979-005 (MPXVZR79, DQ011155).

A.

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                                20                  40                  60                  80
VACVWR52  MWPFASVPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
VACVCO90  MWPFASVPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
RPXVUTR41 MWPFAPVPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
MPXVZR79  MWPFASVPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EVSEKGIKII 80
MPXVV79   MWPFASVPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EVSEKGIKII 80
VARVBD74  MWPFTSAPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
VARVUK52  MWPFTSAPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
VARVIN67  MWPFTSAPAG AKCRLVETLP ENMDFRSDHL TTFECFNEII TLAKKYIYIA SFCCNPLSTT RGALIFDKLK EASEKGIKII 80
                               100                 120                 140                 160
VACVWR52  VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
VACVCO90  VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
RPXVUTR41 VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
MPXVZR79  VLLDERGKRN LGELQSHSPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
MPXVV79   VLLDERGKRN LGELQSHSPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
VARVBD74  VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
VARVUK52  VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
VARVIN67  VLLDERGKRN LGELQSHCPD INFITVNIDK KNNVGLLLGC FWVSDDERCY VGNASFTGGS IHTIKTLGVY SDYPPLATDL 160
                               180                 200                 220                 240
VACVWR52  RRRFDTFKAF NSAKNSWLNL CSAACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLKSA KTSIDIEHLA 240
VACVCO90  RRRFDTFKAF NSAKNSWLNL CSAACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLRSA KTSIDIEHLA 240
RPXVUTR41 RRRFDTFKAF NSAKNSWLNL CSAACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLKSA KTSIDIEHLA 240
MPXVZR79  RRRFDTFKAF NSAKNSWLNL CSAACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLKSA KTSIDIEHLA 240
MPXVV79   RRRFDTFKAF NSAKNSWLNL CSAACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLKSA KTSIDIEHXA 240
VARVBD74  RRRFDTFKAF NSVKNSWLNL YSSACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLRSA KTSIDIEHLA 240
VARVUK52  RRRFDTFKAF NSVKNSWLNL YSSACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLRSA KTSIDIEHLA 240
VARVIN67  RRRFDTFKAF NSVKNSWLNL YSSACCLPVS TAYHIKNPIG GVFFTDSPEH LLGYSRDLDT DVVIDKLRSA KTSIDIEHLA 240
                               260                 280                 300                 320
VACVWR52  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
VACVCO90  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
RPXVUTR41 IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
MPXVZR79  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
MPXVV79   IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
VARVBD74  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
VARVUK52  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA RSLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
VARVIN67  IVPTTRVDGN SYYWPDIYNS IIEAAINRGV KIRLLVGNWD KNDVYSMATA ESLDALCVQN DLSVKVFTIQ NNTKLLIVDD 320
                               340                 360
VACVWR52  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
VACVCO90  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
RPXVUTR41 EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
MPXVZR79  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
MPXVV79   EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
VARVBD74  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
VARVUK52  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
VARVIN67  EYVHITSANF DGTHYQNHGF VSFNSIDKQL VSEAKKIFER DWVSSHSKSL KI 372
```

B.

Number of amino acid differences

		1	2	3	4
VACVWR52	1		1	2	6
RPXVUTR41	2	99.73		3	7
MPXVZR79	3	99.46	99.19		8
VARVBD74	4	98.39	98.12	97.85	

Percent identity

Figure 2. Comparison of VP37 amino acid reference sequences used for NGS analyses (DAVP analyses). **A.** An amino acid multiple alignment of orthopoxvirus VP37 from several reference viruses. **B.** A percent identity matrix showing that all of the orthopoxvirus reference strains analyzed were closely related. The following sequences were compared: Monkeypox viruses: monkeypox virus Zaire '79 (MPXVV79; HQ857562 and ADX22692.1); Monkeypox virus strain Zaire_1979-005 (MPXVZR79; DQ011155 and AAY97243.1); Rabbitpox virus: Rabbitpox virus Utrecht (RPXVUTR41; AY484669 and AAS49754.1); Vaccinia viruses: Vaccinia virus Western Reserve (VACVWR52; P04021); Vaccinia virus Copenhagen (VACVCO90; M35027 and AAA48031.1) and Variola viruses: Variola virus Ind3/1967 (VARVIN67; P33815); Variola virus Bangladesh 1974 (VARVBD74; DQ441422 and ABF24006.1) and Variola virus United Kingdom 1952 Butler (VARVUK52; DQ441447 and ABF29026.1).

NGS Resistance Data Overview

The sponsor provided 24 fastq files as part of their resistance analysis for 10 animals (4 NZW rabbits and 6 NHPs) from 3 animal studies and two fastq files from NGS controls. The sponsor included an

NGS Tecovirimat Resistance Monitoring report, which contained the resistance analyses conducted for animals that were challenged with either RPXV (rabbits) or MPXV (NHPs). The NGS data provided by the sponsor included: 1) frequency tables showing amino acid variation that occurred at each position of the viral target protein (MPXV C19L and RPXV RPXV041) for each animal at the time of failure for all samples that were successfully sequenced using Illumina MiSeq™ technology; 2) raw sequence data in fastq format for all animals that were deep sequenced; 3) summary resistance data for each study; and 4) cross study comparisons of resistance data.

Given that next generation sequencing is an evolving technology with no current standards for analysis, the division requested raw data so that an independent analysis could be performed on the NGS data. The sponsor's summary NGS data were compared to the results generated by DAVP following these criteria:

1. The sponsor's frequency tables were used to generate a summary and do a direct comparison of the results reported by the sponsor;
2. Frequency tables were generated by DAVP using an independent mapping of reads to a reference for each sample and using an optimized NGS analysis pipeline in the High-Performance Integrative Virtual Environment (HIVE). A second optimized NGS analysis pipeline using CLC Genomics Workbench (CLC) was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency) between the sponsor's results and the HIVE pipeline. The results of the independent assessment were compared with those reported by the sponsor and those generated using the sponsor's frequency table; and
3. The conclusions from the NGS data were compared to the results reported by the sponsor.

The analysis pipeline used by DAVP has been described previously (Donaldson et al., 2015; Donaldson et al., 2015) and can be viewed in detail in a previous NDA review for NDA209939 SDN 000 003.

NGS RESISTANCE ANALYSIS OF ANIMAL STUDIES

The sponsor attempted to perform next generation sequencing analysis of samples taken from several animals across multiple studies. In total, viral DNA was isolated from 129 blood and tissue samples from NHP/MPXV and rabbit/RPXV studies and processed for PCR amplification and NGS analysis of the VP37 target gene (C19L gene in MPXV, RPXV041 gene in RPXV). These samples came from NHP/MPXV studies SR10-037F and SR10-038F; rabbit/RPXV studies SR13-025F, SR14-008F, and SR13-007F; and an untreated rabbit from RPXV study SR14-015F. However, of the 129 blood and tissue samples processed for NGS analysis, the sponsor reported that (b) (4) was only able to successfully PCR amplify and sequence 11 (9%) samples, including 7 MPXV samples from 6 animals and 4 RPXV samples from 4 animals (all from blood).

Prior to the NDA submission, Clinical Virology was made aware of this poor success rate, and requested that the sponsor provide more details regarding the low success rate for PCR amplification and sequencing of these samples. In response, the sponsor stated that for successful amplification and sequencing library generation, the assay used by (b) (4) requires an input DNA concentration of $>10^6$ genome copies per PCR reaction, and with an input volume of 5 µL this requires a very high starting viral DNA concentration of $\geq 2 \times 10^8$ copies/mL. In addition, the sponsor also stated that during NGS method development, (b) (4) . Repeat attempts only confirmed the requirement for a concentration of 2×10^8 genome copies/mL. The study director at (b) (4) speculated that the reason for this was (b) (4) . The PCR reaction conditions were

designed to ensure high-fidelity over amplification efficiency so that nucleotide changes were not introduced during the reaction possibly resulting in the report of nucleotide variants not present in the template DNA (see Clinical Virology reviews of IND 69019 SDN 398 and IND 69019 SDN 402 for more details and a list of all samples attempted for analysis).

Comment (not to be communicated): The requirement of ≥2x10^8 genome copies/mL for samples to be successfully amplified for sequencing library generation seems to be excessive, given that many assay limitations are several orders of magnitude lower (10^3 genome copies/mL). However, there is not likely enough material for the sponsor to perform additional attempts at sequencing the samples. We note that the sponsor could have employed several additional steps in an attempt to increase sample amplification, including adding a step to remove host DNA, nested PCR, and re-assaying samples.

Although the available NGS data were limited, the overall results were consistent with tecovirimat having a low resistance barrier and multiple potential resistance pathways, as observed in cell culture resistance analysis studies (please see the primary virology review of NDA 208627 SDN 000). NGS data were obtained for 7 MPXV samples from 6 animals in 2 studies (SR10-037F and SR10-038F), and 4 RPXV samples from 4 animals in 1 study (SR13-007F). All of these data came from blood samples. In the sponsor's analyses, genotypic changes were identified relative to published GenBank Accession numbers HQ857562 (MPXV) and AY484669 (RPXV). DAVP used the same AY484669 (RPXV) reference sequence but opted to use the DQ011155 (MPXVZR79) as the monkeypox virus reference instead of HQ857562 (MPXV), as the sponsor's reference sequence contains an 'X' indicating an ambiguous amino acid call at position 239. Of the 11 samples derived from 10 animals (6 NHPs and 4 rabbits), the sponsor reported that genotypic changes were observed in the VP37 protein in 4 samples (3 MPXV, 1 RPXV) from 4 animals. The other 7 samples from 6 animals did not have any reported genetic changes. Of note, there were two samples collected and sequenced for one NHP and two of the NHP samples for which there were no detected substitutions were from monkeys receiving placebo.

Table 1 summarizes the amino acid substitutions detected in the viral isolates from the 10 animals; Table 2 provides additional details regarding the specific nucleotides, codons, and variant frequencies observed; and Table 3 provides a summary of additional characteristics for the 4 animals with reported VP37 amino acid substitutions.

Table 1. Number of tecovirimat-treated animals with indicated VP37 amino acid substitutions (Table 1, page 11, Study Report 16-039f).

Virus	Substitutions	Subjects with Change
MPXV Zaire '79	H238Q	1/6
	N267del/K/S/I	2/6
	N267D	1/6
	R268G	1/6
	D280Y	1/6
	A290V	1/6
	A295E	2/6
	L297ins	1/6
	I372N	2/6
	I372 ILKIKNRK	1/6
RPXV Utrecht	N179T	1/4

Despite the limited data, amino acid substitutions were detected at several positions previously shown to be associated with tecovirimat resistance for vaccinia virus, including positions H238, N267, A290, A295 and I372 (Table 1); note that these positions are highly conserved among orthopoxviruses (see Clinical Virology review of IND069019 SDN 373). Macaque 4819 had monkeypox virus with resistance-

associated substitutions at both Day 9 and Day 11, with evidence of further resistance emergence between these timepoints while the animal was still receiving tecovirimat: A295E enriched from ~10% to ~53%, and N268del enriched from ~2% to ~17% with several additional substitutions detected at this position on Day 11 (Table 2).

Amino acid substitutions were also observed at positions R268, D280, L297 and N179 (Table 1). According to the sponsor, substitutions at these 4 positions have not been previously demonstrated to affect tecovirimat susceptibility. The significance of the substitutions at these positions is unclear as only a single animal had one substitution for each of these positions, and in all 4 cases the substitutions were detected at a low frequency (2.32-3.98%). The monkeypox virus R268G and L297ins changes were observed in the virus from the same animal (4768), which had other more prevalent changes at known resistance-associated positions, in particular with multiple different substitutions observed at resistance-associated position N267. Similarly, the macaque with the D280Y substitution (4770) had a predominant I372N resistance-associated substitution, as well as an unusual insertion at this same position (See Figure 3 for more details). However, the N179T substitution was the only substitution observed in rabbitpox virus-infected rabbits (Table 2).

Table 2. Nucleotide and codon changes, and variant frequency for tecovirimat-treated animals with VP37 substitutions (Table 2, pages 12-13, Study Report 16-039f).

STUDYID	USUBJID	NGSPL	VISIT	NTPOS	NTREF	NTSub	P37AAPOS	AAREF	AASub	TCOV	VCOV	PERCENT
Monkeypox virus Zaire '79 (GenBank Accession HQ857562) P37 Variant Frequencies												
SR10037F	4768	Illumina	Day 12	41100	A	T	372	I	N	65748	10026	15.25
SR10037F	4768	Illumina	Day 12	41325	C	CACA	297	-	L (insert)	129609	3011	2.32
SR10037F	4768	Illumina	Day 12	41331	G	T	295	A	E	124235	2704	2.18
SR10037F	4768	Illumina	Day 12	41412	CTAT	C	267	N	- (delete)	187758	86196	45.91
SR10037F	4768	Illumina	Day 12	41413	T	C	268	R	G	92577	3573	3.86
SR10037F	4768	Illumina	Day 12	41414	A	T	267	N	K	98443	6408	6.51
SR10037F	4768	Illumina	Day 12	41415	T	C	267	N	S	101349	9347	9.22
SR10037F	4768	Illumina	Day 12	41415	T	A	267	N	I	101349	10515	10.38
SR10037F	4768	Illumina	Day 12	41417	A	G	266	I	I (silent)	193220	7805	4.04
SR10037F	4768	Illumina	Day 12	41501	A	C	238	H	Q	221444	15802	7.14
SR10037F	4770	Illumina	Day 11	41097	T	A	372	I	ILKIKNRK	23018	902	3.92
SR10037F	4770	Illumina	Day 11	41100	A	T	372	I	N	23231	14617	62.92
SR10037F	4770	Illumina	Day 11	41346	G	A	290	A	V	48530	1182	2.44
SR10037F	4770	Illumina	Day 11	41377	C	A	280	D	Y	62471	2487	3.98
SR10038F	4819	Illumina	Day 9	41331	G	T	295	A	E	43539	4227	9.71
SR10038F	4819	Illumina	Day 9	41412	CTAT	C	267	N	- (delete)	65637	1335	2.03
SR10038F	4819	Illumina	Day 11	41331	G	T	295	A	E	145936	77585	53.16
SR10038F	4819	Illumina	Day 11	41412	CTAT	C	267	N	- (delete)	220307	36893	16.75
SR10038F	4819	Illumina	Day 11	41414	A	T	267	N	K	184180	3744	2.03
SR10038F	4819	Illumina	Day 11	41415	T	C	267	N	S	188221	5784	3.07
SR10038F	4819	Illumina	Day 11	41415	T	A	267	N	I	188221	8294	4.41
SR10038F	4819	Illumina	Day 11	41416	T	C	267	N	D	229679	7666	3.34
SR10038F	4819	Illumina	Day 11	41417	A	G	266	I	I (silent)	227652	6587	2.89
Rabbitpox Utrecht (GenBank Accession AY484669) P37 Variant Frequency												
SR13007F	751	Illumina	Day 7	46145	T	G	179	N	T	374702	13819	3.69

STUDYID (b) (4) study number; USUBJID, unique subject identification code; NGSPL, next generation sequencing platform; VISIT, visit day relative to virus infection on Day 0; NTPOS, genomic nucleotide position based on the reference sequence of monkeypox virus Zaire '79 (GenBank Accession HQ857562) or the reference sequence of rabbitpox virus Utrecht (GenBank Accession AY484669); NTREF, reference nucleotide at the position indicated; NTSub, nucleotide variant at the indicated position; P37AAPOS, amino acid position in the poxvirus p37 protein affected by the nucleotide variation; AAREF, wild-type amino acid at the position indicated; AASub, amino acid variant at the indicated position; TCOV, total next generation sequencing coverage at the indicated nucleotide position; VCOV, next generation sequencing coverage of the variant nucleotide at the indicated nucleotide position; PERCENT, frequency at which the variant nucleotide occurs (x100).

Table 3. Characteristics of tecovirimat-treated animals with VP37 substitutions (Table 3, page 14, Study Report 16-039f).

Study	Virus	Animal	Tecovirimat	Disposition	P37 Variants
SR10-037F	MPXV	4768	10 mg/kg Initiated Day 4 (intended for 14 daily doses) to Day 17 Received 8 doses	•Found dead Day 12 •Moderate dehydration •Mild depression •Mild dyspnea •Mild weakness •Severely reduced food consumption	I372N, 15.25% L297ins, 2.32% A295E, 2.18% N267del, 45.91% R268G, 3.86% N267K, 6.51% N267S, 9.22% N267I, 10.38%
		4770	10 mg/kg Initiated Day 6 (intended for 14 daily doses)to Day 19 Received 5 doses	•Euthanized Day 11 •Severe dehydration •Mild depression •Mild dyspnea •Mild nasal discharge •Moderate ocular discharge •Severely reduced food consumption	I372ILKIKNRK, 3.92% I372N, 62.92% A290V, 2.44% D280Y, 3.98%
SR10-038F	MPXV	4819	10 mg/kg Initiated Day 4 (intended for 10 daily doses) to Day 13 Received 7 doses	•Euthanized Day 11 •Moderate dehydration •Mild depression •Mild dyspnea •Mild nasal discharge •Moderate general observations – excessive drooling and continuous salivation •Mild weakness •Mildly reduced food consumption	A295E, 53.16% N267del, 16.75% N267K, 2.03% N267S, 3.07% N267I, 4.41% N267D, 3.34%
SR13-007F	RPXV	751	80 mg/kg Initiated Day 6 (intended for 14 daily doses) to Day 19 Received 2 doses	•Euthanized Day 7 •Hypothermic •11.8% weight loss •Cyanotic	N179T, 3.69%

Resistance Analysis Methodologies Used by the Sponsor

The sponsor outsourced the NGS analyses to (b) (4) which performed the sequencing using the Illumina MiSeq™ platform. The sponsor provided the details of the (b) (4) analysis process and analysis pipeline (see the methods section in Appendix I). Overall, the (b) (4) analysis process appeared to be robust, and in many cases was able to align more reads/per position increasing the coverage than the algorithms used by DAVP.

DAVP Assessment of NGS Data

The independent assessment of the NGS data was performed by mapping the reads from each sample to a nucleotide reference gene sequence specific for the VP37 protein (Rabbitpox virus strain Utrecht [RPXVUTR41; AY484669.1] and Monkeypox virus strain Zaire 1979-005 [MPXVZR79, DQ011155]) for each sample using an optimized NGS analysis pipeline in the High-Performance Integrative Virtual Environment (HIVE). A second optimized NGS analysis pipeline using CLC Genomics Workbench was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency) between the sponsor's results and the HIVE pipeline (for details on both analysis pipelines please see NDA209939 SDN 000 003). Variant call files were produced from each pipeline, and these were analyzed and modified to generate frequency tables that identified potential resistance-associated

substitutions associated with resistance to tecovirimat. The results of the independent assessment were compared with those reported by the sponsor and those generated using the sponsor's frequency table and the conclusions from the NGS data were compared to the results reported by the sponsor.

In general, there was good agreement between the results generated by the sponsor (Table 2 and Table 3) and those derived by DAVP (Table 4), although there were some discrepancies among variant calls below a frequency of ~5%. For example, in study SR10-038F from the Day 11 sample from NHP 4819, there were several low frequency variants (<5%) identified at amino acid position 267 by the sponsor's analysis, including N267K at 2.03%, N267S at 3.07%, N267I at 4.41% and N267D at 3.34%, for a cumulative total of ~13%. Of these, only N267D at 3.39% was detected by CLC Genomics Workbench. These discrepancies are likely due to coverage differences reported by each algorithm used to perform the mappings. For the NHP 4819 sample from Day 11 at the nucleotide position for N267D, the sponsor reported a total coverage 229,652, whereas, HIVE reported a coverage of 212,272 and CLC Genomics a coverage of 143,983. The coverage differences observed between the two optimized analysis pipelines used by DAVP and the one used by the sponsor illustrate the unique challenges of using NGS data to inform regulatory decisions, and provide further support for performing independent analyses of NGS data used for regulatory purposes.

Table 4. Independent assessment of NGS data (DAVP analysis).

Study	NHP	ARM	Outcome	SAMPLE	TYPE	Titer	No. files	No. Read Pairs	Sponsor	DAVP -HIVE	DAVP-CLC
	4803	PBO	Death D12	D12	BLOOD	410000000	2	2,338,544			
SR10-038F	4819	10 mg/kg	Death D11	D11	BLOOD	886000000	2	2,519,966	A295E, 53.16% N267del, 16.75% N267K, 2.03% N267S, 3.07% N267I, 4.41% N267D, 3.34%	N267del, 20% A295E, 53%	N267del, 18.06% A295E, 60.24% N267D, 3.39%
	4819	10 mg/kg	Death D11	D9	BLOOD	165100000	2	1,244,800	A295E, 9.71%	A295E, 10%	
	4762	PBO	Death D7	D7	BLOOD	306000000	2	2,743,136			
SR10-037F	4768	10 mg/kg	Death D12	D12	BLOOD	3630000000	2	2,861,944	I372N, 15.25% L297ins, 2 32% A295E, 2.18% N267del, 45.91% R268G,3.86% N267K, 6.51% N267S, 9.22% N267I,10.38% H238Q, 7.14%	N267del, 55% I372N, 17%	H238Q, 7.06% N267del, 48.97% A295E, 2.18% I372N, 15.22%
	4770	10 mg/kg	Death D11	D11	BLOOD	348000000	2	2,478,352	I372ILKIKNRK, 3.92% I372N, 62.92% A290V, 2.44% D280Y, 3.98%	I372N, 59%	D280Y, 3.98% A290V, 2.36% I372N, 53.85%
	4773	10 mg/kg	Death D8	D8	BLOOD	176700000	2	2,460,248			

Study	RABBIT	ARM	Outcome	SAMPLE	TYPE	Titer	No. files	No. Read Pairs	Sponsor	DAVP -HIVE	DAVP-CLC
SR13-007F	714	80 mg/kg	Death D7	D7	BLOOD	1177672533	2	2,886,580			
	719	80 mg/kg	Death D7	D7	BLOOD	481691080	2	2,630,570			
	741	80 mg/kg	Death D7	D7	BLOOD	407785307	2	2,839,218			
	751	80 mg/kg	Death D7	D7	BLOOD	390790327	2	2,635,430	N179T, 3.69%	N179T, 3.00%	n.d.

NHP, nonhuman primate; DAVP, Division of Antiviral Products; HIVE, high-performance integrative virtual environment; CLC, CLC Genomics Workbench; PBO, placebo; D, study day; del, deletion; n.d., not determined.

Of note, the I372ILKIKNRK variant detected at a frequency of 3.92% (Table 2 and Table 4), which was reported by the sponsor for NHP 4770 from the Day 11 sample, was not detected by either HIVE or CLC Genomics, so this substitution was analyzed in further detail. In this case, the stop codon at the end of the C19L gene was mutated from TAA (stop codon,*) to TTA (leucine), which extended the ORF for six additional amino acids resulting in ILKIKNRK* instead of the wild type version I*KIKNRK*. This was not detected by HIVE or CLC Genomics because the reference sequence used for mapping reads did not include any additional sequence beyond the stop codon used to define the gene (Figure 3).

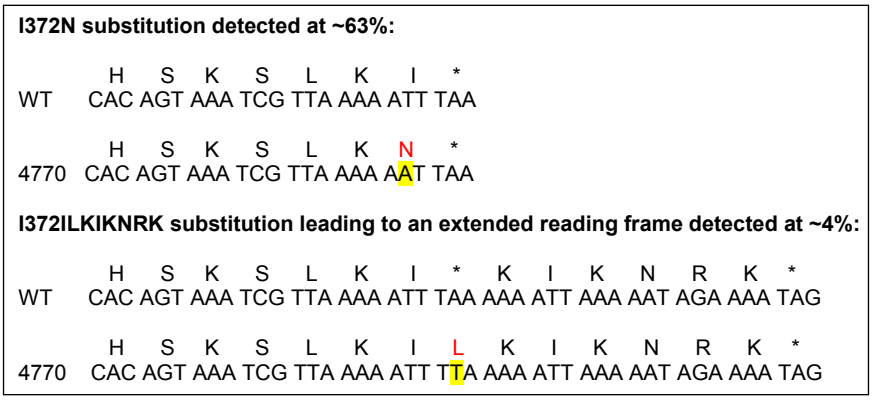

Figure 3. Coding diagrams for position 372 and the substitutions identified at this position for NHP 4770 (DAVP Analysis). Red indicates an amino acid substitution that differed from the wildtype challenge strain; yellow indicated a nucleotide mutation that differed from the wildtype challenge strain. WT, wildtype C129L sequence; 4770, sample collected from NHP 4770 at Day 11; *, stop codon.

Despite these differences, the comparison of the data generated by DAVP to the results provided by the sponsor were in agreement for the resistance pathways identified, and no novel resistance-associated substitutions were detected.

Resistance Conclusions from the Sponsor

The sponsor acknowledged that low quantities of DNA prevented the sequencing of the majority of the samples sent to [redacted] (b) (4) However, from the 11 samples that could be sequenced, 5 samples from 4 animals yielded resistance-associated substitutions in the VP37 gene. Amino acid substitutions at positions H238, N267, A290, A295 and I372 are known tecovirimat resistance-associated substitution sites that have been described in the literature; however, the sponsor stated that changes at R268, D280, L297 and N179 were novel to this analysis.

DAVP Resistance Conclusions

The overall results of the independent analysis of NGS data were in agreement with those reported by the sponsor. Tecovirimat resistance-associated substitutions were identified at the following sites: N267 (n=2), A295 (n=2), I372 (n=2), H238 (n=1), R268 (n=1), D280 (n=1), A290 (n=1), and L297 (n=1) in NHPs challenged with MPXV and N179 (n=1) in rabbits challenged with RPXV. No additional substitutions were detected.

CONCLUSIONS

DAVP performed an independent analysis of the NGS data submitted in support of the approval of tecovirimat for the treatment of smallpox disease caused by VARV. Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few minor variations in sequencing depth and frequency. No novel amino acid substitutions (not detected by the sponsor's analyses) were detected by DAVP in any sample from any animal treated with tecovirimat. The tecovirimat resistance-associated substitutions detected in two animals were at the following sites: 267 (n=2; N267del/I/D/S/K in one and N267del/I/S/K in the other), 295 (n=2; A295E), 372 (n=2; I372N in one and I372N and I372ILKIKNRK in the other). Single tecovirimat resistance-associated substitutions were detected at the following sites: 238 (n=1, H238Q), 268 (n=1, R268G), 280 (n=1, D280Y), 290 (n=1, A290V), and L297 (n=1, L287ins) in NHPs challenged with MPXV and 179 (n=1, N179T) in rabbits challenged with RPXV. Of note, phenotypic shifts in tecovirimat susceptibility were noted for substitutions H238Q, N267D, N267S, A290V, A295E, and I372N indicating that these

positions are associated with resistance to tecovirimat. Additional phenotype resistance analysis will be required as a post-marketing requirement (see the primary virology review of NDA 208627 SDN 000 for specific details.

POST MARKETING RECOMMENDATIONS

1. No recommendations based on the independent analysis of NGS data.

ADMINISTRATIVE

Reviewer's Signature(s)

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

Concurrence(s)

_____Date: _____
HFD-530/Clin Micro TL/J O'Rear

cc:
HFD-530/NDA
HFD-530/Division File
HFD-530/RPM/Gentles

4 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page

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/s/

ERIC F DONALDSON
05/08/2018

PATRICK R HARRINGTON
05/08/2018

JULIAN J O REAR
05/08/2018

DIVISION OF ANTIVIRAL PRODUCTS / VIROLOGY REVIEW
NDA: 208627 **SDN**: 000 (Original NDA) **REVIEW COMPLETED:** 5/4/2018
Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 208627 **SDN:** 000 (Original NDA)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

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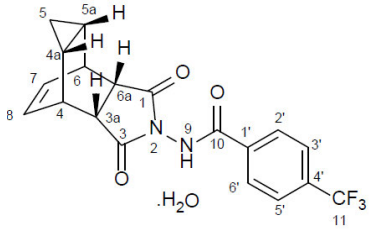
Correspondence Date: 12/8/2017
CDER Receipt Date: 12/8/2017
Review Complete Date: 5/4/2018 (primary review goal date: 5/8/2018)
PDUFA Date: 8/8/2018

Proprietary Name	**TPOXX™**
Drug Names	tecovirimat, ST-246
IND #	69019
Chemical Name	Benzamide, N-[(3aR,4R,4aR,5aS,6S,6aS)-3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl]-4-(trifluoromethyl), rel-(monohydrate)
Structure	
Molecular Formula	$C_{19}H_{15}F_3N_2O_3 \cdot H_2O$
Molecular Weight	376.33 Daltons (monohydrate=394.35 Daltons)

Related/Supporting Documents: SDNs 1 (pre-submission), 3 (completed submission), 7 (updated labeling/indication), 8 (updated resistance data), 12 (labeling), 13 (response to Virology request), 16 (response to comments on field study protocol), 18 (response to Virology question), 19 (response to proposed virology post-marketing study), 23 (response to Virology question), 27 (phenotype data), 30 (updated clinical field trial protocol); Virology review by Dr. Donaldson (N208627.000.0004)

Dosage Form and Route of Administration: 200 mg capsules / oral
Dispensed: Rx x OTC__
Proposed Indication: Treatment of human smallpox disease caused by variola virus

Abbreviations: ATCC, American Type Culture Collection; [redacted] (b) (4); EC, effective concentration; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GLP, good laboratory practice; GT, genotype; IV, intravenous(ly); LLOQ, lower limit of quantification; LOD, limit of detection; MOI, multiplicity of infection; MPXV, monkeypox virus; MVA, modified vaccinia Ankara; NHP, nonhuman primate; NZW, New Zealand white (rabbits); ORF, open reading frame; PCR, polymerase chain reaction; PFU, plaque-forming unit; PK, pharmacokinetics; PRNT, plaque reduction neutralization assay; RPXV, rabbitpox virus; SIV, simian immunodeficiency virus; SOP, standard operating procedure; SRV, simian retroviruses; STLV, simian T-cell leukemia virus; USAMRIID, U.S. Army Research Institute of Infectious Diseases; VACV, vaccinia virus

DIVISION OF ANTIVIRAL PRODUCTS / VIROLOGY REVIEW
NDA: 208627 **SDN**: 000 (Original NDA) **REVIEW COMPLETED:** 5/4/2018
Virology Reviewer: Patrick R. Harrington, Ph.D.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

This Original NDA for TPOXX™ (tecovirimat, ST-246), an orthopoxvirus VP37 envelope wrapping protein inhibitor, is 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). See Section 2.4 of this review for specific VP37 amino acid polymorphisms of interest. The final PMC wording and study milestones were still under negotiation with the sponsor at the time of finalization of this review.

> Conduct cell culture studies to characterize tecovirimat antiviral activity against an expanded panel of variola virus isolates and recombinant vaccinia viruses. These studies should capture the known VP37 amino acid heterogeneity in variola viruses, as well as a common orthopoxvirus VP37 polymorphism, and should also include multiple independent isolates with identical VP37 amino acid sequences.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

Tecovirimat (ST-246) is a small molecule antiviral drug that targets the highly conserved orthopoxvirus VP37 protein (also referred to as p37) within an infected cell to block a viral envelope wrapping step, which results in inhibition of viral spread to uninfected cells. In cell culture assays, tecovirimat had broad and consistent activity against a panel of different orthopoxviruses, including 8 isolates of variola virus (VARV), with EC_{50} values of 9-68 nM and a therapeutic index >600. Tecovirimat resistance could be selected in multiple different orthopoxviruses in cell culture, and was associated with the emergence of amino acid substitutions in VP37. There are several potential independent pathways for the generation of orthopoxviruses with high-level phenotypic resistance to tecovirimat, which in many cases require only a single amino acid substitution. To this reviewer's knowledge, some but not necessarily all tecovirimat resistance pathways have been described publicly. ***Therefore, due to the potential threat of variola virus being developed as a biological weapon, specific tecovirimat resistance pathways are not described in this Executive Summary, and we recommend that any mention of specific resistance pathways in the body of the review be redacted before the review is publicly posted.***

Tecovirimat efficacy was evaluated under the Animal Rule (21 CFR part 314, subpart I). The ideal animal model for VARV would involve infection with VARV itself, but natural VARV infection and disease is specific to humans. Use of an NHP/VARV model was explored, and there was evidence of tecovirimat activity in small scale studies using this model, but due to scientific and practical challenges the NHP/VARV model was not used in pivotal efficacy studies. The 6 pivotal efficacy studies of tecovirimat were conducted using two different non-VARV, immunocompetent animal models: monkeypox virus (MPXV)-infected nonhuman primates (NHP/MPXV model) and rabbitpox virus (RPXV)-infected rabbits (rabbit/RPXV model). Use of these models to establish tecovirimat 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.

Tecovirimat efficacy was demonstrated in 4 pivotal studies using the NHP/MPXV model, which were conducted by three independent contract research organizations/laboratories. In this model, NHPs were lethally challenged intravenously with 5 x 10^7 plaque-forming units of the MPXV Zaire '79 strain, which was originally isolated from scab material from a 1-year-old boy with severe MPXV disease. Treatment efficacy was based on survival from death or moribund disease. Studies AP-09-026G and FY10-087 evaluated a range of tecovirimat oral doses between 0.3 and 20 mg/kg/day for 14 days starting on Day 4 post-challenge, which is a time when all animals typically have developed pox lesions in this model. Study SR10-037F evaluated a tecovirimat 10 mg/kg/day dose, with 14 days of dosing starting on Day 4, 5 or 6 post-challenge. Study SR10-038F again evaluated a tecovirimat 10 mg/kg/day dose, with dosing starting on Day 4 post-challenge, for a total duration of 3, 5, 7 or 10 days. Collectively, these studies demonstrated that tecovirimat doses ≥3 mg/kg/day starting on Day 4 were associated with improved survival, delaying the start of tecovirimat 10 mg/kg/day to Day 6 post-challenge was associated with reduced treatment efficacy, and tecovirimat 10 mg/kg/day remained effective when dosed for as little as 5 days. Tecovirimat treatment was associated with reduced MPXV DNA levels in whole blood collected throughout the disease course, and also in tissues collected at necropsy.

Tecovirimat efficacy was also demonstrated in 2 pivotal studies using the rabbit/RPXV model. In these studies, rabbits were lethally challenged intradermally with 1,000 PFU of the RPXV Utrecht strain. In study SR14-008F, animals received a range of tecovirimat oral doses of 20-120 mg/kg/day, starting on Day 4 post-challenge, which is typically when fever develops in this model. Study SR13-025F was an uncontrolled, PK-focused study that similarly evaluated doses of 40-120 mg/kg/day starting on Day 4 post-challenge. All tecovirimat doses evaluated (≥20 mg/kg/day) were associated with a high rate of survival (≥80%), with no apparent dose response, while all animals that received placebo in study SR14-008F succumbed to the infection. Tecovirimat treatment resulted in reduced RPXV DNA levels in whole blood throughout the disease course, and also in tissues collected at necropsy, across all tecovirimat dose groups.

Resistance analyses were conducted for a subset of animal efficacy/activity studies, and included PCR amplification and next generation nucleotide sequencing (NGS) of the VP37 target gene (C19L gene in MPXV, RPXV041 gene in RPXV) from blood and tissue samples. Unfortunately, due to sensitivity limitations of the assay, of the 129 blood and tissue samples processed for NGS analysis, results were successfully obtained only for 11 (9%) samples, all blood, including 7 MPXV samples from 6 NHPs (4 received tecovirimat and 2 received placebo) and 4 RPXV samples from 4 rabbits (all 4 received tecovirimat). All data came from animals that died or were euthanized prior to study completion. Despite the low assay success rate, emergence of tecovirimat resistance-associated amino acid substitutions in VP37 was observed in 3 of the 4 tecovirimat-treated NHPs with available data. Among the 4 rabbits with available data, only a single VP37 amino acid substitution was detected at a low level in one rabbit, and phenotypic analyses indicated that the substitution likely does not affect tecovirimat susceptibility.

Tecovirimat could play an especially important role in the treatment of smallpox for individuals with immune deficiencies or who are on immunosuppressive therapy, for whom live vaccinia virus (VACV)-based smallpox vaccines are contraindicated. Tecovirimat antiviral activity was evaluated in multiple different immune deficient mouse models, and in general, these studies demonstrated that tecovirimat had reduced anti-orthopoxvirus efficacy in the setting of severe immune deficiency, particularly in mice lacking functional T and B cells.

Although outside the treatment indication being considered for this NDA, it is possible that tecovirimat will be used in combination with VACV-based vaccine in some circumstances, and because tecovirimat has

anti-VACV activity, it could potentially interference with vaccine immunogenicity or efficacy. The sponsor conducted multiple studies characterizing the potential for tecovirimat interference with VACV vaccine. Although vaccine interference was not observed consistently across all studies, there was clear evidence from at least one NHP/MPXV study that co-administration of live VACV vaccine plus tecovirimat modestly reduced vaccine immunogenicity. Compared to animals that received VACV vaccine alone, animals that received VACV plus tecovirimat had modestly enhanced disease (i.e., reduced vaccine-mediated protection) following MPXV challenge, as evidenced by higher blood MPXV DNA levels and higher lesion counts. Ultimately, the clinical relevance this potential interaction is unclear, and likely varies depending on the specific circumstance in which vaccine and tecovirimat are used.

In summary, tecovirimat has a well-established, virus-targeted mechanism of action, has broad and consistent activity against orthopoxviruses, and has a low resistance barrier with multiple potential resistance pathways. Tecovirimat efficacy has been demonstrated, to the extent feasible, in pivotal efficacy studies using the NHP/MPXV and rabbit/RPXV models, and antiviral activity was also demonstrated in several additional animal studies of variola and non-variola virus infection. Collectively, results from the sponsor's nonclinical development program indicate tecovirimat is likely to be an effective treatment for human smallpox.

2.2 Clinical Virology

As described above, tecovirimat efficacy was evaluated under the Animal Rule. No clinical studies of tecovirimat were conducted to assess antiviral activity and efficacy. Tecovirimat was provided for emergency use for the treatment of non-variola virus infections for 5 cases in the U.S., and 1 case in Finland. The best characterized case from a virology perspective was from an individual patient with acute myelogenous leukemia who developed progressive vaccinia following VACV vaccination and initiation of chemotherapy for his malignancy. Multiple antiviral and immunologic treatments were administered, including tecovirimat, and the individual contribution of tecovirimat towards the eventual viral clearance and healing of the vaccine and satellite lesions was unclear, except that tecovirimat alone did not appear to be sufficient to clear the VACV infection. Furthermore, emergence of tecovirimat-resistant VACV occurred in the patient, reflecting both the antiviral activity of tecovirimat as well as its low resistance barrier, further illustrating the potential limitation of tecovirimat efficacy in the setting of severe immune deficiency.

3. ADMINISTRATIVE

3.1 Reviewer's Signature

_____
Patrick R. Harrington, Ph.D.
Senior Clinical Virology Reviewer, Division of Antiviral Products

3.2 Concurrence

_____
Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antiviral Products

OND CLINICAL VIROLOGY REVIEW

1. INTRODUCTION AND BACKGROUND

1.1 Important Milestones in Product Development and Prior FDA Reviews

This is the Original NDA submission for tecovirimat (ST-246). Tecovirimat was developed under Pre-IND/IND 69019. The Pre-IND was first opened in 2004, and the Original IND was submitted in 2005. 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 tecovirimat under the Animal Rule (21 CFR part 314, subpart I). Pre-NDA discussions were held with the sponsor in 2017. The NDA was submitted on a rolling submission basis, with the first submission including most nonclinical study reports (Module 4) received on 6/14/2017, and the final complete NDA package was received on 12/8/2017. Virology reviews throughout product development under Pre-IND/IND 69019 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 tecovirimat is the treatment of human smallpox disease caused by variola virus in adults and pediatric patients. Variola virus (VARV) 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. No antiviral drugs are approved for the treatment of VARV infection.

1.3 Methodology

Tecovirimat was developed under the Animal Rule (21 CFR part 314, subpart I) to support an indication for the treatment of VARV infection. Cell culture virology studies were used to characterize the mechanism of action of tecovirimat, as well as tecovirimat antiviral activity against VARV and other orthopoxviruses used in animal model studies. Numerous animal studies were conducted to assess the effect of tecovirimat treatment on orthopoxvirus disease outcomes. These animal models included, but were not limited to, monkeypox virus (MPXV) infection of nonhuman primates (NHPs), rabbitpox virus (RPXV) infection of rabbits, VARV infection of NHPs, vaccinia virus (VACV) infection in mice, and orthopoxvirus infections in immune deficient animals. During development, tecovirimat was also used under emergency IND (E-IND) in a small number of human patients with complications related to VACV-based vaccination, and limited clinical and laboratory data were collected from these cases.

Six "pivotal" animal studies, including 4 studies using the NHP/MPXV model and 2 studies using the rabbit/RPXV model, were conducted to characterize the efficacy and pharmacokinetics of tecovirimat when administered at times when animals had developed disease signs. These orthopoxvirus animal models and their relevance to human smallpox disease are described in greater detail in Section 3.1. Various permutations of the study designs were used to identify tecovirimat dose(s), duration(s) and treatment initiation times that provided optimal efficacy. All but one of the studies were conducted in a 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 various times post-challenge before and after initiation of tecovirimat treatment. Viral DNA levels were measured using a quantitative PCR assay targeting the conserved orthopoxvirus hemagglutinin gene. The lower limit of quantification for the PCR assays varied by study, but were in the range of 1,000-10,000 copies/mL for whole blood; for this reviewer's independent analyses, viral DNA levels reported below the assay LLOQ were assigned the LLOQ value. For a subset of studies viral DNA was also quantified in tissue samples collected at necropsy.

Blood and tissue samples were also collected and processed to investigate the emergence of tecovirimat-resistant virus. Resistance analyses included next generation nucleotide sequencing (NGS) analyses to identify tecovirimat treatment-emergent amino acid substitutions in the viral VP37 target protein (RPXV and MPXV studies), as well as cell culture phenotypic resistance analyses of virus isolates from treated animals (RPXV only).

Additional methodology details for virology studies are included as needed in the following review sections. This reviewer conducted independent analyses of data submitted from the pivotal animal efficacy studies, focusing particularly on viral DNA and drug resistance data. Please see the Clinical, Pharmacology/Toxicology and/or Biostatistics reviews for additional detailed independent FDA analyses of the sponsor's efficacy results based on survival and clinical disease signs. A pooled analysis was conducted for resistance results reported from the pivotal animal studies; see Section 3.8. In addition, FDA/DAVP Virology Reviewer Dr. Eric Donaldson, Ph.D., independently analyzed raw NGS fastq files used by the sponsor to produce VP37 amino acid substitution data; see Dr. Donaldson's review for more details (N208627.000.0004). Study reports for cell culture studies and other "non-pivotal" animal model studies were also reviewed, and key findings are summarized throughout this review.

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

2.1 Mechanism of Action

Tecovirimat is a small molecule antiviral drug that targets the orthopoxvirus VP37 protein (also referred to as p37) within an infected cell, ultimately resulting in inhibition of viral spread to uninfected cells. The VP37 protein is encoded by the VACV *F13L* gene, as well as homologous genes in other orthopoxviruses including VARV (*C17L*), MPXV (*C19L*), RPXV (*RPXV041*), CPXV (*V061*), and ectromelia virus (ECTV) (*EVM036*).

During the orthopoxvirus assembly process, intracellular mature virus (IMV) particles, which are surrounded by a single lipid bilayer membrane, are produced in intracellular "virus factories" and transported via microtubules to endosomes or the trans-Golgi network where they are wrapped by a double-bilayer membrane to form intracellular enveloped virus (IEV) particles, which are sometimes also referred to as "wrapped virus" (Figure 1; modified from Smith and Law, 2004). The IEV particles are then transported to the cell surface and the outermost IEV membrane fuses to the cellular plasma membrane

to expose a viral particle on the cell surface that initially remains associated with the cell, termed a cell-associated enveloped virus (CEV) particle. The CEV particles can then be released as extracellular enveloped virus (EEV), or may induce the formation of long actin "tails" that facilitate transfer of virus to uninfected cells.

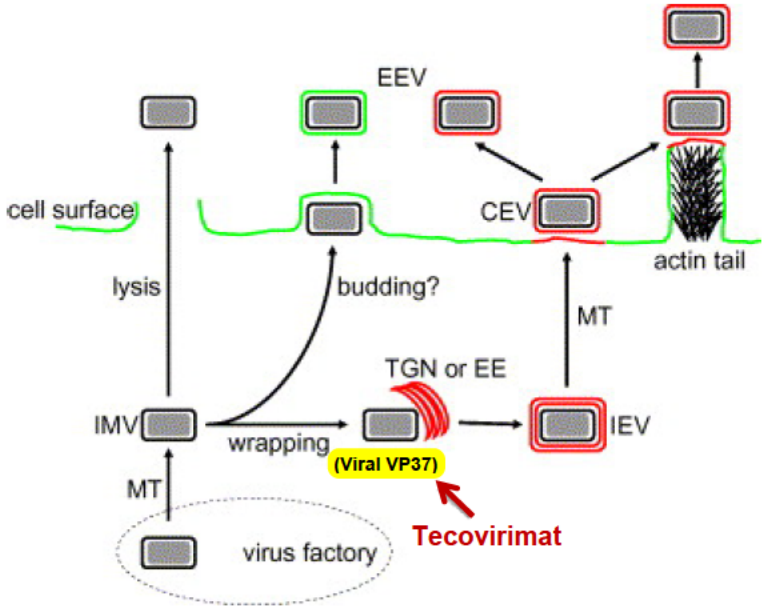


Figure 1. Schematic of orthopoxvirus assembly and release, and the step targeted by tecovirimat. CEV, cell-associated enveloped virus; EE, early endosome; EEV, extracellular enveloped virus; IEV, intracellular enveloped virus; IMV, intracellular mature virus; MT, microtubules; TGN, *trans*-Golgi network.

The VP37 protein is one of multiple orthopoxvirus proteins required for the membrane wrapping step to produce IEV particles, which subsequently results in production of infectious CEV and EEV. In the absence of any further processing, the IMV particles themselves are also infectious after release from infected cells, but IMV release occurs only late in the infection process as a result of cellular lysis, or possibly by inefficient budding. Furthermore, in contrast to CEV/EEV particles, IMV particles are not efficiently transferred to uninfected cells within the host and are also more easily recognized by neutralizing antibody. On the other hand, owing to their robust environmental stability outside of hosts, IMV particles may play a role in viral transmission to a new host.

Deletion of the VACV *F13L* gene (Blasco and Moss, 1991) or other genes required for IEV and subsequent CEV/EEV formation results in a small plaque phenotype in cell culture reflective of impaired viral spread. Viruses lacking the *F13L* gene are also attenuated *in vivo*. For example, in one study conducted by the sponsor (246-PC-020), the 50% lethal dose (LD_{50}) of the VACV-WR strain administered to weanling BALB/c mice by intranasal challenge was 4.5×10^4 PFU. In contrast, with a VACV-WR mutant virus in which the *F13L* gene was replaced with a reporter gene, no lethality was observed up to the highest challenge dose of 2.4×10^7 PFU, reflecting a >533-fold reduction in virulence.

As further support for therapeutically targeting viral envelope wrapping and egress, a paper by Payne (1980) demonstrated from an investigation of 13 different VACV strains that virulence in mice was correlated with the relative amount of EEV produced, as well as the ability to form "comet-shaped" plaques in liquid overlay cultures that are indicative of EEV-driven virus spread. One exception was the VACV-WR strain, which was highly pathogenic in mice yet produces significantly less EEV relative to other pathogenic VACV strains. It was later found that the VACV-WR strain produces high levels of enveloped virus and normal-sized plaques in cell culture, but more of the extracellular virus remains associated with cells (CEV) rather than being released into the media as EEV, and thus the virus was

slower to produce "comet-shaped" plaques (Blasco and Moss, 1992). Variability in EEV production has also been demonstrated between different orthopoxvirus species (Duraffour et al. 2008), and even between different VARV clades without a clear correlation between VARV EEV production and smallpox case fatality rates (Olson et al., 2009). While EEV can contribute to long-range, cell-free spread within a host, CEV also likely contributes to viral dissemination as it drives rapid cell-to-cell spread of the infection (Doceul et al., 2010; Pickup 2015). Thus, inhibition of wrapping of IMV particles to block downstream production of both CEV and EEV particles is a well-supported strategy to inhibit orthopoxvirus spread within infected hosts.

The initial evidence that tecovirimat targets the viral VP37 protein came from (1) an observation that tecovirimat selectively inhibits VACV extracellular but not intracellular virus production, and (2) tecovirimat resistance in CPXV maps to the CPXV *V061* gene, which is homologous to VACV *F13L* (Yang et al., 2005). Subsequent experiments with other orthopoxviruses similarly mapped tecovirimat resistance to amino acid coding substitutions in the *F13L* gene (or equivalent homologs), while viruses with a deleted *F13L* gene had a small plaque phenotype as expected but were resistant to further inhibition by tecovirimat (Duraffour et al., 2015). Tecovirimat resistance mechanisms are discussed in greater detail in Sections 2.3 and 2.4.

Several other experiments provided additional evidence that tecovirimat targets the orthopoxvirus VP37 protein to inhibit IMV wrapping and subsequent release. Analyses of viral particles produced from VACV infected cells demonstrated that tecovirimat strongly inhibited the formation of IEV, CEV and EEV particles, while IMV formation was unaffected (Chen et al., 2009). Co-immunoprecipitation analyses demonstrated that tecovirimat inhibits membrane wrapping complex formation by blocking interactions between VP37, the viral B5R protein that is also involved in IEV production, and host cell late endosome proteins Rab9 and TIP47 (Chen et al., 2009; Duraffour et al., 2015). Tecovirimat did not inhibit these interactions when cells were infected with a virus carrying a tecovirimat resistance-associated substitution in VP37. Furthermore, hypothetical molecular modeling was used to predict a tecovirimat binding pocket in VP37 (Duraffour et al., 2015).

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

In cell culture assays, tecovirimat had broad and consistent activity against a variety of orthopoxviruses, including 8 isolates of VARV, with EC_{50} values of 9-68 nM (Table 1; 246-PC-007; SIGA-246-MET-001; Smith et al., 2009; Yang et al., 2005; and Original IND 69019 review by Dr. Jules O'Rear). These assays were conducted using Vero (CCL-81) or BSC40 African green monkey kidney cell lines, and viral replication was measured based on the degree of cytopathic effect in cultures, measured by crystal violet staining 2-3 days after infection. None of the tecovirimat metabolites M4, M5, or TFMBA had measurable anti-RPXV activity (EC_{50} values >5 µM). Consistent with its targeting of orthopoxvirus VP37, tecovirimat had no measurable antiviral activity (EC_{50} values >40 µM) against a panel of DNA and RNA viruses including herpes simplex virus type 1, cytomegalovirus, respiratory syncytial virus, rotavirus, Rift Valley Fever virus, Tacaribe virus and lymphocytic choriomeningitis virus.

Table 1. Tecovirimat cell culture antiviral activity against a panel of orthopoxviruses. Genbank IDs are shown for the VARV strains, as reported in SDN 13. *Bangladesh strain, specific isolate unknown (SDN 18).

Virus (Strain or Isolate)	EC_{50} Value (nM)
Vaccinia Virus (NYCBH)	9
Cowpox Virus (Brighton Red)	50
Cowpox Virus (Brighton Red, CDV-resistant)	30
Ectromelia Virus (Moscow)	68
Camelpox Virus	12
Rabbitpox Virus (Utrecht)	14
Monkeypox Virus (Zaire '79)	14-39
Monkeypox Virus (V78-I-3945, Benin)	23
Monkeypox Virus (V81-I-179, Ivory Coast)	32
Monkeypox Virus (2003-USA-039)	36
Monkeypox Virus (V77-I-823, Zaire)	30
Variola Virus (Butler-ABF29026.1; ABF29026.1)	16
Variola Virus (BSH*)	46
Variola Virus (BRZ66-39, Brazil; ABF23410.1)	67
Variola Virus (SLN68-258, Sierra Leone; ABF27026.1)	37
Variola Virus (SUD47-juba, Sudan; ABF27625.1)	19
Variola Virus (BSH74-sol, Bangladesh; ABF24006.1)	28
Variola Virus (NEP73-175, Nepal; ABG44827.1)	21
Variola Virus (Minor, SOM77-ali, Somalia; ABG45232.1)	28

When dosed as recommended, tecovirimat blood exposures are expected to exceed by several-fold the EC_{50} values for all of these tested orthopoxviruses. The geometric mean tecovirimat C_{min} levels on Day 1 (i.e., after first dose) were 356 nM (134 ng/mL; MW=376.33) in MPXV-infected NHPs that received the 10 mg/kg/day "fully effective dose," and 477 ng/mL (1.3 µM) and 162 ng/mL (430 nM) in humans who received tecovirimat 600 mg BID in the fed and fasted state, respectively. Tecovirimat exposures were modestly higher on Day 14 in both NHPs and humans, but drug exposures during the earliest stages of treatment are likely most critical for rapidly inhibiting viral spread. Please see the Clinical Pharmacology review by Dr. Su-Young Choi, Pharm.D., Ph.D., for further details on tecovirimat pharmacokinetics.

The sponsor did not provide cell culture data on the activity of tecovirimat against other poxviruses outside of the orthopoxvirus genus, such as molluscum contagiosum virus (molluscipoxvirus genus) and orf virus (parapoxvirus genus), which cause human disease. According to the sponsor, molluscum contagiosum and orf viruses encode homologs to the orthopoxvirus VP37 protein with approximately 40% amino acid similarity. The sponsor noted that most VP37 amino acids that are essential for tecovirimat anti-orthopoxvirus activity, based on resistance analyses, are not conserved in molluscum contagiosum or orf viruses, indicating that neither of these viruses would likely be susceptible to tecovirimat (Sponsor's Nonclinical Overview).

Tecovirimat is predicted to have non-antagonistic antiviral activity when combined with other anti-orthopoxvirus agents with different mechanisms of action. No other small molecule antiviral drugs are currently approved to treat an orthopoxvirus infection, and to this reviewer's knowledge, the sponsor did not submit data on the cell culture combination antiviral activity of tecovirimat with other investigational anti-orthopoxvirus agents. However, results from a published study indicated tecovirimat had non-antagonistic anti-orthopoxvirus activity when combined with CMX001 (prodrug of cidofovir, a viral DNA

polymerase inhibitor), both in cell culture assays and also in a CPXV mouse model (Quenelle et al., 2007). In addition, the sponsor conducted multiple studies using a highly pathogenic ectromelia virus mouse model, which also demonstrated non-antagonistic activity of tecovirimat in combination with cidofovir or CMX001 (Appendix A).

Tecovirimat had a CC_{50} value of >40 µM in Vero cell cultures, reflecting a therapeutic index of >4,000 for VACV and >600 for other orthopoxviruses (Study Report 246-PC-007). Cytotoxicity was measured after 2 days of drug exposure by alamar blue assay, which measures cellular oxidation-reduction activity as a measure of cell viability. In another study quantifying cell growth over 3 days of drug exposure, tecovirimat had CC_{50} values of >50 µM for a variety of different cell lines including BSC-40 (African green monkey kidney), HEK-293 (human embryonic kidney), L929 (mouse fibroblast), MRC5 (human lung fibroblast), SIRC (rabbit cornea), and Vero cells (African green monkey kidney) (Study Report 246-PC-021).

The presence of increasing human serum concentrations up to 10% had a modest and somewhat inconsistent impact on the cell culture antiviral activity of tecovirimat against VACV-WR (Study Report 246-PC-005). The sponsor extrapolated results from this study to estimate a VACV-WR EC_{50} value of 28 nM in the presence of 100% human serum, which was approximately 2.5-fold higher than the EC_{50} value in the absence of human serum. However, these results are somewhat confounded as the correlation between EC_{50} values and human serum concentrations was poor, and the assay signal to noise ratio was reduced to <3 in the presence of human serum concentrations ≥2%. Plasma protein binding based on equilibrium dialysis is similar between different relevant species; tecovirimat is approximately 87-89% bound to mouse, rabbit and monkey plasma protein, and 77-82% bound to human plasma protein (Study Reports 246-AD-001 and 17SIGAP1).

2.3 Resistance Selection in Cell Culture

In an initial study characterizing the mechanism of action of tecovirimat, selection of CPXV for resistance to tecovirimat resulted in the emergence of a virus with a G277C amino acid substitution in the CPXV VP37 protein (Study Report 246-PC-015). The tecovirimat EC_{50} value shifted from 0.01 µM for wild-type virus to >40 µM for the resistant virus (>4,000-fold increase in EC_{50} value). Re-engineering the specific G277C substitution into the CPXV or VACV genomes resulted in viral recombinants that were resistant to tecovirimat, consistent with the VP37 protein (encoded by CPXV *V061* and VACV *F13L* genes) being the viral protein target of tecovirimat.

In a follow-up study (246-PC-035), random PCR-mediated mutagenesis of the VACV *F13L* gene sequence was conducted to create a library of *F13L* mutant gene sequences, which were then introduced into a wild-type VACV genome. Progeny viruses were then harvested and plated on cells in the presence or absence of 5 µM tecovirimat, and virus-containing plaques that formed in the presence of tecovirimat were further plaque purified and the *F13L* genes were sequenced to identify genetic changes that were associated with reduced tecovirimat susceptibility. A total of 16 plaques were observed in the presence of tecovirimat out of an estimated 2.2×10^4 PFU subjected to compound selection, corresponding to a resistant virus frequency of 7.2×10^{-4} after PCR mutagenesis (compared to an estimated 1.2×10^{-6} pre-existing resistant virus frequency [fluctuation analysis] in the absence of PCR mutagenesis; Study ST-246-PC-034).

Genotypic and phenotypic resistance results from this study confirmed the low resistance barrier of tecovirimat, and indicated several potential independent pathways to high level (>1,000-fold) tecovirimat resistance, even in the absence of PCR mutagenesis of the *F13L* gene. Table 2 (Study Report 246-PC-035; pg. 6) summarizes the VACV VP37 amino acid substitutions detected in the tecovirimat-selected

viruses, along with the corresponding tecovirimat EC_{50} values. Although an R291K substitution emerged in several tecovirimat-selected VACV clones, the sponsor did not consider R291K to be associated with tecovirimat resistance because it was also observed in multiple VACV isolates in the absence of tecovirimat drug selection; R291K is a common polymorphism in several VACV and CPXV isolates (see Section 2.4).

(b) (4)

Table 2. VACV VP37 amino acid substitutions detected in tecovirimat-selected viruses. The top 16 rows indicate isolates from the PCR-mutagenized viral population, and the bottom 4 rows indicate isolates obtained directly from the platting of non-mutagenized virus in the presence of tecovirimat. Yellow shading indicates single amino acid changes that correlate with resistance. N239Q is an error and should refer to H238Q (see Clinical Virology review of IND 69019.281 by Dr. Jules O'Rear). Also, column heading '268' should be '267'.

aa pos.	2	68	178	179	225	239	258	268	280	283	291	294	295	302	308	309	317	EC50, uM
wt																		0.01
211		K68N					Y258C								T308S			50
215			L178S				Y258C											50
216	W2C				D225A		Y258C		D280G									5
217										D283G	R291K							50
311								N267D			R291K							18.9
321								N267S			R291K							49.7
331														L302P				17
318								N267S			R291K						I317V	32
319											R291K			L302Q				50
320				N179H						D283G	R291K							50
322										D283G	R291K							50
423											R291K	D294V						50
425								N267D								I309T		50
427									D280G		R291K	D294G						22
530								N267S			R291K							1.5
531											R291K			L302P				50
911								N267D										50
921						N239Q												6
1011														L302P				n.d.
1021													A295E					n.d.

In a separate published study by Duraffour et al., 2015, tecovirimat resistance selections were conducted with VACV, CPXV and two different strains of CMLV (strains Iran [CML1] and Dubai [CML14]), and drug resistant viral clones were characterized genotypically and phenotypically. Five viral genes (*A27L*, *B5R*, *F13L*, *A33R*, *A34R*; VACV nomenclature) involved in IMV wrapping or viral release were sequenced, and among 6 mutant viral clones, all amino acid substitutions detected mapped to the *F13L* gene encoding VP37 (Figure 2; Duraffour et al., 2015). All of these viruses had reduced susceptibility to tecovirimat, but remained fully susceptible to cidofovir, which acts by a different mechanism of action by inhibiting the viral DNA polymerase. Analyses of recombinant viruses carrying certain specific VP37 substitutions (G277C, I372N, G277C+I372N, or F25V+I372N) confirmed these substitutions conferred tecovirimat resistance. Note that although tecovirimat resistance has consistently mapped to VP37 across multiple studies, an earlier abstract by Duraffour et al., 2014: Abstract 24 claimed the identification of one tecovirimat-resistant CMLV isolate without any VP37 amino acid substitutions and with a frameshift mutation in the B5R protein also associated with wrapping; thus, it remains theoretically possible that other genome regions could contribute to tecovirimat resistance.

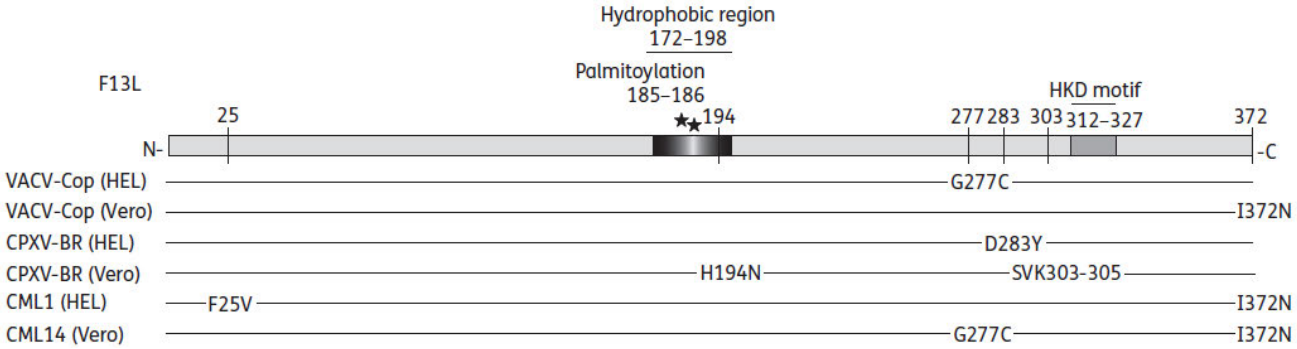


Figure 2. VP37 amino acid substitutions observed in tecovirimat-selected vaccinia virus (VACV), cowpox virus (CPXV) and camelpox virus (CMLV; strains CML1 and CML14).

Of note, results from the Duraffour et al., 2015 study revealed multiple tecovirimat resistance pathways in VP37 that are apparently conserved across multiple different orthopoxviruses. The G277C substitution, which was originally identified in tecovirimat-selected CPXV (Study Report 246-PC-015), also emerged in tecovirimat-selected VACV and CMLV. Furthermore, an I372N substitution emerged in tecovirimat-selected VACV and CMLV, and this same substitution also emerged in MPXV from two tecovirimat-treated NHPs that succumbed to MPXV infection (see Section 3.8).

2.4 Compiled Resistance Summary and Analyses of Published VP37 Amino Acid Sequences

Table 3 (adapted from study report SR16-039F-Amendment 1) provides a compiled summary of available phenotype analysis results for tecovirimat resistance-associated substitutions observed across various cell culture, animal and human studies. These compiled results clearly indicate that there are several potential independent pathways for the generation of viruses with high-level resistance to tecovirimat, which in many cases require only a single amino acid substitution. This summary includes updated data on the N179T substitution, reported in SDN 27.

Table 3. Phenotypic analyses of orthopoxviruses with VP37 amino acid substitutions. Note that results are reported without consideration of the presence or absence of the R291K polymorphism.

Virus	VP37 AA Change	Tecovirimat EC_{50} Value (µM)	Tecovirimat EC_{50} FC[1]	Recomb. Virus?[2]	Reference(s)
VACV WR, COP, ACAM2000	n/a (WT)	0.003-0.01	n/a (WT)	n/a	Yang et al., 2005, Duraffour et al., 2007; Duraffour et al., 2015; Lederman et al., 2012
VACV-WR	N179T	<0.1	(susceptible)	Y	15201.01-P2 (See Section 3.8)
VACV-WR	H238Q	6->50	600 to >5000	N	246-PC-035, Byrd et al., 2012[3]
VACV-WR	N267D	>50	>5000	Y	246-PC-035
VACV-WR	N267S	5	500	Y	246-PC-035
VACV-COP	G277C	0.29	97	Y	Duraffour et al., 2015
VACV-WR	D283G	>50	>5000	Y	246-PC-035
VACV-?	A288P	"resistant"[4]	n/a	?	Duraffour et al., 2015
VACV-WR	A290V	8.8	220	Y	Byrd et al., 2012[3]
VACV-ACAM2000	A290V	2.5	31	Y	Byrd et al., 2012[3]
VACV-WR	D294V	>50	>5000	Y	246-PC-035
VACV-WR	A295E	16	1600	N	246-PC-035
VACV-WR	L302P	>50	>5000	Y	246-PC-035
VACV-WR	L302Q	>50	>5000	Y	246-PC-035
VACV-WR	L315M	0.19	5	Y	Byrd et al., 2012[3]
VACV-ACAM2000	L315M	0.06	1	Y	Byrd et al., 2012[3]
VACV-COP	I372N	0.08	9	N	Duraffour et al., 2015
VACV-WR	L178S+Y258C	>50	>5000	Y	246-PC-035
VACV-WR	N179H+D283G	>50	>5000	Y	246-PC-035
VACV-WR	N267D+I309T	>50	>5000	Y	246-PC-035
VACV-WR	N267S+I317V	32	3200	Y	246-PC-035
VACV-WR	D280G+D294G	22	2200	Y	246-PC-035
VACV-WR	A290V+L315M	>25	>625	Y	Byrd et al., 2012[3]
VACV-ACAM2000	A290V+L315M	7.6	94	Y	Byrd et al., 2012[3]
VACV-WR	K68N+Y258C+T308S	>50	>5000	Y	246-PC-035
VACV-WR	W2C+D225A+Y258C+D280G	5	500	Y	246-PC-035
CPXV-BR	n/a (WT)	0.013-0.16	n/a (WT)	n/a	Yang et al., 2005; Duraffour et al., 2007; Duraffour et al., 2015
CPXV-BR	G277C	>40	>800	N	Yang et al., 2005, Duraffour et al., 2015
CPXV-BR	D283Y	6	44	N	Duraffour et al., 2015
CPXV-BR	H194N+303insSVK	>53	>333	N	Duraffour et al., 2015
CMLV Iran (CML1), Dubai (CML14)	n/a (WT)	0.005-0.03	n/a (WT)	n/a	Duraffour et al., 2007; Duraffour et al., 2015
CMLV-CML14	G277C	2.3	459	Y	Duraffour et al., 2015
CMLV-CML1	F25V+I372N	>40	>5714	Y	Duraffour et al., 2015
CMLV-CML14	G277C+I372N	>53	>10600	Y	Duraffour et al., 2015

[1]Fold-change (FC) in EC_{50} value relative to wild-type virus based on results in referenced study(ies).
[2]Phenotypic results based on (Y) recombinant viruses with VP37 substitutions engineered by site-directed mutagenesis or by random mutagenesis (as in study 246-PC-035), or (N) drug-selected, non-recombinant clonal viruses.
[3]Reference is for a poster included in the amended SR16-039F study report (SDN 8).
[4]A288P briefly noted in Duraffour et al., 2015 paper to confer resistance to tecovirimat ("unpublished results").

Table 4 (FDA compilation) provides a high-level summary of all tecovirimat resistance-associated or treatment-emergent amino acid substitutions observed across cell culture and animal model studies, and

also in a human case study; see Sections 3.8 and 8.1 for additional details from the animal models and human case, respectively.

Table 4. Summary of VP37 amino acid substitutions that confer tecovirimat phenotypic resistance and/or were selected by tecovirimat in cell culture, treated animals, or in a human VACV infection case. Results are compiled from all strains of the indicated orthopoxviruses.

VP37 Amino Acid Substitution(s)	Vaccinia Virus	Cowpox Virus	Camelpox Virus	Monkeypox Virus	Rabbitpox Virus
W2C[1]	Y				
F25V[1]			Y		
K68N[1]	Y				
L178S[1]	Y				
N179H/T	Y				Y
H194N[1]		Y			
D225A[1]	Y				
H238Q	Y			Y	
Y258C[1]	Y				
N267del/D/I/K/S	Y			Y	
R268G[1]				Y	
G277C	Y	Y	Y		
D280G/Y[1]	Y			Y	
D283G/Y	Y	Y			
A288P	Y				
A290V	Y			Y	
D294G/V	Y				
A295E	Y			Y	
L297ins[1]				Y	
L302P/Q	Y				
SVK303ins[1]		Y			
T308S[1]	Y				
I309T[1]	Y				
L315M	Y				
I317V[1]	Y				
I372N/ILKIKNRK[2]	Y		Y	Y	

[1]Substitution(s) at these positions were only observed in combination with other VP37 substitutions.
[2]Insertion caused by mutation of VP37 stop codon and extension of the open reading frame; see Dr. Donaldson's review for details.

As noted above, several VP37 tecovirimat resistance-associated substitutions have been identified across multiple different orthopoxviruses. Therefore, any tecovirimat resistance pathway in one orthopoxvirus should be considered as potentially conserved across multiple orthopoxviruses, including VARV, unless demonstrated otherwise. On the other hand, it is also important to recognize that specific substitutions may have varying effects on tecovirimat susceptibility in the context of different orthopoxviruses, as evidenced by the varying susceptibilities of VACV and CPXV carrying the key G277C resistance-associated substitution (Table 3). Different effects of certain viral DNA polymerase inhibitor resistance-associated substitutions have also been observed in VACV versus CMLV (Duraffour et al., 2012).

Despite the apparently low barrier of orthopoxvirus resistance to tecovirimat, no well-established resistance-associated substitutions are known to predominate as natural polymorphisms in any orthopoxviruses in the absence of drug selective pressure. No clear tecovirimat resistance-associated

substitutions were observed in 41 unique F13L/VP37 amino acid sequences (from a total of 166 nucleotide sequences analyzed) from 7 different orthopoxvirus species, consistent with the broad anti-orthopoxvirus activity of tecovirimat (Figure 3; adapted from Study Report 712 and Duraffour et al., 2015).

Across 29 different VP37 amino acid positions of interest, only positions R268 and R291 showed amino acid variability across these orthopoxvirus sequences, and neither position is clearly associated with tecovirimat resistance. A single VARV isolate (GBR44/UNK44 1946-Harvey) has an R268K polymorphism. To this reviewer's knowledge, R268K has not been observed in any tecovirimat resistance analyses, although an R268G substitution emerged in a single tecovirimat-treated, MPXV-infected NHP (see Section 3.8). Nevertheless, the R268G substitution was detected only in a small minority (~4%) of sequences from this animal, and multiple other well-established tecovirimat resistance-associated substitutions were present in a larger proportion sequence reads, so it remains unclear if R268G or R268K affect tecovirimat antiviral activity. As noted above, the R291K polymorphism is present in several VACV and CPXV sequences, and a single VARV isolate (India 1967 IND3) has an R291E polymorphism. Neither of these changes at position R291 are known to be associated with tecovirimat resistance, but this position is noted because of its polymorphic nature and the presence of R291K in several tecovirimat-selected VACV clones (see Table 2 above). All other positions evaluated, including the known key resistance-associated positions H238, N267, G277, D283, A290, D294, A295, L302, and I372, are 100% conserved across all orthopoxvirus VP37 sequences.

From these alignments, a total of 7 unique VARV VP37 amino acid sequences have been identified (Figure 3). Four VP37 amino acids (T5, A7, V173, Y181) differ from other orthopoxviruses but are 100% conserved in known VARV sequences. Polymorphisms among VARV sequences exist at 6 other VARV amino acid positions: R268K and R291E noted above, as well as R89K, S183L, S229L and F343L. This reviewer independently conducted an alignment of 52 VARV VP37 amino acid sequences from the NCBI database, and confirmed the presence of these VARV polymorphisms, and no additional VARV VP37 polymorphisms were identified (Appendix B). All of the VARV VP37 polymorphisms were infrequently observed among the 52 sequences analyzed; note that some of the NCBI VP37 sequences are possibly duplicates of the same isolates.

Of the 8 VARV isolates (7 with VP37 amino acid data) evaluated for tecovirimat susceptibility (Table 1), all of the VP37 amino acid sequences were identical except for an F343L polymorphism present in two isolates, Somalia/SOM77 and Sudan/SUD47 (alignment provided in SDN 13). The remaining known VARV polymorphisms R89K, S183L, S229L, R268K and R291E were not represented in these cell culture studies.

Based on these findings, additional phenotypic analyses of a larger panel of variola virus isolates and/or recombinant vaccinia viruses are recommended to characterize the impact of the following VP37 substitutions/polymorphisms on tecovirimat antiviral activity: R89K, S183L, S229L, R268K, and R291E. In addition, we recommend analyzing R291K given that it is a common polymorphism in orthopoxviruses.

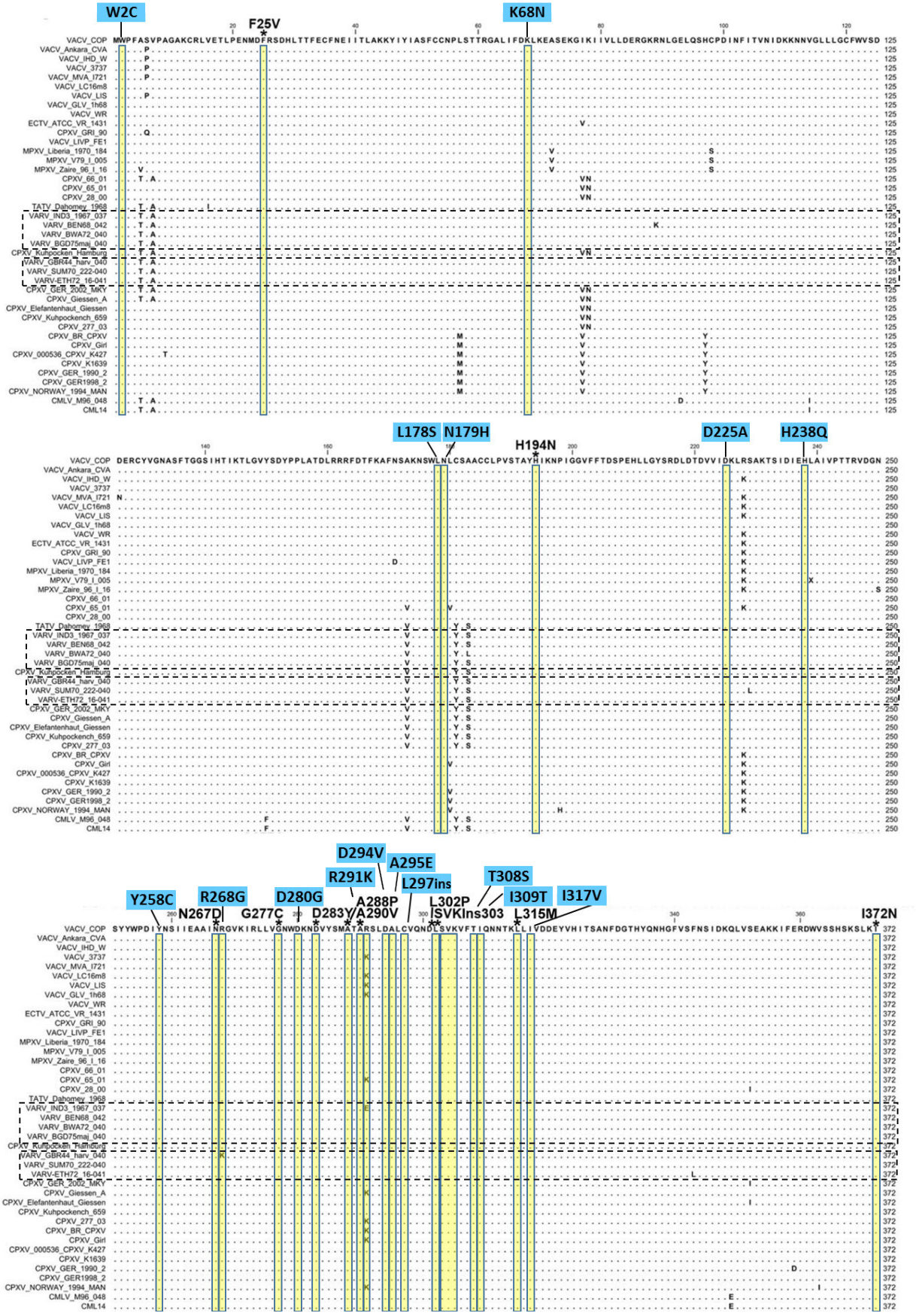


Figure 3 (above). Alignment of 41 unique F13L/VP37 amino acid sequences from 7 different orthopoxvirus species (adapted from Study Report 712 and Duraffour et al., 2015). Amino acid substitutions/positions of interest are noted, with those in blue added to the sponsor's list by this reviewer; note that the single substitutions indicated are examples, and for some positions (e.g., N267) multiple different amino acid substitutions are associated with resistance. Position R291 is not a known resistance-associated position but is noted because of its polymorphic nature. The dashed boxes indicate variola virus (VARV) VP37 sequences.

3. "REGISTRATIONAL" ANIMAL MODEL STUDIES

3.1 Orthopoxvirus Animal Models Used to Establish Tecovirimat Efficacy

The sponsor's summary and rationale for the animal models used to demonstrate tecovirimat efficacy were provided in a white paper titled, "TPOXX® Development Under the Animal Rule, Selection of Animal Models for Human Smallpox for Demonstration of TPOXX® Efficacy." In general, the sponsor's position and perspectives on acceptable animal models for the evaluation of antiviral drugs against variola virus (VARV) are in alignment with conclusions from the 2011 Antiviral Drugs Advisory Committee meeting on this topic.

For the purpose of establishing tecovirimat efficacy under the Animal Rule, the sponsor's NHP/MPXV and rabbit/RPXV model studies are acceptable from a virology perspective. The sponsor's white paper on animal models is summarized in this review section, with a focus on the key virology-related features of these models, including the sources of challenge viruses and typical disease courses. Please also see the reviews by Drs. Kirk Chan-Tack, M.D. and Peyton Myers, Ph.D., for the clinical and pharmacology/toxicology perspectives on these animal models, respectively.

Introduction to Smallpox, and Rationale for Animal Rule Development Pathway
Smallpox is caused by infection with VARV. Following exposure to VARV, typically by inhalation of what is thought to be a low number of infectious units, there is an incubation period of 7-19 days before disease onset. During this period, there is a primary viremia in which the virus replicates in lymph nodes and begins to spread to other tissues. A secondary viremia then occurs and initiates the "prodrome phase" of disease, during which symptoms such as high fever, headaches, and joint pain occur, and is associated with high levels of viremia. The virus then spreads to the skin and oropharyngeal mucous membranes leading to the "eruptive" phase of disease, during which the characteristic smallpox rash begins to form. If the infected individual does not succumb to disease, pocks on the skin are fully resolved within approximately 21 days after their initial appearance.

The clinical diagnosis of smallpox was historically based on the prodromal fever, the order of appearance of a centrifugal rash, and the characteristic evolution of individual lesions (including on palms of hands and soles of feet). In non-endemic regions, laboratory or epidemiological investigations were used to exclude other diseases. Laboratory diagnosis of presumptive smallpox was made only after the onset of clinical disease, and included laborious and time consuming procedures to detect virus or viral antigens. It is assumed that modern molecular techniques that have emerged since the eradication of smallpox, such as real-time PCR, would allow for earlier detection of VARV infection. The sponsor believes that in a current outbreak situation, it is likely that at least for the index cases the diagnosis will still be made after the eruption of pocks, but secondary cases will likely be identified earlier in the pre-eruptive phase using modern analytical methods, possibly allowing for initiation of therapy in the prodromal or pre-eruptive phase of disease.

The Animal Rule is appropriate for the development of antiviral drugs to treat VARV infection on the basis of the following:

- Naturally occurring smallpox has been eradicated, and therefore clinical trials are not feasible.
- Because of the high lethality of VARV infection, it would be unethical to conduct human challenge clinical trials with VARV for the purpose of assessing the efficacy of therapeutic or prophylaxis approaches.
- Other naturally occurring orthopoxviruses such as VACV, CPXV, or MPXV are less pathogenic in humans than VARV, and may have differing pathophysiology mechanisms. Also in the case of MPXV, although the frequency of MPXV outbreaks may be on the rise due to waning immunity from cessation of smallpox vaccination (Rimoin et al., 2010), mortality is typically low (1-2%), and outbreaks are typically small and infrequent and occur in remote regions in which controlled clinical trials would be challenging. VACV infections are seen rarely and can occur in vaccinated servicemen and servicewomen (or their close contacts) who are immunocompromised. CPXV infections are also uncommon.

The ideal animal model for VARV would involve infection with VARV itself, but natural VARV infection and disease is specific to humans. In NHPs, VARV challenge doses as high as 10^9 plaque-forming units (PFU) have been evaluated and do not cause uniform lethality or disease that resembles typical human smallpox (Jarhling et al., 2004). Furthermore, experiments with live VARV have significant feasibility challenges due to the requirement to use a World Health Organization (WHO)-approved biosafety level-4 laboratory, a resource that is limited.

There is evidence of tecovirimat antiviral activity in the NHP/VARV model (Huggins et al., 2009; Mucker et al., 2013; also see Review Section 4), but for the reasons noted above the sponsor used other animal models of surrogate orthopoxviruses for pivotal efficacy studies, specifically the NHP/MPXV intravenous (IV) challenge and rabbit/RPXV intradermal challenge models. An amino acid alignment conducted by Dr. Eric Donaldson, Ph.D., confirmed that the tecovirimat VP37 protein targets are highly conserved between MPXV, RPXV, and VARV. The VP37 amino acid sequences of the specific MPXV Zaire '79 and RPXV-UTR strains used in the pivotal animal models are approximately 98% identical to a prototypical VARV Bangladesh '74 strain; see Dr. Donaldson's review for details.

Table 5 (Integrated Summary of Efficacy, pg. 18) includes the sponsor's summary of the NHP/MPXV and rabbit/RPXV disease models in comparison to human smallpox. Each of these models is discussed in greater detail in the following text.

Table 5. NHP/MPXV and rabbit/RPXV disease models in comparison to human smallpox.

	Monkeys	**Rabbits**	**Humans**
Orthopoxvirus	Monkeypox	Rabbitpox	Smallpox
Time to Onset of Disease	Clinical signs appear within 2 days post-inoculation by the intravenous route	Clinical signs appear by Days 2-4 post-inoculation by the intradermal route	Incubation period following primary exposure to is 7-19 days
Time Course of Disease Progression	Fever is usually evident by the second day post-infection which is coincident with circulating monkeypox virus genomic DNA, skin rash, and lesions appear within 3 to 4 days post-inoculation	Fever is usually evident 2 to 3 days post-infection followed by circulating rabbitpox virus DNA within 24 hours of the first observation of fever. Respiratory distress, with lung lesions appearing by Days 3-6 post-inoculation	Primary viremia develops during incubation period of 7 to 19 days
Disease Manifestations	Fever, skin rash, lesions, and high blood titers of MPXV	Fever, high blood titers of RPXV, skin lesions, obvious signs of respiratory distress and lesions of the lung, liver, and adrenal cortex	Increase in viral load (i.e., secondary viremia) leading to development of generalized pustular rash on the head and/or extremities, with mortality rate of up to 30-40%. Sudden onset of prodrome, with fever, malaise, headache, and backache.
Trigger for Intervention	Pock formation; Day 3-4 post-infection	Fever coincident with viremia; Day 3-4 post-infection	Pock formation (historically), disease confirmation by molecular diagnostics
Time to Death	Approximately 10-14 days post-infection	Approximately 6-10 days post-infection	Occurs between 22 to 28 days post-infection
Lesion Counts	Between 500 and 1,500 total body count by the time of death. Distributed centrifugally on head, arms, and legs	Few to many, may manifest within 3-7 days post-exposure on mouth, nose, eyes, anus, genitourinary regions, back, ears	100s–1000s distributed centrifugally on head, arms, legs. Fewer on the trunk

NHP/MPXV Model

The sponsor used an NHP/MPXV model in which NHPs (specifically cynomolgus monkeys) were challenged intravenously (IV) with a high viral challenge dose of 5 x 10^7 PFU MPXV. The MPXV challenge strain used in key efficacy studies is referred to as the "Zaire '79" strain (also referred to as V79-I-005, ZAI-1979-005, or Zaire-79-I-05). Viral challenge stocks were derived from scab material originally collected on 1/11/1979 from a severely ill 1-year-old boy living in Zaire (now Democratic Republic of Congo) during a 1978-1979 monkeypox outbreak (Zaire case 1134, monkeypox case #38; Breman et al., 1980). Note that the sponsor originally reported this boy as case #39, but the sponsor corrected this to case #38 (SDN 23). The infected boy, who first developed a rash on 11/16/1978, did not die from the infection, but experienced severe disease characterized by >100 lesions and severe incapacity requiring medical care. Of the 11 reported MPXV cases from this 1978 Zaire outbreak, "severe" illnesses associated with >100 lesions, severe incapacity and requiring medical care, were reported in 9 (82%) cases, including 3 (27%) deaths; the other 2 cases were reported as "moderate" in severity (>25 lesions, moderate incapacity, usually requiring medical care).

Although the MPXV-infected boy in Zaire was the original source of challenge virus used in all key tecovirimat NHP/MPXV efficacy studies, the final challenge stocks used in each study were not uniformly produced (Figure 4; Animal Models White Paper, pg. 14).

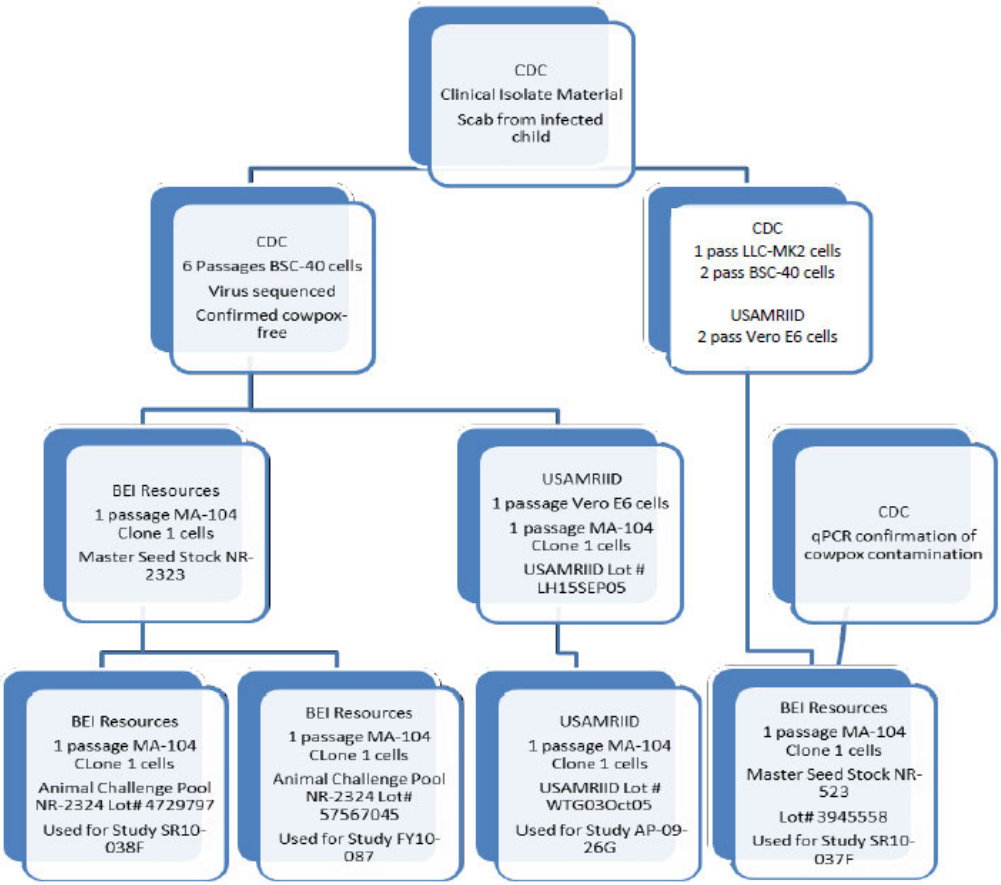


Figure 4. Schematic of MPXV Zaire '79 stocks used for NHP challenge studies.

The MPXV challenge stock for NHP study SR10-037F was amplified from the boy's scab material in LLC-MK2 cells (rhesus macaque kidney cell line), passaged twice in BSC-40 cells (African green monkey kidney cell line) to make a master seed stock, and then passaged twice in Vero cells (African green monkey kidney cell line) at a multiplicity of infection (MOI) of 3 PFU/cell to produce a working seed stock (NR-523). As part of release testing, a sample of the NR-523 virus stock was evaluated at Dr. Inger Damon's laboratory at the CDC, which revealed contamination with CPXV (~1 part per million).

To produce a virus stock that was not contaminated with CPXV, and ultimately used for NHP studies SR10-038F, FY10-087 and AP-09-026G, virus was obtained from another scab from the same clinical case, passaged 6 times in BSC-40 cells (5th passage virus sequenced; Likos et al., 2005), passaged one time in MA-104 cells (African green monkey kidney cell line) to produce a "Master Bank" stock (NR-2323), and finally passaged one additional time in MA-104 cells to produce a "Working Bank" stock (NR-2324). The MOI(s) used for all of the rounds of virus passaging were not described. Testing at the CDC showed that the NR-2324 stock was free from contamination by CPXV and VACV under conditions where CPXV contamination was observed in the NR-523 stock. The production of these stocks was conducted by the Biodefense and Emerging Infections Research Resources Repository (BEI Resources), and a separate stock of the BSC-40-passaged virus was made at USAMRIID (Figure 4).

Additional testing of each stock included infectivity, viral titer, nucleotide sequence analysis of the viral hemagglutinin gene for consistency with MPXV Zaire '79 sequence, sterility testing (no microbial grown), mycoplasma testing (none detected), endotoxin testing (<0.03 EU/mL), and pH (~8.0).

Of note, the MPXV Zaire '79 challenge stocks used in these studies were never plaque purified. The sponsor assumed that the virus in the scab was derived from a single infectious unit. Passage of poxviruses is known to generate deletions which may include viral genes encoding functions that compromise the host immune system. As reported by Likos et al., 2005, the passaged MPXV Zaire-79 isolate has a truncation in the interleukin-1β receptor orthologue gene that in theory could reduce viral pathogenesis (e.g., at lower viral challenge dose levels), although it is unclear if this is a natural genetic variant or a cell-culture artifact. These authors proposed the interleukin-1β receptor orthologue gene as a virulence factor based on a comparison of isolates from Central and Western Africa. Nevertheless, across all 4 key efficacy studies using the NHP/MPXV model, MPXV infection following the high dose viral challenge resulted in death or euthanasia due to moribund disease in 95% (19/20) of untreated control animals.

The disease onset in this model is rapid and leads to disease signs such as fever, rash and lesions that are similar to human smallpox in the secondary viremia phase (i.e., after the initial incubation period) (Figure 5; Animal Models White Paper, pg. 12). On average, the viral DNA copy number in blood increases from ~10^3 copies/mL on Day 1 to a peak of ~10^7 copies/mL by Day 12. Plaque assays can also measure viral titers, but according to the sponsor interfering substances in blood complicate technical steps, and viral DNA quantification by PCR is more reproducible. Both blood viral DNA level and lesion number correlate with mortality, and a higher viral DNA level correlates with a shorter time to death. In the absence of treatment, mortality in this model is high (>99% according to the sponsor) with a mean time to death of approximately 14 days post-challenge. The sponsor noted that MPXV respiratory challenge routes (PreIND 109254: aerosol, intranasal, intratracheal) were also explored and resulted in lethality due to lung complications in some animals. However, the disease following IV MPXV challenge was more reproducible and consistently lethal, and the systemic lesional disease observed more closely resembles late stage human smallpox disease.

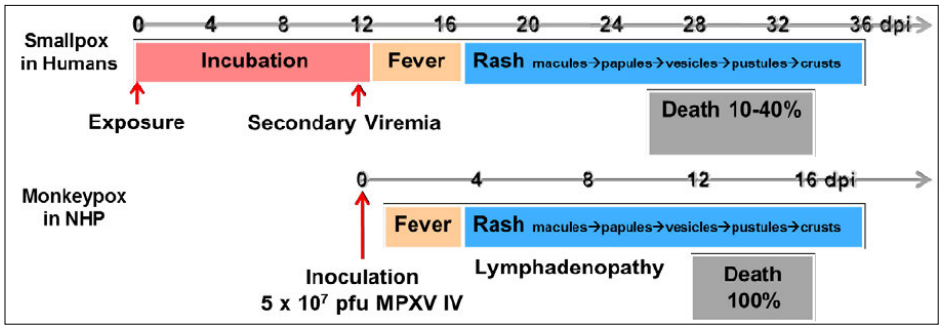


Figure 5. Comparison of clinical course of smallpox in humans and MPXV infection in NHPs.

As noted above, diagnosis of smallpox was primarily based on clinical symptoms, particularly the development of a centrifugal rash and lesions after a few days of severe fever, which would likely be used as a trigger for treatment in clinical practice. In the NHP/MPXV model, the appearance of lesions first occurs 3-4 days post-challenge, and therefore initiation of treatment around this timeframe would be relevant to treatment of VARV infection of humans.

Rabbit/RPXV Model

The RPXV Utrecht (RPXV-UTR) strain, first isolated in 1941, causes a rapid and consistently fatal disease in rabbits with a relatively low viral challenge dose. The sponsor noted that the RPXV-UTR isolate is much more pathogenic than a "Rockefeller" isolate that was obtained earlier in 1932, and

therefore RPXV-UTR has been the primary RPXV isolate used in published studies over the last 40+ years.

The early passage and transfer history of RPXV-UTR is not well-documented. The virus was transferred to multiple international academic laboratories before it was obtained by (b) (4) in 1963 and subsequently transferred to the American Type Culture Collection (ATCC) (Figure 6; Animal Models White Paper, pg. 19). The ATCC stock virus was obtained by (b) (4) who produced the RPXV (b) (4)-01 stock. This virus stock had been passaged at least 23 times at an unknown multiplicity of infection and NGS analysis indicated heterogeneity (see review of BARDA Pre-IND 118305 SDN 017 by Dr. Eric Donaldson). Finally, a clonal stock was produced from RPXV-(b) (4)-01 by three rounds of plaque purification under GLP conditions, and this clonal stock (#090314) was used in studies SR14-008F and SR13-025F. The clonal viral stock was tested for sterility (no microbial growth) endotoxin levels (<(b) (4) EU/mL), mycoplasma (negative) and pH (b) (4)). Genomic sequence comparison with the RPXV-UTR reference sequence (GenBank accession #AY484669; Li et al., 2005) showed 100% identity for the RPXV041 (encoding VP37, tecovirimat target), RPXV054 (encoding DNA polymerase) and RPXV163 (encoding viral hemagglutinin) genes.

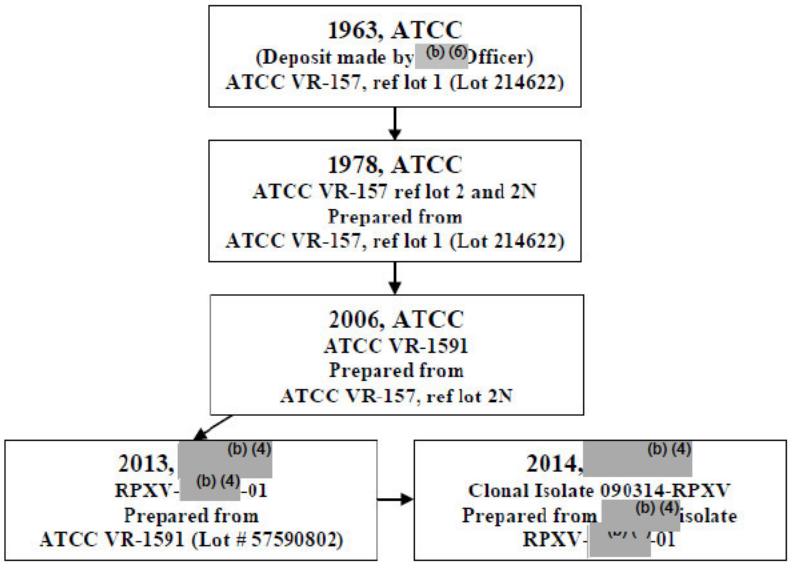


Figure 6. Transfer history of RPXV isolates used in challenge studies. Note that the virus obtained by ATCC in 1963 had previously been transferred to multiple different international academic laboratories.

The sponsor considered both aerosol (Nalca and Nichols, 2011) and intradermal viral challenge routes for rabbit/RPXV studies; however, the intradermal challenge model ultimately was chosen because in the aerosol model lethality appears to be lung-related and not associated with a systemic infection as observed in human smallpox. The intradermal challenge route causes a smallpox-like disease that results in uniform mortality at low viral challenge doses, allows for a more reproducible viral challenge dose, and is simpler and more scalable (Adams et al., 2007).

The sponsor's key rabbit/RPXV efficacy studies used 16-week-old rabbits challenged intradermally with 1,000 PFU of virus. The sponsor preferred 16-week-old rabbits over 9-week-old rabbits, which had been used in previous RPXV studies (e.g., Adams et al., 2007) because they believe: (1) the 16-week rabbit model is more humane as it requires the sacrifice of fewer animals to achieve study endpoints since younger rabbits are generally more susceptible to unrelated stressors that are detrimental to their general health, (2) the 16-week-old rabbit model is more amenable to experimental procedures (e.g., blood sampling), and (3) the model is more reproducible.

The disease course in RPXV-infected rabbits is shown in Figure 7 (Animal Models White Paper, pg. 21). Clinical signs of RPXV infection include changes in respiration rates and redness, edema, scabbing and necrosis at the injection site. Body temperatures increase on Day 3-4 post-challenge and remain elevated until just prior to death/euthanasia when animals become hypothermic. Low levels of viral DNA are detectable in the blood in 83-100% of animals by the time fever is evident, with levels increasing to 10^5-10^9 genome copies/mL by the time of death. Fever and detectable viral DNA is observed in all infected rabbits by Day 4 post-challenge, which the sponsor uses as the basis for initiating treatment on Day 4 for efficacy studies. Secondary lesions may occur later between Days 4-10 in most but not necessarily all animals prior to death. Unambiguous lesions can usually be seen on the back of shaved animals by Days 4-6. Ear lesions may occur prior to death in a fraction of animals, and similarly lesions may form in other sites such as the eye, nose, mouth and ano-genital area. However, because lesions emerge relatively late in disease and are not necessarily always observed makes the appearance of lesions an imperfect trigger for treatment. Weight loss is observed, but not necessarily in all animals. Mortality with this model is nearly universal, occurring in 6-10 days post-challenge, and is typically attributed to severe respiratory distress.

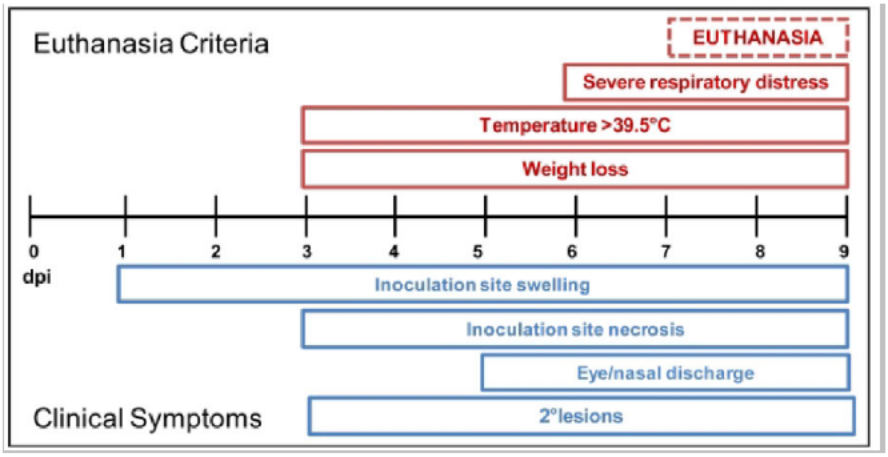


Figure 7. Clinical disease course and euthanasia criteria in rabbits (New Zealand white rabbits) infected intradermally with RPXV-UTR.

A study led by BARDA and conducted by (b) (4) (see Clinical Virology review of Pre-IND 118305 SDN 26 by Dr. Jules O'Rear for details) confirmed that the plaque-purified RPXV-UTR strain was highly virulent in 9- and 16-week-old NZW rabbits, with a 50% lethal dose (LD_{50}) of <4.66 PFU in the 9-week-old rabbits. For the 16-week-old rabbits, because a few rabbits that received higher challenge doses survived, the probit regression analysis could not estimate an LD_{50}; however, lethality was 100% for those that received 4.66 PFU and therefore it is reasonable to assume that the true LD_{50} is in this range or lower. The 1,000 PFU challenge dose in pivotal efficacy studies was chosen to ensure reproducible mortality, and may accelerate the appearance of swelling at the primary infection site 12-24 hours relative to lower challenge doses, but the overall clinical disease course follows the same time line.

3.2 Study AP-09-026G (NHPs/Monkeypox Virus)

Title
AP-09-026G, "Double-Blind, Randomized, Placebo-Controlled, Repeat-Dose Efficacy Study of the Minimum Effective Therapeutic Dose of Oral ST-246® Polyform I in Cynomolgus Monkeys Infected with Monkeypox Virus"

Summary of Design

Study AP-09-026G was a blinded, randomized, placebo-controlled study to evaluate tecovirimat ("ST-246 Polyform I") efficacy in cynomolgus monkeys infected with MPXV, conducted at USAMRIID. The purpose of the study was to determine the minimum effective dose of oral tecovirimat for the treatment of MPXV infection in the lesional cynomolgus monkey model, with study drug dosing starting on the day of onset of pox lesions in each animal.

Prior to virus challenge, sera from all study animals were tested and found to be free of antibodies to orthopoxviruses (VACV or MPXV), simian immunodeficiency virus (SIV), simian T-cell leukemia virus (STLV) and simian retroviruses (SRV). Twenty-seven male cynomolgus monkeys were randomized into two study "iterations" with five treatment groups per iteration, and with 5 animals total assigned to each dosing group (Table 6; adapted from pg. 58). Two extra monkeys, one per iteration, served as potential replacements and were assigned to the placebo control group.

Table 6. Study AP-09-026G study group assignments and tecovirimat dose levels.

	Group	N	Tecovirimat Dose (mg/kg)
Iteration #1	1	3	10
	2	3	3
	3	3	1
	4	3	0.3
	5	4	0
	Group	**N**	**Tecovirimat Dose (mg/kg)**
Iteration #2	1	2	10
	2	2	3
	3	2	1
	4	2	0.3
	5	3	0

On Day 0, animals were anesthetized and challenged IV with 1 mL of virus inoculum at a target dose of 5.0×10^7 PFU (actual dose 5.55×10^7 PFU) of the MPXV Zaire '79 strain (lot WTG03Oct05). Monkeys then received placebo or tecovirimat once daily by orogastric gavage beginning on the day pox lesions first appeared, which was Day 4 post-challenge for all animals, and continued every 24 ± 2 hours for 14 consecutive days. Body weights, clinical observations, lesion counts and clinical laboratory analyses were conducted throughout the post-challenge period. The primary efficacy endpoint was the proportion of animals that survived until Day 28 post-challenge. Secondary endpoints included time to death (including euthanasia), viral DNA levels, pox lesion counts and other laboratory values. All surviving animals were euthanized on post-challenge Day 42 or 43. No drug resistance assessments were conducted for this study.

Efficacy Results (Survival and Clinical Disease)

The sponsor's analysis of survival through Post-Challenge Day 28 is summarized in Figure 8 (Report pg. 92). According to the sponsor's analysis, no animals in the Placebo or Tecovirimat 1 mg/kg groups survived to Post-Challenge Day 28, and 20% (1/5) of animals in the Tecovirimat 0.3 mg/kg group survived. In contrast, 80% (4/5) of animals in each of the Tecovirimat 3 mg/kg and 10 mg/kg groups survived, which was a significantly greater survival rate (p=0.0101) compared to the Placebo. There were no unscheduled deaths or euthanasia after Post-Challenge Day 27. The sponsor noted that the two deaths in the Tecovirimat 3 mg/kg and 10 mg/kg groups (Animals 27 and 21, respectively) occurred under anesthesia and were not associated with "typical" MPXV disease. Among the remaining 16 animals that died prematurely in the study, 5 (31%) were found dead and 11 (69%) were euthanized due to moribund disease.

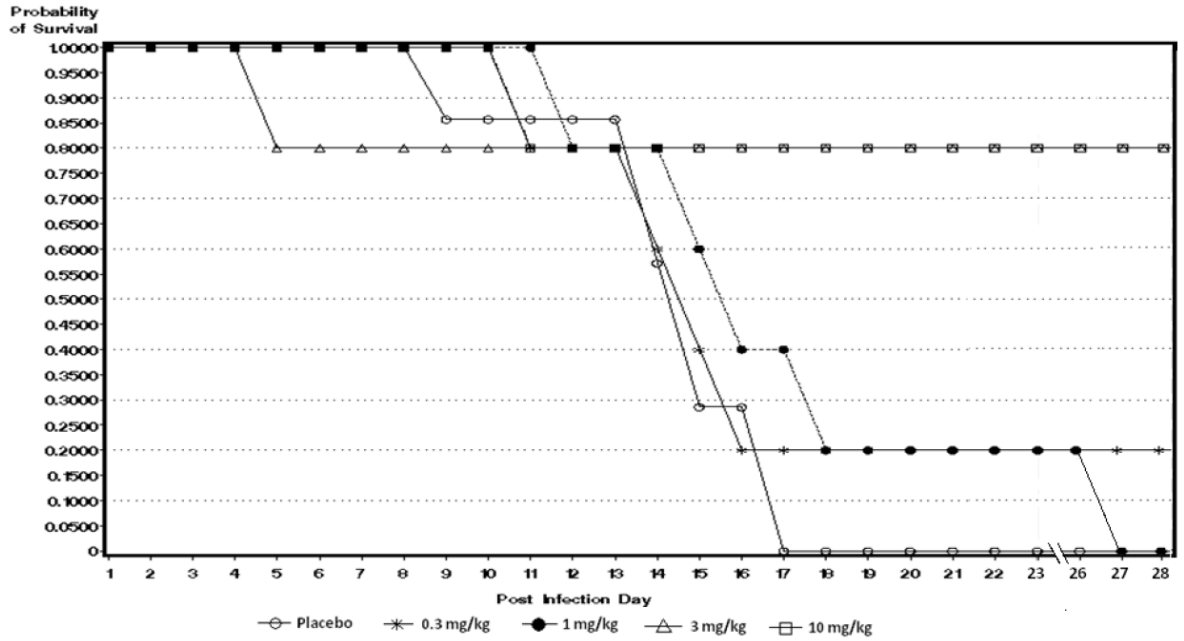


Figure 8. Kaplan-Meier survival curve through Day 28 in NHP/MPXV study AP-09-026G.

The sponsor's analyses of pox lesion counts across treatment groups are summarized in Figure 9 (Report pg. 97). According to the sponsor's analyses, the mean time-weighted average of total pox lesion counts for Treatment Day 1 through 14 and Post-Challenge Day 1 through 23 were significantly decreased ($p<0.05$) in the Tecovirimat 10 mg/kg group, but no significant differences were noted between the Placebo and the Tecovirimat 0.3 mg/kg, 1 mg/kg, and 3 mg/kg groups.

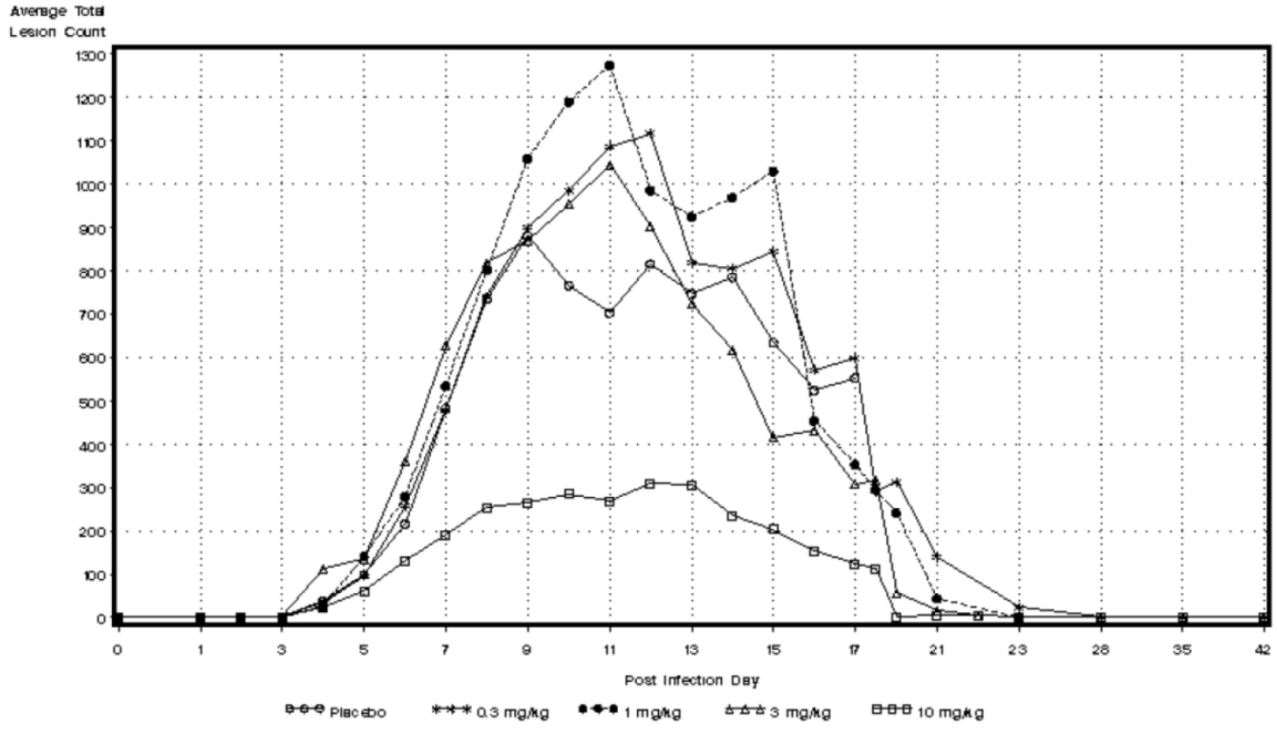


Figure 9. Pox lesion counts over time in NHP/MPXV study AP-09-026G.

Virology Results

Whole blood MPXV DNA levels over time for individual animals are shown in Figure 10 (FDA analysis). Group mean and median DNA levels are shown in Figure 11 (FDA analysis), and summarized further in Table 7 (FDA analysis).

All animals had evidence of infection as demonstrated by increasing blood viral DNA levels during the initial days post-challenge. Animals treated with tecovirimat at the 3 mg/kg or 10 mg/kg dose level had reduced viral DNA levels over time starting around Post-Challenge Day 7-9 (i.e., treatment days 3-5). During this time period viral DNA levels were lowest on average in the tecovirimat 10 mg/kg dose group, with median viral DNA levels 1 to 3 $\log_{10}$ copies/mL lower relative to placebo-treated animals.

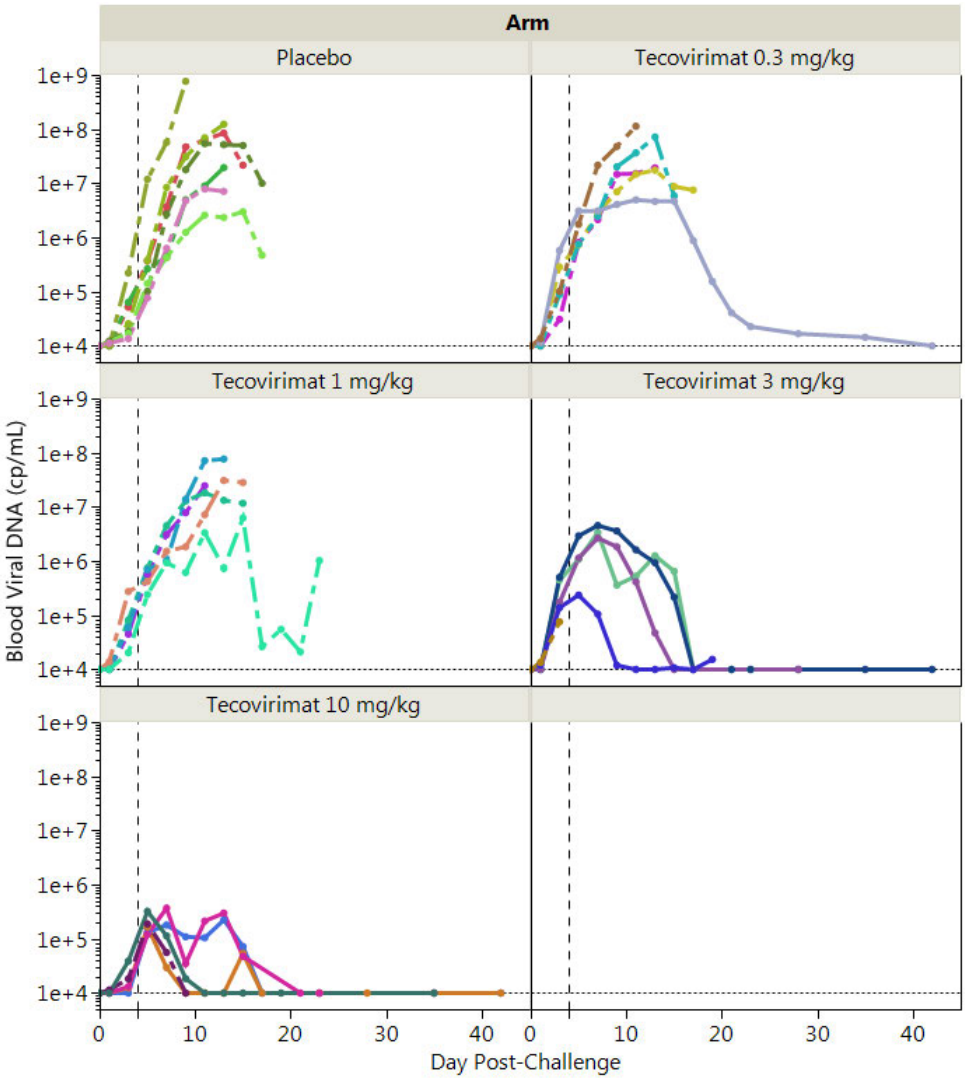


Figure 10. Viral DNA levels in whole blood for individual animals in NHP/MPXV study AP-09-026G. Animals that did not survive through the end of the observation period are indicated by dashed lines. The assay LLOQ was 10,000 copies/mL (horizontal reference line), and all animals were dosed with tecovirimat or placebo starting on Post-Challenge Day 4 (vertical reference line). Day 0 viral DNA levels quantifying viral challenge delivery are not shown.

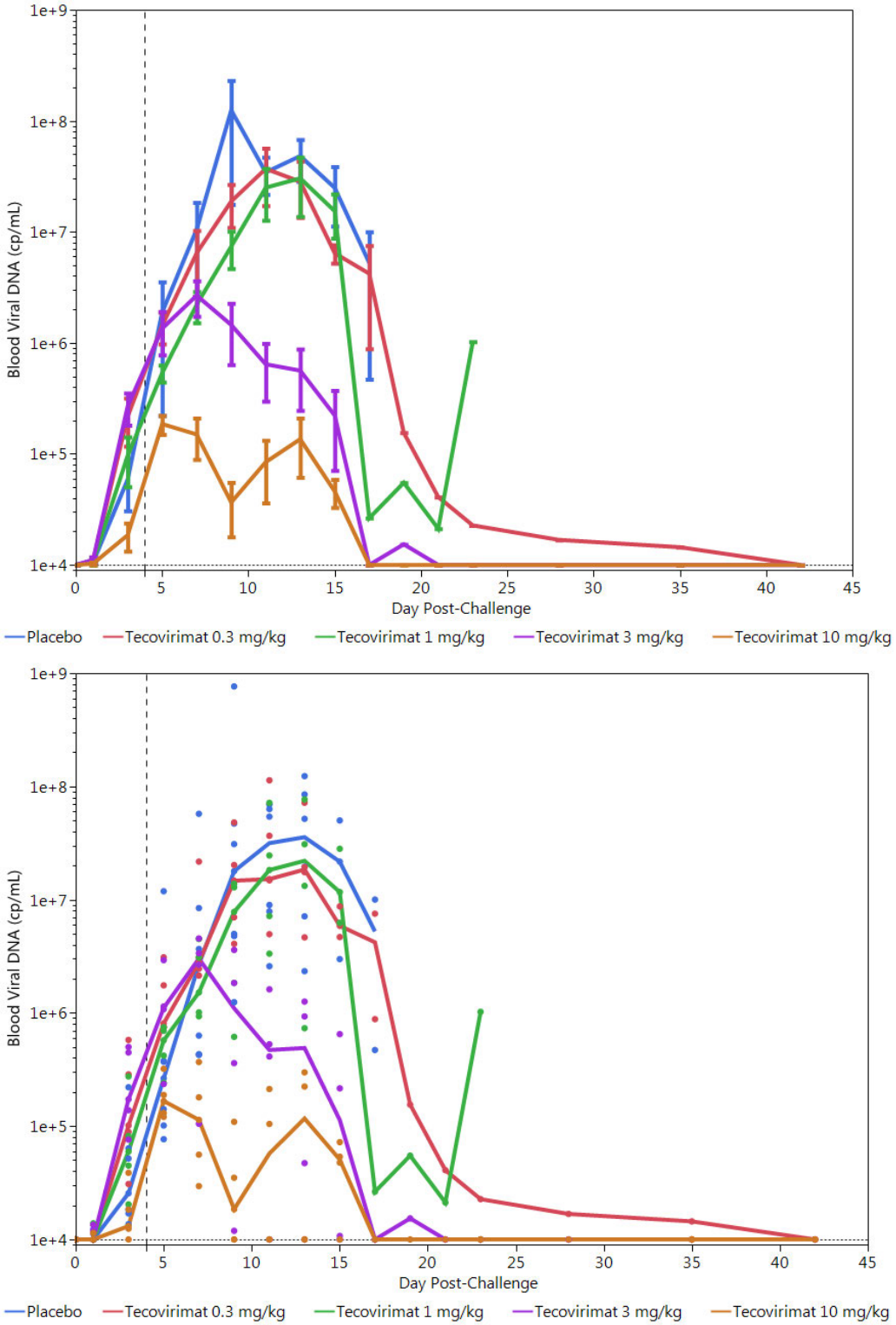


Figure 11. Mean +/- standard error (top) and median w/individual (bottom) viral DNA levels in whole blood in NHP/MPXV study AP-09-026G. The assay LLOQ was 10,000 copies/mL (horizontal reference line), and all animals were dosed with tecovirimat or placebo starting on Post-Challenge Day 4 (vertical reference line). Day 0 viral DNA levels quantifying viral challenge delivery are not shown.

Table 7. Summary of median (range) viral DNA levels in whole blood in NHP/MPXV study AP-09-026G. The assay LLOQ was 4.0 log_{10} copies/mL. Differences from placebo ≥1 log_{10} copies/mL are highlighted.

	MPXV DNA Levels (Log_{10} copies/mL)													
	Placebo		Tecovirimat 0.3 mg/kg			Tecovirimat 1 mg/kg			Tecovirimat 3 mg/kg			Tecovirimat 10 mg/kg		
Day Post-Challenge	N	Median (Range)	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO
1	6	4.0 (4.0-4.1)	5	4.0 (4.0-4.1)	0	5	4.0 (4.0-4.1)	0	5	4.0 (4.0-4.1)	0	5	4.0 (4.0-4.1)	0
3	7	4.4 (4.1-5.3)	5	5.0 (4.5-5.8)	0.6	5	4.8 (4.3-5.4)	0.4	5	5.2 (4.9-5.7)	0.8	5	4.1 (4.0-4.6)	-0.3
5	7	5.4 (4.9-7.1)	5	5.9 (5.9-6.5)	0.5	5	5.8 (5.4-5.9)	0.4	4	6.0 (5.4-6.5)	0.6	5	5.2 (5.1-5.5)	-0.2
7	7	6.4 (5.6-7.8)	5	6.4 (6.3-7.3)	0	5	6.2 (6.0-6.7)	-0.2	4	6.5 (5.0-6.7)	0.1	5	5.1 (4.5-5.6)	-1.3
9	7	7.3 (6.1-8.9)	5	7.2 (6.6-7.7)	-0.1	5	6.9 (5.8-7.1)	-0.4	4	5.9 (4.1-6.6)	-1.4	5	4.3 (4.0-5.0)	-3.0
11	6	7.3 (6.4-7.8)	5	7.2 (6.7-8.1)	-0.1	5	7.3 (6.5-7.9)	0	4	5.7 (4.0-6.2)	-1.6	4	4.5 (4.0-5.3)	-2.8
13	6	7.5 (6.4-8.1)	4	7.3 (6.7-7.9)	-0.2	4	7.3 (5.9-7.9)	-0.2	4	5.3 (4.0-6.1)	-2.2	4	4.7 (4.0-5.5)	-2.8
15	3	7.3 (6.5-7.7)	3	6.8 (6.7-6.9)	-0.5	3	7.1 (6.8-7.5)	-0.2	4	4.7 (4.0-5.8)	-2.6	4	4.7 (4.0-4.9)	-2.6
17	2	6.3 (5.7-7.0)	2	6.4 (5.9-6.9)	0.1	1	4.4 (n/a)	-1.9	4	4.0 (4.0-4.0)	-2.3	2	4.0 (4.0-4.0)	-2.3
23	0		1	4.4 (n/a)	n/a	1	6.0 (n/a)	n/a	2	4.0 (4.0-4.0)	n/a	3	4.0 (4.0-4.0)	n/a
28	0		1	4.2 (n/a)	n/a	0		n/a	2	4.0 (4.0-4.0)	n/a	1	4.0 (n/a)	n/a
Peak Viral DNA	7	7.7 (6.5-8.9)	5	7.3 (6.7-8.1)	-0.4	5	7.4 (6.8-7.9)	-0.3	5	6.4 (4.9-6.7)	-1.3	5	5.4 (5.2-5.6)	-2.4

As shown in Figure 11, just prior to tecovirimat treatment initiation whole blood viral DNA levels in the tecovirimat 10 mg/kg group appeared to be lower, on average, relative to all other treatment groups. This observation raises a concern that the MPXV infection in the tecovirimat 10 mg/kg group was not as robust relative to the other treatment groups, confounding the differences observed in viral DNA levels during the treatment period in this group relative to other groups.

To investigate this issue further, pre-treatment viral DNA levels on Post-Challenge Day 3 (i.e., last available timepoint prior to treatment initiation) were analyzed in greater detail across the treatment groups. In addition, the sponsor collected blood samples from animals within ~2 minutes after viral challenge for viral DNA analysis to confirm and quantify viral delivery into the bloodstream of each animal, and these viral DNA levels were also compared across treatment groups.

As shown in Figure 12 (FDA analysis), viral DNA titers immediately after viral challenge were generally comparable across treatment groups, with DNA levels in all but one animal within a 0.8 log_{10} copies/mL range. At Post-Challenge Day 3 viral DNA titers trended lower in the tecovirimat 10 mg/kg group relative to all other groups. Nevertheless, there was no evidence of a lower pre-treatment viral DNA titer overall in tecovirimat-treated animals relative to placebo-treated animals at Day 0 or Day 3 Post-Challenge ($p=0.98$ and $p=0.21$, respectively; Wilcoxon Rank-Sum test). These results indicate that the viral challenges and early dynamics of viral replication prior to treatment were generally similar between the placebo and pooled tecovirimat treatment groups.

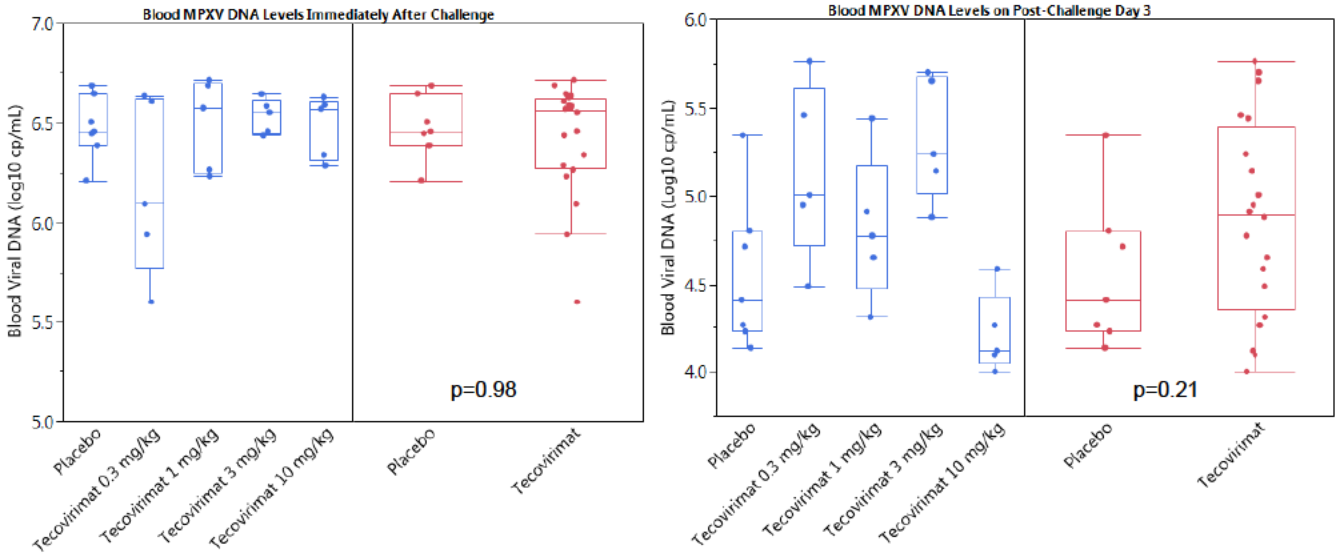


Figure 12. Whole blood viral DNA levels immediately after challenge (left) or on Day 3 Post-Challenge (right, i.e., 1 day prior to treatment start) in NHP/MPXV study AP-09-026G. P values shown are based on Wilcoxon Rank-Sum test, and only considered the placebo versus pooled tecovirimat comparisons (red box plots) due to small sample sizes of individual treatment groups. The assay LLOQ was 4.0 $\log_{10}$ copies/mL.

It is theoretically possible that the ~1 $\log_{10}$ lower pre-treatment viral DNA level in the tecovirimat 10 mg/kg group relative to the 3 mg/kg group contributed to the lower viral DNA levels observed in the 10 mg/kg group during the treatment period. However, it is important to note that this does not call into question tecovirimat efficacy in this study, as antiviral activity was demonstrated based on both survival and blood viral DNA results even in the tecovirimat 3 mg/kg group, which had the highest median Post-Challenge Day 3 viral DNA levels.

Viral DNA levels in tissues collected at necropsy are shown in Figure 13 (FDA analysis). Viral DNA levels were lowest in the tecovirimat 3 mg/kg and 10 mg/kg groups generally across all tissues examined.

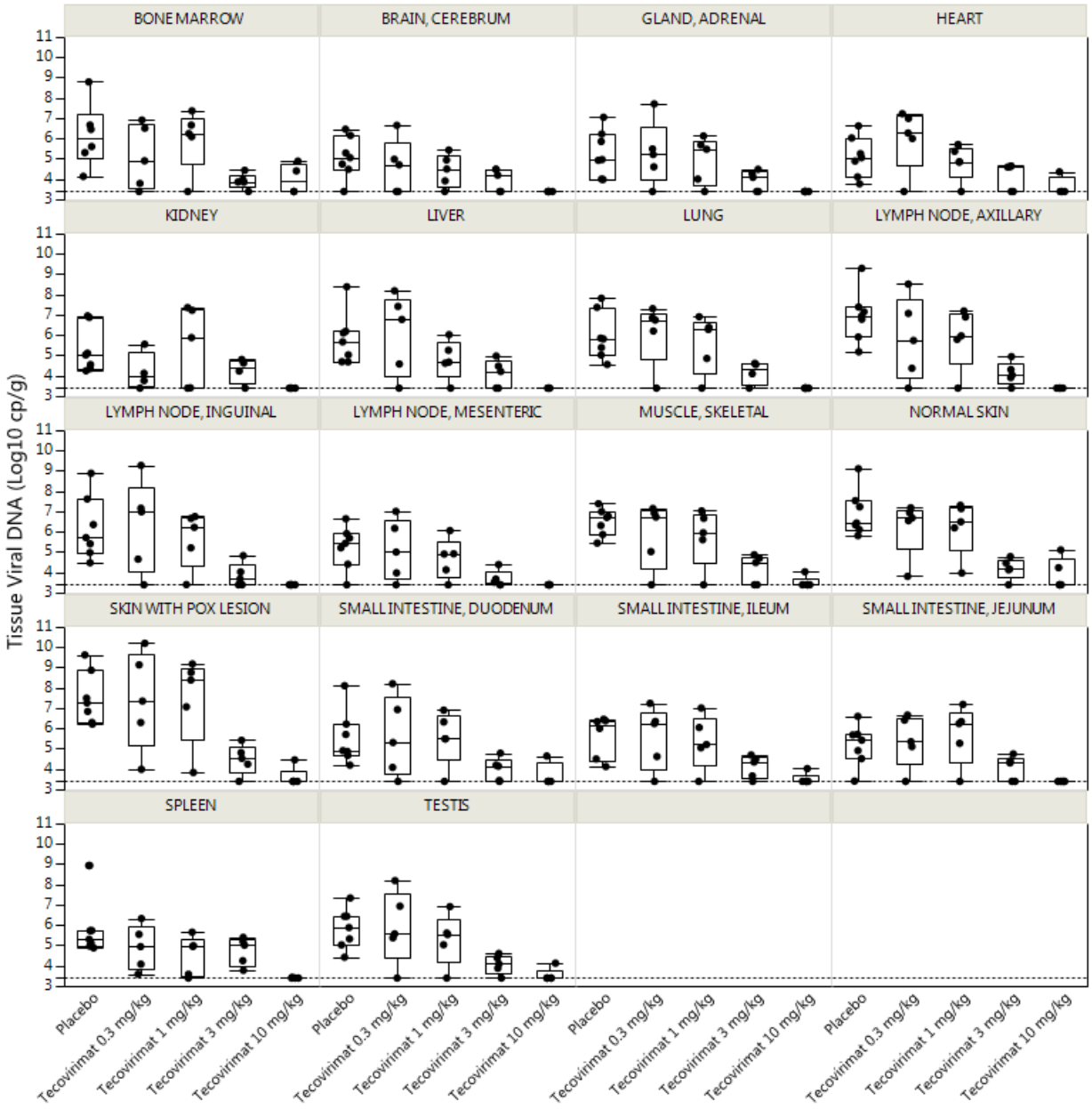


Figure 13. Viral DNA levels in tissue samples collected at necropsy in NHP/MPXV study AP-09-026G, according to treatment group.

Tissue viral DNA levels were higher in animals that died or were euthanized due to moribund disease, relative to those that survived until the end of study (Figure 14, FDA analysis), which is not surprising given the low systemic viral DNA levels at the end of study in suviving animals. Among non-surviving animals, there was a weak correlation of higher viral DNA levels in tissues for animals that died earlier in disease, although the correlation was not consistent across all tissues (analyses not shown).

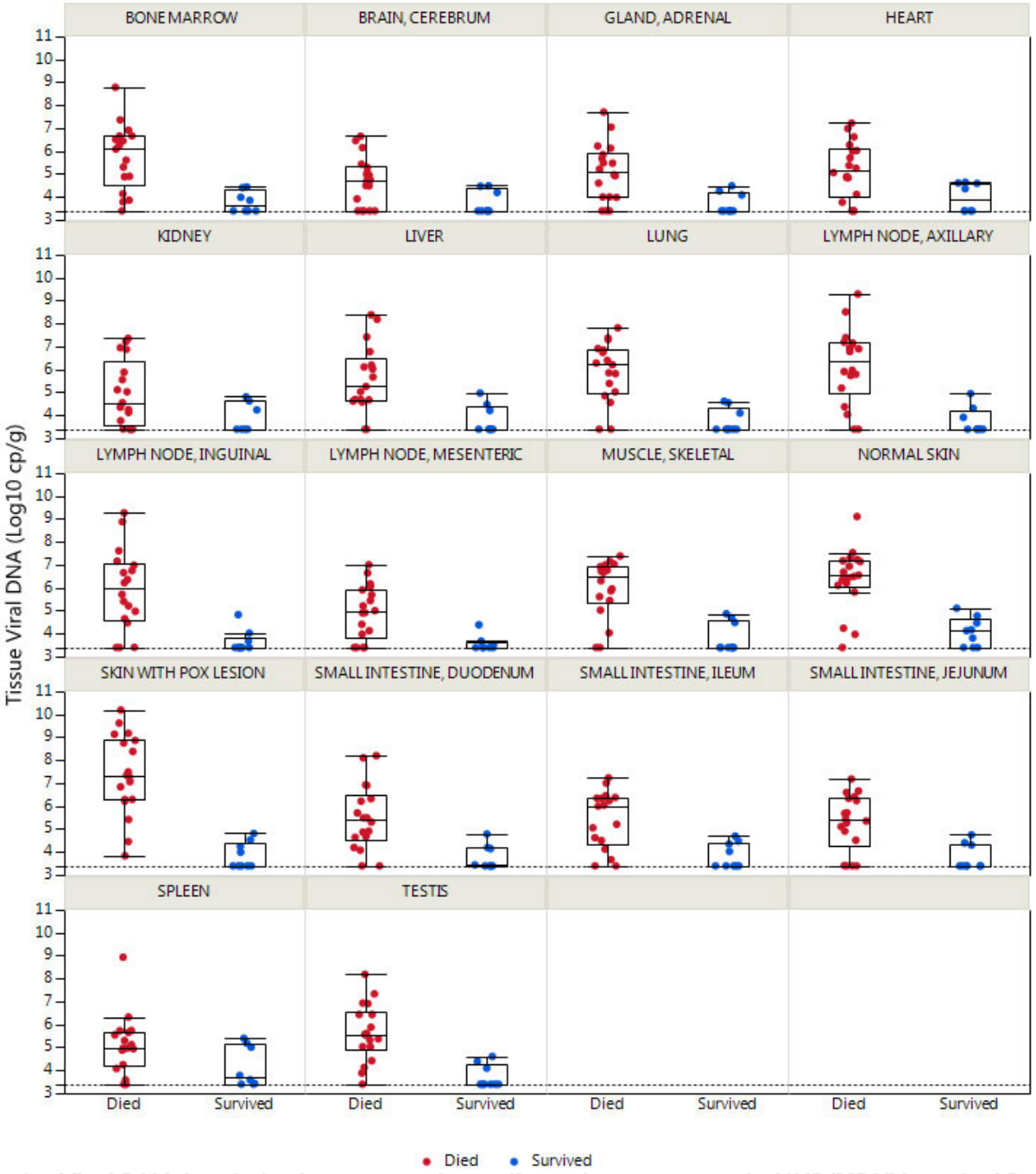


Figure 14. Viral DNA levels in tissue samples collected at necropsy in NHP/MPXV study AP-09-026G, according to survival outcome irrespective of treatment group.

Among animals that died or were euthanized due to moribund disease, tissue MPXV DNA levels were highest in skin with pox lesions (Figure 15, FDA analysis). Further supporting the utility of the NHP/MPXV model, in the VARV NHP model viral DNA levels at later stages of infection were similarly highest in skin lesions (see Section 4.1).

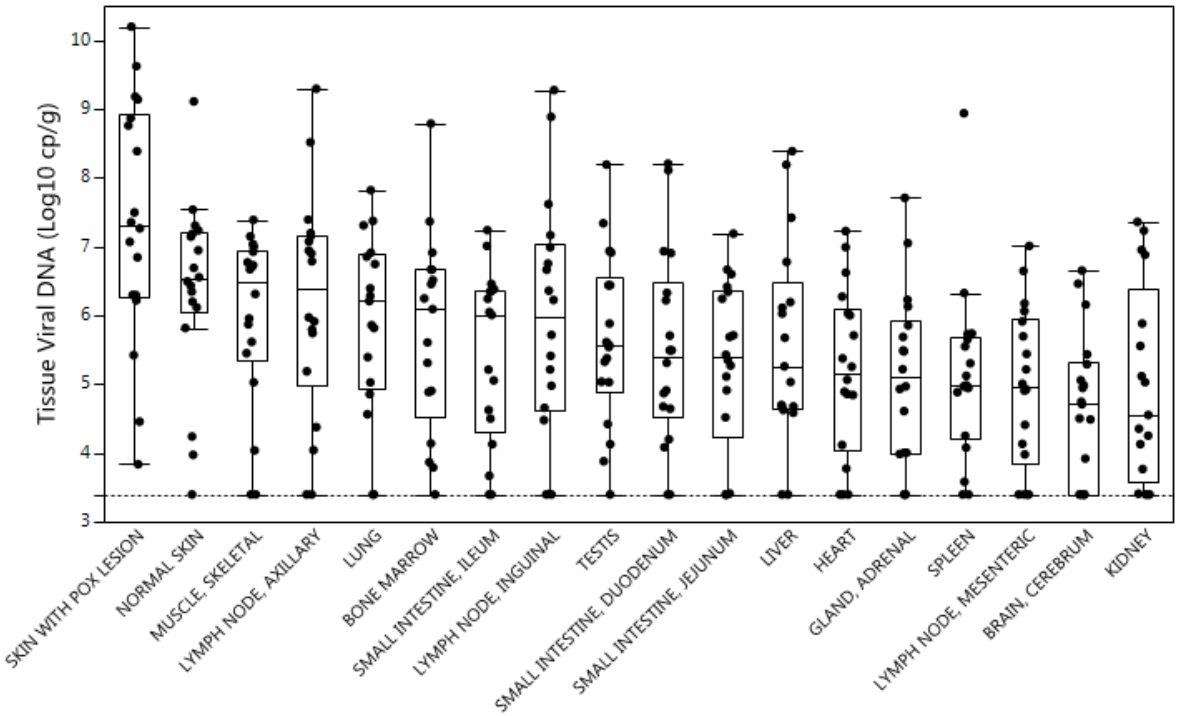


Figure 15. Tissue MPXV DNA levels in animals that died (or were euthanized due to moribund disease), irrespective of treatment group. Tissues in x-axis are sorted by median viral DNA results.

The AP-09-026G study report noted 3 animals in tecovirimat treatment groups that died prior to the end of study, apparently without typical MPXV disease at the time of death:

- Animal 19 (tecovirimat 1 mg/kg): found dead on Day 27
- Animal 27 (tecovirimat 3 mg/kg): died under anesthesia on Day 5
- Animal 21 (tecovirimat 10 mg/kg): died under anesthesia on Day 11

Virology characteristics of these 3 animals are summarized in Figure 16 (FDA analysis). In general, these 3 animals had blood viral DNA levels that were comparable or lower relative to other tecovirimat-treated animals that died or were euthanized prematurely, although Animal 19 had an interesting rebound in blood viral DNA level on Day 23, which was the last available blood viral DNA assessment. These animals had relatively low viral DNA levels in tissues at necropsy.

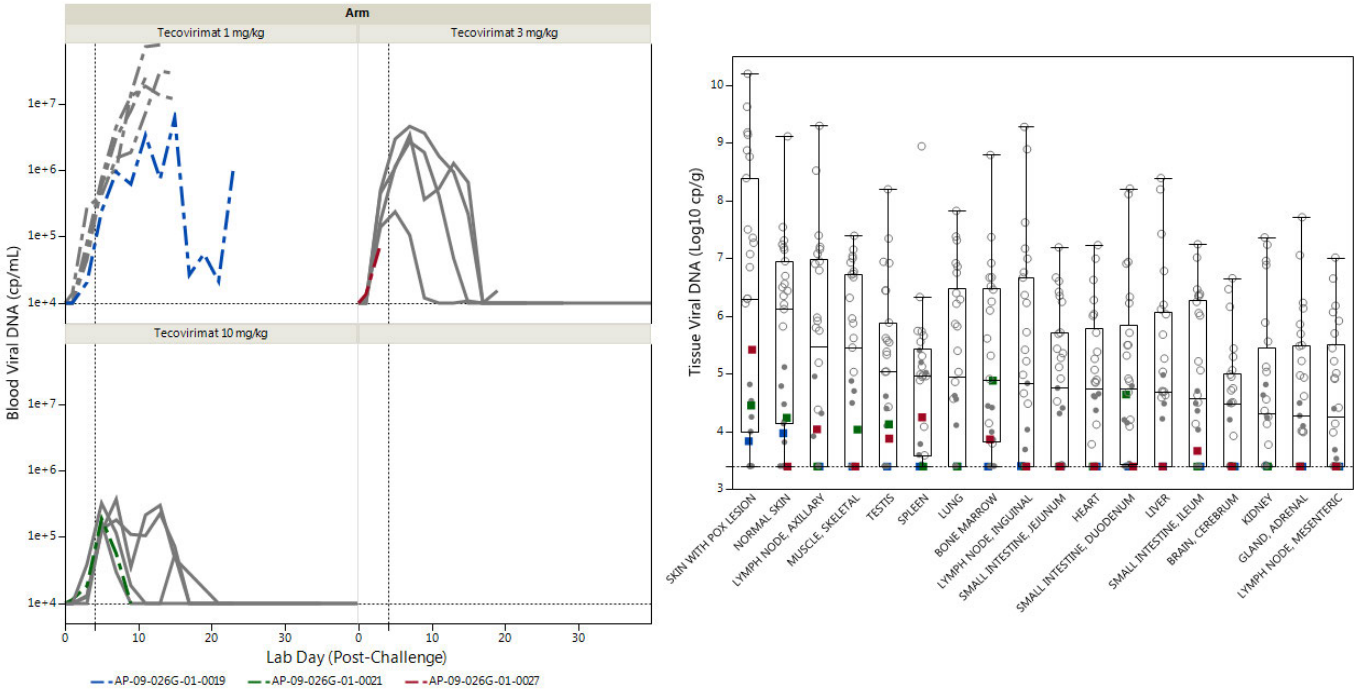


Figure 16. Blood (left) and tissue (right) viral DNA levels in 3 animals (19, 21, 27) that died of atypical disease in study AP-09-026G, relative to other animals in the study. Viral DNA levels for these 3 animals are shown in colored lines or symbols; all other animals are shown in gray. Dashed lines and open circles indicate other animals that died or were euthanized prematurely. The right panel also includes data from placebo-treated animals.

3.3 Study SR10-037F (NHPs/Monkeypox Virus)

Title

SR10-037F, "Double-Blind Placebo Controlled Study to Evaluate Effect of Delayed ST-246 Treatment on Efficacy Following Lethal Monkeypox Virus Challenge in Cynomolgus Macaques"

Summary of Design

Study SR10-037F was a randomized, double-blind, placebo-controlled study of tecovirimat administered orally to cynomolgus monkeys infected with MPXV, conducted at (b) (4). The primary purpose of this study was to determine the effect of delayed initiation of treatment on the efficacy of tecovirimat in MPXV-infected cynomolgus monkeys.

A total of 21 cynomolgus monkeys that were negative for pre-existing antibodies against orthopoxviruses were randomly assigned to four groups to receive either placebo or tecovirimat 10 mg/kg for 14 days starting either on Day 4, Day 5 or Day 6 post-MPXV-challenge (Table 8; adapted from pg. 30). All monkeys were challenged on Day 0 with 5.0 x 10^7 PFU of the MPXV Zaire '79 strain (NR-523 stock).

Table 8. Treatment groups in NHP/MPXV study SR10-037F.

Group	# Animals		MPXV Challenge (Day 0)	Tecovirimat Dose	Dosing Schedule (designed to maintain blind)	
	M	F			Tecovirimat	Placebo
Placebo	1	2	5 x 10^7 PFU IV	0 mg/kg	N/A	Days 4-19
Tecovirimat Day 4	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-17	Days 18 + 19
Tecovirimat Day 5	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 5-18	Days 4 + 19
Tecovirimat Day 6	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 6-19	Days 4 + 5

Body weights, clinical observations, lesion counts and clinical laboratory analyses were conducted throughout the post-challenge period. The primary efficacy endpoint was the proportion of animals that survived after MPXV challenge for up to 56 days. Secondary endpoints included analyses of blood viral DNA levels over time, lesion counts, body weights, and other clinical and laboratory observations. All surviving animals were euthanized and necropsied on Post-Challenge Day 56, 60 or 62. Genotypic (i.e. NGS) resistance data were obtained from a subset of animals; results are summarized in Section 3.8.

Efficacy Results (Survival and Clinical Disease)

The sponsor's analysis of survival through Post-Challenge Day 56 is summarized in Figure 17 (Report pg. 50). All 3 animals in the Placebo group died by Post-Challenge Day 10, while survival rates in the Tecovirimat Day 4, 5 and 6 groups were 83% (5/6), 83% (5/6) and 50% (3/6), respectively. According to the sponsor's analyses, survival rates were significantly greater (p=0.0476) in the Tecovirimat Day 4 and Tecovirimat Day 5 groups relative to the Placebo group. Of the 8 animals that died prematurely in the study, 3 (37.5%) were found dead and 5 (62.5%) were euthanized due to moribund disease.

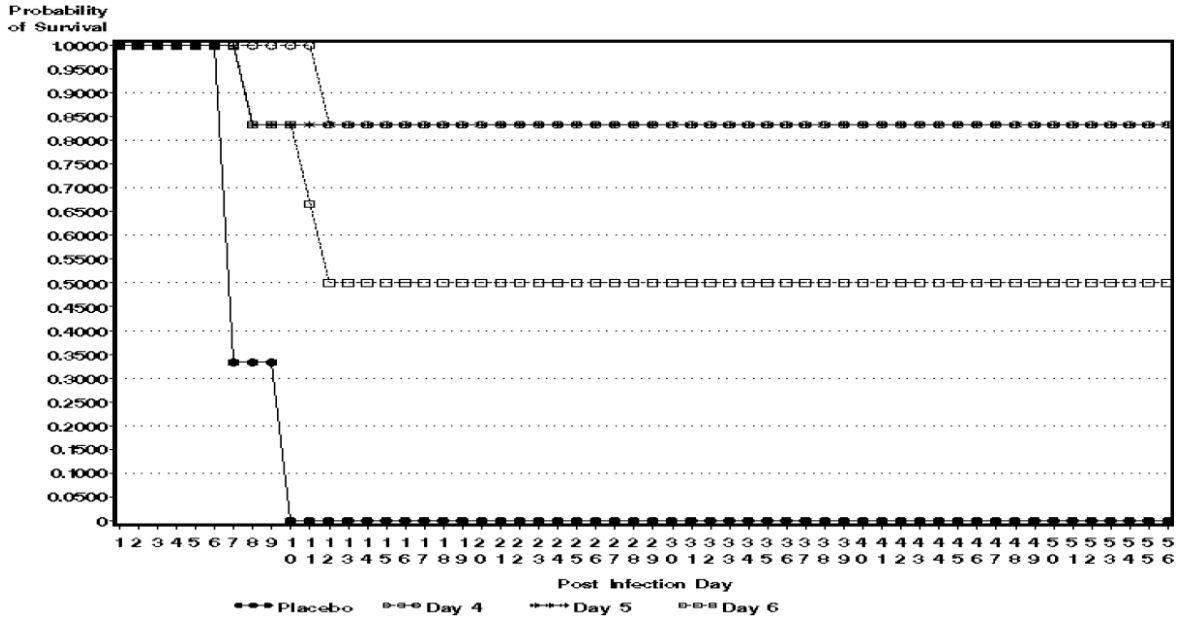


Figure 17. Kaplan-Meier survival curve through Day 56 in NHP/MPXV study SR10-037F.

The sponsor's analyses of pox lesion counts across treatment groups are summarized in Figure 18 (Report pg. 56). Although the sponsor concluded there were no statistically significant differences in lesion counts between tecovirimat- and placebo-treated animals, lesion counts trended lower in animals that started tecovirimat treatment earlier in infection.

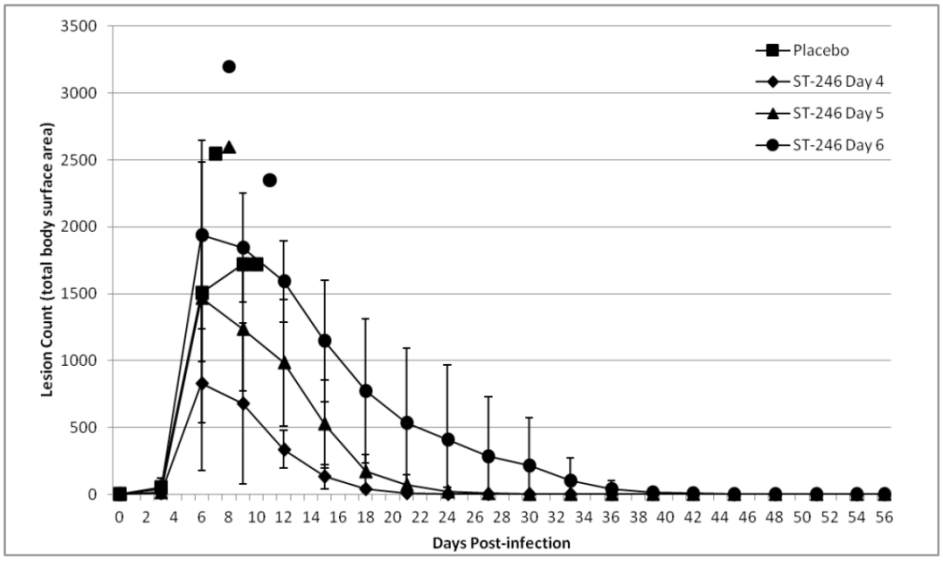


Figure 18. Pox lesion counts over time (mean ± standard deviation) in NHP/MPXV study SR10-037F. Stand-alone symbols indicate individual animal lesion counts off-schedule at time of euthanasia.

Virology Results

Whole blood MPXV DNA levels over time for individual animals are shown in Figure 19 (FDA analysis). Group mean and median levels are shown in Figure 20 (FDA analysis), and summarized further in Table 9 (FDA analysis). In general, viral DNA levels were consistent with clinical efficacy observations, with reduced levels (e.g., lower peak and more rapid decline) in animals that initiated tecovirimat treatment at Post-Challenge Day 4 or Day 5 relative to those that started tecovirimat at Post-Challenge Day 6.

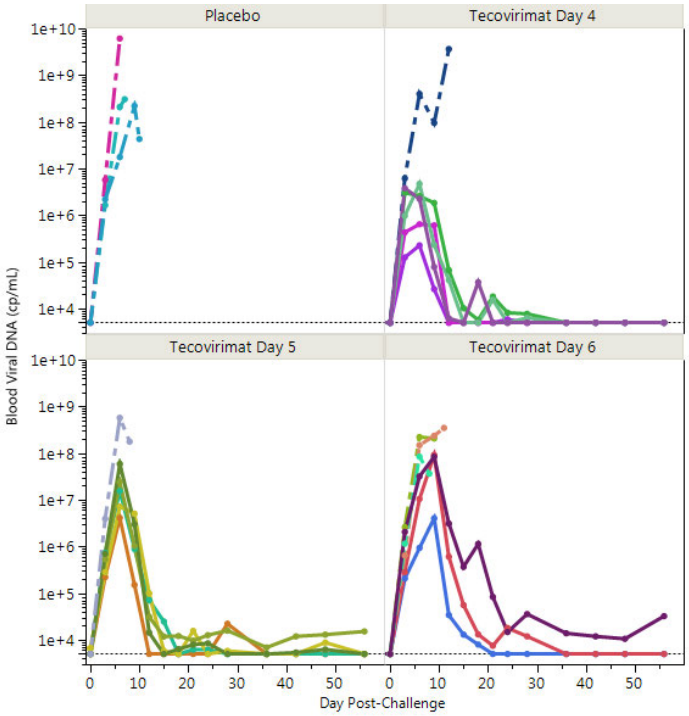


Figure 19. Viral DNA levels in whole blood for individual animals in NHP/MPXV study SR10-037F. Animals that did not survive through the end of the observation period are indicated by dashed lines. The assay LLOQ was 5,000 copies/mL (horizontal reference line). Day 0 viral DNA levels quantifying viral challenge delivery are not shown.

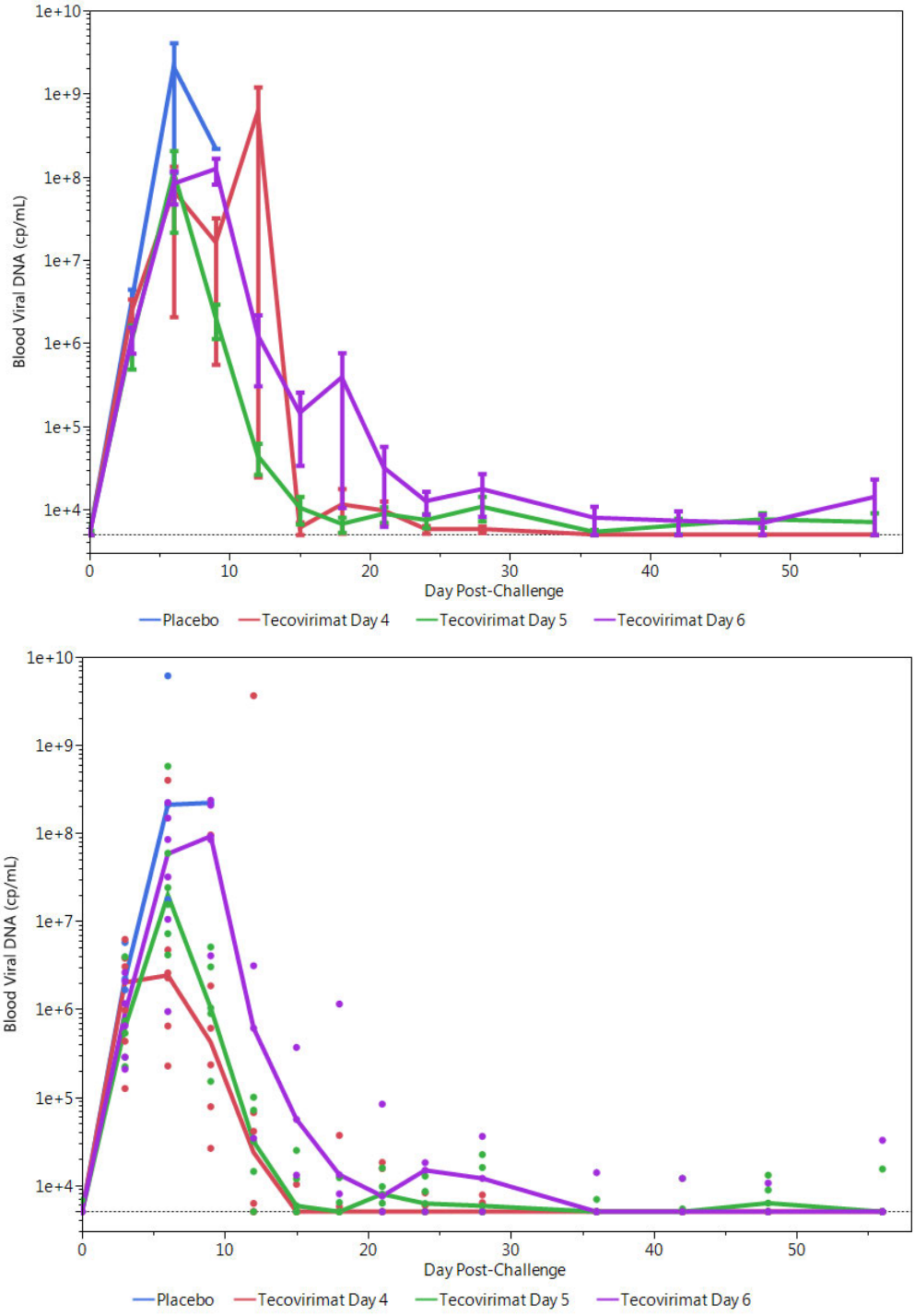


Figure 20. Mean +/- standard error (top) and median w/individual (bottom) viral DNA levels in whole blood in NHP/MPXV study SR10-037F. The graphs censor data for Post-Challenge Days 7 (n=1), 8 (n=2), 10 (n=1) and 11 (n=1) due to data being reported for only a small subset of animals. Day 0 viral DNA levels quantifying viral challenge delivery also are not shown. The assay LLOQ was 5,000 copies/mL (horizontal reference line). The large spike in the mean viral DNA level on Day 12 for the Tecovirimat Day 4 group is driven by a single animal (4768) with a titer of 9.6 $\log_{10}$ copies/mL.

Table 9. Summary of median (range) viral DNA levels in whole blood in NHP/MPXV study SR10-037F. The assay LLOQ was 3.7 log_{10} copies/mL. Differences from placebo ≥1 log_{10} are highlighted.

	MPXV DNA Levels (Log_{10} copies/mL)										
	Placebo		Tecovirimat Day 4			Tecovirimat Day 5			Tecovirimat Day 6		
Day Post-Challenge	N	Median (Range)	N	Median (Range)	Δ Rel. to PBO	N	Median (Range)	Δ Rel. to PBO	N	Median (Range)	Δ Rel. to PBO
3	3	6.3 (6.2-6.8)	6	6.2 (5.1-6.8)	-0.1	6	5.8 (5.3-6.6)	-0.6	6	5.9 (5.3-6.4)	-0.4
6	3	8.3 (7.2-9.8)	6	6.4 (5.4-8.6)	-1.9	6	7.3 (6.6-8.8)	-1.0	6	7.7 (6.0-8.3)	-0.6
9	1	8.3 (n/a)	6	5.6 (4.4-8.0)	-2.8	5	6.0 (5.2-6.7)	-2.3	5	8.0 (6.6-8.4)	-0.4
12	0	n/a	6	4.2 (3.7-9.6)	n/a	5	4.5 (3.7-5.0)	n/a	3	5.8 (4.5-6.5)	n/a
15	0	n/a	5	3.7 (3.7-4.0)	n/a	5	3.8 (3.7-4.4)	n/a	3	4.7 (4.1-5.6)	n/a
18	0	n/a	5	3.7 (3.7-4.6)	n/a	5	3.7 (3.7-4.1)	n/a	3	4.1 (3.9-6.1)	n/a
21	0	n/a	5	3.7 (3.7-4.3)	n/a	5	3.9 (3.7-4.2)	n/a	3	3.9 (3.7-4.9)	n/a
28	0	n/a	5	3.7 (3.7-3.9)	n/a	5	3.8 (3.7-4.3)	n/a	3	4.1 (3.7-4.6)	n/a
36	0	n/a	5	3.7 (3.7-3.7)	n/a	5	3.7 (3.7-3.8)	n/a	3	3.7 (3.7-4.1)	n/a
Peak Viral DNA	3	8.5 (8.3-9.8)	6	6.5 (6.1-9.6)	-2.0	6	7.3 (6.6-8.8)	-1.2	6	7.9 (6.6-8.5)	-0.5

Viral DNA levels at Post-Challenge Day 3, corresponding to 1-, 2-, and 3-days prior to tecovirimat treatment initiation in the Tecovirimat Day 4, Day 5 and Day 6 groups, respectively, were higher in animals that eventually died from MPXV infection relative to those that survived (Figure 21, FDA analysis). Interestingly, for all 3 non-surviving animals in the Tecovirimat Day 6 group, Day 3 viral DNA levels were similar to those from survivors in the Tecovirimat Day 4 group, indicating that earlier treatment may have prevented the deaths that occurred in the Day 6 group. Collectively, the survival and viral DNA results indicate that tecovirimat is likely most efficacious when started closest to the time of challenge and when circulating viral DNA levels are lowest.

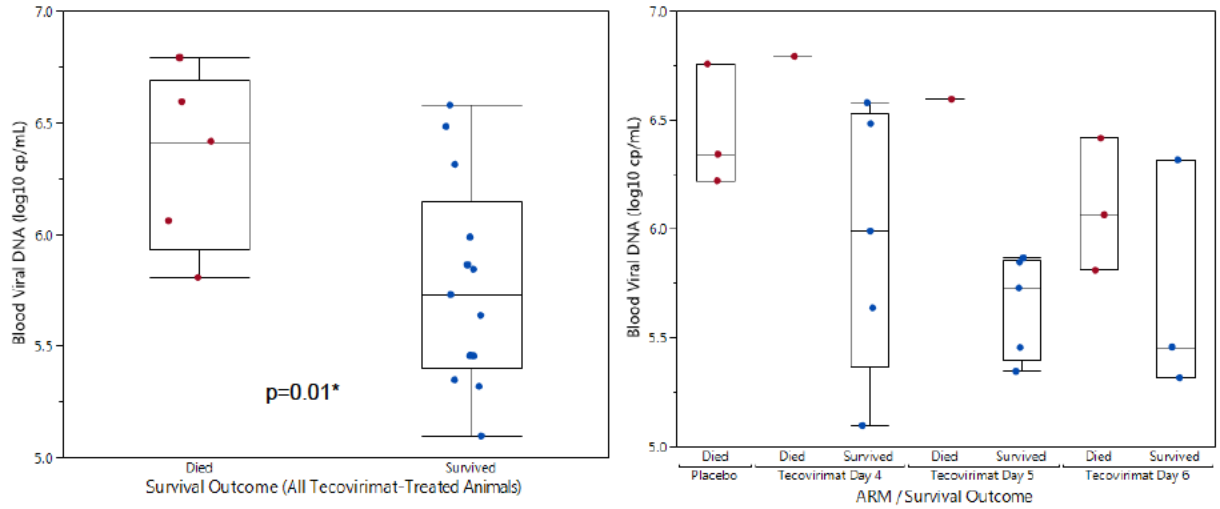


Figure 21. MPXV DNA levels in whole blood on Post-Challenge Day 3 (i.e., 1-3 days pre-tx) in NHP/MPXV study SR10-037F according to survival outcome. *Wilcoxon Rank-Sum test.

Analyses of viral DNA levels in blood samples collected immediately post-challenge indicated comparable viral challenge delivery (median 6.2 log_{10} copies/mL) across different treatment groups, and

with no differences between animals that ultimately survived or died from the infection (FDA analyses, not shown). Not surprisingly, and consistent with the results from study AP-09-026G, viral DNA levels in tissues collected at necropsy were higher in animals that died or were euthanized due to moribund condition, relative to animals that survived to the scheduled termination of the study (sponsor's analyses, see study report pg. 68-69).

Serum samples collected at various timepoints throughout the study were analyzed for anti-orthopoxvirus antibody using binding (i.e., ELISA) and neutralization (i.e., PRNT) assays; detailed methods were not provided. Results from the PRNT assays are summarized in Table 10; study report pg. 73. Anti-orthopoxvirus antibodies were generally detected by Post-Challenge Day 9, with titers increasing at later times Post-Challenge. All animals that survived to the end of study developed high titers of binding and neutralizing antibody.

Table 10. Sponsor's analyses of anti-poxvirus neutralizing antibody (PRNT) in NHP/MPXV study SR10-037F. Data were not provided in an independently analyzable format.

Monkey Number	Study Day														
	-7	3	6	7	8	9	10	11	12	15	28	36	42	48	56
Group 1 (Placebo)															
4762	<10	<10	<10	<10								ND[a]			
4763	<10	<10	<10									ND[b]			
4765	<10	<10	480	ND	ND	1792	1707					ND[c]			
Group 2 (Day 4 Treatment Onset)															
4756	<10	<10	<10	ND	ND	60	ND	ND	931	4960	>10,240	>10,240	9387	9076	9992
4757	<10	<10	264	ND	ND	280	ND	ND	4754	4754	5120	>10,240	8121	6489	5258
4760	<10	<10	200	ND	ND	640	ND	ND	234	580	2432	3328	7360	4096	4923
4767	<10	<10	116	ND	ND	1231	ND	ND	1280	>10,240	10,240	>10,240	5303	6051	5120
4768	<10	<10	<10	ND	ND	<10	ND	ND	<10			ND[d]			
4769	<10	<10	91	ND	ND	640	ND	ND	>10,240	>10,240	>10,240	>10,240	>10,240	>10,240	>10,240
Group 3 (Day 5 Treatment Onset)															
4758	<10	<10	553	ND	ND	2987	ND	ND	1792	4655	7040	>10,240	4230	2464	2347
4759	<10	<10	140	ND	ND	2211	ND	ND	>10,240	>10,240	>10,240	>10,240	>10,240	>10,240	>10,240
4761	<10	<10	<10	ND	ND	23	ND	ND	1920	>10,240	>10,240	>10,240	>10,240	>10,240	>10,240
4766	<10	<10	40	ND	ND	280	ND	ND	2368	>10,240	>10,240	>10,240	7078	5899	6949
4771	<10	<10	30	ND	ND	544	ND	ND	1493	>10,240	>10,240	>10,240	8320	8960	6144
4773	<10	<10	<10	ND	<10							ND[e]			
Group 4 (Day 6 Treatment Onset)															
4754	<10	<10	160	ND	ND	815	ND	ND	5632	6144	>10,240	>10,240	>10,240	8448	7360
4755	<10	<10	64	ND	ND	80	ND	ND	>10,240	>10,240	10,240	>10,240	>10,240	>10,240	>10,240
4764	<10	<10	306	ND	ND	261						ND[d]			
4770	15	<10	64	ND	ND	160	ND	<10				ND[f]			
4772	<10	<10	40	ND	160							ND[e]			
4774	<10	<10	60	ND	ND	108	ND	ND	3520	>10,240	>10,240	>10,240	4923	6400	5514

[a]Euthanized on Day 7
[b]Found dead on Day 7
[c]Euthanized on Day 10
[d]Found dead on Day 12
[e]Euthanized on Day 8
[f]Euthanized on Day 11
KEY: Limit of quantification = 10; ND = Not determined

3.4 Study SR10-038F (NHPs/Monkeypox Virus)

Title
SR10-038F, "Double-Blind, Placebo-Controlled Study to Evaluate Effect of Duration of ST-246 Treatment on Efficacy Following Lethal Monkeypox Virus Challenge in Cynomolgus Macaques"

Summary of Design
Study SR10-038F was a double-blind, randomized, placebo-controlled study of oral tecovirimat in cynomolgus monkeys infected with MPXV, conducted at (b) (4). The primary purpose of this study was to determine the effect of varying the duration of treatment on the efficacy of tecovirimat in MPXV-infected cynomolgus monkeys.

A total of 25 cynomolgus monkeys that were negative for pre-existing antibodies against poxviruses were randomly assigned to five groups to receive either placebo or 3, 5, 7 or 10 days of tecovirimat starting on Post-Challenge Day 4 (Table 11; adapted from study report pg. 29). All monkeys were challenged on Day 0 with 5.0 x 10^7 PFU of the MPXV Zaire strain (NR-2324 stock).

Table 11. Treatment groups in study SR10-038F.

Group	# Animals		MPXV Challenge (Day 0)	ST-246 Dose Level	Dosing Schedule (designed to maintain blind)	
	M	F			Tecovirimat	Placebo
Placebo	2	2	5 x 10^7 PFU IV	0 mg/kg	N/A	Days 4-13
Tecovirimat 3-Dose	2	2	5 x 10^7 PFU IV	10 mg/kg	Days 4-6	Days 7-13
Tecovirimat 5-Dose	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-8	Days 9-13
Tecovirimat 7-Dose	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-10	Days 11-13
Tecovirimat 10-Dose	2	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-13	N/A

Body weights, clinical observations, lesion counts and clinical laboratory analyses were conducted throughout the post-challenge period. The primary efficacy endpoint was the proportion of animals that survived after MPXV challenge for up to 28 days. Secondary and other endpoints included analyses of blood viral DNA levels over time, lesion counts, body weights, and other clinical and laboratory observations. All surviving animals were euthanized on Post-Challenge Day 28. Necropsies were not performed and no tissues were preserved. Limited genotypic (i.e. NGS) resistance data were obtained from two animals; results are summarized in Section 3.8.

Efficacy Results (Survival and Clinical Disease)

The sponsor's analyses of survival through Post-Challenge Day 28 are summarized in Figure 22 (study report pg. 46) and Table 12 (study report pg. 45). Survival rates were 25% (1/4) in the Placebo group and 86% (18/21) in the pooled Tecovirimat treatment groups; all 6 premature deaths were by euthanasia due to moribund disease. There was a trend of reduced efficacy in the Tecovirimat 3-Dose group relative to other Tecovirimat groups, although sizes of the treatment groups for comparison were small.

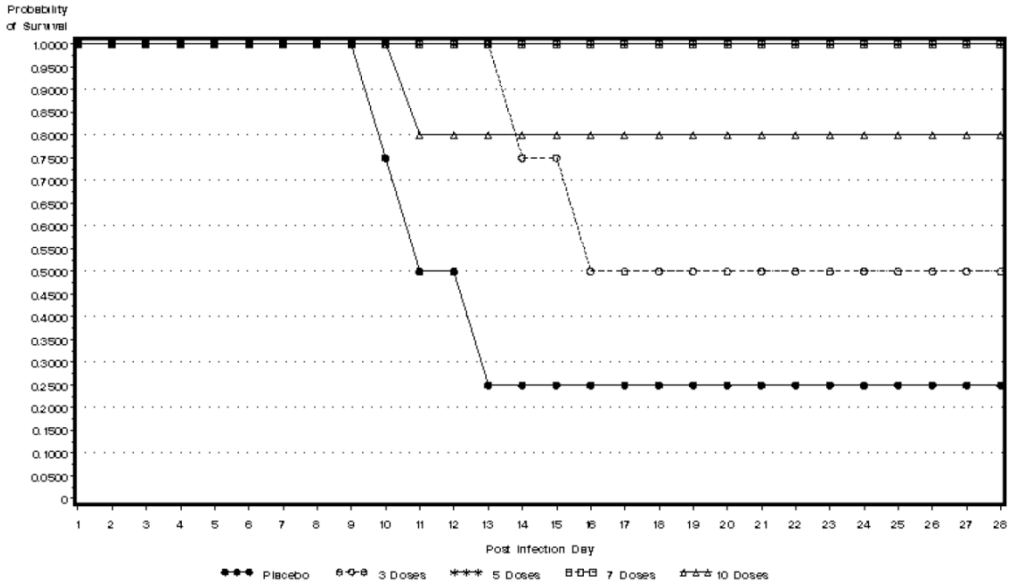


Figure 22. Kaplan-Meier survival curve through Day 28 in NHP/MPXV study SR10-038F.

Table 12. Summary of survival results through Day 28 in NHP/MPXV study SR10-038F.

Group	Number of Animals		Statistics[a]
	Study Start	Study End	
Placebo	4	1 (25%)	--
All ST-246 Treated[b]	21	18 (86%)	0.0312[c]
ST-246, 3 doses	4	2 (50%)	1.0000[d]
ST-246, 5 doses	6	6 (100%)	0.0333[d]
ST-246, 7 doses	6	6 (100%)	0.0333[d]
ST-246, 10 doses	5	4 (80%)	0.2063

[a]p-value obtained using Fisher's Exact Test (2-sided) vs. Placebo
[b]3-, 5-, 7-, and 10-Dose groups combined
[c]Significant at p = 0.05
[d]p-values are nominal; sequential comparisons of ST-246 regimens less than 10 doses in duration could not be rigorously evaluated in the closed-test procedure since p-value in the 10-Dose group was not statistically significant

Pox lesion counts were variable and not statistically different between treatment groups, but trended higher in the Placebo and Tecovirimat 3-Dose groups relative to the Tecovirimat 5-Dose, 7-Dose and 10-Dose treatment groups (Figure 23; study report pg. 52). The sponsor noted that among surviving animals, there was also a tendency for observed clinical signs to be generally less frequent and of lower severity with increasing length of tecovirimat treatment.

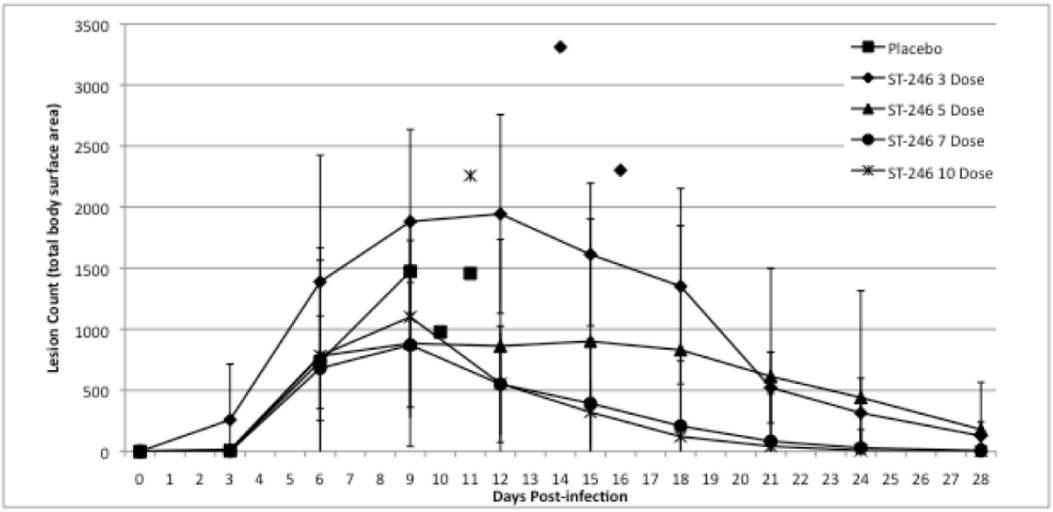


Figure 23. Sponsor's analyses of pox lesion counts over time in NHP/MPXV study SR10-038F. Symbols connected by lines indicate the mean (+/- standard deviation) of each group at each scheduled interval. Stand-alone symbols not connected by lines indicate individual animal lesion counts performed off-schedule at the time of euthanasia.

Virology Results

Whole blood MPXV DNA levels over time for individual animals are shown in Figure 24 (FDA analysis). Group mean and median DNA levels are shown in Figure 25 (FDA analysis), and median and peak DNA levels are summarized in Table 13 (FDA analysis). In general, animal groups that received a greater number of tecovirimat doses tended to have lower viral DNA levels over the course of infection, with the lowest viral DNA levels observed in the Tecovirimat 7-Dose and 10-Dose groups.

Interestingly, viral DNA levels in the Tecovirimat 5-Dose group rebounded quickly after the last tecovirimat dose on Post-Challenge Day 8, and dropped again off-treatment starting around Post-

Challenge Day 15, and all 6 animals survived. These results indicate that even a transient decline in viral replication at the time when viremia and disease typically peak in untreated animals may provide a survival benefit.

Also of note, viral DNA levels in the Tecovirimat 3-Dose group were comparable to those in the Placebo group throughout the disease course. Relatively high viral DNA levels might be predicted for this group for timepoints beyond Post-Challenge Day 6 (i.e., after treatment cessation); however, viral DNA levels in the Tecovirimat 3-Dose group were also curiously higher relative to other Tecovirimat groups at Post-Challenge Day 6, a time when animals in all Tecovirimat groups would have received the same course of Tecovirimat treatment. Consistent with this observation, lesion counts tended to be greater throughout the disease course in the Tecovirimat 3-Dose relative to other study groups, including the Placebo group (Figure 23).

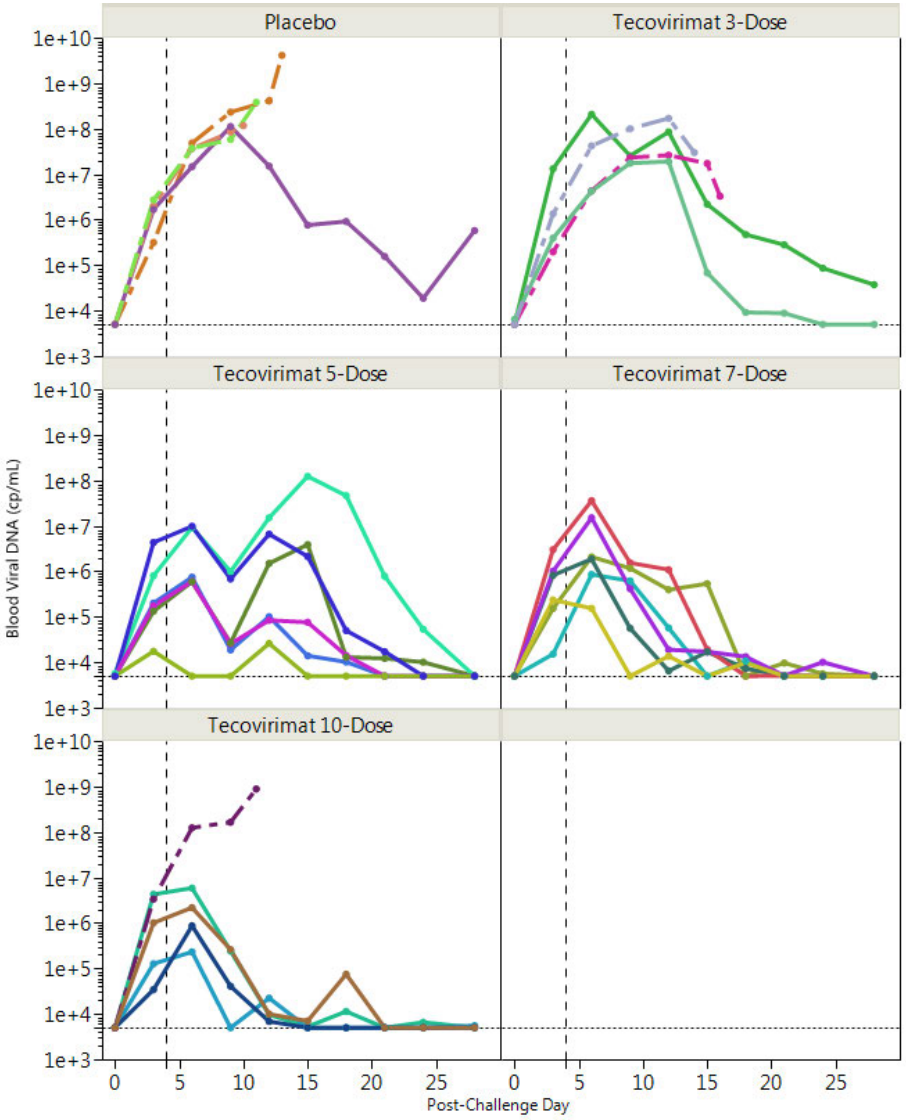


Figure 24. Viral DNA levels in whole blood for individual animals in NHP/MPXV study SR10-038F. Animals that did not survive through the end of the observation period are indicated by dashed lines. The assay LLOQ was 5,000 copies/mL (horizontal reference line), and all animals started tecovirimat or placebo QD on Post-Challenge Day 4 (vertical reference line) for the indicated number of doses/days. Day 0 viral DNA levels quantifying viral challenge delivery are not shown.

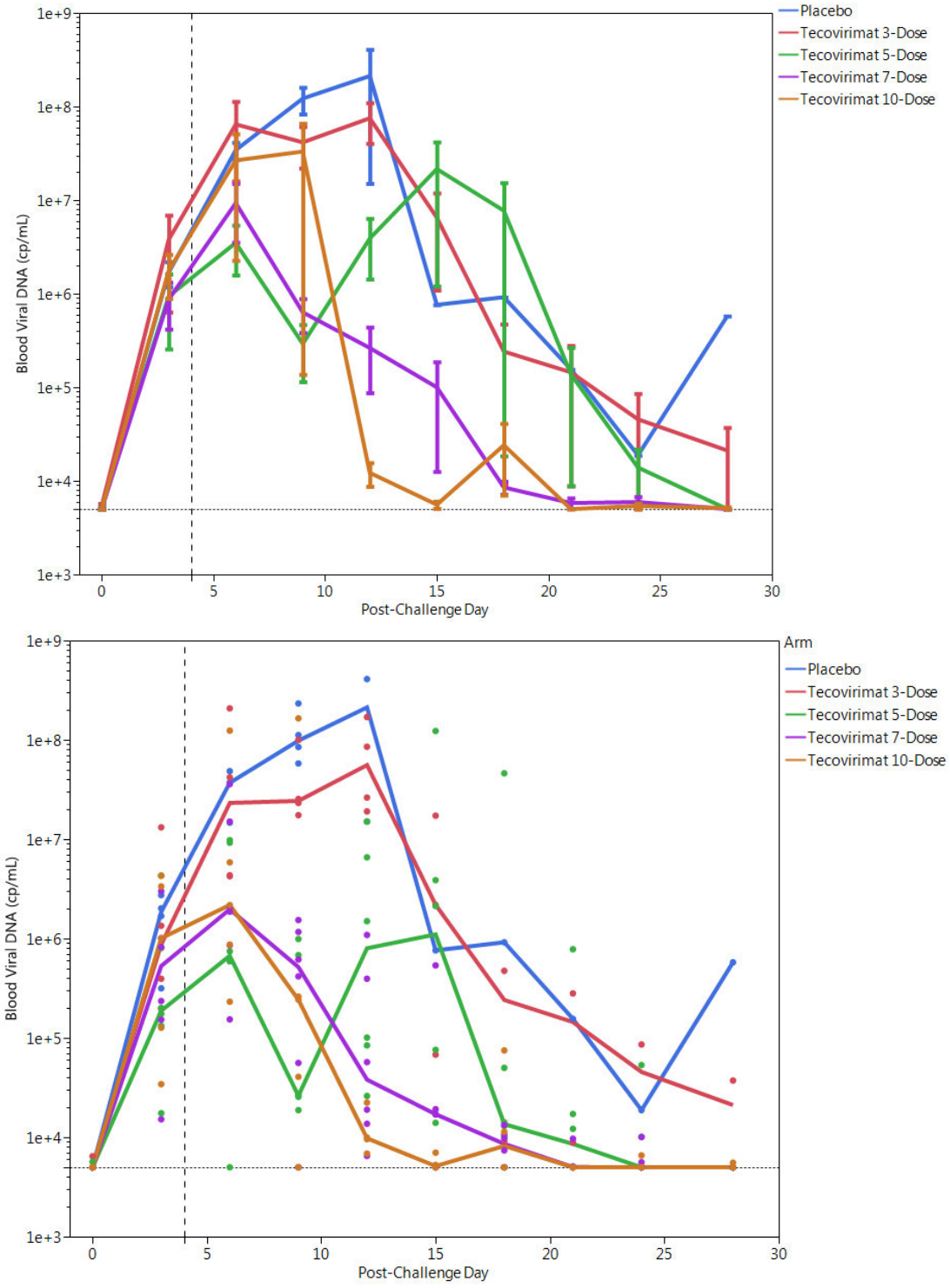


Figure 25. Mean +/- standard error (top) and median w/individual (bottom) viral DNA levels in whole blood in NHP/MPXV study SR10-038F. The graphs censor data for Post-Challenge Days 10 (n=1), 11 (n=2), 13 (n=1), 14 (n=1) and 16 (n=1) due to data being reported for only a small subset of animals. Day 0 viral DNA levels quantifying viral challenge delivery also are not shown. The assay LLOQ was 5,000 copies/mL (horizontal reference line), and all animals started tecovirimat or placebo on Post-Challenge Day 4 (vertical reference line) for the indicated number of doses/days.

Table 13. Summary of median (range) viral DNA levels in whole blood in study SR10-038F. The assay LLOQ was 3.7 log_{10} copies/mL. Differences from placebo ≥1 log_{10} copies/mL are highlighted.

	MPXV DNA Levels (Log_{10} copies/mL)												
	Placebo		Tecovirimat 3-Dose			Tecovirimat 5-Dose			Tecovirimat 7-Dose			Tecovirimat 10-Dose		
Day Post-Challenge	N	Median (Range)	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO
3	4	6.3 (5.5-6.4)	4	5.9 (5.3-7.1)	-0.4	6	5.3 (4.2-6.6)	-1.0	6	5.6 (4.2-6.5)	-0.6	5	6.0 (4.5-6.6)	-0.3
6	4	7.6 (7.2-7.7)	4	7.1 (6.6-8.3)	-0.4	6	5.8 (3.7-7.0)	-1.7	6	6.3 (5.2-7.6)	-1.3	5	6.3 (5.4-8.1)	-1.2
9	4	8.0 (7.8-8.4)	4	7.4 (7.2-8.0)	-0.6	6	4.4 (3.7-6.0)	-3.6	6	5.7 (3.7-6.2)	-2.3	5	5.4 (3.7-8.2)	-2.6
12	2	7.9 (7.2-8.6)	4	7.7 (7.3-8.2)	-0.2	6	5.6 (4.4-7.2)	-2.3	6	4.5 (3.8-6.0)	-3.4	4	4.0 (3.8-4.4)	-3.9
15	1	5.9 (n/a)	3	6.3 (4.8-7.2)	0.5	6	5.6 (3.7-8.1)	-0.3	6	4.2 (3.7-5.7)	-1.6	4	3.7 (3.7-3.8)	-2.2
18	1	6.0 (n/a)	2	4.8 (4.0-5.7)	-1.1	6	4.1 (3.7-7.7)	-1.8	6	3.9 (3.7-4.1)	-2.0	4	3.9 (3.7-4.9)	-2.1
21	1	5.2 (n/a)	2	4.7 (3.9-5.4)	-0.5	6	3.9 (3.7-5.9)	-1.3	6	3.7 (3.7-4.0)	-1.5	4	3.7 (3.7-3.7)	-1.5
24	1	4.3 (n/a)	2	4.3 (3.7-4.9)	0.0	6	3.7 (3.7-4.7)	-0.6	6	3.7 (3.7-4.0)	-0.6	4	3.7 (3.7-3.8)	-0.6
28	1	5.8 (n/a)	2	4.1 (3.7-4.6)	-1.6	6	3.7 (3.7-3.7)	-2.1	6	3.7 (3.7-3.7)	-2.1	4	3.7 (3.7-3.7)	-2.1
Peak Viral DNA	4	8.3 (8.0-9.6)	4	7.8 (7.3-8.3)	-0.5	6	6.5 (5.8-8.1)	-1.8	6	6.4 (6.2-7.6)	-2.0	5	6.5 (6.2-8.9)	-1.9

Viral DNA levels at selected timepoints were further analyzed to compare the kinetics of viremia at early timepoints prior to and during treatment across different treatment groups. As shown in Figure 26 (FDA analysis), viral DNA levels in samples collected at the time of virus challenge indicate that a comparable amount of virus was delivered to all animals, but on Treatment Day 3 viral DNA levels were elevated in the Tecovirimat 3-Dose group relative to other tecovirimat-treated animals. Although it is not possible to draw firm conclusions from these data due to the small sample sizes, a more intense initial infection among animals in the Tecovirimat 3-Dose group may have inflated the differences in clinical and virologic outcomes between this group and the other Tecovirimat treatment groups.

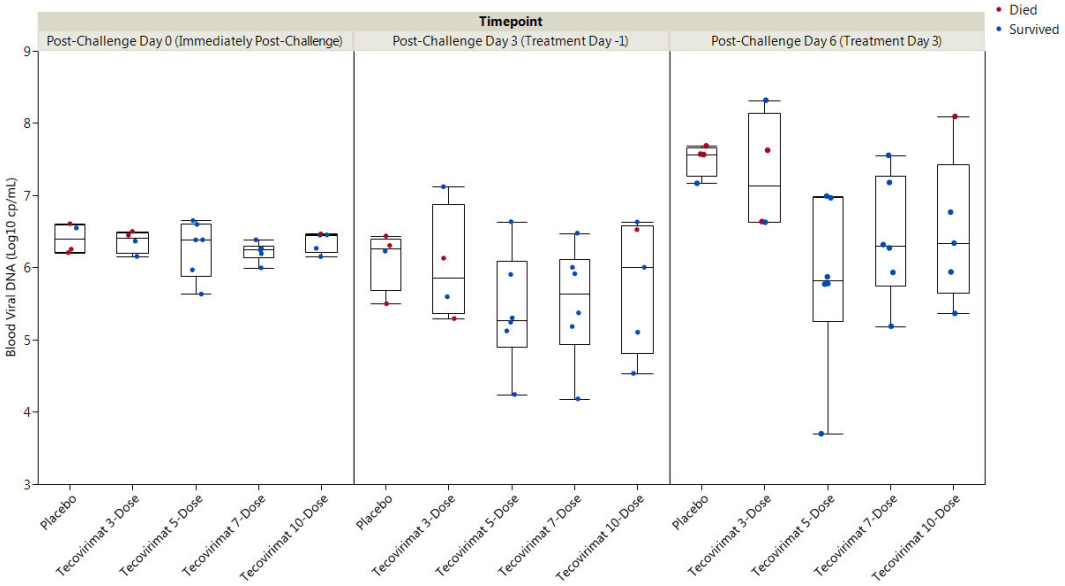


Figure 26. Whole blood viral DNA levels at early timepoints in NHP/MPXV study SR10-038F.

As in study SR10-037F, serum samples collected at various timepoints throughout the study were analyzed for anti-orthopoxvirus antibody using binding (i.e., IgG ELISA) and neutralization (i.e., PRNT)

assays; detailed methods were not provided. Results from the PRNT assays are summarized in Table 14; study report pg. 68. Anti-orthopoxvirus antibodies were generally detected by Post-Challenge Day 6, with titers increasing at later times Post-Challenge. All animals that survived to the end of study developed high titers of binding and neutralizing antibody.

Table 14. Anti-poxvirus neutralizing antibody (PRNT) in NHP/MPXV study SR10-038F.

Monkey Number	Study Day								
	-7	6	10	11	12	13	14	16	28
Group 1 (Placebo)									
4803	<10	124	ND	ND	240	297	ND[a]		
4814	<10	107	ND	ND	3520	ND	ND	ND	>10240
4815	<10	67	120	ND[b]					
4821	<10	366	ND	267	ND[c]				
Group 2 (3 Doses)									
4801	<10	100	ND	ND	291	ND	ND	ND	>10240
4808	<10	640	ND	ND	2560	ND	ND	6949	ND[d]
4812	<10	1431	ND	ND	2080	ND	ND	ND	6400
4818	<10	304	ND	ND	1331	ND	4608	ND[e]	
Group 3 (5 Doses)									
4799	<10	914	ND	ND	1024	ND	ND	ND	10240
4805	<10	345	ND	ND	3584	ND	ND	ND	8704
4809	<10	1040	ND	ND	1092	ND	ND	ND	2240
4816	<10	49	ND	ND	2560	ND	ND	ND	>10240
4817	<10	80	ND	ND	>10240	ND	ND	ND	>10240
4823	<10	70	ND	ND	610	ND	ND	ND	6400
Group 4 (7 Doses)									
4800	<10	105	ND	ND	520	ND	ND	ND	5632
4802	<10	416	ND	ND	4096	ND	ND	ND	>10240
4806	<10	123	ND	ND	160	ND	ND	ND	>10240
4807	<10	560	ND	ND	3200	ND	ND	ND	>10240
4811	<10	640	ND	ND	3328	ND	ND	ND	6400
4822	<10	640	ND	ND	2080	ND	ND	ND	4096
Group 5 (10 Doses)									
4804	<10	103	ND	ND	>10240	ND	ND	ND	>10240
4810	<10	100	ND	ND	640	ND	ND	ND	2560
4813	<10	553	ND	ND	1600	ND	ND	ND	2240
4819	<10	149	ND	40	ND[c]				
4820	<10	560	ND	ND	2816	ND	ND	ND	>10240

[a]Euthanized on Day 13
[b]Euthanized on Day 10
[c]Euthanized on Day 11
[d]Euthanized on Day 16
[e]Euthanized on Day 14
KEY: Limit of quantification = 10; ND Not determined

3.5 Study FY10-087 (NHPs/Monkeypox Virus)

Title
FY10-087, "Evaluation of the Pharmacokinetics of ST-246 in Cynomolgus Macaques Infected Intravenously with Monkeypox Virus"

Summary of Design
Study FY10-087 was designed to evaluate the pharmacokinetics (PK) of tecovirimat in cynomolgus macaques infected with MPXV, conducted at (b) (4) Twenty-four NHPs were randomized into 4 different treatment groups (Table 15; adapted from study report pg. 17). On Day -10 relative to challenge, animals were dosed with tecovirimat for PK analysis. Animals were then challenged IV with a target dose of 5 x 10^7 PFU of the MPXV Zaire '79 strain (NR-2324 stock). Starting on Post-Challenge Day 4, tecovirimat treatment by orogastric gavage was initiated and continued for 14 days. Animal weights, clinical observations, viral load and lesion progression were monitored throughout the study.

Table 15. Study FY10-087 study group assignments and tecovirimat dose levels.

Treatment Group (starting Day 4 post-challenge)	Number of Animals	MPXV Target Challenge
1-Vehicle Control	6 (3 male, 3 female)	5 x 10^7 PFU IV
2-Tecovirimat 3 mg/kg	6 (3 male, 3 female)	5 x 10^7 PFU IV
3-Tecovirimat 10 mg/kg	6 (3 male, 3 female)	5 x 10^7 PFU IV
4-Tecovirimat 20 mg/kg	6 (3 male, 3 female)	5 x 10^7 PFU IV

Because the primary objective of the study was to evaluate tecovirimat PK in MPXV-infected NHPs, only limited independent FDA virology analyses were conducted. The purpose of these analyses was to confirm active MPXV infection and tecovirimat anti-MPXV activity.

Summary of Efficacy and Virology Results

According to the sponsor, all 6 animals in the Vehicle Control group were euthanized due to moribund condition between Post-Challenge Days 12 and 16. All tecovirimat-treated animals survived to the planned end of study on Post-Challenge Day 28.

Whole blood MPXV DNA levels for individual animals are shown in Figure 27 (FDA analysis). Group median DNA levels are shown in Figure 28 (FDA analysis). All animals had evidence of infection as demonstrated by increasing blood viral DNA levels during the initial days post-challenge. Consistent with study AP-09-026, tecovirimat anti-MPXV activity was evident in all Tecovirimat Groups (≥3 mg/kg/day). There was little evidence of a tecovirimat dose-response relationship with viral DNA dynamics, with the possible exception of a slightly shallower decline in viral DNA levels in the 3 mg/kg group.

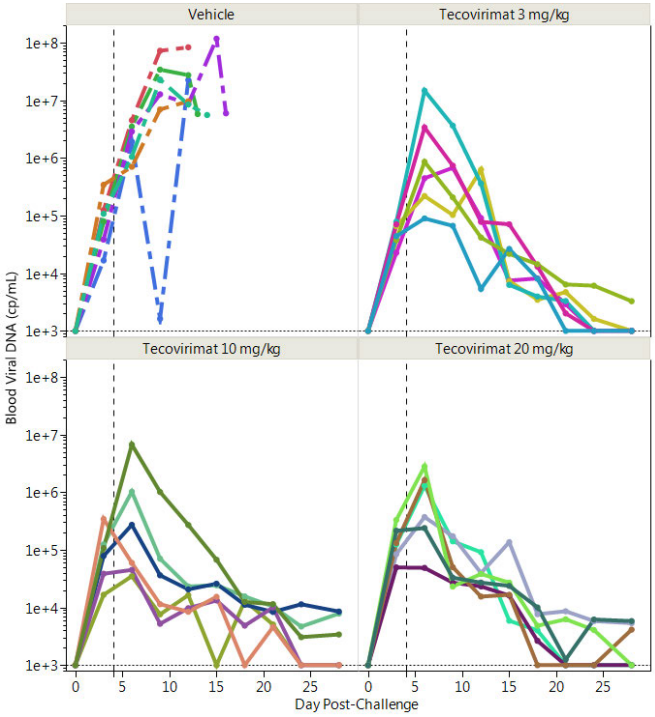


Figure 27. Viral DNA levels in whole blood for individual animals in NHP/MPXV study FY10-087. Animals that did not survive through the end of the observation period are indicated by dashed lines. The assay LLOQ was 1,000 copies/mL (horizontal reference line), and all animals were dosed with tecovirimat or vehicle starting on Post-Challenge Day 4 (vertical reference line). Day 0 viral DNA levels quantifying viral challenge delivery are not shown. The sponsor excluded the Day 9 low viral DNA outlier in the Placebo group from their analyses but could not determine a reason for the result.

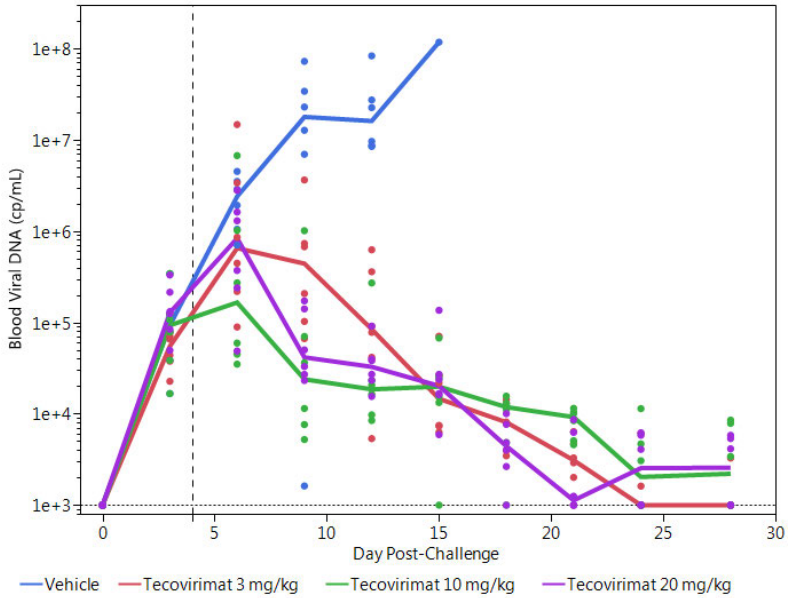


Figure 28. Median w/individual viral DNA levels in whole blood in NHP/MPXV study FY10-087. The assay LLOQ was 1,000 copies/mL (horizontal reference line), and all animals were dosed with tecovirimat or placebo starting on Post-Challenge Day 4 (vertical reference line). The graphs censor data for Post-Challenge Days 13 (n=1), 14 (n=1), and 16 (n=1) due to data being reported for only individual animals. Day 0 viral DNA levels quantifying viral challenge delivery also are not shown.

3.6 Study SR14-008F (Rabbits/Rabbitpox Virus)

Title
SR14-008F, "Evaluation of the Dose-Response Relationship between Tecovirimat Plasma Exposure and Therapeutic Efficacy in NZW Rabbits Intradermally-Infected with a Lethal Dose of Rabbitpox Virus"

Summary of Design
Study SR14-008F was a blinded, placebo-controlled, GLP study designed to evaluate various doses of tecovirimat for efficacy against lethal intradermal RPXV challenge, conducted at (b) (4) Fifty (50, 25 male/25 female) NZW rabbits, ~16-weeks old at time of challenge (2.0-2.8 kg), were randomized into 5 groups of 10 animals per group (5 male, 5 female) based on gender and body weight. All animals were challenged intra-dermally in both thighs with the clonal RPXV-UTR stock (Lot #090314) at a target dose of 1,000 PFU (1,160 actual PFU).

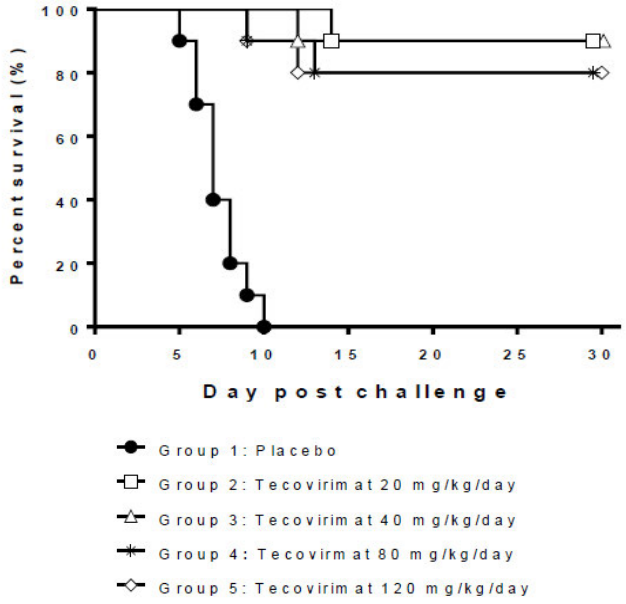
On Day 4 post-challenge (a day by which all RPXV-infected animals are historically expected to exhibit fever and viral DNA in the blood as a therapeutic trigger), all animals started once daily dosing of tecovirimat or placebo by oral gavage, and dosing continued for 14 days. All animals were confirmed as having fever on Day 4 post-challenge. Tecovirimat dosing level was based on the previous day's animal body weight. The dosing groups were as follows:

- Group 1: Placebo
- Group 2: Tecovirimat 20 mg/kg/day
- Group 3: Tecovirimat 40 mg/kg/day
- Group 4: Tecovirimat 80 mg/kg/day
- Group 5: Tecovirimat 120 mg/kg/day

Following challenge and for the duration of the study, animals were monitored for clinical signs of disease, including temperature and weight changes and appearance of secondary lesions, and survival for up to 30 days. Animals that displayed disease symptoms satisfying prospectively defined euthanasia criteria were euthanized. Viral DNA levels were measured in whole blood samples collected throughout the study, as well as lung and spleen tissue samples collected at necropsy. In addition, plaque assays were conducted for terminal blood, lung, and spleen samples. Resistance analyses were also to be conducted, but no genotypic resistance data and only very limited phenotypic resistance data were obtained (see Section 3.8).

Efficacy Results (Survival)

Tecovirimat treatment at any of the evaluated doses (20-120 mg/kg/day) was associated with a survival advantage (Figure 29; Report SR14-008F pgs. 35-37). Nine of 10 rabbits in Group 1 that received placebo were euthanized or found dead between Day 6 and Day 10 post-challenge. Overall, of the 16 animals that died prematurely, 6 (37.5%) were found dead and 10 (62.5%) were euthanized due to moribund disease. One animal (990) died on Day 5 from possible complications from blood collection procedures. In contrast, 80-90% of animals in the tecovirimat dosing groups survived until the end of the observation period, with no apparent tecovirimat dose-response relationship. The cause of death for one animal in Group 4 (989, tecovirimat 80 mg/kg/day) was inconclusive; according to the sponsor, the animal appeared healthy and did not have a high cumulative disease score, but was found dead on Day 13.



Group	Treatment	Survival Rate
1	Placebo	0% (0/10)
2	Tecovirimat 20 mg/kg/day	90% (9/10)
3	Tecovirimat 40 mg/kg/day	90% (9/10)
4	Tecovirimat 80 mg/kg/day	80% (8/10)
5	Tecovirimat 120 mg/kg/day	80% (8/10)

Figure 29. Animal survival rates in rabbit/RPXV study SR14-008F.

Virology Results

Whole blood RPXV DNA levels over time for individual animals are shown in Figure 30 (FDA analysis). Group mean and median DNA levels are shown in Figure 31 (FDA analysis), and median and peak DNA levels are summarized in Table 16 (FDA analysis).

Most animals had a rapid spike in RPXV DNA levels in blood during the first 2-6 days post-challenge, with peak viral DNA levels occurring around Post-Challenge Day 6. Tecovirimat-treated animals had an approximately 2-$\log_{10}$ copies/mL lower viral DNA level during the time of peak viremia. Consistent with the sponsor analyses of clinical efficacy, there was not a clear relationship between tecovirimat dose and whole blood viral DNA levels over time.

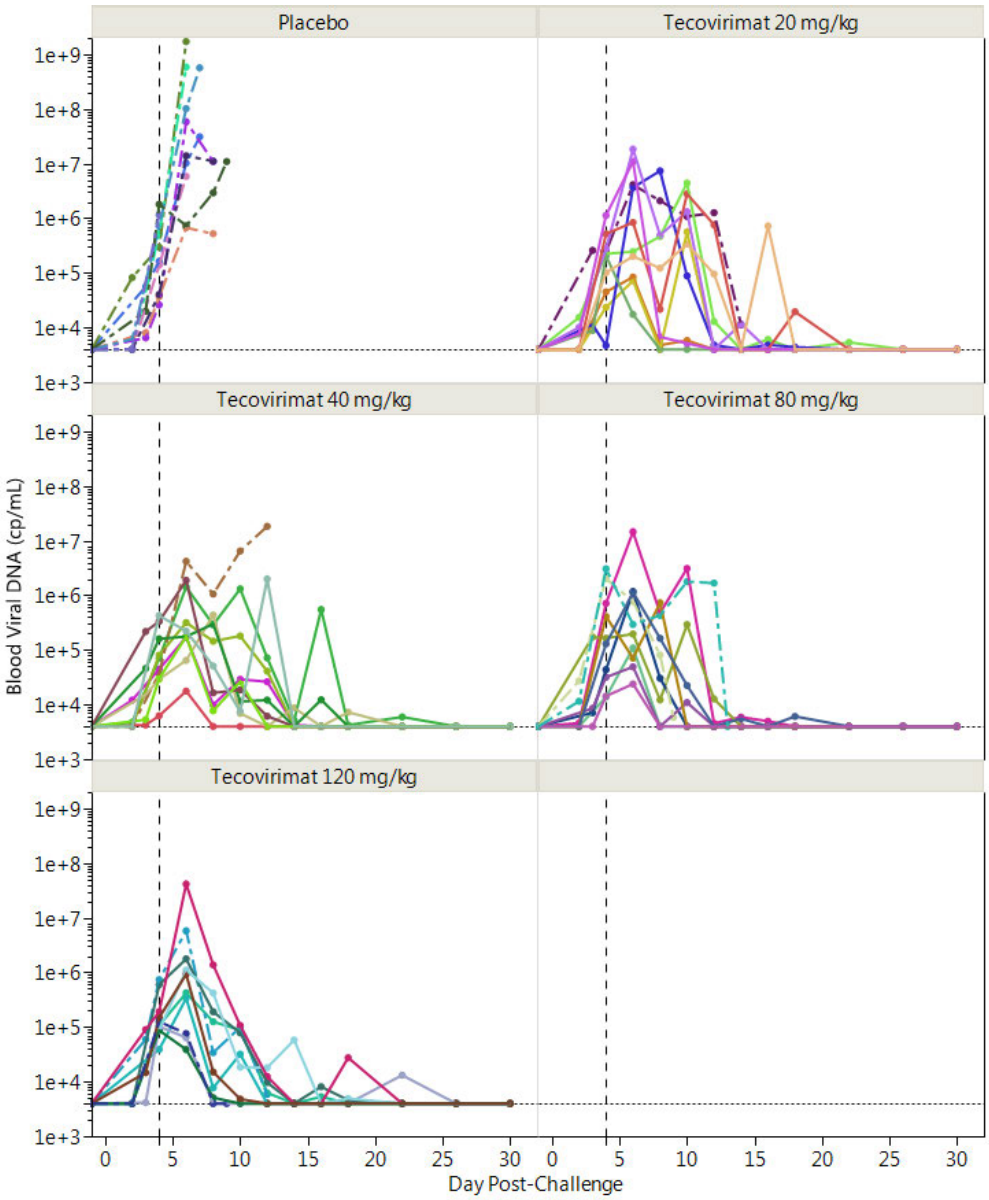


Figure 30. Viral DNA levels in whole blood for individual animals in rabbit/RPXV study SR14-008F. Animals that did not survive through the end of the observation period are indicated by dashed lines. The assay LLOQ was 4,000 copies/mL (horizontal reference line), and all animals were dosed with tecovirimat or placebo starting on Post-Challenge Day 4 (vertical reference line).

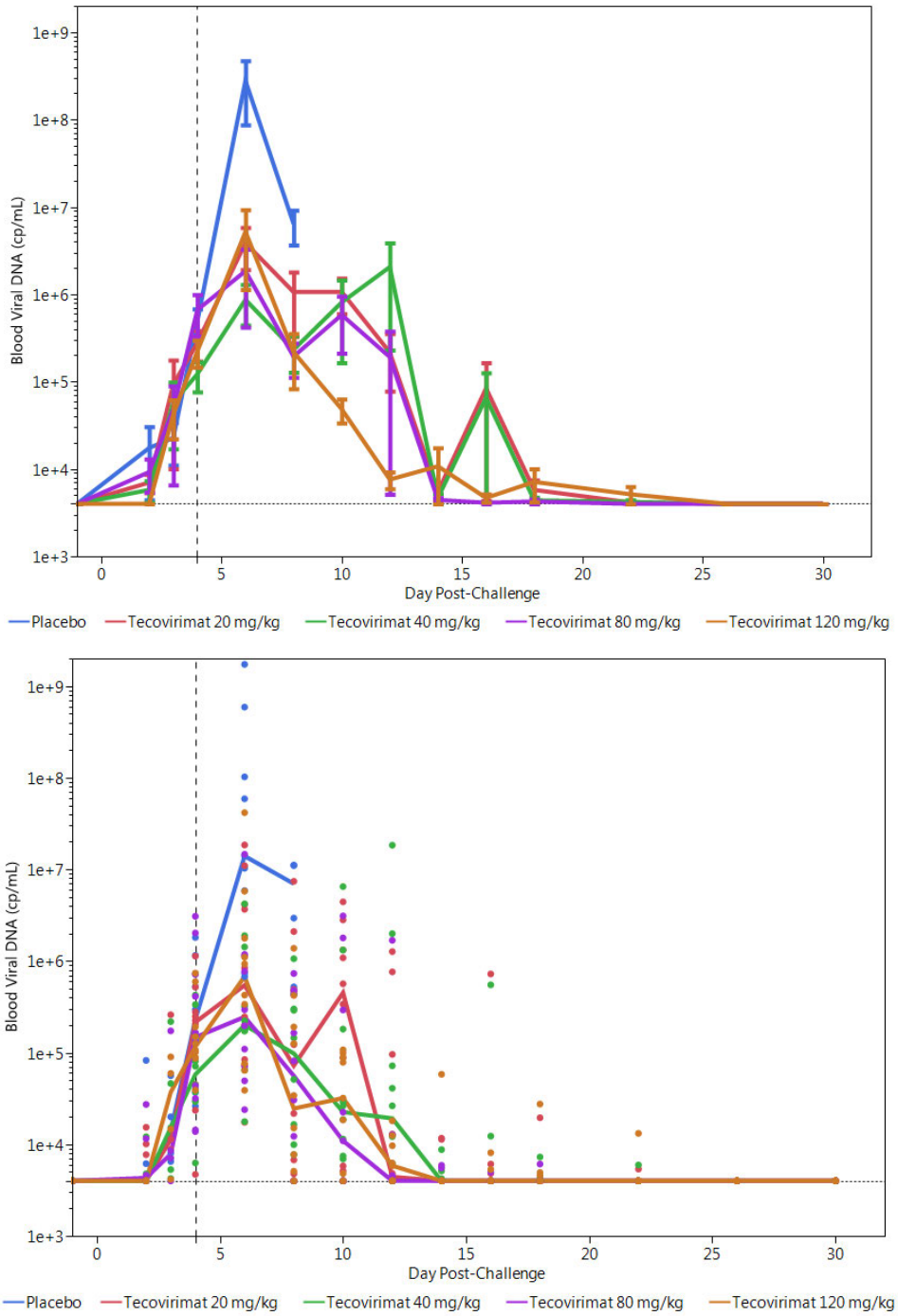


Figure 31. Mean +/- standard error (top) and median w/individual (bottom) viral DNA levels in whole blood in in rabbit/RPXV study SR14-008F. The graphs censor data for Post-Challenge Days 7 (n=2), 9 (n=3) and 13 (n=1) due to data being reported for only a small number of animals. The assay LLOQ was 4,000 copies/mL (horizontal dashed line), and all animals were dosed starting on Post-Challenge Day 4 (vertical dashed line).

Table 16. Summary of RPXV DNA levels (log_{10} copies/mL) in whole blood over time in rabbit/RPXV study SR14-008F. The assay LLOQ was 3.6 log_{10} copies/mL. Differences from placebo ≥1 log_{10} copies/mL are highlighted.

	RPXV DNA Levels (Log_{10} copies/mL)													
	Placebo		Tecovirimat 20 mg/kg			Tecovirimat 40 mg/kg			Tecovirimat 80 mg/kg			Tecovirimat 120 mg/kg		
Day Post-Challenge	N	Median (Range)	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO	N	Median (Range)	Δ Rel. PBO
2	6	3.6 (3.6-4.9)	7	3.6 (3.6-4.2)	0	5	3.6 (3.6-4.1)	0	6	3.6 (3.6-4.4)	0	5	3.6 (3.6-3.6)	0
3	4	4.1 (3.8-4.8)	3	4.1 (3.9-5.4)	0	5	4.2 (3.6-5.3)	0.1	4	3.9 (3.6-5.2)	-0.2	4	4.5 (3.6-5.0)	0.4
4	10	5.3 (4.4-6.3)	10	5.3 (3.7-6.1)	0	10	4.8 (3.8-5.6)	-0.5	10	5.2 (4.1-6.5)	-0.1	10	5.1 (4.6-5.9)	-0.2
6	9	7.1 (5.8-9.2)	10	5.7 (4.2-7.3)	-1.4	10	5.3 (4.2-6.6)	-1.8	10	5.4 (4.4-7.2)	-1.7	10	5.8 (4.6-7.6)	-1.3
8	4	6.8 (5.7-7.0)	10	4.7 (3.6-6.9)	-2.1	10	4.9 (3.6-6.0)	-1.9	10	4.7 (3.6-5.9)	-2.1	10	4.4 (3.6-6.1)	-2.4
10	0	n/a	10	5.6 (3.6-6.6)	n/a	10	4.3 (3.6-6.8)	n/a	9	4.0 (3.6-6.5)	n/a	9	4.5 (3.6-5.0)	n/a
12	0	n/a	10	3.6 (3.6-6.1)	n/a	10	4.3 (3.6-7.3)	n/a	9	3.6 (3.6-6.2)	n/a	9	3.8 (3.6-4.3)	n/a
Peak Viral DNA	10	7.3 (5.8-9.2)	10	6.5 (4.9-7.3)	-0.8	10	5.6 (4.2-7.3)	-1.7	10	6.0 (4.4-7.2)	-1.3	10	5.8 (4.9-7.6)	-1.5

As further shown in Figure 32A (FDA analysis), viral DNA levels in blood collected on Day 4, just prior to initiation of tecovirimat treatment, were similar between rabbits that received tecovirimat or placebo (p=0.28; Wilcoxon Rank-Sum test). These results indicate that both groups had a comparable viral burden at the time of initiation of treatment.

Among tecovirimat-treated rabbits, Day 4 viral DNA levels trended lower in those that survived infection relative to those that died or were euthanized due to their disease (Figure 32B; FDA analysis) (p=0.051, p=0.15 if excluding animal 989 that died of unknown cause; Wilcoxon Rank-Sum test). Thus, consistent with studies in the NHP/MPXV model and with a mechanism targeting viral spread, tecovirimat may be more effective when treatment is initiated in the presence of a relatively lower viral burden.

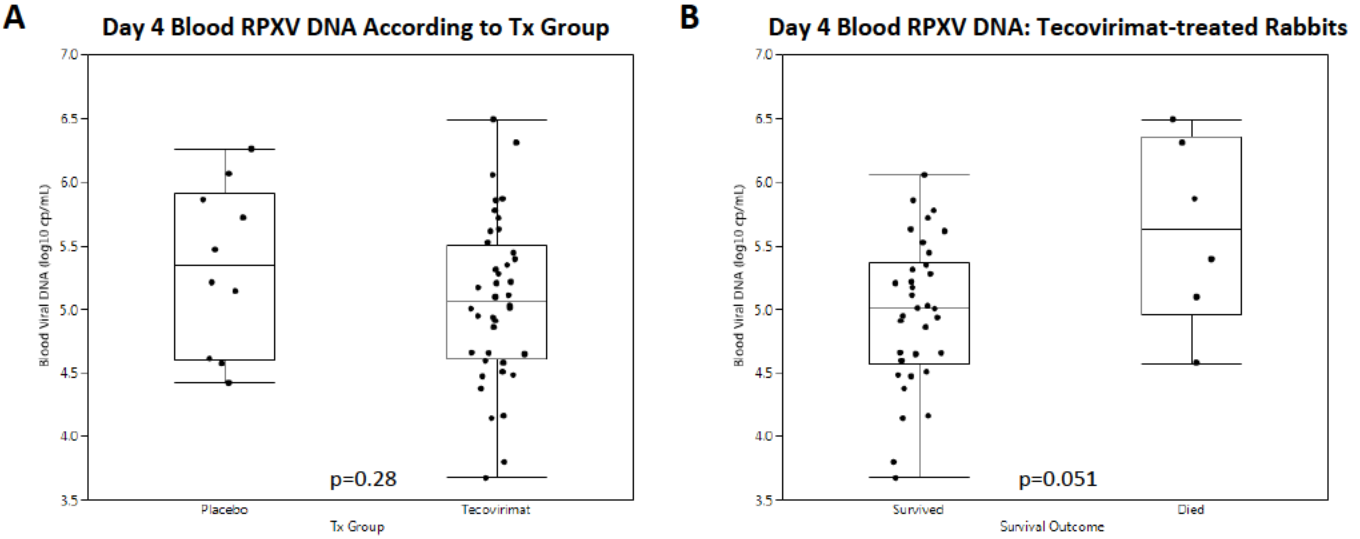


Figure 32. Viral DNA levels at Day 4 (just prior to initiation of treatment) in rabbit/RPXV study SR14-008F. P values shown are based on Wilcoxon Rank-Sum test.

Viral DNA levels in lung and spleen tissues collected at the time of necropsy were lower among tecovirimat-treated animals relative to those that received placebo (Figure 33; FDA analysis). Much of the difference in tissue viral DNA levels between treatment groups may be attributed simply to the timing of necropsy rather than a difference in tissue viral burden throughout the disease course. Animals that survived until the planned euthanasia (Day 30-31), comprised entirely of tecovirimat-treated animals, had much lower tissue viral DNA levels relative to those that died or were euthanized at earlier timepoints (Figure 34; FDA analysis). Furthermore, among animals that did not survive until the end of study, including both tecovirimat- and placebo-treated animals, there was a linear correlation between timing of death/necropsy and tissue $\log_{10}$ viral DNA level ($p<0.0001$, FDA analysis). Plaque assay results were generally consistent with viral DNA results, with all placebo-treated animals having quantifiable titers in terminal lung and spleen tissues (mean ~10^7 PFU/mg), and little or no detection of viral PFU from tecovirimat-treated animals (sponsor's analyses, report pgs. 226-228).

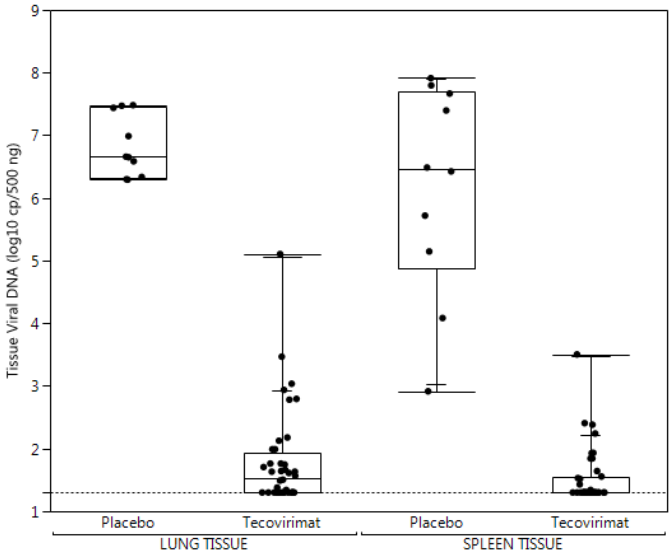


Figure 33. Tissue RPXV DNA levels at the time of necropsy in rabbit/RPXV study SR14-008F.

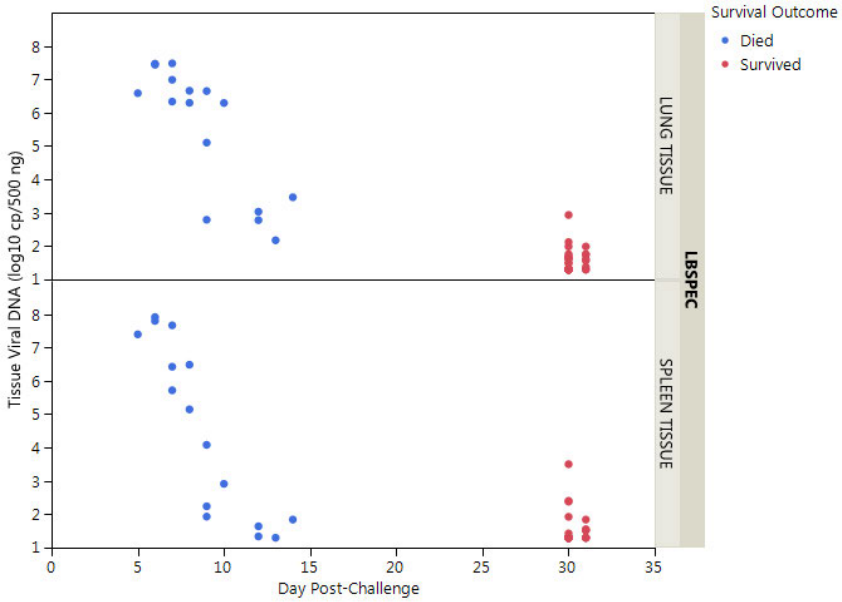


Figure 34. Tissue RPXV DNA levels according to timing of necropsy for all animals regardless of treatment group.

3.7 Study SR13-025F (Rabbits/Rabbitpox Virus)

Title
SR13-025F, "Evaluation of the Impact of Rabbitpox Virus Infection on Oral Pharmacokinetics of Tecovirimat in Male and Female New Zealand White Rabbits"

Summary of Design
Study SR13-025F was a small, double-blinded, GLP study intended to evaluate the effect of RPXV infection on the oral PK of tecovirimat in New Zealand White rabbits, conducted at (b) (4) Twenty-four (24) rabbits, aged ~16 weeks at time of challenge (2.3-2.6 kg when randomized, 1-week prior to challenge) were randomized into 3 groups of 8 animals each based on sex and body weight. On Day -7 (7 days prior to challenge), animals in each group received a single dose of tecovirimat at dose levels of 40 mg/kg (Group 1), 80 mg/kg (Group 2) and 120 mg/kg (Group 3), and initiated 7 days of wash-out period before viral challenge. On Day 0, all animals were challenged intra-dermally in both thighs with RPXV (Lot #090314) at a target dose of 1,000 PFU (actual 940 PFU). On Day 4 post-challenge, all animals started once-daily dosing of tecovirimat by oral gavage corresponding to each dose group, and the dosing continued for 14 days. Animals were monitored for clinical signs of disease and survival for up to 18 days post-challenge. All surviving animals were euthanized at the end of the study on Post-Challenge Day 18.

Viral DNA levels were measured in whole blood samples collected throughout the study, as well as lung, spleen and brain samples collected at necropsy. In addition, plaque assays were conducted for terminal blood, lung, and spleen samples. Resistance analyses were also to be conducted, but no resistance data were obtained from this study (see Section 3.8). This study was not placebo-controlled and evaluated the same tecovirimat treatment doses that were evaluated in study SR14-008F, with the goal primarily to evaluate the impact of rabbitpox virus infection on tecovirimat PK. Therefore, only a brief summary of efficacy and virology results is included below to characterize tecovirimat activity in a second 'pivotal' rabbit/RPXV study.

Efficacy Results (Survival)
The sponsor's analyses of survival are summarized in Figure 35 (Report SR13-025F pg. 37). Overall, 92% (22/24) of the tecovirimat-treated rabbits survived through the end of the 18-day observation period. As noted above, no placebo-treated or untreated animals were included as controls. Two animals were found dead: Animal 1011 (tecovirimat 40 mg/kg) found dead on Day 16 post-challenge, and Animal 993 (tecovirimat 80 mg/kg) found dead on Day 17 post-challenge. According to the sponsor, these two animals were found dead immediately after the dosing procedure and it was determined that this mortality was a result of the gavage procedure and not caused by RPXV infection.

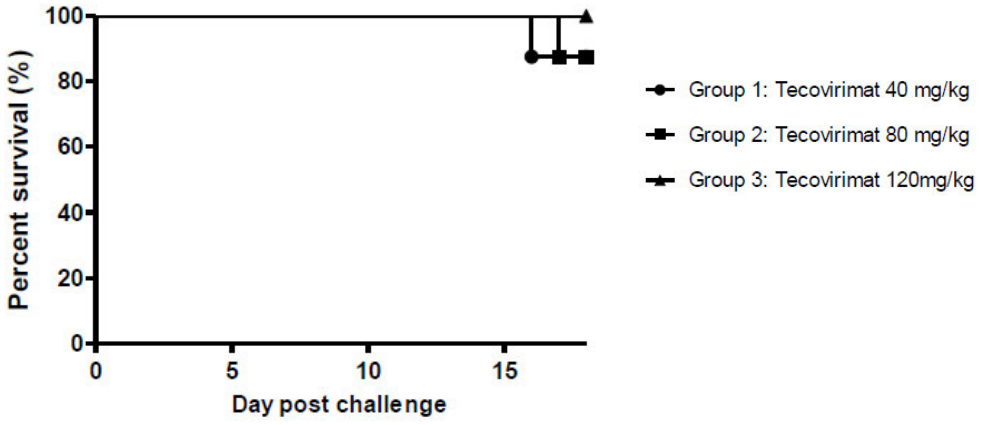


Figure 35. Animal survival rates in rabbit/RPXV study SR13-025F.

Virology Results

Similar to study SR14-008F, all tecovirimat dose levels in study SR13-025F appeared to reduce RPXV DNA levels in whole blood (Figure 36; FDA analysis). Somewhat in contrast to study SR14-008F, a slight delay in median viral DNA declines was observed in the tecovirimat 40 mg/kg dose group relative to the other dose groups starting at Post-Challenge Day 7.

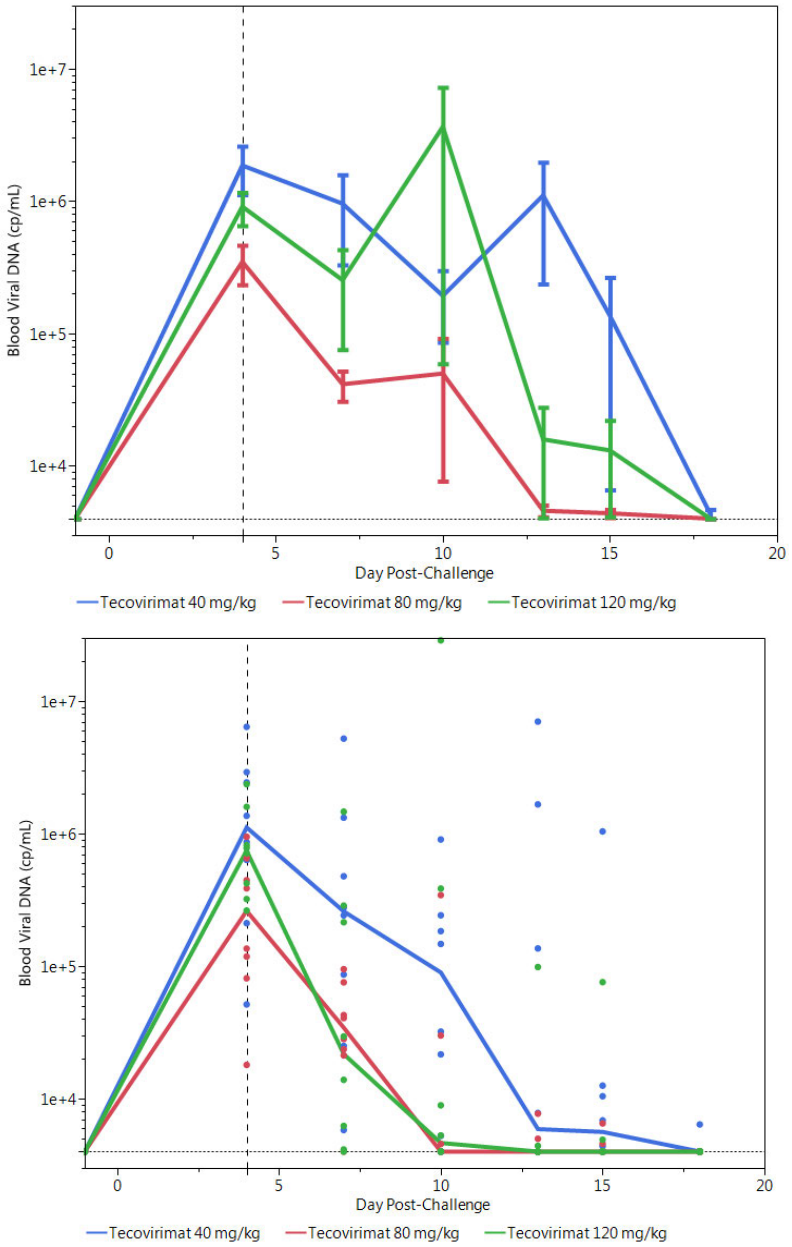


Figure 36. Mean +/- standard error (top) and median w/individual (bottom) viral DNA levels in whole blood in rabbit/RPXV study SR13-025F. The graphs censor data for Post-Challenge Days 16 (n=1) and 17 (n=1) due to data being reported for only a limited number of animals. The assay LLOQ was 4,000 copies/mL (horizontal dashed line), and all animals were dosed starting on Post-Challenge Day 4 (vertical dashed line).

Although the timing of blood samples obtained for viral DNA quantification varied, the general trends of viral DNA changes over time in tecovirimat-treated animals were similar across RPXV studies SR13-025F and SR14-008F (Figure 37; FDA analysis). Of note, blood viral DNA levels at the time of treatment

(Day 4) among animals treated with the 40 mg/kg dose were higher for animals in study SR13-025F relative to SR14-008F, which may have contributed to the slight delay in viral DNA declines in this dosing group relative to the other dose groups that was observed in SR13-025F, but not in SR14-008F.

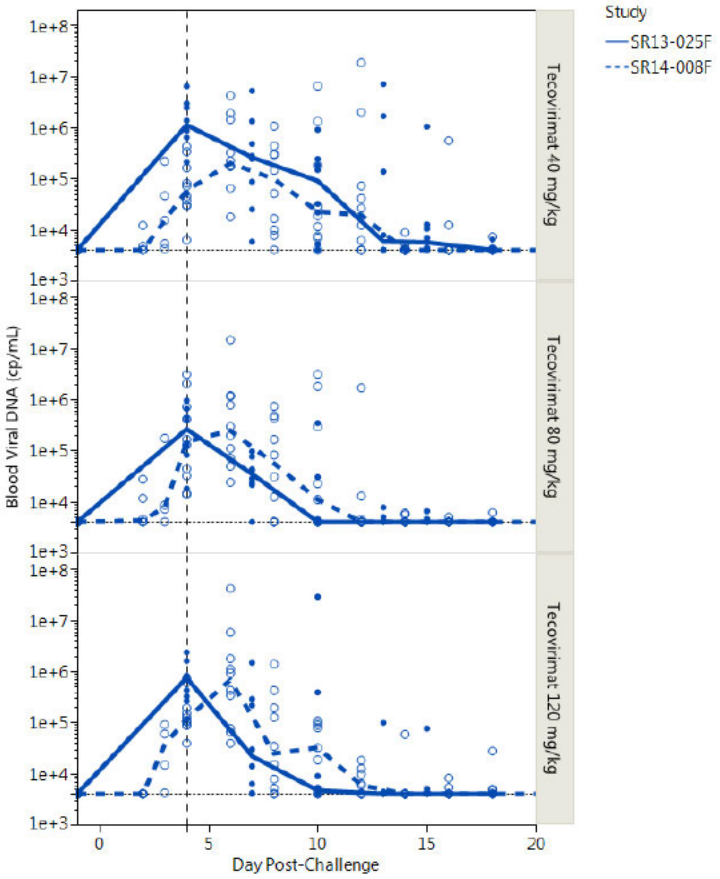


Figure 37. Median and individual viral DNA levels in whole blood in rabbit/RPXV study SR13-025F, compared to results from rabbit/RPXV study SR14-008F. Data censored for Post-Challenge Days 16 (n=1) and 17 (n=1) from study SR13-025F, and Post-Challenge Days 9 (n=2) and 13 (n=1) from study SR14-008F, due to data being reported for only a small number of animals. The assay LLOQ was 4,000 copies/mL (horizontal dashed line), and dosing started on Post-Challenge Day 4 (vertical dashed line).

3.8 Analyses of Drug Resistance in Registrational Animal Model Studies

Overview of Methods and Available Data

Two approaches were used to monitor for the development of drug resistant virus in NHP/MPXV and rabbit/RPXV studies (Study Report SR16-039F). These studies were conducted by (b) (4) (b) (4)

In the first approach, the testing facility conducted plaque assays in an attempt to isolate and quantify the frequency of tecovirimat-resistant viruses in blood and tissue samples from rabbit/RPXV studies SR14-008F and SR13-025F; these analyses were not conducted for any NHP/MPXV studies. Stored samples of blood, lung and spleen from infected rabbits treated with tecovirimat were subjected to plaque assays in the presence or absence of 0.5 µM (~35x EC_{50} value) tecovirimat. A total of 40 samples from 31 animals were analyzed, including 34 samples from 29 tecovirimat-treated animals, and 6 samples from 2 untreated animals; note that the report mistakenly noted animal 937 was treated with tecovirimat, when it in fact was in a placebo group. Of the 40 samples analyzed, plaques were recovered in the absence of

tecovirimat from only 3 samples (all lung) from 3 tecovirimat treated animals and 5 samples from the untreated animals, and the plaque titers were low for the samples from tecovirimat treated animals (≤900 PFU/mL). No plaques were obtained in the presence of tecovirimat for any samples. Given the lack of successful recovery of virus from most samples even in the absence of tecovirimat, no conclusions can be drawn regarding the frequency of tecovirimat-resistant versus tecovirimat-sensitive virus in these tissue samples/compartments based on these results. Therefore, results from these analyses are not discussed in detail in this review.

In the second resistance monitoring approach, viral DNA was isolated from a total of 129 blood and tissues samples from NHP/MPXV and rabbit/RPXV studies and processed for PCR amplification and next generation nucleotide sequencing (NGS) analysis of the VP37 target gene (C19L gene in MPXV, RPXV041 gene in RPXV). These samples came from NHP/MPXV studies SR10-037F and SR10-038F, rabbit/RPXV studies SR13-025F, SR14-008F, and SR13-007F, and an untreated rabbit from RPXV "potency" study SR14-015F. No samples were analyzed from key NHP study AP-029-026G; according to Clinical Virology review of IND 69019 SDN 389 by Dr. Jules O'Rear, the sponsor has not been able to analyze samples from this study.

Unfortunately, of the 129 blood and tissue samples processed for NGS analysis, results were successfully obtained only for 11 (9%) samples, including 7 MPXV samples from 6 animals and 4 RPXV samples from 4 animals (all from blood). Prior to NDA submission, we requested that the sponsor explain why there was such a low success rate for PCR amplification and sequencing of these samples. The sponsor responded that for successful amplification and sequencing library generation, the assay requires an input of $>10^6$ genome copies per PCR reaction, and with an input volume of only 5 µL this requires a very high starting viral DNA concentration of $\geq 2 \times 10^8$ copies/mL (see Clinical Virology reviews of IND 69019 SDN 398 and IND 69019 SDN 402 for more details and a list of all samples attempted for analysis). Although the available NGS data were limited, the overall results were consistent with tecovirimat having a low resistance barrier and multiple potential resistance pathways, as observed in cell culture resistance analysis studies. The following sub-sections summarize the NGS results for the 10 animals with available data, based on the analysis data obtained and reported by the sponsor. It appears that the sponsor used a 2% variant frequency cutoff for reporting amino acid substitutions.

The raw NGS fastq files were also independently analyzed by Dr. Eric Donaldson. According to Dr. Donaldson's draft review, the NGS analyses results reported by the sponsor were confirmed with his independent analyses, with a few minor variations in sequencing depth and frequency. No additional substitutions were determined to be associated with resistance to tecovirimat. Please see Dr. Donaldson's review for details.

Genotypic Resistance Analyses in MPXV-Infected NHPs

Genotypic resistance results were obtained from blood samples from 4 NHPs in Study SR10-037F and 2 NHPs in Study SR10-038F. Of these 6 NHPs, 4 received tecovirimat and 2 received placebo:

- SR10-037F NHP blood samples w/NGS resistance data (Treatment Group):
  - Animal 4762-Day 7 (Placebo); sacrificed/died on Day 7
  - Animal 4768-Day 12 (Tecovirimat 10 mg/kg Day 4-17); sacrificed/died on Day 12
  - Animal 4770-Day 11 (Tecovirimat 10 mg/kg Day 6-19); sacrificed/died on Day 11
  - Animal 4773-Day 8 (Tecovirimat 10 mg/kg Day 5-18); sacrificed/died on Day 8
- SR10-038F NHP blood samples w/NGS resistance data (Treatment Group):
  - Animal 4803-Day 12 (Placebo); sacrificed/died on Day 13
  - Animal 4819-Days 9 and 11 (Tecovirimat 10 mg/kg Day 4-13); sacrificed/died on Day 11

Viral DNA levels and treatment-emergent VP37 substitutions observed in the 4 tecovirimat-treated NHPs with available NGS analysis data are summarized in Figure 38 (FDA analysis).

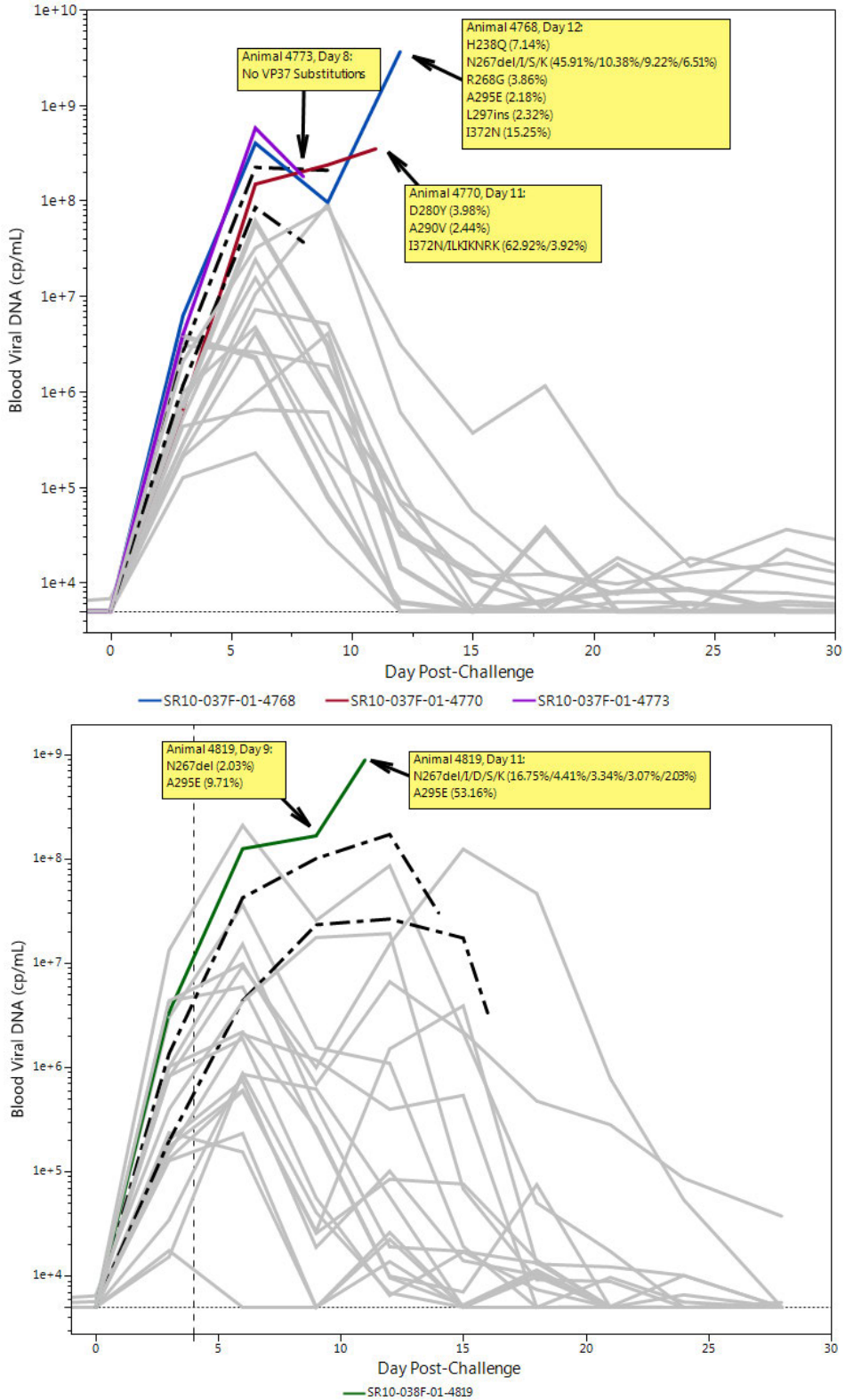


Figure 38. Whole blood viral DNA levels and available VP37 gene NGS analysis data for tecovirimat-treated animals in NHP/MPXV studies SR10-037F (top) and SR10-038F (bottom). Colored lines show viral DNA levels for animals with available NGS data. Black lines indicate other tecovirimat-treated animals that died or were euthanized prematurely, for which no resistance data were available. All other animals represented by grey lines survived to the end of study.

Emergence of tecovirimat VP37 resistance-associated substitutions was clearly demonstrated in 3 of the 4 tecovirimat-treated NHPs with available data. Viral DNA levels observed in all 4 NHPs with available NGS data were among the highest levels observed for all tecovirimat-treated NHPs in these studies. NGS analysis data were not available for 4 other tecovirimat-treated NHPs that received suboptimal tecovirimat treatment regimens and died prematurely (black dashed lines in Figure 38): NHPs 4764 and 4772 in SR10-037F received tecovirimat starting on Day 6 post-challenge, and NHPs 4808 and 4818 in SR10-038F were in the tecovirimat 3-dose group.

Table 17 (FDA analysis) summarizes the specific VP37 substitutions observed in these NHPs, and available phenotype data that describe their effect on tecovirimat antiviral activity in cell culture. Several of these substitutions are known to confer large phenotypic reductions in tecovirimat anti-orthopoxvirus activity. Not surprisingly, no VP37 amino acid substitutions were reported for the 2 NHPs that received placebo.

Table 17. Summary of VP37 substitutions observed in tecovirimat-treated NHPs that died prematurely in studies SR10-037F and SR10-038F.

VP37 Position	Substitution	# (%) of NHPs (n=4 animals w/data)	Fold-Change in EC_{50} Value Conferred Single Substitutions (Virus)
H238	H238Q	1 (25%)	600 to >5,000 (VACV)
N267	N267-any	2 (50%)	n/a
	N267del	2 (50%)	No Data
	N267D	1 (25%)	>5,000 (VACV)
	N267I	2 (50%)	No Data
	N267K	2 (50%)	No Data
	N267S	2 (50%)	500 (VACV)
R268	R268G	1 (25%)	No Data
D280	D280Y	1 (25%)	No Data
A290	A290V	1 (25%)	31-220 (VACV)
A295	A295E	2 (50%)	1600 (VACV)
L297	L297ins	1 (25%)	No Data
I372	I372N	2 (50%)	9 (VACV)
	I372ILKIKNRK[1]	1 (25%)	No Data
Any (from list above)		3 (75%)	n/a

[1]Insertion caused by mutation of VP37 stop codon and extension of the open reading frame; see Dr. Donaldson's review for details.

Genotypic Resistance Analyses in RPXV-Infected Rabbits

Genotypic resistance results were obtained from blood samples from 4 rabbits in Study SR13-007F, which was an early exploratory RPXV study (no viral load datasets were provided to conduct an independent analysis). The 4 blood samples are as follows (SR13-007F Treatment Group):

- Animal 714-Day 7 (Tecovirimat 80 mg/kg Day 6-19); sacrificed/died on Day 7
- Animal 719-Day 7 (Tecovirimat 80 mg/kg Day 6-19); sacrificed/died on Day 7
- Animal 741-Day 7 (Tecovirimat 80 mg/kg Day 6-19); sacrificed/died on Day 7
- Animal 751-Day 7 (Tecovirimat 80 mg/kg Day 6-19); sacrificed/died on Day 7

As noted above, all 4 animals were in the same treatment group in which tecovirimat was initiated on Day 6 post-challenge. All 5 animals in this dosing group died by Day 7, and their blood viral DNA levels are summarized in Figure 39 (Study Report SR13-007F, pg. 61). In contrast, only 1 of 5 animals that started tecovirimat on Day 5 died in the study, and no animals that started tecovirimat on Day 2, 3 or 4 died (6 per group). Therefore, the animals that died and were subjected to resistance analyses likely received tecovirimat too late in the infection for the drug to provide an efficacy benefit.

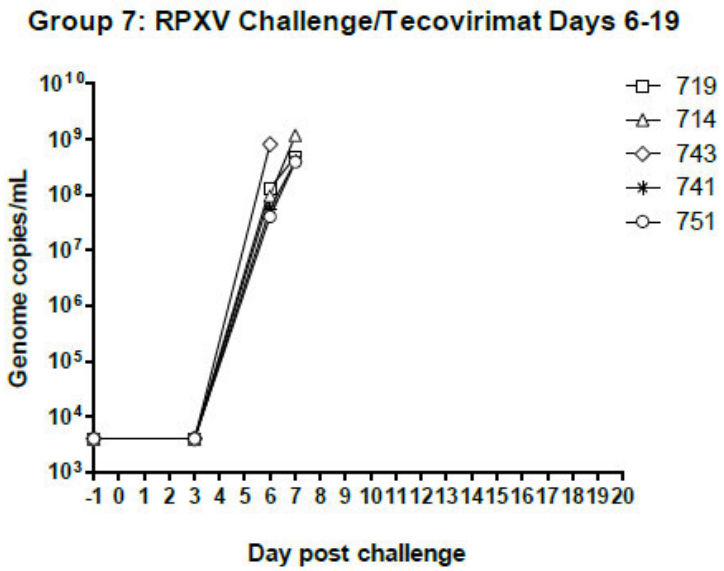


Figure 39. RPXV DNA levels in blood for rabbits that received tecovirimat starting on Day 6 post-challenge. NGS analysis data were provided for animals 714, 719, 741 and 751.

Among these four rabbits, only a single VP37 amino acid substitution was detected in one rabbit, Animal 751. The VP37 amino acid substitution was N179T, and was detected in only 3.69% of NGS reads. No phenotype data were available in the initial NDA submission to assess the potential impact of N179T on tecovirimat anti-RPXV activity. However, the sponsor reported in SDN 27 that from the phenotypic analysis of the Day 7 blood sample (methods described above), 112 plaques were observed with a 1:50 dilution of the sample cultured without tecovirimat, while no plaques were observed at any sample dilution cultured in the presence of tecovirimat (Report 15201.01-P1). Furthermore, an N179T substitution did not confer tecovirimat phenotypic resistance (no plaques observed in presence of ≥0.1 µM tecovirimat) when engineered into VACV-WR (Report 15201.01-P2). Thus, the low frequency of the N179T substitution detected only in one sample of one rabbit, coupled with the lack of a clear phenotypic effect on tecovirimat susceptibility, indicate that N179T is unlikely a tecovirimat resistance-associated substitution.

4. VARIOLA VIRUS ANIMAL MODEL STUDIES

4.1 Study 1400JAHMONC (Natural History Study of Variola Virus Infection in NHPs)

Title
1400JAHMONC, "Evaluation of the Progression of Pathogenic Events in Cynomolgus Macaques Infected with Variola Virus"

Summary of Design
Study 1400JAHMONC was an observational study of cynomolgus macaques infected with VARV, conducted at the CDC. The objectives of the study were (1) to evaluate gross, microscopic, and histopathologic changes of the experimentally smallpox-infected cynomolgus macaque monkeys until they die or are euthanized, and (2) to characterize the relationship of virological events and host immune responses following smallpox virus infection in these monkeys.

Eighteen male NHPs were challenged IV with a high quantity of variola virus, 1 x 10^8 PFU (Harper strain, and presumably mostly IMV particles; Jahrling et al., 2004). The study consisted of two phases. In Phase 1, 3 NHPs were challenged on Day 0, and then on Day 5 post-challenge (if still alive) they were to be anesthetized, exsanguinated and euthanized, with a full necropsy performed for pathological examination. In Phase 2, 15 NHPs were challenged and randomly grouped into 5 cohorts of 3 NHPs each. For each cohort, if still alive, NHPs were to be anesthetized, exsanguinated and euthanized at selected times post-challenge: Days 1, 3, 7, 9 and 11. Again, for each cohort a full necropsy was to be performed for pathological examination. Clinical signs of disease, blood chemistry and hematology, and poxvirus lesions were monitored throughout the study. Viral DNA levels were measured by quantitative PCR for whole blood samples collected at various times post-challenge, as well as for tissues collected at necropsy.

Note that a previously published study (Jahrling et al., 2004) evaluated two different strains of VARV (Harper and India 7124) administered to cynomolgus monkeys at various IV challenge doses, including in combination with aerosol challenge. The authors observed that with either strain, 10^9 PFU IV challenge was consistently lethal, but reducing the IV challenge dose to 10^8 PFU or less resulted in reduced or no lethality. The high VARV inoculum required for lethal infection of NHPs indicates the virus replicates poorly in this species, potentially lowering the bar for demonstrating activity of an antiviral drug.

Results

The progression of VARV viremia in cynomolgus monkeys was modest compared to the NHP/MPXV and rabbit/RPXV models. As shown in Figure 40 (study report pg. 22), the mean viral DNA level in whole blood was approximately 4.5 $\log_{10}$ copies/mL on Days 1-2 post-challenge, and peaked at approximately 6 $\log_{10}$ copies/mL on Days 7-11.

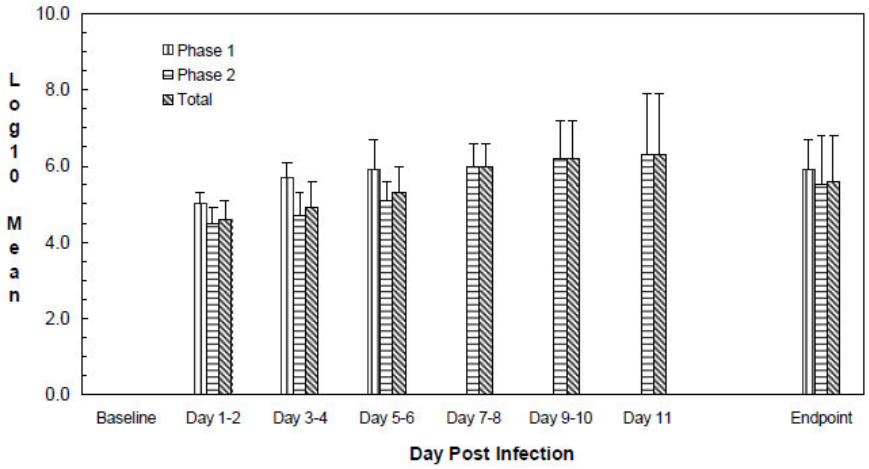


Figure 40. VARV DNA levels in whole blood (mean $\log_{10}$ copies/mL) in untreated cynomolgus monkeys in study 1400JAHMONC.

In general, tissue VARV DNA levels also increased only modestly or fluctuated as the infection progressed between Day 1-2 and Day 11 post-challenge (Figure 41; study report pg. 23). It appears that tissue viral DNA levels were normalized to the volume of extracted DNA solution analyzed, and not necessarily the weight or host DNA content of the tissue, and therefore modest differences in tissue viral DNA levels across timepoints or different tissues should be interpreted cautiously (detailed methods not provided). Nevertheless, the observation that viral DNA levels at later stages in infection were highest in skin lesions is consistent with the NHP/MPXV model (see Section 3.2).

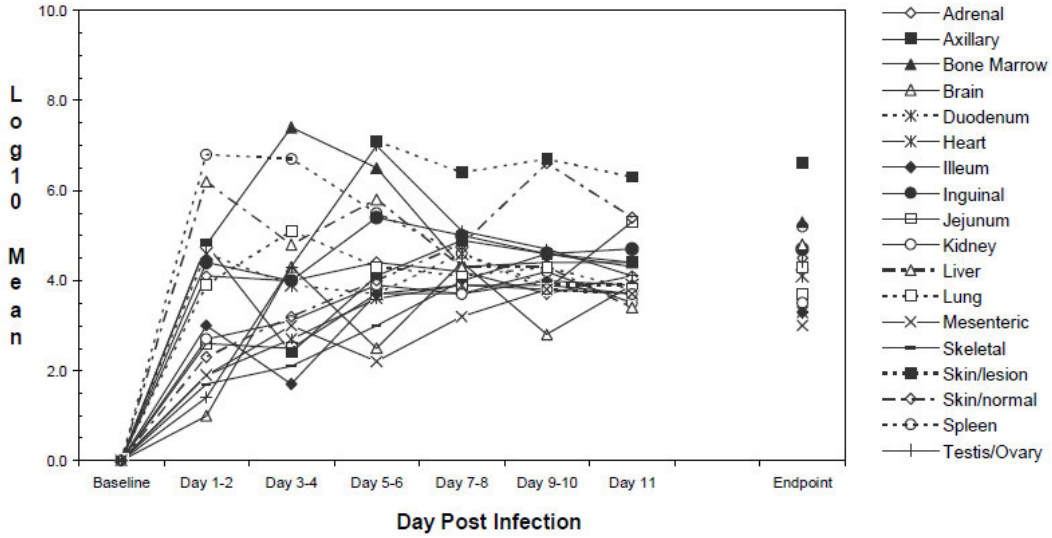


Figure 41. VARV DNA levels in tissues collected at necropsy (mean log_{10} copies/mL) in untreated cynomolgus monkeys in study 1400JAHMONC.

Regarding clinical disease signs, all animals were euthanized on the planned day of euthanasia. No changes in clinical score were observed using a General Distress and Humane Endpoint Scoring (GDHES) system, which considered clinical signs of dyspnea, recumbency and unresponsiveness. Similarly, an Orthopoxvirus Primate Clinical Observation Scoring (OPCOS) system, which considered clinical signs such as cough, dehydration, edema, lymphadenopathy, nasal discharge, stool condition and nonspecific rash, did not detect statistically significant changes from baseline, with the exception of lymphadenopathy and some dehydration.

Lesions characteristic of human smallpox were first observed on Day 3-4 post-challenge, and peaked in number around Day 5-10 (Figure 42, study report pg. 48). Beyond the body sites shown in the figure, lesions were also noted on the face/lips, hands and feet. The sponsor concluded that lesion formation in this model is reproducible and quantifiable, and may serve as a useful maker for evaluating efficacy of smallpox therapeutics.

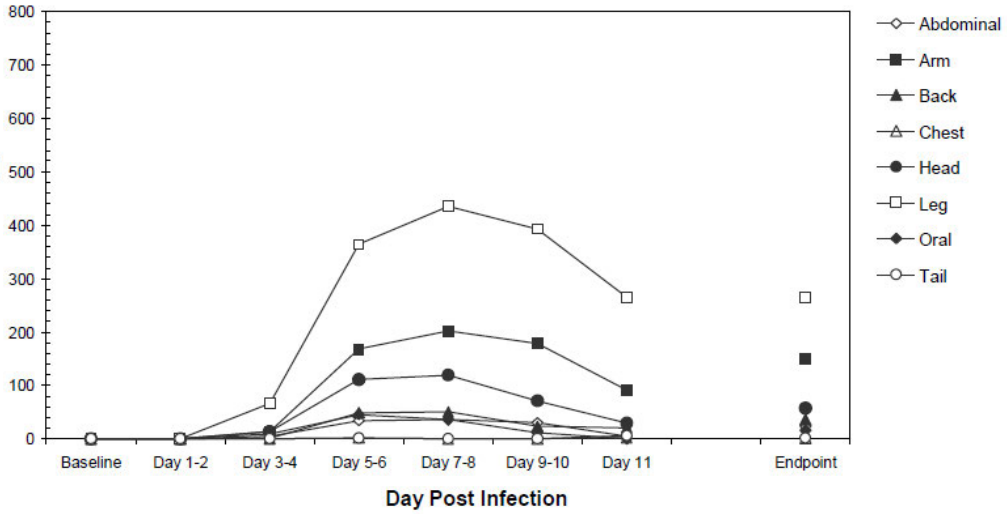


Figure 42. Mean pox lesion count over time for specific body regions in study 1400JAHMONC.

4.2 Study 2238GOFMONC (Tecovirimat Activity in Variola Virus NHP Model)

Title
2238GOFMONC, "Double-Blind, Randomized, Placebo-Controlled, Repeat-Dose Efficacy Study of the Therapeutic Window of the Proposed Primate Equivalence of the Human Dose of Oral ST-246 in Cynomolgus Monkeys Infected with Variola Virus"

Summary of Design
Study 2238GOFMONC was a randomized, double-blinded, placebo-controlled study of tecovirimat administered orally to cynomolgus monkeys challenged with VARV. Eighteen male cynomolgus monkeys were randomized to receive either placebo or tecovirimat 10 mg/kg orally for 14 days in one of three different treatment groups (Table 18; study report pg. 24). Animals in Groups 2 and 3 started tecovirimat on Day 2 or Day 4 post-VARV-challenge, respectively. All monkeys were IV challenged with 1.0 x 10^8 PFU of the VARV Harper strain (CDC Lot MBAPR00) on Day 0. Viral DNA levels, lesion counts, clinical signs, and clinical laboratory results were monitored throughout the study. Surviving animals were euthanized on Day 28-30 post-challenge. The primary efficacy endpoint was based on total lesion count.

Table 18. Treatment groups in NHP/VARV study 2238GOFMONC.

Group	No. Animals (all male)	VARV Challenge Dose (Day 0)	ST-246 Dose	Dosage Schedule[a]	
				ST-246	Placebo
1) Placebo[b]	6	1 x 10^8 pfu IV	0 mg/kg	N/A	Day 2-17
2) ST-246 Day 2	6	1 x 10^8 pfu IV	10 mg/kg	Day 2-15	Day 16-17
3) ST-246 Day 4	6	1 x 10^8 pfu IV	10 mg/kg	Day 4-17	Day 2-3

Results
According to the sponsor's analyses, no animals succumbed naturally to the disease, but 3/6 (50%) placebo-treated animals were euthanized on Post-Challenge Day 13 (n=1) or 14 (n=2) due to moribund disease. All tecovirimat-treated animals survived to the end of study on Day 28.

The sponsor's analyses of lesion counts are summarized in Figure 43 (study report pg. 37). According to the sponsor, lesions were apparent for all animals by Post-Challenge Day 3, first appearing focally and then progressing to all body areas prior to death or resolution. Tecovirimat dosing was associated with significant reductions in total lesion counts by Day 4 ($p<0.02$) for the animals started on treatment on Day 2, and by Day 6 ($p<0.04$) for animals started on treatment on Day 4.

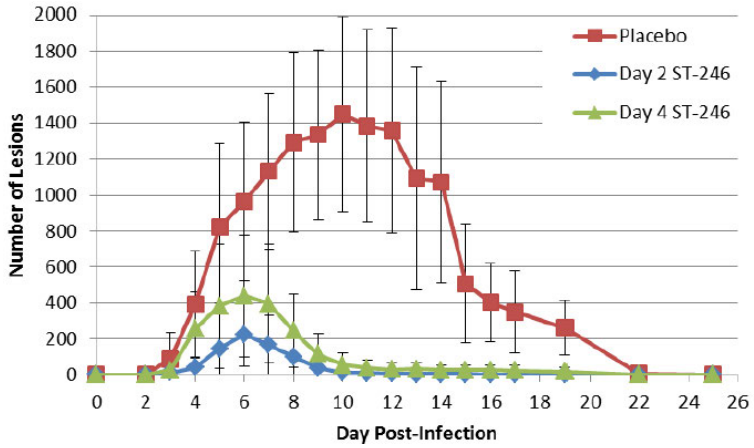


Figure 43. Average total body lesion counts in NHP/VARV study 2238GOFMONC.

Analyses of viral DNA levels in whole blood also demonstrated an antiviral effect of tecovirimat treatment (Figure 44; study report pg. 45). Although not statistically significant according to the sponsor's analyses, viral DNA levels trended lower in the Tecovirimat Day 2 group relative to the Tecovirimat Day 4 group, indicating that earlier treatment initiation resulted in an earlier viral DNA decline.

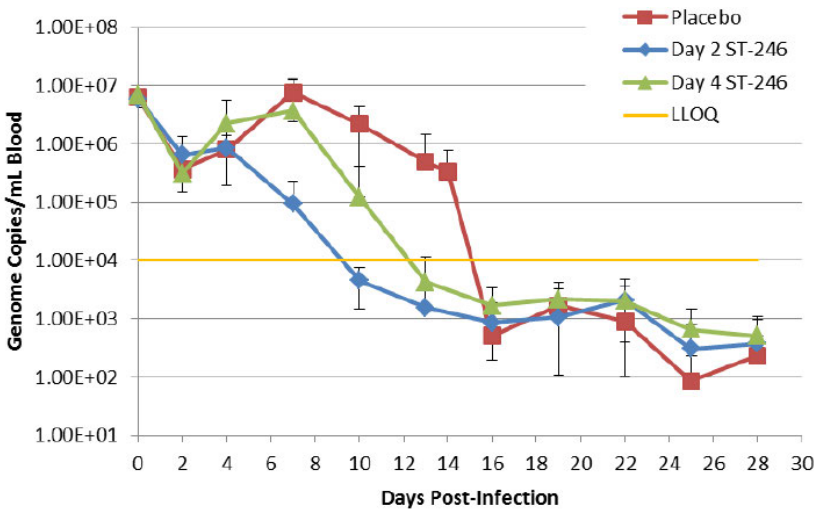


Figure 44. Whole blood viral DNA levels over time in NHP/VARV study 2238GOFMONC.

Analyses of viral DNA levels and PFU in throat mucosal swabs also demonstrated an antiviral effect of tecovirimat treatment (Figure 45; study report pg. 46). The sponsor noted that virus shedding in throat mucosa was significantly lower in tecovirimat-treated animals at multiple timepoints, although the differences across treatment groups are modest.

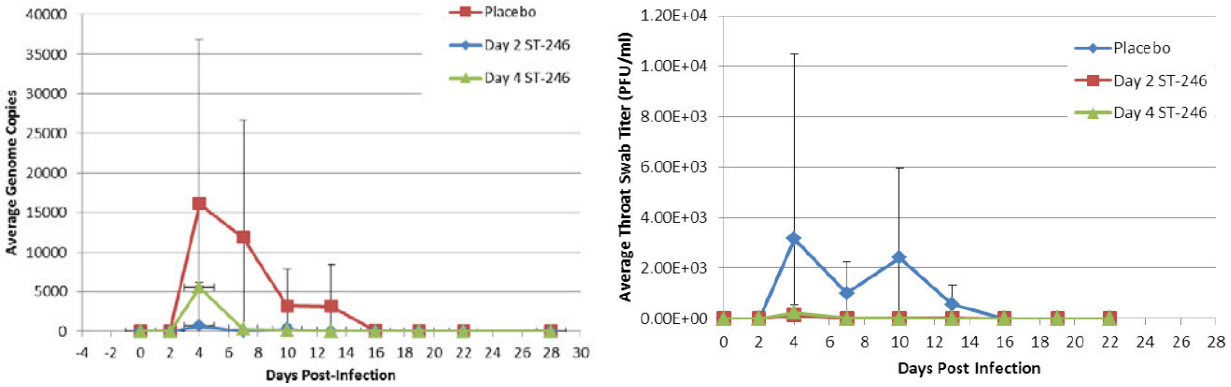


Figure 45. Viral DNA levels (left) and plaque forming units (PFU, right) in throat mucosal swabs over time in NHP/VARV study 2238GOFMONC. Note that the colors/symbols for the different treatment groups are not consistent between both panels, and the y-axes are in linear scale.

In terms of clinical disease signs, tecovirimat-treated animals had reduced weight loss relative to placebo-treated animals. Clinical signs such as recumbency, unresponsiveness bleeding, lymphadenopathy and dehydration were also elevated in the placebo group relative to tecovirimat-treated animals. Body temperatures in all groups were elevated by approximately 1-2ºC from Baseline on Post-Challenge Days 3-5, and were generally similar between tecovirimat- and placebo-treated animals, with the exception of the three placebo-treated animals that were euthanized due to disease and had a large drop in body temperature on the day of euthanasia.

4.3 Study ST246-1745 (Tecovirimat Activity in Variola Virus NHP Model)

Title
ST246-1745, "Double Blind, Randomized, Placebo-Controlled, Repeat-Dose Efficacy Study of the Therapeutic Window of the Proposed Primate Equivalence of the Human Dose (400 mg/day) of Oral ST-246 Polyform I in Cynomolgus Monkeys Infected with Variola Virus"

Summary of Design
Study ST246-1745 was a randomized, double-blinded study of tecovirimat administered orally to cynomolgus monkeys challenged with VARV. Fourteen male cynomolgus monkeys were randomized into two treatment groups of 7 animals each to receive either tecovirimat 10 mg/kg or placebo QD for 14 days. One additional animal was included as a possible alternate in the event that an animal scheduled for treatment did not meet infection criteria; this animal substituted one animal that died prior to treatment initiation. All monkeys were challenged IV with 1.0 x 10^8 PFU of the VARV Harper strain on Day 0. Treatment with tecovirimat or placebo started on the day that lesions first appeared, which occurred on Study Day 3 or 4. Viral DNA levels, lesion counts, clinical signs, and clinical laboratory results were monitored throughout the study. Surviving animals were euthanized on Day 24-27 post-challenge. The primary efficacy endpoint was the proposition of animals surviving through Study Day 23.

Results
Excluding the one animal that died prior to treatment start, only a single animal died across both the tecovirimat and placebo treatment groups. The animal that died was in the tecovirimat treatment group and died under anesthesia, and while the animal had signs of VARV infection the severity of lesions was considered by the sponsor to be insufficient to account for death.

Whole blood viral DNA levels were generally similar between the tecovirimat and placebo groups for most study timepoints, although viral DNA levels were transiently ≥1.5 $\log_{10}$ copies/mL lower in the tecovirimat-treated animals on Days 8-9 post-challenge (Figure 46; study report pg. 44). It is possible that much of the detected viral DNA reflects residual DNA or inactive viral particles given the high initial viral inoculum; analyses of virus (i.e. PFU/mL) were not conducted or provided.

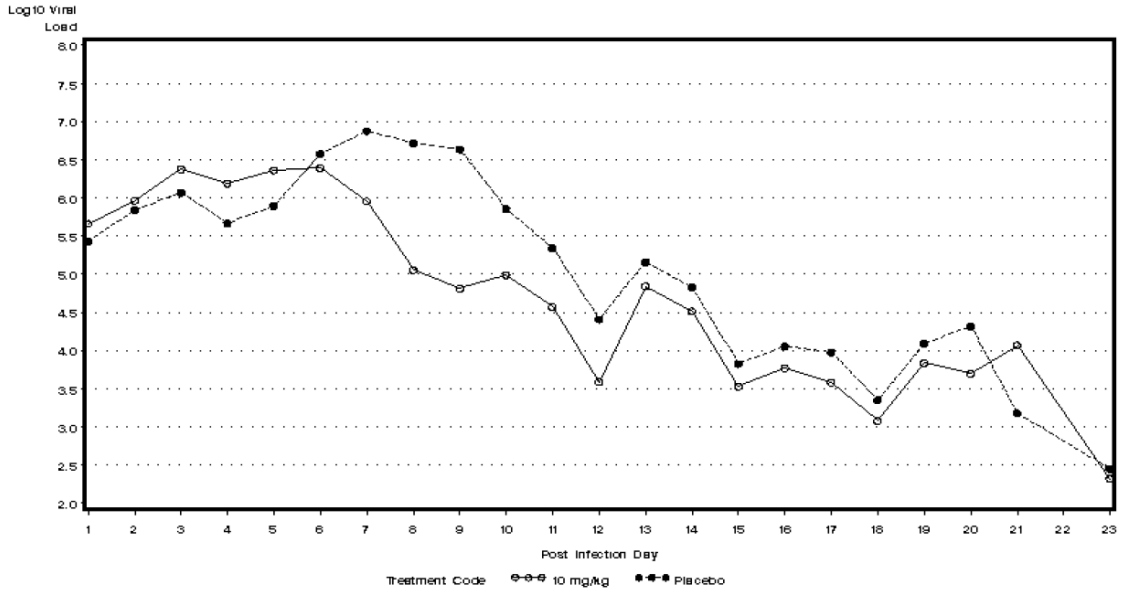


Figure 46. Whole blood viral DNA levels in study ST246-1745. It is unclear if these results indicate median or mean viral DNA levels for the treatment groups. Figure image quality is low in study report.

In contrast to the survival and viral DNA results, analyses of pox lesion counts clearly indicated a tecovirimat treatment effect. As shown in Figure 47 (study report pg. 48), average lesion counts were lower in tecovirimat- versus placebo-treated animals throughout the study after Day 4 post-challenge. According to the sponsor, clinical signs were mild and similar between both treatment groups.

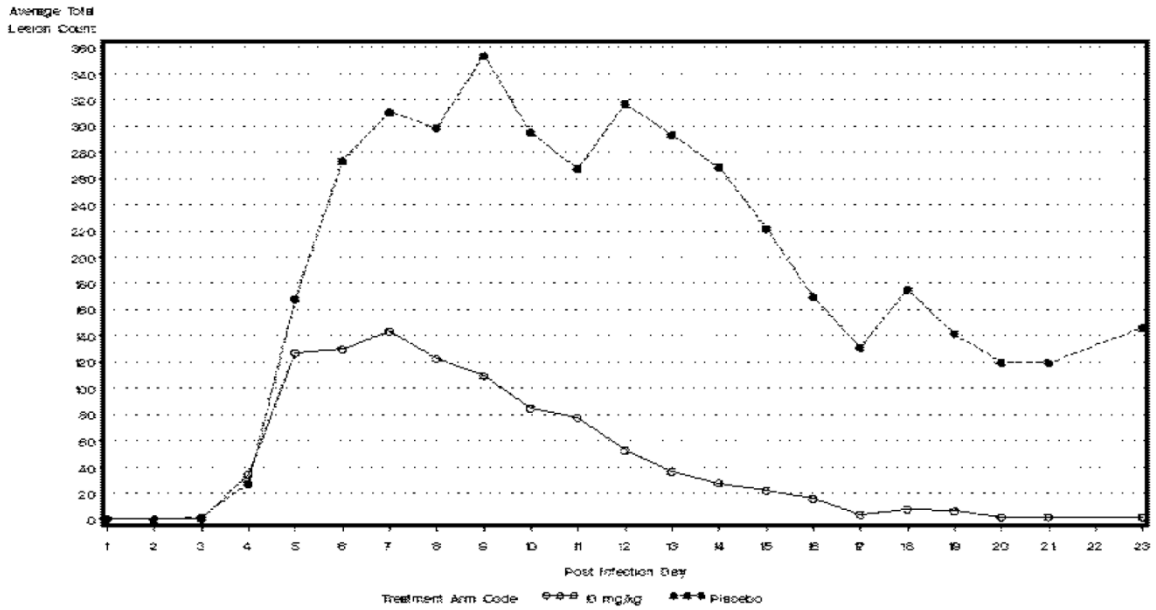


Figure 47. Total pox lesion counts in study ST246-1745. Figure image quality is low in study report.

5. TECOVIRIMAT ACTIVITY IN IMMUNE DEFICIENT MICE

5.1 Summary and Reviewer's Perspective

Tecovirimat could play an especially important role in the treatment of smallpox for individuals with immune deficiencies or who are on immunosuppressive therapy. In this population, live VACV-based smallpox vaccines are contraindicated due to safety concerns, and vaccines in general may be less immunogenic. Furthermore, multiple proteins encoded in orthopoxvirus genomes directly interfere with the immune response to infection, and it is possible that certain proteins in VARV in particular may strongly inhibit the human immune response (e.g., see Rosengard et al., 2002). The characteristics of tecovirimat, including its low resistance barrier and mechanism targeting a late step in the viral replication cycle, also theoretically could limit its efficacy.

This review section summarizes studies of tecovirimat antiviral activity in various immune deficient mouse models. In general, these studies demonstrated that tecovirimat had reduced anti-orthopoxvirus efficacy in the setting of severe immune deficiency, particularly in mice lacking functional T and B cells. These results were consistent with an anecdotal human case in which tecovirimat was used to treat an individual with acute myelogenous leukemia who developed progressive vaccinia following vaccination and initiation of chemotherapy for his malignancy. In this individual, treatment only with tecovirimat and VACV immune globulin did not appear to be sufficient to clear the VACV infection (see Section 8.1). It is important to appreciate the potential limitation of tecovirimat efficacy in the setting of severe immune deficiency, and recognize the public health need for continued countermeasure development or optimization (e.g., combination antiviral therapy, optimized vaccines) for this population.

5.2 Study 246-PC-009 (Tecovirimat Activity in Athymic-nu/nu Mice/VACV Challenge)

Study 246-PC-009 evaluated the activity of tecovirimat in immunocompromised, athymic (nu/nu) mice challenged with a lethal dose (5 x 10^5 PFU) of VACV strain Lister administered by lumbosacral scarification. Athymic "nude" mice lack functional T-cell immune responses as a result of a deletion of the FOXN1 gene. Intradermal infection of these mice with VACV results in a persistent infection, and is thus used as a model of progressive VACV infection in humans. According to the sponsor, following VACV inoculation, virus replicates locally at the site of inoculation to form a visible lesion that spreads over time, and the infection ultimately spreads to other organs and mice succumb to infection within 60-100 days post-challenge.

A total of 25 mice were divided into 5 different treatment groups of 5 mice each to receive either vehicle (Group 1), or tecovirimat 50 mg/kg oral BID for 15, 10 or 5 total days administered over 5-day intervals (Groups 2, 3, and 4, respectively). Mice in Group 5 received CDV 50 mg/kg by i.p. injection (presumably QD) for 10 total days, again administered in two 5-day intervals. Treatments started 2-hours post-challenge. Viral lesions were graded on a scale of 1 to 5 based on appearance at four different timepoints, and moribund animals were euthanized and the mean time to death was calculated for each treatment group. Animals were followed for up to 107 days post-challenge.

The sponsor's analyses of lesion counts and mean time to death are summarized in Table 19 (adapted from study report pg. 21). Treatment with tecovirimat or cidofovir was associated with reduced lesion scores and delayed death from infection, although tecovirimat did not necessarily prevent eventual death. The sponsor did not comment on any resistance analysis results that may have explained whether deaths in treated animals were due to drug resistance selection versus insufficient antiviral activity or treatment duration.

Table 19. Sponsor's analyses of lesion scores and mean time to death in study 246-PC-009. P values are based on the sponsor's analyses using Student's *t*-test for comparisons to control group.

Tx Group (n=5/group)	Tx Days	Day4	Day 7		Day 14		Day 22		Time to Death	
		Mean±SD	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*
1-Vehicle Control	0-4 7-11 14-18	2 ± 0	3.6 ± 1.1	n/a	4.0 ± 1.4	n/a	4.0 ± 1.4	n/a	57.4 ± 11.8	n/a
2-Tecovirimat 50 mg/kg	0-4 7-11 14-18	0	1 ± 0.6	0.004	1.4 ± 0.5	0.011	1.1 ± 0.8	0.006	74.0 ± 6.1 (2/5 survived)	0.04
3-Tecovirimat 50 mg/kg	0-4 7-11	0	0.8 ± 0.3	0.004	0.6 ± 0.5	0.004	0.6 ± 0.4	0.004	69.0 ± 17.4 (2/5 survived)	0.38
4 Tecovirimat 50 mg/kg	0-4	0	0.8 ± 0.8	0.003	1.1 ± 0.8	0.006	1.6 ± 1.8	0.048	52.0 ± 4.8	0.38
5-Cidofovir 50 mg/kg	0-4 7-11	0	0.3 ± 0.3	0.002	0.3 ± 0.4	0.003	0.7 ± 1.8	0.003	76.0 ± 13.4	0.05

5.3 Grosenbach et al., 2010 (Tecovirimat Activity in Immunodeficient Mice/VACV Challenge)

In this detailed published study, tecovirimat activity was assessed in a variety of different immunodeficient mouse models following lethal VACV-WR challenge. Note that the VACV-WR strain produces enveloped virus and is highly pathogenic in mice, but compared with other VACV strains more

of the extracellular virus remains associated with cells (CEV) rather than being released as EEV (see Section 2.1).

In one experiment, nude (athymic, lack T cells) and severe combined immune deficiency (SCID, lack functional B and T cells) mice were challenged intranasally with 10^3 to 10^5 PFU VACV-WR and oral tecovirimat ~100 mg/kg was initiated at the time of challenge (i.e. post-exposure prophylaxis) and continued for 21 consecutive days. Tecovirimat treatment delayed disease progression initially, but then the mice developed increasingly severe disease and all mice eventually succumbed to the infection.

Interestingly, the authors also challenged nude and SCID mice with a recombinant VACV-WR strain with a deletion in the F13L gene (challenge doses up to 10^6 PFU), and all of the mice survived and did not show any signs of disease. While these results further validated the F13L/VP37 protein as a rational drug target, they also indicated that tecovirimat treatment in this model was not equivalent to complete knockout of the VACV F13L gene. The authors speculated that tecovirimat was less effective than F13L genetic knockout due to inefficient tissue penetration of the drug, and that drug resistance may have also contributed to lack of tecovirimat efficacy in these models.

In another set of experiments, tecovirimat anti-VACV activity was evaluated in six other mouse models of immune deficiency (n=5 mice per experimental group): BALB/c mice depleted of $CD4^+$ or $CD8^+$ T cells (via monoclonal antibodies), J_H knockout mice (lack mature B cells), and J_H knockout mice with additional depletion of T cells ($CD4^+$ alone, $CD8^+$ alone, or combined $CD4^+$/$CD8^+$ T cell depletion). Mice were challenged intranasally with 2.5 x 10^5 PFU of VACV-WR, which is 10x the 50% lethal dose and results in 100% mortality in normal immunocompetent BALB/c mice. Tecovirimat ~100 mg/kg was administered in these mice starting either at the time of challenge or at 72 hours post-challenge, and continued for 14 daily doses.

Tecovirimat treatment starting on Day 0 resulted in 100% survival for immunocompetent BALB/c mice, as well as all other immune-deficient mice except for J_H mice with combined $CD4^+$/$CD8^+$ T cell depletion, which had delayed disease but ultimately succumbed to the infection. Other immune-deficient mice had varying degrees of weight loss and other disease signs but ultimately survived to the end of study (Day 30). Importantly, all mice that survived were re-challenged with a higher dose of VACV-WR and ultimately survived the infection in the absence of any tecovirimat treatment, indicating that immune responses developed in these partially immune deficient mice and contributed to viral clearance and protection upon re-challenge.

Delaying tecovirimat to 72 hours post-challenge resulted in increased morbidity and substantial weight loss in all groups, although the drug still provided protection from death in immunocompetent BALB/c mice as well as BALB/c mice depleted of $CD4^+$ or $CD8^+$ T cells, J_H mice depleted of $CD8^+$ T cells, and J_H mice depleted of $CD4^+$ T cells (80% survival). All J_H mice and J_H mice with combined $CD4^+$/$CD8^+$ T cell depletion succumbed to the infection. It was not clear why disease was greater in J_H mice without any T cell depletion relative to J_H mice singly depleted $CD4^+$ or $CD8^+$ T cells (the authors speculated about a potential immunopathology-related mechanism). Additional exploratory analyses indicated that in the absence of an effective immune response, viral replication may still be inhibited in tissues during tecovirimat treatment, but the virus can persist or rebound following cessation of treatment.

Collectively, results from this study indicate that the immune response likely plays an important role in controlling orthopoxvirus disease during and following tecovirimat treatment. Tecovirimat may be effective in the setting of some partial immune deficiencies, but efficacy may be reduced in the setting of severe immune deficiencies such as SCID or the absence of both $CD4^+$ and $CD8^+$ T cells.

5.4 Berhanu et al., 2011 (Tecovirimat + ACAM2000™ Vaccine in Immunodeficient Mice)

In this published study, the effect of co-administration of tecovirimat plus ACAM2000™ vaccine on vaccine reactogenicity, immunogenicity and efficacy was evaluated in immunocompetent mice as well as mice with varying types of immunodeficiency. These included immunocompetent BALB/c mice and J_H mice (lacking mature B cells) depleted of $CD4^+$ T cells, $CD8^+$ T cells, or both $CD4^+$ and $CD8^+$ T cells (n=4 to 5 mice per analysis group). ACAM2000™ vaccine was administered on the tails of mice by epidermal scarification, and tecovirimat 100 mg/kg or vehicle were administered orally starting at the time of vaccination and continuing for 14 days. Vaccine efficacy against lethal VACV-WR challenge was evaluated at 1 and 6 months post-vaccination.

In all groups of mice, with the exception of $CD4^+$/$CD8^+$-depleted BALB/c and $CD4^+$/$CD8^+$-depleted J_H mice, tecovirimat treatment was associated with reduced vaccine lesion severity and accelerated resolution of lesions. Nevertheless, the anti-VACV activity of tecovirimat did not significantly impact vaccine efficacy in this study. Other than modestly lower antibody responses in tecovirimat-treated mice relative to comparable vehicle-treated mice, cellular immune responses and overall vaccine efficacy against VACV-WR challenge were comparable between tecovirimat- and vehicle-treated mice across all mouse groups. However, consistent with results from the Grosenbach et al., 2010 study, even following ACAM2000™ vaccination, mice depleted of both $CD4^+$ and $CD8^+$ T cells were not fully protected from lethal VACV-WR challenge, further illustrating the importance of T cell immune responses in VACV infection. Other animal studies of tecovirimat +/- vaccine are summarized in review Section 6.

5.5 Tecovirimat + Cyclophosphamide in SKH1 Mice/VACV Challenge

The sponsor provided a brief summary (Tecovirimat Efficacy in Immunodeficient Animal Hosts pg. 20-21) of a study of tecovirimat in cyclophosphamide-immunosuppressed SKH1 mice challenged with VACV-WR, which has been developed as a model for progressive VACV infection (Smee et al., 2014). SKH1 mice are immunocompetent but hairless, making them suitable for evaluation of lesional disease progression following vaccination. Cyclophosphamide is a cytotoxic cancer chemotherapeutic agent that acts as a DNA alkylating agent, and has potent humoral and cellular immunosuppressive activity.

Mice (number not specified) received cyclophosphamide by intraperitoneal injection 100 mg/kg every 4 days, and were vaccinated with 10^6 PFU of VACV-WR by dermal scarification on the back. Groups of vaccinated mice received either oral placebo, tecovirimat starting at 12 hours post-vaccination, or tecovirimat starting on Day 3 post-vaccination after establishment of infection and formation of a primary vaccination lesion. Tecovirimat was administered at 100 mg/kg/day for 14 days.

Consistent with the studies summarized above, the severely immunosuppressed mice were not capable of controlling VACV infection, and while tecovirimat clearly had antiviral activity based on reduced lesion spread during treatment, it was not sufficient to prevent advanced disease following treatment cessation. In placebo-treated mice, vaccine lesions increased in size beyond the initial site of inoculation, and satellite lesions became evident within 14 days post-vaccination, increasing in number and severity until disease progression warranted euthanasia (Days 17-27 post-vaccination). Tecovirimat treatment starting at 12 hours post-vaccination reduced lesion severity and resulted in apparent resolution of lesions by Day 14 post-vaccination. Delayed tecovirimat treatment starting on Day 3 resulted in the formation of a primary lesion that was restricted to the site of inoculation without evidence of dissemination or the formation of satellite lesions. However, according to the sponsor's summary, following cessation of tecovirimat, mice began to display evidence of disease, and due to advanced morbidity required euthanasia at approximately 35 days post-vaccination. The sponsor speculated that a longer course of tecovirimat treatment or additional therapies may be necessary for complete resolution of progressive vaccinia disease in severely immunodeficient hosts.

6. TECOVIRIMAT +/- VACCINE ANIMAL STUDIES

6.1 Summary and Reviewer's Perspective on a Potential Tecovirimat-Vaccine Interaction

Although outside the treatment indication being considered for this NDA, it is possible that tecovirimat will be used in combination with VACV-based vaccine in some circumstances. This review section summarizes NHP studies that evaluated the potential for tecovirimat interference with vaccine immunogenicity or efficacy.

The NHP studies summarized below indicate that tecovirimat, as a result of its anti-VACV activity, has the potential to interfere with live, replicating VACV-based smallpox vaccines when tecovirimat is started at the time of vaccination. The clinical relevance of this potential interaction is unclear, and likely varies depending on the specific circumstance in which vaccine and tecovirimat are used. Any vaccine interference observed in the NHP studies was only partial, as animals that received vaccine + tecovirimat consistently had reduced MPXV disease and improved survival compared to non-vaccinated animals. However, the impact of tecovirimat co-administration on longer term vaccine-generated immunity was not evaluated in these studies.

Evidence from other animal studies described throughout this review indicate tecovirimat 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, studies in immune deficient mouse models demonstrated that the immune response plays a critical role in clearing the viral infection during tecovirimat treatment (see Section 5). Furthermore, tecovirimat-treated animals that survived a lethal viral challenge were protected from disease when re-challenged, indicating the development of an effective immune response during tecovirimat treatment (e.g., studies SR12-005F [Appendix A] and Grosenbach et al., 2010 [section 5.3]). Pivotal NHP/MPXV efficacy studies SR10-037F and SR10-038F also demonstrated tecovirimat-treated animals developed high titers of orthopoxvirus neutralizing antibody.

In the setting of a VARV outbreak, it is possible that tecovirimat will be co-administered with vaccine in individuals with suspected exposure to VARV, and it is challenging to predict if tecovirimat would have a clinically significant impact on vaccine immunogenicity in this setting. It is possible that tecovirimat would be effective even in the absence of a vaccine-elicited immune response. Animal studies SR12-005F and 246-PC-031 (summarized in Appendix A) demonstrated that tecovirimat +/- vaccine was effective as a post-exposure prophylaxis/treatment, but vaccine alone was only effective when administered prior to viral challenge. On the other hand, the more protracted VARV incubation period in humans compared to orthopoxvirus animal models presumably provides a greater window of opportunity for effective vaccination. This reviewer is not aware of any evidence that co-administration of vaccine with tecovirimat would negatively impact tecovirimat efficacy as a post-exposure prophylaxis or treatment.

6.2 Study 1218-100004544 (Tecovirimat +/- ACAM2000™ Vaccine +/- FK-506, NHPs/MPXV Challenge)

Title
1218-100004544, "Effect of ST-246, an Anti-Poxvirus Therapeutic, in Preventing Vaccinia Virus Disease in FK-506-immunosuppressed Non-Human Primates"

Summary of Design
Study 1218-100004544 intended to evaluate the antiviral activity of tecovirimat in ameliorating reactogenicity of the ACAM2000™ live VACV vaccine in cynomolgus monkeys immunosuppressed with FK-506 (tacrolimus). This unblinded study also assessed the effect of tecovirimat on vaccine

immunogenicity and survival following subsequent MPXV challenge. FK-506 is an FDA-approved immunosuppressive drug used in human organ transplant settings to prevent transplant rejection. According to prescribing information, FK-506 inhibits T-lymphocyte activation and can also suppress humoral immunity in animals, although the exact mechanism of action is not known.

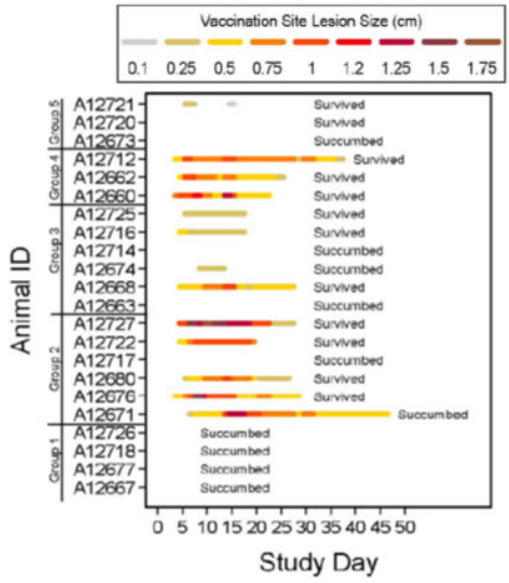
A total of 22 cynomolgus monkeys without detectable MPXV neutralizing antibody were randomized into 5 groups as shown in Table 20 (adapted from study report pg. 3). Vaccine (or mock vaccine) was administered by scarification on Day 0, and tecovirimat or vehicle were administered QD for 14 days starting on Day 0. FK-506 was administered in Groups 1-3 from Day -10 (10 days prior to vaccination) through Day 60. All animals were challenged with 2.1 x 10^7 PFU MPXV (Zaire '79 strain) IV on Day 30 (challenge dose slightly less than pivotal NHP efficacy studies). Viral DNA levels, neutralizing antibody titers, lesion counts/analyses, and clinical signs were monitored throughout the study. Surviving animals were euthanized on Day 60. Datasets for independent review were not provided, although for improved clarity of the sponsor's results in some cases independent FDA analyses were conducted using data appended to the study report (in .pdf format).

Table 20. 1218-100004544 study groups.

Group	# Animals	Male/ Female	FK-506 (2X daily)	Vaccine	Treatment	Target MPXV Challenge (PFU)
1	4	2M/2F	0.05mg/kg	Mock	Vehicle	2.1 x 10^7
2	6	3M/3F	0.05mg/kg	ACAM2000™	Vehicle	2.1 x 10^7
3	6	3M/3F	0.05mg/kg	ACAM2000™	Tecovirimat 10 mg/kg	2.1 x 10^7
4	3	2M/1F	None	ACAM2000™	Vehicle	2.1 x 10^7
5	3	1M/2F	None	ACAM2000™	Tecovirimat 10 mg/kg	2.1 x 10^7

Results

Vaccine lesions were smaller and resolved more quickly in animals that received co-administration of tecovirimat with vaccine, with or without FK-506, reflecting the anti-VACV activity tecovirimat (Figure 48; adapted from study report pgs. 28 and 426). However, results on the impact of FK-506 immunosuppression on the activity of tecovirimat during vaccination were generally inconclusive, as there was not an obvious impact of FK-506 treatment alone on the size or duration of vaccine lesions.



Group	Avg. Lesion Size (cm)	Avg. Lesion Duration (days)
1 (FK-506 only)	none	none
2 (FK-506+Vaccine)	0.45	23.8
3 (FK-506+Vaccine+Tecovirimat)	0.11	12.3
4 (Vaccine only)	0.47	23.7
5 (Vaccine+Tecovirimat)	0.01	9.0

Figure 48. Sponsor's analyses of vaccine site lesion size and duration in study 1218-100004544.

Following MPXV challenge, all animals in Group 1 (non-vaccinated) succumbed to infection by Day 12 post-challenge, while all animals in Group 4 (vaccine only) survived (Figure 49; study report, pg. 21). A subset of animals in Groups 2, 3 and 5 died following MPXV challenge, although after adjusting for multiple comparisons no statistical differences in mortality were detected between any pair of groups according to the sponsor's analyses.

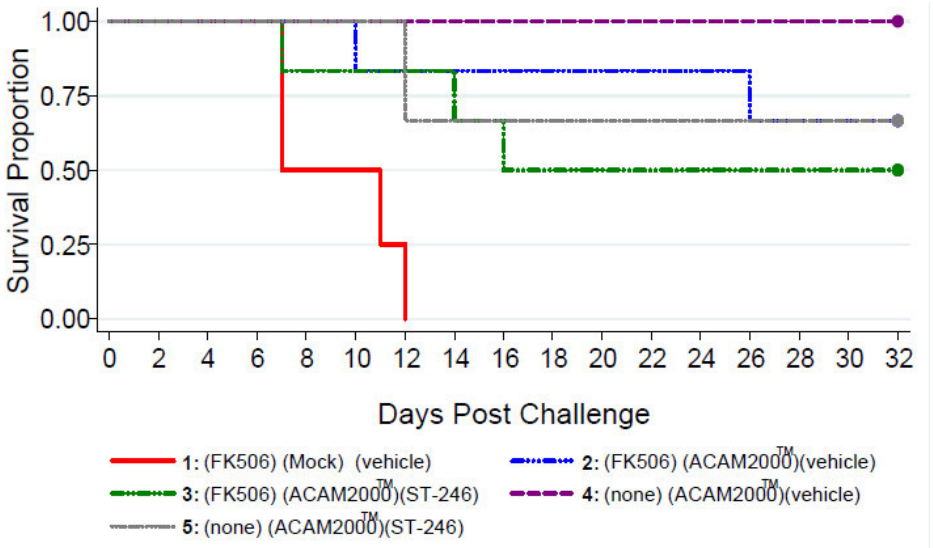


Figure 49. Kaplan-Meier survival analysis in study 1218-100004544, following MPXV challenge.

Although differences in survival rates between animals that received vaccine +/- tecovirimat were not statistically significant based on the sponsor's analyses, it is notable that co-administration of tecovirimat plus vaccine was associated with a numerically lower survival rate compared to animals that received vaccine alone: 7/9 (78%, Groups 2+4) versus 5/9 (56%, Groups 3+5), respectively. The sponsor did not comment on the one animal in Group 2 that died later on Day 26 post-MPXV challenge (Animal A12671). Compared to other animals, Animal A12671 had an unusual disease course with few lesions following MPXV challenge and only a late spike in whole blood viral DNA (shown below). While the small numbers of animals in these groups makes it challenging to draw firm conclusions about the impact of tecovirimat on vaccine efficacy, the survival outcome results were consistent with other measures indicating possible tecovirimat interference with vaccine immunogenicity.

As shown in Figure 50 (FDA analysis), during the first 9 days post-challenge median MPXV DNA levels in blood were 1-4 $\log_{10}$ copies/mL greater in animals that had received vaccine with tecovirimat (±FK-506, Groups 3 and 5) compared to those that received vaccine without tecovirimat (±FK-506, Groups 2 and 4), indicating a reduction in vaccine immune response. Interestingly, a subset of animals that received vaccine without tecovirimat (5/9, 56%) had transiently quantifiable VACV DNA levels in blood on Day 7 following vaccination, while none of the animals that received vaccine with tecovirimat had quantifiable DNA levels in blood following vaccination, likely reflecting the anti-VACV activity of tecovirimat.

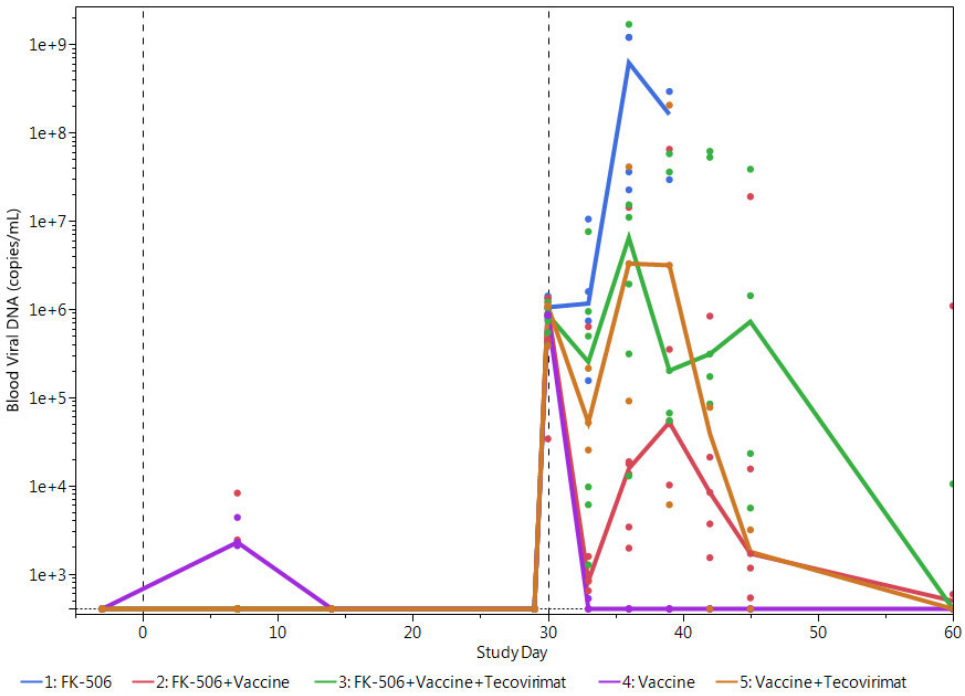


Figure 50. Median and individual viral DNA levels in whole blood in study 1218-100004544. Animals were vaccinated (or mock vaccinated) on Day 0, with or without concomitant FK-506 and/or tecovirimat, and challenged with MPXV on Day 30.

Across all groups, early death/euthanasia was associated with higher MPXV DNA levels in whole blood (Figure 51; FDA analysis), which illustrates the potential significance of differences in blood MPXV DNA levels between animals that received vaccine with versus without tecovirimat. The one exception was animal A12671, which died later than all other non-surviving animals, although interestingly the animal had a late and rebound in viral DNA levels.

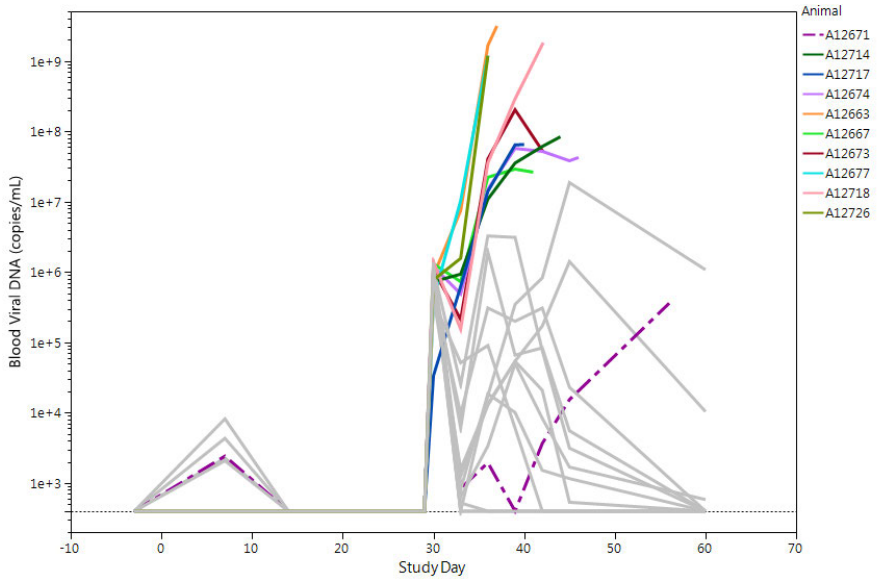


Figure 51. Whole blood viral DNA levels in animals that died/euthanized (colored lines) relative to animals that survived (gray lines) in study 1218-100004544. Animal A12671, which died later than all other non-surviving animals, is shown in the dashed line. Animals were vaccinated or mock vaccinated Day 0, with or without FK-506 and/or tecovirimat, and challenged with MPXV on Day 30.

Consistent with viral DNA levels in blood, pox lesion counts post-challenge also trended higher in the groups that received vaccine plus tecovirimat. For example, on Day 39 (Post-Challenge Day 9) around the time when lesion counts were near their peak, on nearly all body sites pox lesion counts were higher for animals that had received vaccine with tecovirimat (±FK-506, Groups 3 and 5) compared to those that received vaccine without tecovirimat (±FK-506, Groups 2 and 4) (Figure 52; FDA analysis). The vaccine-only group (Group 4) did not develop any pox lesions at any timepoint following MPXV challenge.

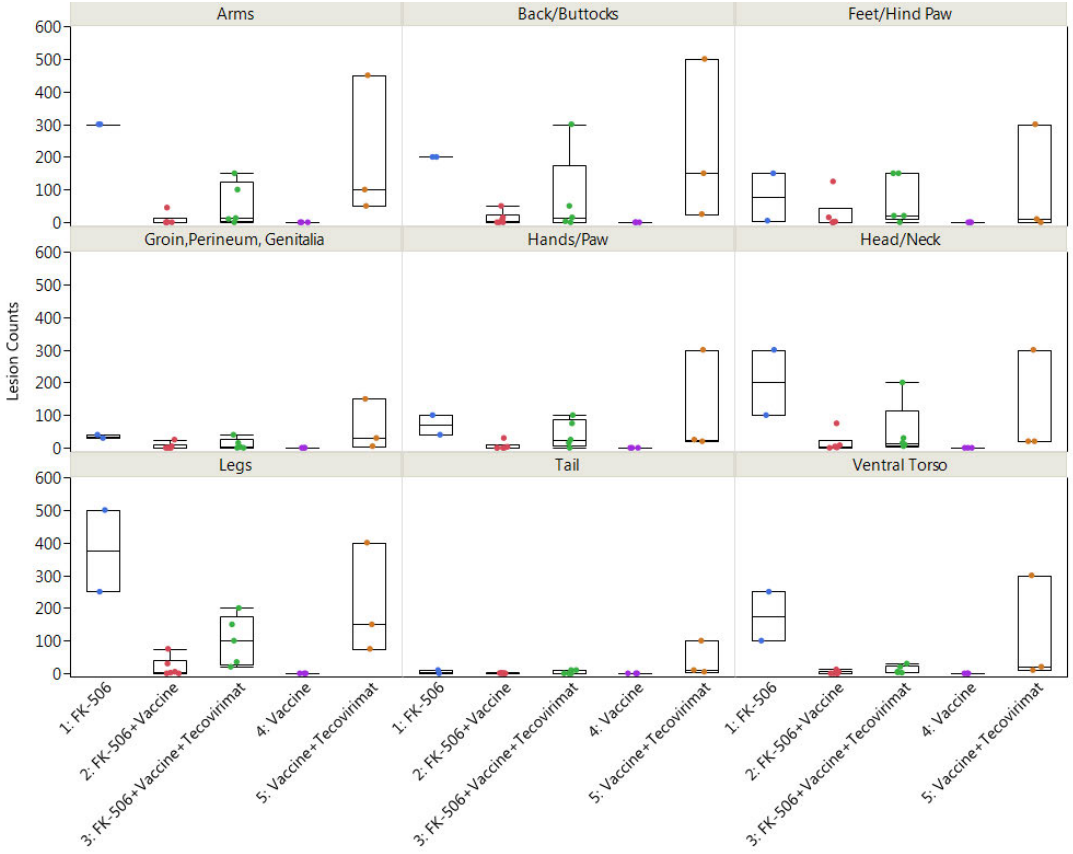


Figure 52. Pox lesion counts on Day 39 (Day 9 post-challenge) in study 1218-100004544.

Finally, neutralizing antibody titers ($PRNT_{50}$) were also indicative of tecovirimat interference of vaccine immunogenicity. On the day of MPXV challenge (Day 30), 89% (8/9) of animals that received vaccine without tecovirimat (Groups 2+4) had measurable MPXV neutralizing antibody titers, with a median $PRNT_{50}$ titer of 112. Interestingly the single animal in this group without a measurable Day 30 titer succumbed to MPXV infection. In contrast, only 11% (1/9) of animals that received vaccine plus tecovirimat (Groups 3+5) had a measurable Day 30 neutralizing antibody titer ($PRNT_{50}$ of 2290), and this animal survived to the end of study.

Collectively, these results indicate that tecovirimat co-administration with the ACAM2000™ vaccine may have a favorable clinical effect of reduced vaccine reactogenicity, but with the consequence of reduced vaccine immunogenicity that could potentially result in reduced vaccine-mediated protection. Nevertheless, it is important to recognize that any interference with vaccine was only partial in this study. Animals that received vaccine + tecovirimat had lower MPXV DNA levels and improved survival compared to non-vaccinated animals.

Although a key objective of study 1218-100004544 was to evaluate the ability of tecovirimat to ameliorate vaccine reactogenicity in FK-506-immunosuppressed animals, the effect of tecovirimat in the setting of FK-506-mediated immunosuppression was generally inconclusive, as FK-506 by itself did not seem to have a major impact on the size or duration of ACAM2000™ vaccine lesions. In other words, immune suppression with FK-506 apparently was not sufficient to result in the severe disseminating VACV infection that can occur in humans with certain immune disorders, which is a scenario where tecovirimat may have an important role in reducing vaccine-related complications.

6.3 Study SR11051.12-Part 2 (Tecovirimat +/- ACAM2000™ Vaccine, NHPs/MPXV Challenge)

Title

11051.12 Part 2, "Impact of Concurrent ST-246® Administration and Vaccination with ACAM2000™ on Vaccination Efficacy in Cynomolgus Monkeys Study #2 (Task Order D15)

Summary of Design

This study assessed if concurrent administration of tecovirimat plus the ACAM2000™ live VACV vaccine in immunocompetent cynomolgus monkeys affected disease outcome following MPXV challenge. A total of 20 animals were assigned to each of 4 study groups according to sex and body weight. The treatment groups are summarized in Table 21 (adapted from study report pg. 15). Tecovirimat or vehicle was administered by oral gavage for 14 days starting on Day 0. Vaccine or diluent was administered by percutaneous scarification on Day 0. A lethal IV challenge dose of MPXV Zaire '79 strain was administered on Day 45. Vaccine lesion development, clinical disease signs, viral DNA levels and neutralizing antibody titers were monitored throughout the study.

Table 21. 11051.12 Part 2 study groups.

Group	N	Tecovirimat (Day 0-13)	Vaccine (Day 0)	MPXV Challenge PFU (Day 45)
1	3	Vehicle	Mock	5×10^7
2	7	Vehicle	ACAM2000™	5×10^7
3	3	Tecovirimat 10 mg/kg	Mock	5×10^7
4	7	Tecovirimat 10 mg/kg	ACAM2000™	5×10^7

Results

Consistent with study 1218-100004544, vaccine lesions were smaller and resolved more quickly in animals that received tecovirimat with ACAM2000™ vaccine compared to those that received vaccine alone (Figure 53; study report pg. 21).

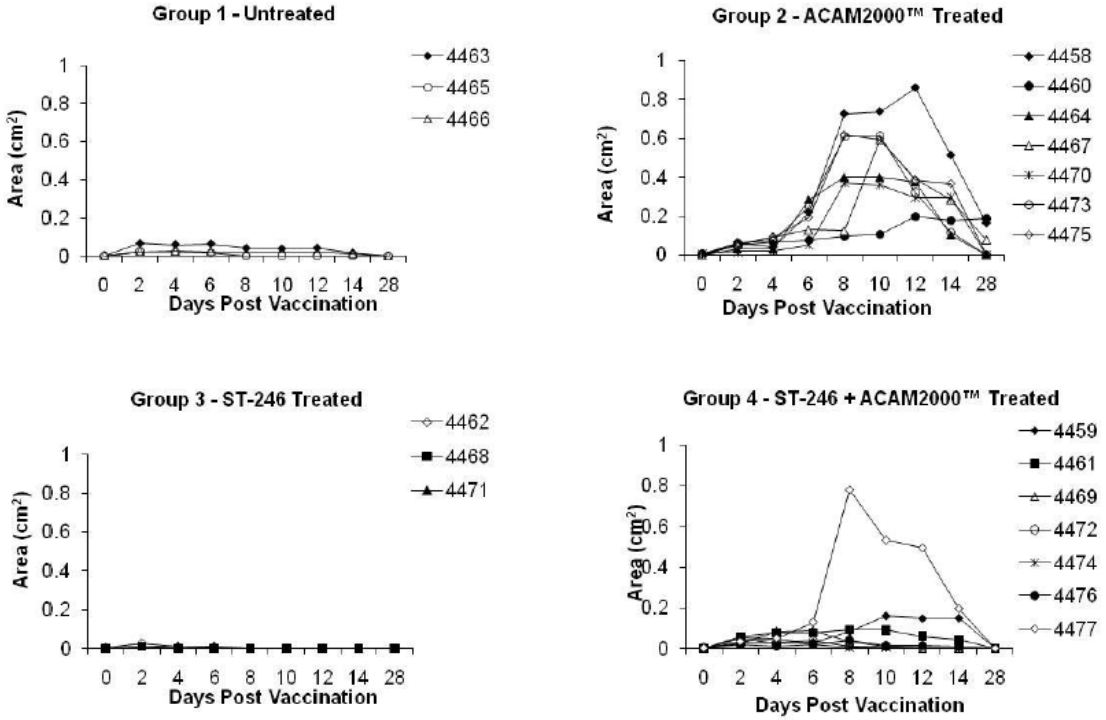


Figure 53. Vaccine site lesion development in study 11051.12 Part 2.

Based on survival alone, there was no evidence that tecovirimat interfered with vaccine efficacy (Table 22; adapted from study report pg. 26). All animals that received vaccine +/- tecovirimat survived to the end of study (28 days post-challenge). In contrast, all non-vaccinated animals were euthanized or found dead between 8 and 11 days post-challenge.

Table 22. Survival following MPXV challenge in study 11051.12 Part 2.

Group	Survival
Group 1: Untreated	0% (0/3)
Group 2: ACAM2000™ only	100% (6/6)*
Group 3: Tecovirimat only	0% (0/3)
Group 4: ACAM2000™ + Tecovirimat	100% (7/7)

*One animal (4475) was euthanized prior to MPXV challenge due to poor health.

Although there was no signal of tecovirimat vaccine interference based on survival, there was other evidence that tecovirimat may have had a modest effect on vaccine immunogenicity. As show in Figure 54 (study report pg. 34), none of the 6 animals that received vaccine alone had detected viral DNA levels in whole blood following MPXV challenge, whereas 5 of the 7 (71%) animals that received vaccine + tecovirimat had transiently quantifiable viral DNA (≥5,000 copies/mL) following MPXV challenge.

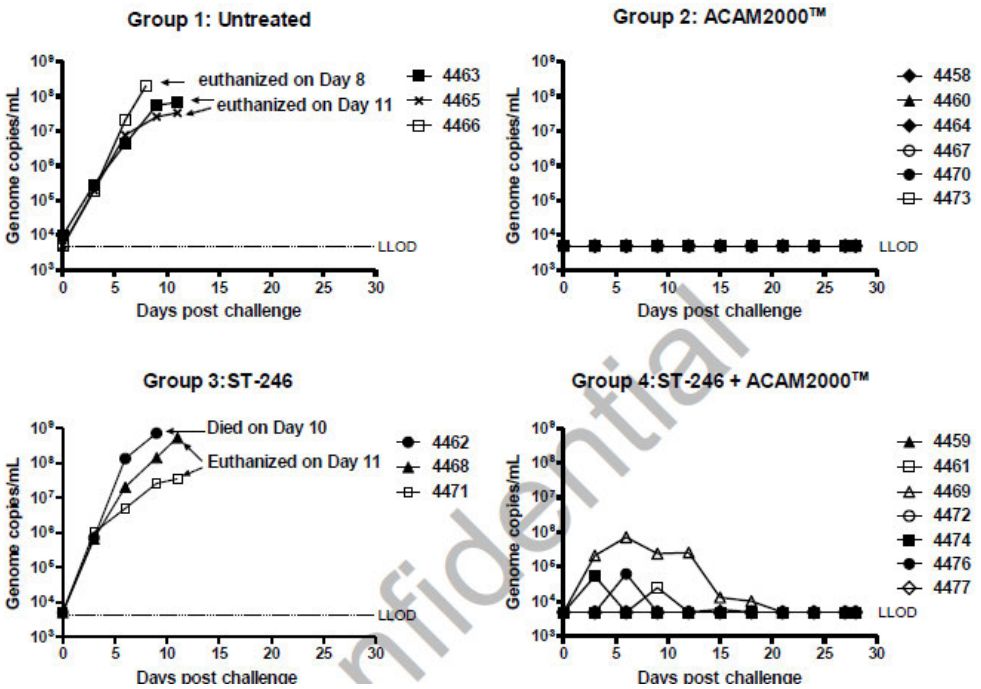


Figure 54. Whole blood MPXV DNA levels in study 11051.12 Part 2.

Pock lesion counts following MPXV challenge also trended higher in animals that received vaccine + tecovirimat compared to those that received vaccine alone (Table 23; report pg. 36). Although the lesions counts were not statistically different between these groups based on the sponsor's analyses, it is notable that 3 of 7 (43%) animals in the vaccine + tecovirimat group had total body lesion counts >300 (or too numerous to count), while the maximum total lesion count at any time in any animal that received vaccine alone was 31.

Table 23. Total lesion counts following MPXV challenge in study 11051.12 Part 2.

	Post-challenge Days												
	26 Sep 2008	29 Sep 2008	02 Oct 2008	04 Oct 2008	05 Oct 2008	07 Oct 2008	08 Oct 2008	11 Oct 2008	14 Oct 2008	17 Oct 2008	20 Oct 2008	23 Oct 2008	24 Oct 2008
Animal No.	D0	D3	D6	D8	D9	D11	D12	D15	D18	D21	D24	D27	D28
Group 1: Untreated													
4463*	0	0	TNTC	n.d.	TNTC	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4465*	0	0	TNTC	n.d.	TNTC	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4466*	0	0	TNTC	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Group 2: ACAM2000 Treated													
4458	0	0	3	n.d.	3	n.d.	3	1	0	0	0	0	0
4460	0	0	0	n.d.	0	n.d.	0	0	0	0	0	0	0
4464	0	0	29	n.d.	31	n.d.	2	0	0	0	0	0	0
4467	0	0	6	n.d.	11	n.d.	10	2	0	0	0	0	0
4470	0	0	7	n.d.	7	n.d.	1	0	0	0	0	0	0
4473	0	0	3	n.d.	3	n.d.	0	0	0	0	0	0	0
Group 3: ST-246 Treated													
4462*	0	0	TNTC	n.d.	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4468*	0	0	TNTC	n.d.	TNTC	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4471*	0	0	TNTC	n.d.	TNTC	TNTC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Group 4: ST-246 + ACAM2000 Treated													
4459	0	0	1	n.d.	1	n.d.	0	0	0	0	0	0	0
4461	0	0	9	n.d.	10	n.d.	7	5	2	2	0	0	0
4469	0	34	TNTC	n.d.	TNTC	n.d.	TNTC	TNTC	TNTC	TNTC	58	20	15
4472	0	0	4	n.d.	5	n.d.	5	1	0	0	0	0	0
4474	0	0	312	n.d.	302	n.d.	0	0	0	0	0	0	0
4476	0	29	TNTC	n.d.	373	n.d.	211	8	0	0	0	0	0
4477	0	0	0	n.d.	0	n.d.	0	0	0	0	0	0	0

Note: The values represent the total lesion counts in designated counting regions (head, arms (left and right), hand (left and right), leg (left and right), foot (left and right), groin, scrotum, genitalia, perianal, tail, chest, back, and abdomen); n.d. = not done; TNTC or Too Numerous To Count was assigned to a given macaque when any one counting region had greater than 200 lesions; Macaque 4466 euthanized on Day 8; 4462 was found dead on Day 10 due to severe MPXV disease; 4463, 4465, 4468, and 4471 were euthanized on Day 11.

Neutralizing antibody titers ($PRNT_{50}$) were not statistically different between animals that received vaccine + tecovirimat compared to those that received vaccine alone. Median (range) neutralizing antibody titers on the day of MPXV challenge (Day 45) were slightly lower at 55 (<10 to 1280) for the vaccine + tecovirimat group, compared to 208 (21 to 571) for the vaccine alone group. All animals that received vaccine +/- tecovirimat developed robust neutralizing antibody titers following MPXV challenge.

Interestingly, only a single animal across both vaccinated groups (Animal 4469, vaccine + tecovirimat) did not develop a measurable neutralizing antibody titer prior to MPXV challenge. Following MPXV challenge, this animal had the highest whole blood viral DNA levels, highest lesion counts, and greatest weight loss of all animals in the vaccine +/- tecovirimat groups. This animal also had the highest tecovirimat exposures of all animals in the vaccine + tecovirimat group, further supporting the interference of tecovirimat with vaccine-generated immune responses.

Despite the evidence of tecovirimat interference with the live ACAM2000™ VACV vaccine, again it is important to recognize that any interference with vaccine was only partial. By all measures (e.g., survival, viral DNA, lesion counts) animals that received vaccine + tecovirimat had reduced MPXV disease compared to non-vaccinated animals.

6.4 Study SR11051.12-Part 3 (Tecovirimat +/- IMVAMUNE® Vaccine, NHPs/MPXV Challenge)

Title
SR11051.12 Part 3, "Impact of Concurrent ST-246 Administration and Vaccination with MVA on Vaccination Efficacy in Cynomolgus Monkeys Study #3 (Task Order D15)"

Summary of Design
This study assessed if concurrent administration of tecovirimat plus the IMVAMUNE® vaccine in immunocompetent cynomolgus monkeys affected disease outcome following MPXV challenge. The study design is similar to study SR11051.12 Part 2, except for the IMVAMUNE® vaccine that was used. This vaccine, which was developed as a safer alternative to fully replication competent VACV vaccines, is based on the highly attenuated, modified vaccinia Ankara (MVA) strain. This strain was passaged more than 570 times in chicken embryo fibroblast cells, and nearly 15% of the VACV genome was lost, rendering the virus incapable of fully replicating in most mammalian cell lines, including human cells (Sánchez-Sampedro et al., 2015). The virus can infect human cells and express viral proteins to stimulate an immune response, but the replication cycle does not complete and the virus cannot spread to other cells, as only immature virions are formed; note that the virus has an intact F13L gene (Sánchez-Puig and Blasco, 2005). Given that tecovirimat prevents viral spread but has no effect on viral protein expression in cells that are already infected, one would not predict tecovirimat would interfere with the IMVAMUNE® vaccine.

A total of 20 animals were assigned to each of 4 study groups according to sex and body weight. The treatment groups are summarized in Table 24 (adapted from study report pg. 16). Tecovirimat or vehicle was administered by oral gavage for 14 days starting on Day 0. Vaccine or diluent was administered by subcutaneous injection on Day 0. A lethal IV challenge dose of MPXV Zaire '79 strain was administered on Day 45. Clinical disease signs, viral DNA levels and neutralizing antibody titers were monitored throughout the study.

Table 24. 11051.12 Part 2 study groups.

Group	N	Tecovirimat (Day 0-13)	Vaccine (Day 0)	MPXV Challenge PFU (Day 45)
1	3	Vehicle	Mock	5×10^7
2	7	Vehicle	IMVAMUNE®	5×10^7
3	3	Tecovirimat 10 mg/kg	Mock	5×10^7
4	7	Tecovirimat 10 mg/kg	IMVAMUNE®	5×10^7

Results

Based on survival, there was no evidence that tecovirimat interfered with vaccine efficacy (Table 25; adapted from study report pg. 25). For both groups of animals that received vaccine +/- tecovirimat, 71% (5/7) survived to the end of study; all 4 deaths occurred by Day 12 post-challenge. In contrast, all non-vaccinated animals were euthanized between Day 8 and Day 11 post-challenge.

Table 25. Survival following MPXV challenge in study 11051.12 Part 3.

Group	Survival
Group 1: Untreated	0% (0/3)
Group 2: IMVAMUNE® only	71% (5/7)
Group 3: Tecovirimat only	0% (0/3)
Group 4: IMVAMUNE® + Tecovirimat	71% (5/7)

Other disease measures did not indicate tecovirimat interference with vaccine efficacy, although it is noted that the IMVAMUNE® vaccine protocol used in this study appeared to be less immunogenic and effective compared to the ACAM2000™ vaccine used in study 11051.12 Part 2. Whole blood MPXV DNA levels were generally similar in the vaccine +/- tecovirimat groups (Figure 55; study report pg. 32). Lesion counts were similar and quite high, with nearly all animals across the 4 groups having lesions too numerous to count on Day 6 and Day 9 post-challenge. Neutralizing antibody levels prior to MPXV challenge were generally low in the vaccine +/- tecovirimat groups.

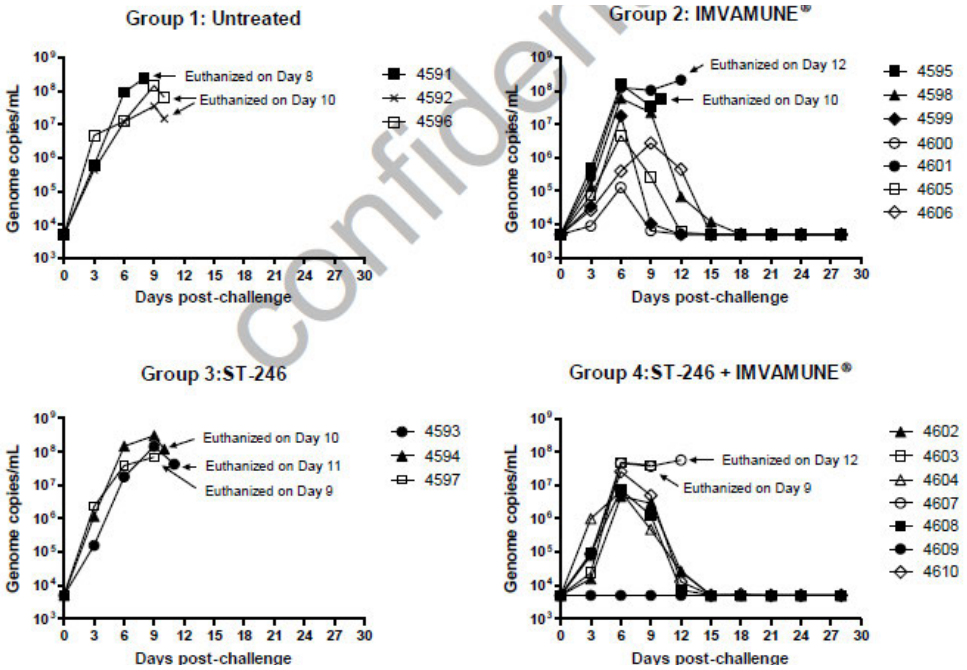


Figure 55. Whole blood MPXV DNA levels in study 11051.12 Part 3.

6.5 Study SR11-011F (Tecovirimat +/- Vaccine in SHIV-Infected NHPs/MPXV Challenge)

Title

SR11-011F, "Effect of ST-246 Co-administration on ACAM2000® Reactogenicity and Immunogenicity in SHIV-Immunocompromised Nonhuman Primates"

Summary of Design

Study SR11-011F evaluated the safety and efficacy of ACAM2000™ vaccine, with or without tecovirimat co-administration, in Indian rhesus macaques infected with Simian Human Immunodeficiency Virus (SHIV) strain 89.6P as a means to induce immunosuppression. Twenty animals were randomized into 5 different treatment groups as shown in Table 26 (study report pg. 16). On Day 0, animals in Groups 1-3 were challenged with SHIV. On Day 28, animals were vaccinated (or mock vaccinated) with the ACAM2000™ vaccine with or without co-administration of tecovirimat (ST-246) or placebo which continued for a total of 14 days. On Day 60, all animals were challenged IV with a target dose of 5 x 10^7 PFU of the MPXV '79 Zaire strain. Immunologic markers, SHIV RNA and MPXV DNA levels, MPXV neutralizing antibody titers, lesion counts/analyses, and clinical signs were monitored throughout the study.

Table 26. Treatment groups in study SR11-011F.

Group	N	[1]SHIV Challenge Dose	[2]Vaccination	[3]ST-246 Dose	[4]Dosage schedule	[5]MPXV Challenge Dose
1 - Unvaccinated	4	1:100 dilution	Mock Vaccination	Placebo	14 Daily Oral Doses	5 x 10^7 PFU IV
2 - ACAM2000® + Placebo	5	1:100 dilution	ACAM2000®	Placebo	14 Daily Oral Doses	5 x 10^7 PFU IV
3 - ACAM2000® + ST-246	5	1:100 dilution	ACAM2000®	ST-246	14 Daily Oral Doses (10 mg/kg)	5 x 10^7 PFU IV
4 - ACAM2000® + Placebo	3		ACAM2000®	Placebo	14 Daily Oral Doses	5 x 10^7 PFU IV
5- ACAM2000® + ST-246	3		ACAM2000®	ST-246	14 Daily Oral Doses (10 mg/kg)	5 x 10^7 PFU IV

[1]The virus challenge inoculum was prepared using a 1:100 dilution of the stock virus in 1 mL of challenge virus diluent and administered to Groups 1 to 3. Groups 4 and 5 remained uninfected and administered the virus diluent for SHIV.
[2]Group 1 was vaccinated via percutaneous scarification with the vaccine diluent supplied with ACAM2000®; Groups 2 to 5 were vaccinated in the same manner as Group 1, but with the reconstituted ACAM2000® vaccine.
[3]Groups 1, 2, and 4 were dosed with the vehicle used in the preparation of ST-246 via oral gavage, thus serving as Placebo controls. Groups 3 and 5 were administered ST-246 at 10 mg/kg via oral gavage.
[4]Dosing of Placebo or ST-246 was administered immediately following vaccination, and thereafter, dosed daily for 13 consecutive days (from Study Days 28 to 41).
[5]All groups were challenged via the intravenous (IV) route with a target dose of 5 x 10^7 PFU of MPXV in 1 mL of challenge inoculum on Study Day 60.

Results

All animals in Groups 1-3 demonstrated an active and pathogenic SHIV infection based on viremia and rapid declines in $CD4^+$ T cell levels. Following vaccination, all animals in Groups 2-5 developed a pock lesion at the site of administration, although lesion sizes were affected both by the immune status of the animals as well as the concomitant exposure to tecovirimat (Figure 56; study report pgs. 31-32). In both the SHIV infected and uninfected animals, vaccine lesion sizes were smaller and resolved more quickly in those that received concomitant tecovirimat treatment. Secondary lesions developed in a subset of SHIV-infected animals (4/5 and 2/5 animals in Groups 2 and 3, respectively).

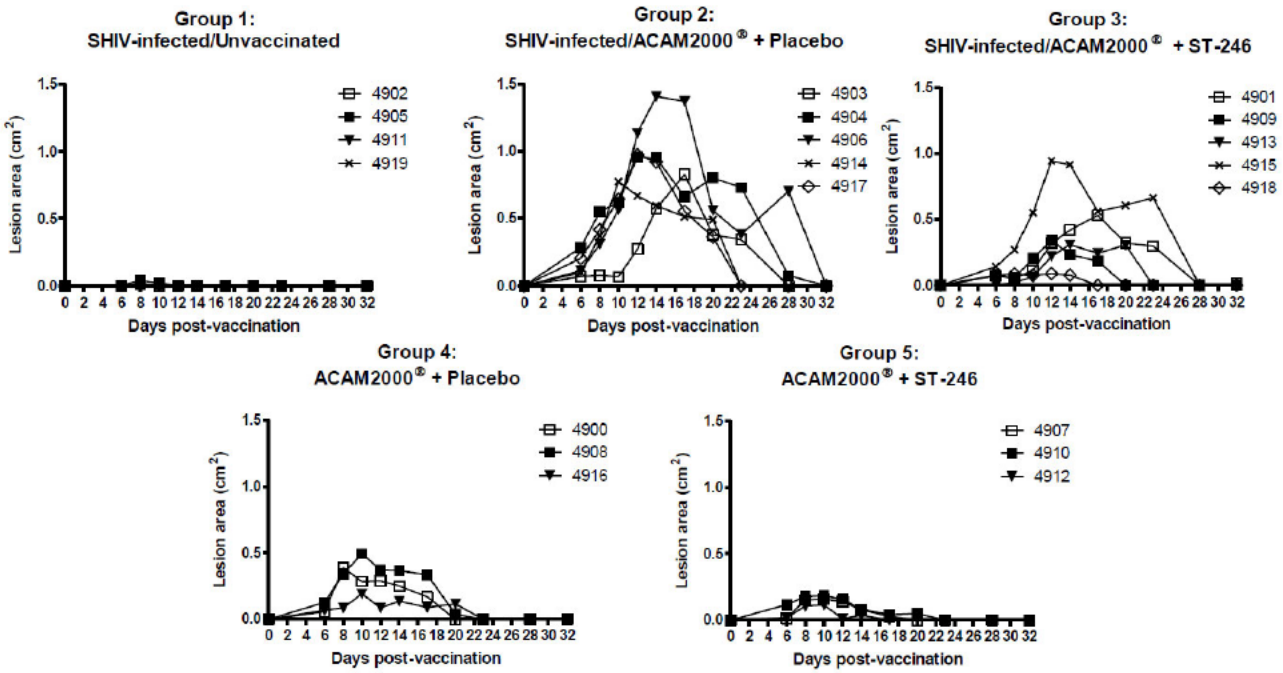


Figure 56. Lesion sizes following ACAM2000™ vaccination (or mock vaccination) in SR11-011F.

Although tecovirimat clearly reduced vaccine reactogenicity, how this might impact vaccine efficacy in the setting of immune deficiency ultimately could not be established, as the vaccine was poorly effective in the SHIV-infected animals regardless of whether tecovirimat was co-administered with vaccine. Only a single SHIV-infected macaque developed a measurable neutralizing antibody titer following vaccination, while all SHIV-uninfected animals developed at least a low-level MPXV neutralizing antibody titer. Similarly, SHIV-infected animals developed weak VACV-specific T cell responses (interferon-γ ELISPOT assay) following vaccination. Following MPXV challenge, all vaccinated, SHIV-uninfected animals (Groups 4+5) survived to the end of study. In contrast, most vaccinated/SHIV-infected animals (Groups 2+3) died or were euthanized as a result of MPXV infection (Figure 57; study report pg. 49). Not surprisingly, none of the unvaccinated animals (Group 1) survived to the end of study.

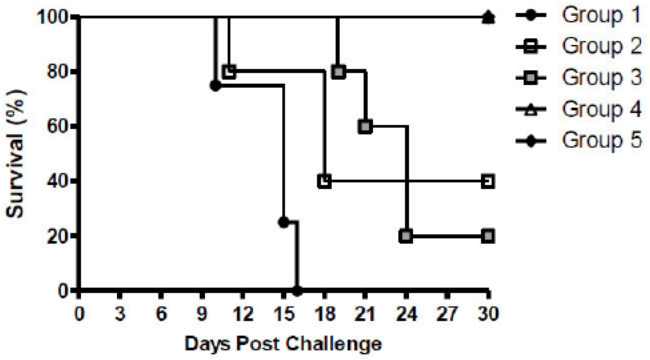


Figure 57. Survival outcomes following MPXV challenge in study SR11-011F.

Among SHIV-uninfected animals in the study (n=3 per group), no clear trends of an impact of tecovirimat co-administration with vaccine were observed based on survival outcomes, MPXV neutralizing antibody titers, or MPXV DNA levels in blood. However, consistent with Study 1218-100004544, at the peak time point of lesion development following MPXV challenge (Day 9 post-challenge), Group 5 animals that had received vaccine plus tecovirimat had a mean (standard error) total body lesion count of 78 (62.8) compared to 3 (0.6) for those in Group 4 that received vaccine alone (Group 4).

7. OTHER SUPPORTING ANIMAL MODEL STUDIES

In addition to the studies summarized above, the sponsor conducted numerous other pilot or exploratory animal model studies of tecovirimat antiviral activity. These additional animal studies are summarized briefly in Appendix A. Although many of these studies were small and exploratory in scope, in general they further confirm the broad anti-orthopoxvirus activity of tecovirimat. Studies 246-PC-030, 246-PC-032 and 246-PC-033 using a highly pathogenic ectromelia virus mouse model (recombinant ECTV Moscow strain expressing interleukin-4) also demonstrated the potential utility of combined treatment with tecovirimat plus cidofovir or CMX001.

8. CLINICAL VIROLOGY

No clinical trials evaluating tecovirimat efficacy have been conducted, although tecovirimat has been provided for emergency use under E-IND for 5 cases in the U.S., and also for 1 case in Finland. Summaries of these cases as reported in the sponsor's Summary of Clinical Pharmacology Studies and related publications are provided below.

8.1 Progressive VACV Infection from Smallpox Vaccine (E-IND 104793)

From a virology perspective, this case study represents the best characterized clinical use of tecovirimat. In (b) (6) a 20-year-old male in the military who recently received the smallpox vaccine developed progressive vaccinia, which is a rare and typically fatal adverse event following smallpox vaccination. The patient received the ACAM2000™ smallpox vaccine on (b) (6) and on (b) (6) presented to a community hospital with fever and headache, and was subsequently diagnosed with acute myelogenous leukemia (AML). At the time, the patient had a 1-cm asymptomatic vesicle at the vaccination site, but then the patient had to start aggressive chemotherapy (cytarabine and idarubicin) for his malignancy. On (b) (6) the patient had a persistent and enlarged vaccine lesion and additional symptoms, and VACV infection was subsequently confirmed by PCR and viral culture from lesion swab material, and the patient was diagnosed with progressive vaccinia. Note that the cytarabine in the chemotherapy regimen is a cytosine analogue (cytosine arabinoside) and has activity in cell culture against VACV, but with inconsistent activity in mouse models (see the Clinical Virology review of IND 116039 SDN 10 by Dr. Jules O'Rear for more details).

The patient's disease and treatment courses, as well as key immunologic and virologic laboratory findings, are summarized in Figure 58 (from Lederman et al., 2012). The patient first received vaccinia immune globulin intravenous (VIGIV) starting on (b) (6), and oral and topical tecovirimat were initiated on (b) (6) and (b) (6), respectively. On (b) (6), the patient became septic due to a bacterial infection and initiated stress-dose corticosteroids, and tecovirimat was withheld for 24 hours. Tecovirimat was subsequently reinitiated and doses were periodically increased following identification of plasma drug concentrations that were lower than expected based on data from healthy volunteers. Reported tecovirimat plasma concentrations ranges were 0.034-0.795 µM (12.9-299 ng/mL) with the 400 mg dose administered (b) (6) 0.143-1.1 µM (53.75-408 ng/mL) with the 800 mg dose administered (b) (6), and 0.291-3.7 µM (109.5-1,395 ng/mL) with the 1,200 mg dose administered (b) (6).

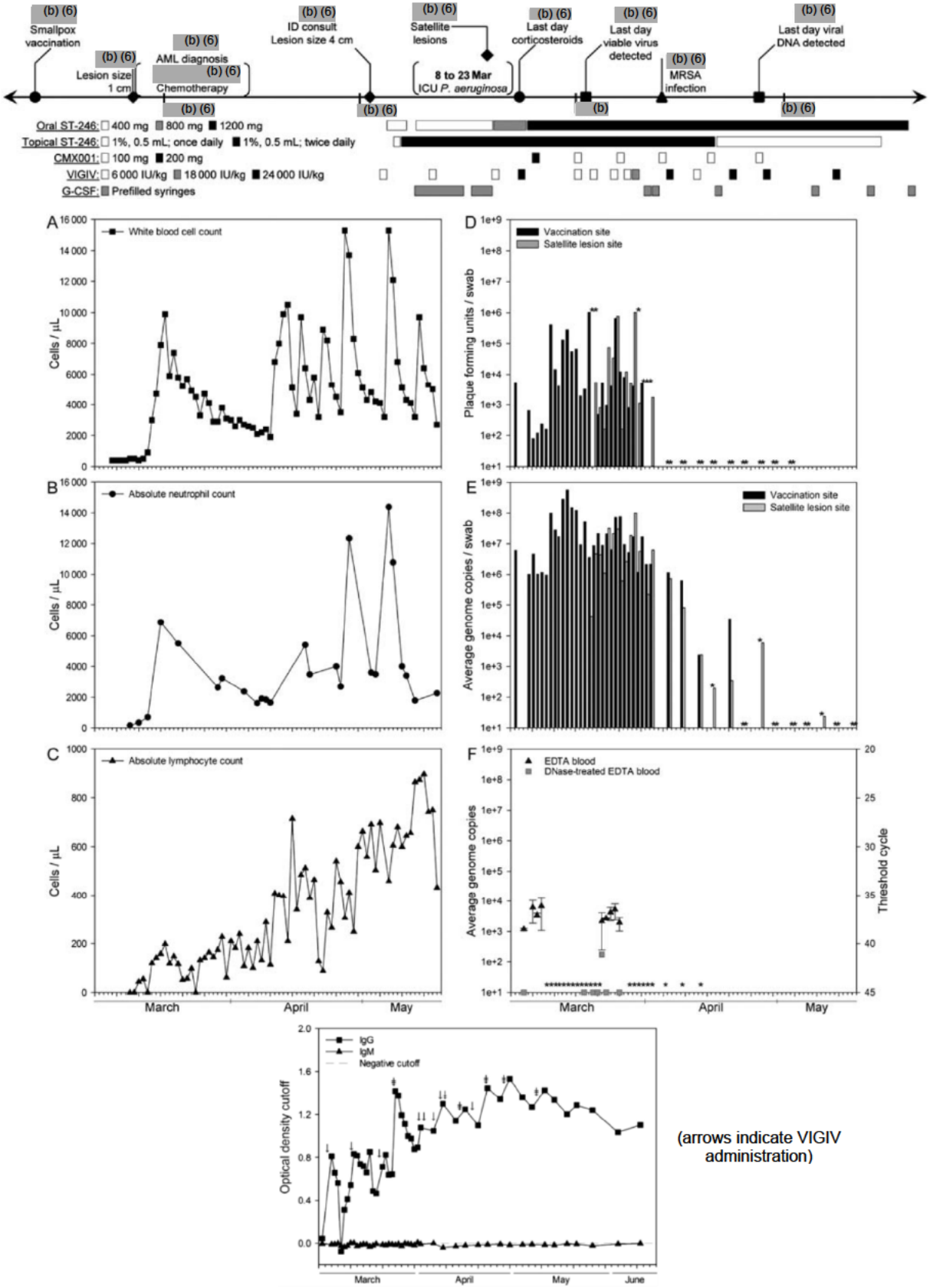


Figure 58. Disease, treatment course, and laboratory results from progressive vaccinia case.

The vaccination site began to respond during treatment but then satellite lesions were noted on (b) (6) Corticosteroid therapy was slowly tapered and periodic granulocyte colony-stimulating factor was administered. On (b) (6), the patient initiated oral CMX001, which is an investigational prodrug of cidofovir (viral DNA polymerase inhibitor). Higher doses of VIGIV and tecovirimat were also administered around this time. The patient was intermittently neutropenic and/or lymphopenic. The vaccine site gradually improved, although a bacterial superinfection developed around (b) (6) but the site resumed healing after treatment with IV vancomycin. On (b) (6) the patient had below-the-knee amputation on both legs due to gangrene as a consequence of pseudomonal sepsis. The patient was eventually discharged in stable condition on (b) (6), subsequently received an allogeneic stem cell transplantation for AML, and remained free of VACV infection.

The individual contribution of the antiviral drugs towards the eventual viral 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 through (b) (6), which was 28-29 days after first starting VIGIV and tecovirimat, and 7 days after starting CMX001. Immune cell counts also improved during this period. Viral DNA continued to remain detected, but at declining levels, through (b) (6). Relatively low levels of viral DNA were detected in whole blood at selected timepoints, and in most cases the detected blood viral DNA was sensitive to DNAse I pre-treatment prior to DNA extraction, indicating that the circulating viral DNA was not likely encapsidated within intact viral particles. Viral DNA was not detected in any oropharyngeal or rectal swab samples.

Of particular interest, there was strong evidence of emergence of tecovirimat-resistant VACV in the patient. The tecovirimat EC_{50} value in cell culture for a viral isolate derived from the vaccine site on (b) (6) (pre-tecovirimat) was 0.07 µM. By (b) (6) and (b) (6) the tecovirimat EC_{50} values for vaccine site isolates increased to 0.95 µM (13.5-fold-change) and 3.55 µM (50.7-fold-change), respectively. Minimal changes in tecovirimat susceptibility were observed for virus isolates from satellite lesions, although only 4 satellite lesions were swabbed. No CMX001 phenotypic resistance was observed following CMX001 treatment. Resistance analyses of viral populations in blood were not conducted or reported. It is important to note that these phenotypic analyses for drug susceptibility were conducted on mixed population viral isolates that were first twice-passaged at an MOI of 0.05. The passaging of virus at a low MOI in the absence of drug could enrich for drug-sensitive virus if any drug resistant viruses have a fitness impairment, and therefore these assessments could under-estimate the true level of phenotypic resistance of viral populations.

Next generation sequencing analyses (0.1% sensitivity cutoff) also identified tecovirimat treatment-emergent genetic changes in the VACV F13L gene, specifically amino acid substitutions A290V and L315M. Neither of these substitutions were detected in a (b) (6) (pre-tecovirimat) vaccine site isolate or in the ACAM2000™ vaccine. However, A290V and L315M were detected in 3.38% and 7.9% of sequences, respectively, from a (b) (6) (18 days on-tecovirimat) vaccine site isolate, and were further enriched at 31.29% and 10.27%, respectively, in a (b) (6) (26 days on-tecovirimat) isolate. Consistent with the phenotypic resistance assessments, these amino acid substitutions were not detected in satellite lesion sites. Subsequent site-directed mutagenesis and phenotypic analyses of recombinant VACV-ACAM2000™ strains with these amino acid substitutions indicated that A290V, L315M and A290V+L315M substitutions reduced the tecovirimat EC_{50} value by 31-, 1- (i.e., no effect) and 94-fold, respectively. Like the clinical phenotypic analyses summarized above, the genotypic analyses conducted for this case could have under-estimated the true level of tecovirimat resistance, as the analyses were restricted to VP37 amino acid positions 259-315; as described in greater detail in Section 2.3, other tecovirimat resistance-associated substitutions have been identified outside of this region.

Taken together, and consistent with studies in immunodeficient animal models (see Section 5), results from this case of progressive vaccinia indicate that tecovirimat alone or in combination with polyclonal VACV immune globulin 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. Nevertheless, the emergence of tecovirimat-resistant virus indicates that the drug was at least partially active against the initial drug-sensitive viral population. In retrospect, combination treatment with tecovirimat and CMX001 ideally would have been initiated at the same time to reduce the risk of tecovirimat resistance selection and increase the overall antiviral durability of the treatment regimen.

8.2 Child Exposed to VACV from Smallpox Vaccine (E-IND 74773)

In [redacted] (b) (6), SIGA provided tecovirimat to treat a 28-month-old child with eczema vaccinatum. The child had a history of "eczema and failure to thrive" and was exposed to VACV through direct contact with his father who had recently received the smallpox vaccine. The child presented to an emergency room with high fever and severe eczema. The child initially received a diagnosis of eczema herpeticum with bacterial superinfection and initiated treatment with clindamycin and acyclovir. On hospital Day 6, VACV was first detected and VIGIV was first administered (along with a variety of other supportive care procedures). Figure 59 (from Vora et al., 2008) summarizes the antiviral treatments administered and antibody and blood viral DNA levels observed starting on hospital Day 6 and throughout the disease course.

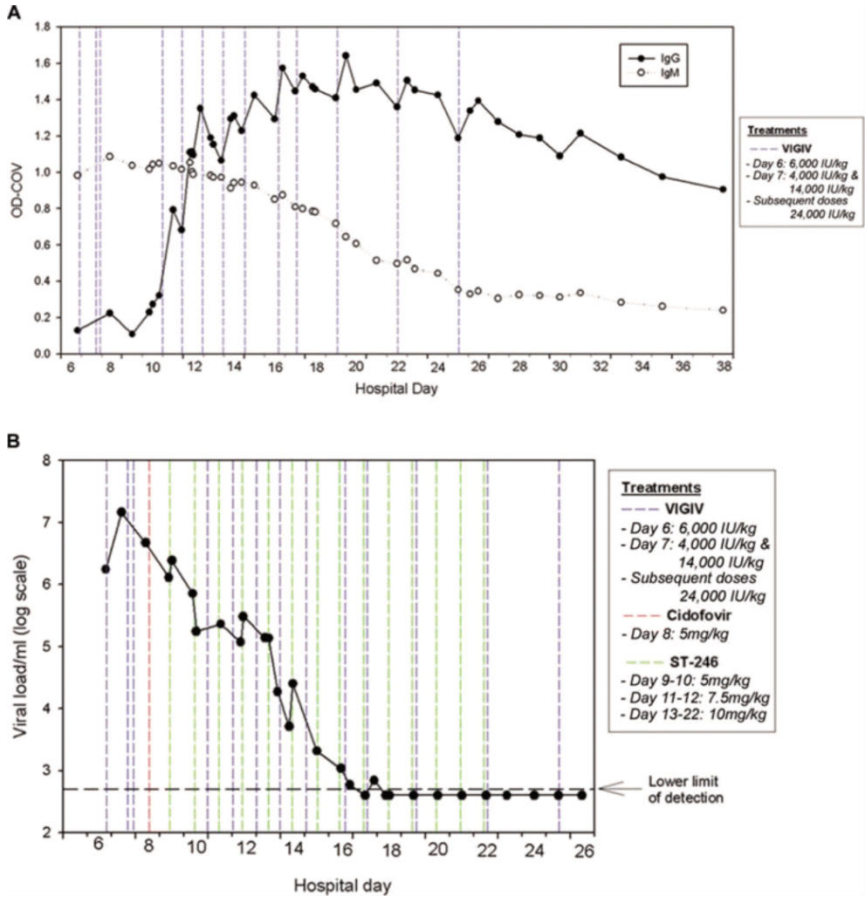


Figure 59. Antibody levels (A) and blood VACV DNA levels (B) in a hospitalized child with eczema vaccinatum. Tecovirimat was first started on Day 9.

On Day 7, skin lesions were continuing to spread and additional VIGIV was administered, and by Day 8 his condition worsened. Cidofovir (viral DNA polymerase inhibitor) was administered on Day 8. Tecovirimat (initially 5 mg/kg by nasogastric tube) was started on Day 9. Both VIGIV and tecovirimat treatment continued for several more days, and the tecovirimat dose was increased based on plasma PK with the aim of reaching a target concentration of 2.7 µM (1,000 ng/mL). Ultimately viral DNA levels in blood peaked around Day 7, the child's condition began to improve after Day 8, and the child was discharged on Day 48. To this reviewer's knowledge, no drug resistance assessments were conducted.

The contribution of tecovirimat to the patient's clinical improvement in this case was unclear because of the administration of 3 different antiviral agents (VIGIV, cidofovir, tecovirimat), along with a decreasing viral DNA load at the time of tecovirimat initiation, intensive supportive care, and apparent induction of the patient's own immune response.

8.3 Other Human Experience with Tecovirimat Treatment

The four other cases of human use of tecovirimat are less well characterized from a Clinical Virology perspective, and again the role of tecovirimat in treating the orthopoxvirus infections was unclear due to confounding treatments and procedures. These cases are summarized briefly as follows:

- E-IND 116039 (additional details from this case are included in the Clinical Virology review of IND 116039 SDN 10 by Dr. Jules O'Rear): A male military service member developed complications after receiving the ACAM2000™ vaccine in (b) (6), including worsening of the vaccine lesion site and the development of additional lesions. The patient was diagnosed with acute myeloid leukemia (AML) on (b) (6). Because of concerns about the patient's AML and expected immunosuppression while receiving chemotherapy, the patient received tecovirimat 600 mg BID starting on (b) (6). The patient received chemotherapy consisting of cytarabine and daunorubicin starting on (b) (6). The patient also received VIGIV (6,000 IU/kg) on (b) (6). Numerous other concomitant medications and procedures to treat AML were conducted over the subsequent months. The patient also received prophylactic administration of the anti-herpesvirus drug acyclovir, which may also have anti-VACV activity. Viral DNA (~10^4-10^8 genome copies/swab) was detected at the vaccine site or other lesions at various times between (b) (6) without sufficient sampling to characterize viral dynamics during treatment. Viral DNA was not detected in blood or bone marrow samples throughout the disease course.

- E-IND 106338: A woman with Crohn's disease undergoing immunosuppressive therapy with Imuran® (azathioprine; cytotoxic purine analogue) and Remicade® (infliximab; anti-TNFα antibody) developed several red papules on her hand after being exposed to a recombinant vaccinia virus-based rabies vaccine in a bait-sponge found the by patient's dog. She was advised to stop taking her immunosuppressive medications, and samples of her papules tested positive for non-variola orthopoxvirus DNA. On Day 6 the papules had increased in number and size, and the patient was administered a dose of VIGIV (6,000 IU/kg), and on Day 12 a second dose of VIGIV (6,000 IU/kg) was administered, and tecovirimat 400 mg QD was initiated and continued for 14 days. On Day 13 the patient began receiving phased reintroduction of her immunosuppressive medications. The patient was discharged on Day 19, and by Day 28 all scabs had separated. Interestingly, the patient remained orthopoxvirus IgM-negative throughout her illness. No further virology-related details are available.

- E-IND 112324: An immunocompetent woman developed suspected VACV lesions after being in contact with a vaccination site of a military contractor. The patient received VIGIV, and 5 days

later started tecovirimat 400 mg QD, and ultimately the lesions healed with minimal scarring. No virology-related details were provided.

- Suspected cowpox virus case in Finland (see also Kinnunen et al., 2015 and Study Report 706 for further details): A woman started tecovirimat 400 mg QD for 14 days in (b) (6) after developing severe keratoconjunctivitis and testing PCR-positive for ocular CPXV. The keratoconjunctivitis did not resolve after tecovirimat treatment, and the patient remained PCR-positive for orthopoxvirus DNA, although an identifiable orthopoxvirus could never be cultured. Concentrations of tecovirimat in tears collected at 1 and 21 hours post-dose on treatment Day 14 were relatively low at 170 nM (64 ng/mL) and 66 nM (25 ng/mL), and ~5-fold lower than in blood at the ~21-hour timepoint. A variety of other treatments and amniotic membrane transplantation procedures were conducted before the patient's condition ultimately improved. The sponsor speculated that active CPXV infection was resolved prior to the start of tecovirimat. Furthermore, herpesvirus-like structures, but not poxvirus-like structures, were observed by histopathology indicating that a herpesvirus may have been the primary cause of disease.

9. 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 sponsor included in the completed NDA submission (SDN 3) a draft protocol synopsis for field trial (b) (4)

(b) (4)

(b) (4)

[redacted]

10. CONCLUSIONS

This Original NDA is approvable from a Virology perspective for the treatment of human smallpox disease caused by variola virus.

11. PACKAGE INSERT

Due to the timing of NDA milestones and PDUFA goal deadlines, the final approved package insert was not available at the time of finalization of this review. Section 12.4 of the proposed TPOXX™ label and suggested edits are shown below (as of 5/4/2018). (b) (4) [redacted] In addition to the edits shown below for Section 12.4, other package insert recommendations included:

- Proposed tecovirimat established pharmacologic class: "orthopoxvirus VP37 envelope wrapping protein inhibitor"

- Section 1-Limitation of Use: "TPOXX efficacy may be reduced in immunocompromised patients based on studies demonstrating reduced efficacy in immunocompromised animal models."
- Section 7.4-Vaccine Interactions: "No vaccine-drug interaction studies have been performed in human subjects. 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."
- Other editorial suggestions in Section 14.

12.4 Microbiology

Mechanism of Action

Tecovirimat (b) (4) targets and inhibits the activity of the orthopoxvirus (b) (4) VP37 protein (encoded by and highly conserved in all members of the orthopoxvirus genus) and blocks its interaction with cellular Rab9 GTPase and TIP47, which prevents the formation of egress-competent enveloped virions necessary for cell-to-cell and long-range dissemination of virus.

Activity in Cell Culture- (b) (4)

(b) (4)

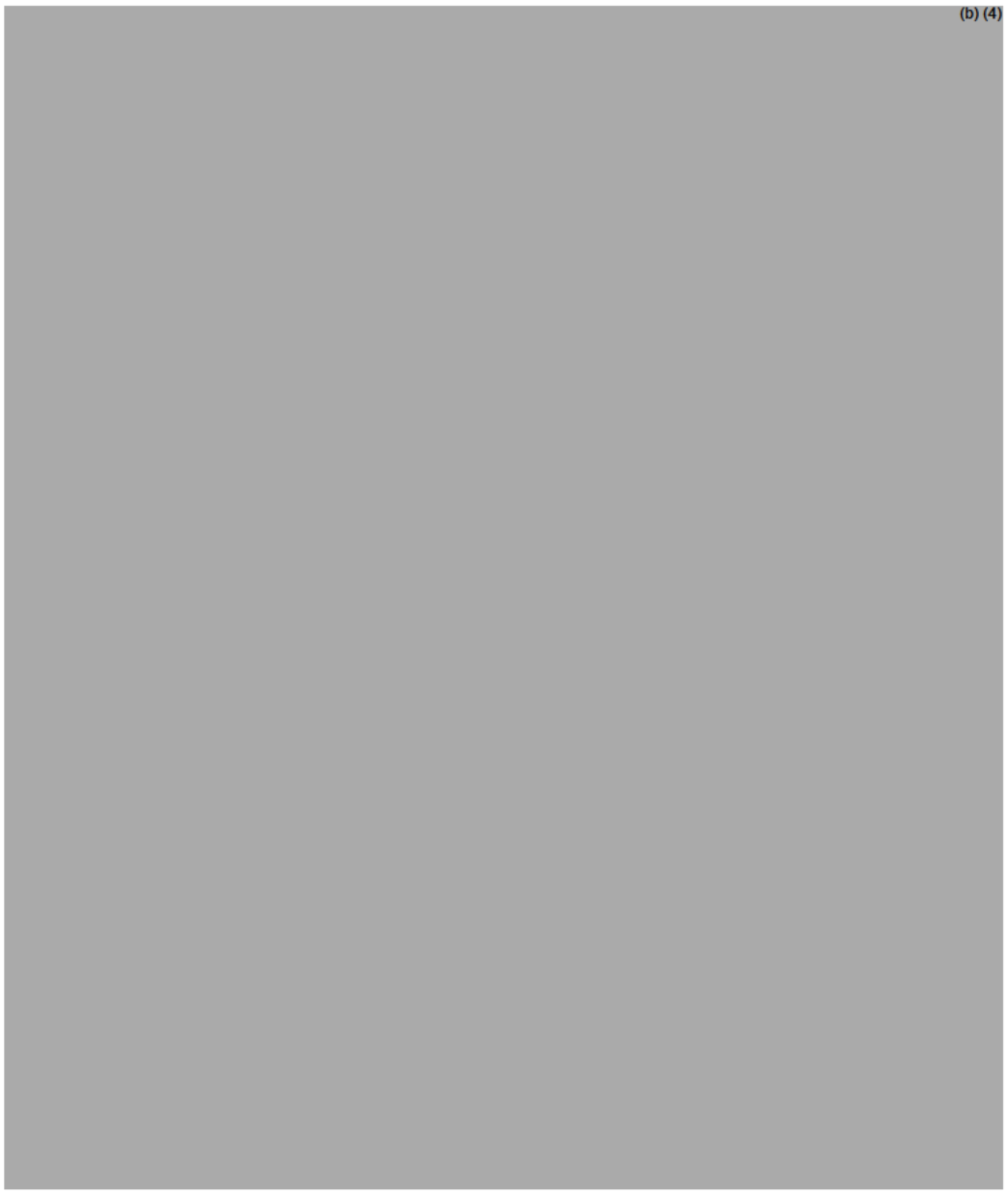
(b) (4) -In cell culture (b) (4) the effective concentrations of tecovirimat resulting in a 50% reduction in virus-induced cytopathic effect (EC_{50}), were 0.016-0.067 µM, 0.014-0.039 µM (b) (4) 0.015 µM, (b) (4) -and (b) (4) 009 µM, for variola, monkeypox, (b) (4) -rabbitpox, (b) (4) -and (b) (4) (b) (4) viruses are reflective of results from multiple strains assayed. (b) (4)

(b) (4)

Resistance

There are no known instances of naturally occurring tecovirimat resistant orthopoxviruses, although tecovirimat resistance may develop (b) (4) under drug selection. Tecovirimat has a relatively low resistance barrier, and certain (b) (4) amino acid substitutions in the target (b) (4) VP37 protein can confer (b) (4) large reductions in tecovirimat antiviral activity. The possibility of resistance to tecovirimat 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: There are no other antiviral drugs approved for the treatment of variola (smallpox) virus infections.

12. RECOMMENDATIONS

We recommend the following post-marketing commitment (PMC). See Section 2.4 of this review for specific VP37 amino acid polymorphisms of interest. The final PMC wording and study milestones were still under negotiation with the sponsor at the time of finalization of this review.

> Conduct cell culture studies to characterize tecovirimat antiviral activity against an expanded panel of variola virus isolates and recombinant vaccinia viruses. These studies should capture the known VP37 amino acid heterogeneity in variola viruses, as well as a common orthopoxvirus VP37 polymorphism, and should also include multiple independent isolates with identical VP37 amino acid sequences.

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14. APPENDICES

Appendix A. Additional supporting animal model studies of tecovirimat

Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
Monkeypox Virus/NHP Studies						
AP-06-021G	Double Blind, Randomized, Placebo-Controlled, Repeat-Dose Efficacy Study of the Therapeutic Window of the Proposed Primate Equivalence of the Human Dose (400 mg/day) of Oral ST-246 Polyform I in Cynomolgus Monkeys Infected With Monkeypox Virus	Monkeypox Virus-Zaire '79, 5 x 10^7 PFU i.v.	Cynomolgus Monkeys	16	Tecovirimat 10 or 20 mg/kg QD oral for 14 days starting on Day 3 or Day 4 post-challenge, or placebo. Randomized, blinded, GLP study.	All tecovirimat-treated animals survived to end of study, and all 4 placebo-treated animals were euthanized early. Lower viral DNA levels in blood and tissues, and reduced lesion counts, in tecovirimat- vs. placebo-treated animals. Deviation of dosing for one animal due to vomiting. Placebo-treated animals did not meet protocol-specified euthanasia criteria, but had moribund disease.
AP-06-21E6	Evaluation of Oral ST-246 in the Lesional Monkeypox Cynomolgus Monkey Model (Pilot Study)	Monkeypox Virus-Zaire '79, 5 x 10^7 PFU i.v.	Cynomolgus Monkeys	6	Tecovirimat 10 mg/kg or placebo QD oral for 14 days, starting on Day 5 post-challenge	All tecovirimat-treated animals survived to the end of study, while the placebo-treated animal was euthanized due to moribund disease. Lower viral DNA levels in blood in tecovirimat-treated animals relative to placebo-treated animal. No clear differences in lesion counts.
AP-06-21-MPX	Evaluation of Oral ST-246® in the Lesional Monkeypox Cynomolgus Monkey Model	Monkeypox Virus-Zaire '79, 5 x 10^7 PFU i.v.	Cynomolgus Monkeys	23	Tecovirimat 3-300 mg/kg QD oral, or placebo, for 14 days starting on Day 1 (300 mg/kg only) or Day 3 (all doses) post-challenge. Blinded study.	All tecovirimat-treated animals survived, while all placebo-treated animals died or were euthanized. Lower blood viral DNA levels, and fewer lesions, in all tecovirimat treatment groups relative to placebo.
FY06-035	ST-246: Efficacy Evaluation of an Antiviral Agent in an Intravenous Monkeypox Virus Challnge(sic) Model Using Cynomolgus Macaques (Macaca fascicularis)	Monkeypox Virus-Zaire '79, 1 x 10^7 PFU i.v.	Cynomolgus Monkeys	12	Tecovirimat 30 or 300 mg/kg QD oral or placebo for 14 days starting 1 hr post-challenge.	Tecovirimat treatment prevented clinical disease, while all 4 placebo-treated animals had clinical disease and 2 were euthanized. Pox lesions not observed in any tecovirimat-treated animals, but were observed in placebo-treated animals. No viral DNA detected in tecovirimat-treated animals.
FY11-096	Evaluation of Aerosol Monkeypox Model for Testing Antivirals	Monkeypox Virus-Zaire '79, ~1 x 10^5 PFU aerosol	Cynomolgus Monkeys	30	Tecovirimat 10 mg/kg QD oral for 14 days, or placebo, starting on Day 1-4 post-challenge	All tecovirimat-treated animals survived to the end of study, while 3/4 placebo-treated animals died. Tecovirimat-treated animals had lower blood viral DNA levels and reduced lesion counts relative to controls. Higher blood viral DNA levels in animals that started tx on Day 3 or 4 relative to those that started treatment on Day 1 or 2. Surviving animals developed MPXV nAb, with the highest titer in the single surviving control animal.
FY11-096A	Evaluation of Aerosol Monkeypox Model for Testing Antivirals: Delayed Treatment Study	Monkeypox Virus-Zaire '79, ~1 x 10^5 PFU aerosol	Cynomolgus Monkeys	32	Tecovirimat 10 mg/kg QD oral for 14 days, or placebo, starting on Day 4-8 post-challenge. Blinded study.	All tecovirimat-treated animals that started treatment on Day 4 or Day 5 survived; 4/6 in Day 6 group, 6/6 in Day 7 group, and 3/6 in Day 8 group survived. 3/4 animals in control group died. No clear trend of treatment affecting viral DNA levels in whole blood, pharyngeal swabs or tissues. Variable lesion counts. Surviving animals developed MPXV nAb, with the highest titer in the single surviving control animal.

Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
Rabbitpox Virus/Rabbit Studies						
246-PC-028	The Effect of ST-246 Treatment Following Aerosol Challenge with Rabbitpox Virus in New Zealand White Rabbits – Proof-of-Concept Study	Rabbitpox Virus-Utrecht, (?) PFU aerosol	NZW Rabbits (age?)	24	Tecovirimat 10 mg/kg or 40 mg/kg QD oral, or placebo, for 14 days starting 4 hours post-challenge.	Tecovirimat 10 mg/kg and 40 mg/kg protected animals from lethal infection, with greater survival in 40 mg/kg group.
246-PC-029	The Effect of Time of ST-246 Treatment Following Aerosol Challenge with Rabbitpox Virus in New Zealand White Rabbits	Rabbitpox Virus-Utrecht, ~2 x 10^3 PFU aerosol	NZW Rabbits (age?)	~48	Tecovirimat 40 mg/kg QD oral for 14 days starting 4-96 hours post-challenge, or placebo	Tecovirimat treatment starting on Day 0-2 post-challenge protected animals from lethal infection, with reduced survival (although improved relative to placebo) in animals that started treatment on Day 3 or 4 post-challenge. Lower blood viral DNA levels in tecovirimat-treated animals relative to placebo.
SR09-005F	Antiviral Efficacy of ST-246® in Juvenile NZW Rabbits Infected with Rabbitpox Virus (Utrecht Strain)	Rabbitpox Virus-Utrecht, 1 x 10^5 PFU i.n.	NZW Rabbits (9wk)	30	Tecovirimat 5-40 mg/kg QD oral for 14 days, or placebo, starting on Day 2 post-challenge.	Tecovirimat 40 mg/kg protected all rabbits from lethal disease, with reduced/variable survival rates for tecovirimat doses of 5, 10 or 20 mg/kg. All control rabbits died or were euthanized. Lower and tx dose-related viral DNA levels in blood and tissues in tecovirimat-treated animals relative to placebo-treated animals. Viral plaques obtained from tissues in absence but not presence of 5 µM tecovirimat.
SR09-014F	Post-Exposure Treatment with ST-246® Starting 72 Hours after Intranasal Challenge of NZW Rabbits with Rabbitpox Virus (RPXV)	Rabbitpox Virus-Utrecht, 1 x 10^5 PFU i.n.	NZW Rabbits (9wk)	42	Tecovirimat 5-40 mg/kg QD oral for 14 days, or placebo, starting on Day 2-3 post-challenge.	Tecovirimat at 40 mg/kg starting on Day 2 or Day 3 post-challenge protected rabbits from lethal disease, and was associated with lower viral DNA levels in blood and tissues. Lower doses less effective. Viral plaques obtained from tissues in absence but not presence of 5 µM tecovirimat.
SR13-007F	Exploratory Pilot Efficacy Study to Evaluate the Impact of Time of Tecovirimat Treatment Initiation on Mortality Following Lethal Intradermal Rabbitpox Virus Challenge of NZW Rabbits	Rabbitpox Virus-Utrecht, 300 PFU i.d.	NZW Rabbits (8wk)	48	Tecovirimat 80 mg/kg QD oral, or placebo, for 14 days starting on Day 2-6 post-challenge. Blinded study.	Tecovirimat starting on Day 2, 3 or 4 protected all animals from lethal disease, with lower viral DNA levels in blood compared to placebo treated animals. Reduced protection when tx started on Day 5 and no protection when started on Day 6. DNase I-treated whole blood had lower viral DNA levels but followed same trends as untreated blood.
Vaccinia Virus/Mouse Studies						
246-PC-001	Efficacy of ST-246 vs. Intranasal Challenge with Vaccinia Virus in BALB/c Mice	Vaccinia Virus-IHD-J, 4 x 10^5 PFU i.n.	BALB/c Mice (4wk)	30	Tecovirimat 50 mg/kg orally BID for 14 days, or cidofovir single-dose i.p. injection 50 mg/kg (or 100 mg/kg?), or vehicle, all starting on 1 hour Pre-Challenge; also included un-challenged or scarified (vaccinated) mice with 8x10^6 PFU VACV. Surviving mice re-challenged i.n. at Day 28 to assess immune protection. Also included VACV i.n. LD50 sub-study.	All tecovirimat- and cidofovir-treated mice survived lethal VACV i.n. challenge. Upon re-challenge, all treated mice, as well as the scarified (vaccinated) mice, survived, whereas previously untreated/unchallenged mice died. Higher VACV neutralizing antibody titers observed in treated/i.n.-challenged mice relative to vaccinated mice.

Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
246-PC-002	Efficacy of ST-246 vs. Intravenous Challenge with Vaccinia Virus in NMRI Mice	Vaccinia Virus-Lister, 4 x 10^3 PFU i.v.	NMRI Mice (age?)	32	Tecovirimat 15 mg/kg or 50 mg/kg oral BID for 5 days, oral placebo, or cidofovir single-dose i.p. 50 mg/kg (or 25 mg/kg?), all started 2 hours post-challenge. VACV lesions on tails quantified on Day 8 post-challenge.	All active drug-treated mice had fewer tail lesions relative to placebo-treated mice. Higher tecovirimat dose (50 mg/kg) resulted in fewer tail lesions.
246-PC-003	Optimal Dosing Duration for Antiviral Efficacy of ST-246 in an Intravenous Challenge Model with Vaccinia Virus in NMRI Mice	Vaccinia Virus-Lister, 4 x 10^3 PFU i.v.	NMRI Mice (age?)	50(?)	Tecovirimat 50 mg/kg BID oral for 2-5 days, or placebo, starting at 2-hours or 1, 2 or 3 days post-challenge; or cidofovir single-dose i.p. 25 mg/kg on Day 0. Tail lesions quantified on Days 7, 11 and 14.	Fewer lesions observed in tecovirimat treatment groups relative to placebo group. Greatest reduction in number of tail lesions observed with tecovirimat administered for 5 days starting at 2-hours post-challenge. Progressively less impact on lesion formation with shorter or delayed tecovirimat dosing.
246-PC-013	Dose Optimization for ST-246 vs. Intranasal Challenge with Vaccinia Virus in BALB/c Mice	Vaccinia Virus-IHD-J, 4.3 x 10^4 PFU i.n.	BALB/c Mice (4wk)	55	Tecovirimat dose-ranging 0.5-100 mg/kg QD or BID or placebo oral for 14 days, or cidofovir single-dose i.p. 50 mg/kg, starting on Day 0. Plus LD50 sub-study with range of VACV challenge doses.	Tecovirimat 100 mg/kg QD, 50 mg/kg BID, and cidofovir protected against death. Lower tecovirimat doses less effective.
246-PC-014	Dose Duration Optimization for ST-246 vs. Intranasal Challenge with Vaccinia Virus in BALC/c Mice	Vaccinia Virus-IHD-J, 4.3 x 10^4 PFU i.n.	BALB/c Mice (4wk)	30	Tecovirimat 100 mg/kg QD for 5-14 days, placebo for 14 days, or cidofovir single-dose i.p. 50 mg/kg, starting 1 hour pre-challenge. Plus LD50 sub-study with range of VACV challenge doses, same as in study -013 (?).	All tecovirimat durations down to 5 days, as well as cidofovir, protected 100% of mice from lethal challenge. Some weight loss in tecovirimat treatment groups.
246-PC-017	Dose Duration Optimization for ST-246 vs. Intranasal Challenge with Vaccinia Virus in BALB/c Mice	Vaccinia Virus-WR, 1 x 10^4 PFU i.n.	BALB/c Mice (3wk)	255	Tecovirimat 100 mg/kg QD or placebo oral for 5-14 days, starting either 4 hours or 24 hours post-challenge. Cidofovir 15 mg/kg QD i.p. for 5 days starting 24 hours post-challenge.	Tecovirimat for 5 or more days starting at 4 or 24 hours post-challenge, or cidofovir for 5 days starting 24 hours post-challenge, prevented mortality. Some inconsistencies between study report text and data tables.
246-PC-019	The Effect of ST-246 on Tissue Infectivity Following Vaccinia Virus Infection of BALB/c Mice	Vaccinia Virus-WR, 1 x 10^4 PFU i.n.	BALB/c Mice (3wk)	90	Tecovirimat 50 mg/kg or placebo QD oral, or cidofovir 15 mg/kg QD i.p., for 9 days starting at 24 hours post-challenge. Serial sacrifice to quantify viral levels in tissues.	Tecovirimat and cidofovir protected mice from lethal infection. Tecovirimat treatment reduced viral titers over time in spleen, liver and kidney, but not in lung. Cidofovir also did not reduce viral titers in lung.
ASM224	Efficacy Evaluation of Two Topical Formulations of ST-246 for the Treatment of Poxvirus Skin Lesions	Vaccinia Virus-WR, 1 x 10^6 PFU dermal scarification	SKH1 Mice (4-6wks)	23	Tecovirimat topical formulation (1% or 5%) applied BID, 100 mg/kg QD oral, or vehicle (topical), for 14 days starting either 10 hours post-challenge or on day of lesion formation.	All mice survived. 1% tecovirimat topical formulation starting at 10 hours post-challenge prevented pox lesions. 5% topical formulation or oral tecovirimat dosing starting at 10 hours less effective but still suppressed lesions relative to controls. Starting treatment on day of lesion formation with topical or oral tecovirimat had very modest or no impact on lesion severity or resolution.

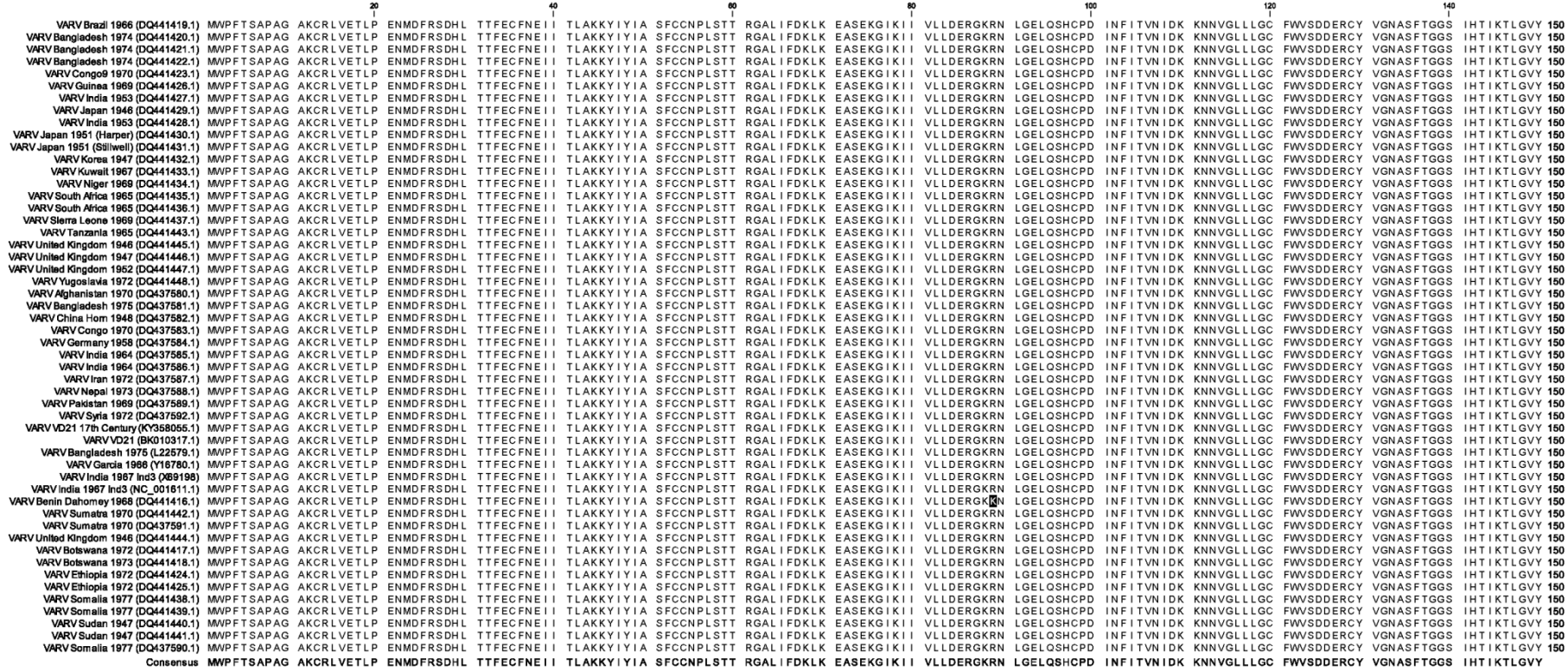
Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
246-PC-012	Antiviral Efficacy of Topical Application of ST-246 in a Model of Progressive Vaccinia Virus Infection in Athymic (nu/nu) Mice.	Vaccinia Virus-Lister, 5 x 10^5 PFU scarification	Athymic-nude (nu/nu) Mice (5wk)	15	Tecovirimat topical formulation (1% or 5%) or placebo applied BID for 10 days starting on Day 0, and photographs of lesions taken on Day 11 post-challenge.	Both topical formulations of tecovirimat inhibited lesion formation. Minimal analyses conducted.
Cowpox Virus/Mouse Studies						
246-PC-016	Dose Duration Optimization for ST-246 vs. Intranasal Challenge with Cowpox Virus in BALC/c Mice	Cowpox Virus-Brighton Red, 3.3 x 10^4 PFU i.n.	BALB/c Mice (4wk)	255	Tecovirimat 100 mg/kg QD or placebo oral for 5-14 days, starting either 4 hours or 24 hours post-challenge. Cidofovir 15 mg/kg QD i.p. for 5 days starting 24 hours post-challenge.	Tecovirimat treatment prevented mortality when administered for at least 7 days starting at 4 hours post-challenge, or for 14 days starting at 24 hours post-challenge. Cidofovir for 5 days starting 24 hours post-challenge prevented mortality.
246-PC-018	The Effect of Time of ST-246 Treatment Following Intranasal Challenge with Cowpox Virus in BALB/c Mice	Cowpox Virus-Brighton Red, 3.3 x 10^4 PFU i.n.	BALB/c Mice (3wk)	~300	Tecovirimat 10, 30 or 100 mg/kg or placebo QD oral, or cidofovir 15 mg/kg QD i.p., for 14 days starting 4-72 hours post-challenge	Tecovirimat 100 mg/kg for 14 days starting as late as 72 hours post-challenge reduced mortality. Some loss of efficacy at 72 hour starting timepoint. Lower tecovirimat doses overall less effective. Cidofovir reduced mortality across all treatment starting points.
246-PC-023	The Effect of ST-246 on Tissue Infectivity Following Cowpox Virus Infection of BALB/c Mice	Cowpox Virus-Brighton Red, 3.3 x 10^4 PFU i.n.	BALB/c Mice (3wk)	90	Tecovirimat 50 mg/kg or vehicle QD oral, or cidofovir 15 mg/kg QD i.p. for 9 days starting 24 hours post-challenge. Serial sacrifice to quantify viral levels in tissues.	Tecovirimat and cidofovir protected animals from lethal disease. Tecovirimat treatment reduced viral titers over time in spleen and liver, but not in lung, with conflicting results in text and figure for kidney viral titers. Cidofovir similarly did not reduce viral titers in lung.
Ectromelia Virus/Mouse Studies						
246-PC-004	Escalating Virus Dose and Optimal Dosing Duration for Antiviral Efficacy of ST-246 in Ectromelia Virus Challenge Model in A/NCR Mice.	Ectromelia Virus-Moscow, varying challenge doses i.n. or i.d.	A/NCR Mice (7wk)	~100(?)	(1) ECTV dose-ranging intranasal challenge: Tecovirimat 50 mg/kg BID oral for 14 days starting on Day 0, Cidofovir single dose 100 mg/kg i.p. on Day 0, or placebo. (2) ECTV i.d. challenge: Tecovirimat 10 mg/kg BID oral for 5 or 14 days starting on Day 0, or cidofovir single dose 100 mg/kg i.p.(?) on Day 0, or placebo. Morbidity and mortality endpoints.	Mice treated with tecovirimat or cidofovir protected from death and morbidity from lethal i.n. ECTV challenge. Both also protected mice from lethal ECTV i.d. challenge, with reduced tecovirimat efficacy with only 5 days tx duration. Issues with study: N's per group not clear, 40x LD50 for ECTV i.n. challenge produced only 20% mortality in placebo group.
246-PC-010	The Effect of ST-246 on Tissue Infectivity Following Infection of Ectromelia Virus Infection of A/NCR Mice	Ectromelia Virus-Moscow, 50 PFU i.n.	A/NCR Mice (7wk)	50	Tecovirimat 20 mg/kg BID oral or placebo for 14 days, cidofovir 100 mg/kg single dose i.p., tx starting 1 hour pre-challenge. Morbidity and mortality endpoints. Serial sacrifice for tecovirimat and placebo treated mice to measure viral titers in tissues over time.	Tecovirimat and cidofovir protected mice from lethal infection. Tecovirimat reduced ECTV titers in various tissues.
246-PC-024	Dose Optimization for ST-246 vs. Intranasal Challenge with Ectromelia Virus in A/NCR Mice	Ectromelia Virus-Moscow, 100 PFU i.n.	A/NCR Mice (4wk)	40	Tecovirimat 1.56-100 mg/kg QD oral for 14 days, placebo, or cidofovir 100 mg/kg (route and duration unclear), starting 4 hours post-challenge.	Tecovirimat doses of at least 12.5 mg/kg, and cidofovir, protected animals from lethal disease. Maximum protection from weight loss with 100 mg/kg tecovirimat dose.

Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
246-PC-025	Dose Duration Optimization for ST-246 vs. Intranasal Challenge with Ectromelia Virus in A/NCR Mice	Ectromelia Virus-Moscow, 700 PFU i.n.	A/NCR Mice (10wk)	56	Tecovirimat 100 mg/kg QD oral for 5-14 days, placebo, or single-dose cidofovir 100 mg/kg i.v., starting 4 hours post-challenge.	Tecovirimat durations of 5 or more days protected mice from lethal disease. Tecovirimat durations of 10 and 14 days resulted in less pronounced weight loss. Cidofovir also protected mice.
246-PC-026	The Effect of Time of ST-246 Treatment Following Intranasal Challenge with Ectromelia Virus in A/NCR Mice	Ectromelia Virus-Moscow, 20 or 200 (?) PFU i.n.	A/NCR Mice (10wk)	128	Tecovirimat 100 mg/kg QD oral for 10 days, or cidofovir 100 mg/kg single dose i.p., starting 4-72 hours post-challenge, or placebo	Both tecovirimat and cidofovir protected mice from lethal disease when dosing was initiated 4-72 hours post-challenge.
Monkeypox Virus/Prairie Dog and Ground Squirrel Studies						
246-PC-022	The Effect of ST-246 on Subcutaneous Challenge with Monkeypox Virus in Ground Squirrels	Monkeypox Virus-strain-Zaire '79, 100 PFU s.c.	Ground Squirrels	59	Tecovirimat 100 mg/kg QD oral until Day 14, starting 0-96 hours post-challenge, or placebo.	Tecovirimat started 0-72 hours post-challenge protected all animals from lethal disease, with reduced efficacy when started at 96 hours post-challenge. Lower or undetected viral levels in blood and tissues in tecovirimat-treated animals. Conflicting information on MPXV strain used.
246-PC-027	The Effect of ST-246 on Intranasal Challenge with Monkeypox Virus in Prairie Dogs	Monkeypox Virus-2003-ROC-358 (Rep. of Congo), 1 x 10^5 (?) PFU i.n.	Prairie Dogs	18	Tecovirimat 30 mg/kg QD oral for 14 days, starting on Day 0, 3 or 10 post-challenge (Day 10 group based on time of rash onset, 10-24 days), or placebo	Tecovirimat treatment starting 0, 3 or 10 days post-challenge protected all animals. For Day 10 group, 3 started on Day 10, 1 on Day 24. Three of 4 placebo-treated animals died, 2 of which died on Day 10, and 1 on Day 12, which confounds results in Tecovirimat Day 10 group in which 4/4 survived. Clinical disease not observed in Tecovirimat Day 0 or Day 3 start groups. Some inconsistencies in description of viral challenge strain and challenge dose.
Monkeypox Virus/NHP or Vaccinia Virus/Mouse Vaccine + Treatment Studies						
SR12-005F	ACAM2000® Vaccination and Concurrent Tecovirimat Administration at Three Days Post-Monkeypox Virus Infection in Cynomolgus Macaques	Monkeypox Virus-Zaire '79, 5 x 10^7 PFU i.v.	Cynomolgus Monkeys	31	ACAM2000™ vaccine alone, tecovirimat 10 mg/kg QD oral for 14 days alone, vaccine + tecovirimat, or mock/placebo, starting Day 3 post-challenge. Surviving animals re-challenged on Day 63.	All animals that received tecovirimat +/- vaccine survived initial challenge and re-challenge, while all animals that were untreated or received vaccine alone died after first challenge. Similar nAb responses, lesion counts, and blood viral DNA levels over time in tecovirimat +/- vaccine groups, with no detected viral DNA in either group after re-challenge.
246-PC-031	Individual or Combined Use of the Smallpox Vaccine and ST-246 as Post-exposure Therapeutics for Lethal Poxvirus Challenge	Vaccinia Virus-WR, 1.2 x 10^6 PFU i.n.	BALB/c Mice (7wks)	100	Vaccine (Dryvax) alone single doses on Day -14 to -2 (pre-challenge), or +0 to 4 (post-challenge); tecovirimat alone 100 mg/kg QD to Day 13 starting Day 0-4 post-challenge; or combined vaccine + tecovirimat starting Day 2-6 post-challenge.	Oral tecovirimat alone starting as late as Day 3 post-challenge resulted in 100% protection from lethal infection, and 20% protection when started on Day 4. Vaccine alone protective only when administered at least 7 days prior to challenge. Oral tecovirimat + vaccine resulted in 80% protection when initiated at Day 4 post-challenge, but not protective when started Day 5 or 6.

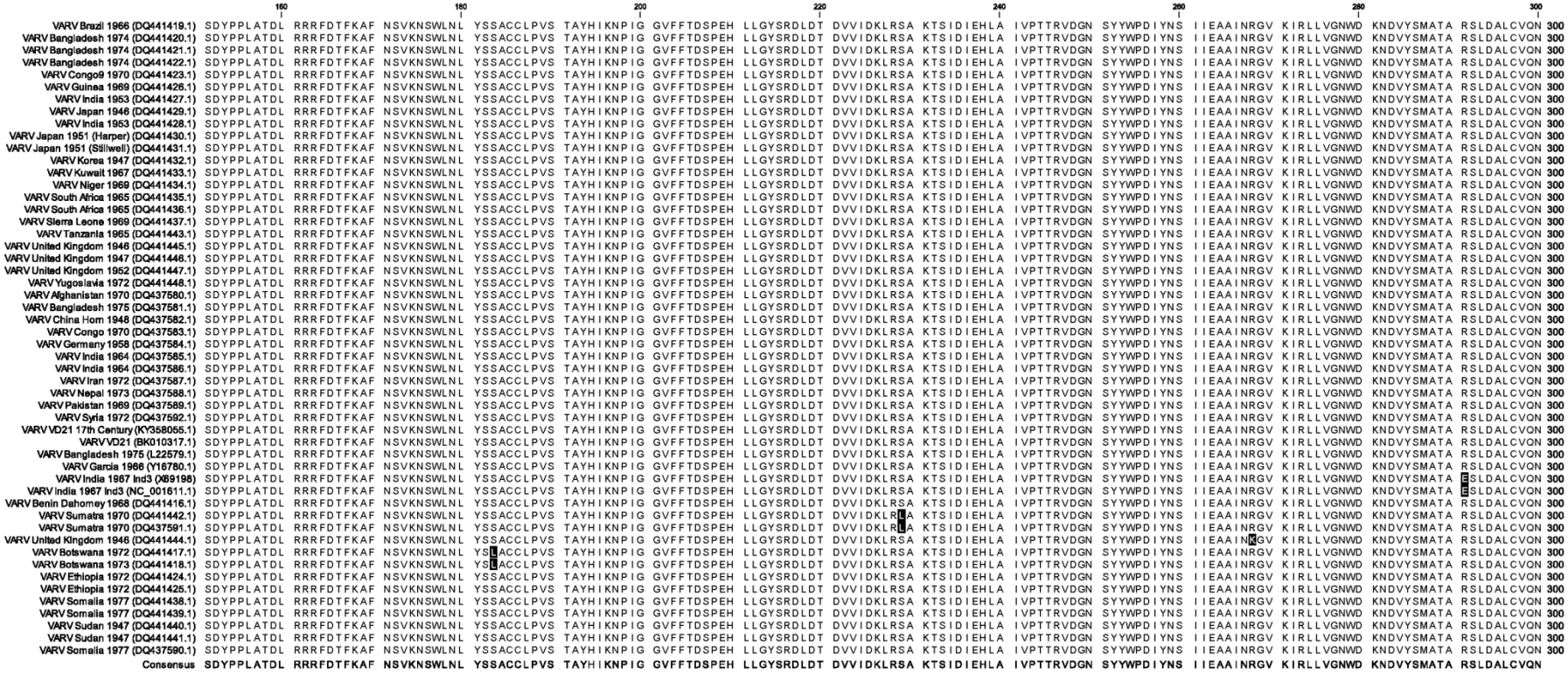
Study w/link to study report	Title	Challenge Virus	Animal (age*)	Total N	Design#/Treatments	Results and Comments
246-PC-035	Isolation and Characterization of Vaccinia Virus Variants with Reduced Suscept bility to ST-246	ACAM2000 vaccine, 2.5 x 10^5 PFU tail inoculation	Nude (nu-/nu-) and SCID mice	21	Tecovirimat 100 mg/kg QD oral or vehicle until day of sacrifice for organ collection or moribund disease.	Virus isolated from spleen samples at selected timepoints to harvest virus and characterize the level of phenotypic resistance by plaque assay in the presence and absence of 5 µM tecovirimat. No tecovirimat-resistant plaques obtained from tissue samples after up to 84 days of treatment.
Ectromelia-IL4 Virus/Mouse Studies of Combinations of Tecovirimat + Other Antivirals						
246-PC-030	The Effect of ST-246 in Combination with CDV to Protect A/NCR Mice Against an Intranasal Challenge with ECTV-11KM-IL-4	Ectromelia Virus-Recombinant Expressing IL-4, 20 or 600 PFU i.n.	A/NCR Mice (5-6wk)	64	Tecovirimat alone 100 mg/kg QD oral for 14 days starting on Day 0 (?), cidofovir alone 100 mg/kg i.p. 5 doses on Day -1 to Day 15, tecovirimat + cidofovir, or placebo	Combination of tecovirimat + cidofovir treatment protected mice from lethal disease. Treatment with either drug alone not protective (some delayed time to death relative to placebo). Timing of tx initiation not entirely clear (Day -1 or Day 0).
246-PC-032	The Effect of ST-246 in Combination with CMX001 to Protect A/NCR Mice Against an Intranasal Challenge with ECTV-11KM-IL-4	Ectromelia Virus-Recombinant Expressing IL-4, 200 PFU i.n.	A/NCR Mice (6-8wk)	64	Tecovirimat alone 100 mg/kg QD oral, CMX001 alone 4 mg/kg QD oral, tecovirimat + CMX001, or placebo, for 14 days starting on day of challenge	Combination of tecovirimat + CMX001 protected mice from lethal disease. Neither drug alone provided significant protection.
246-PC-033	The Effect of ST-246 in Combination with CDV to Protect A/NCR Mice Against an Intradermal Challenge with ECTV-11KM-IL-4	Ectromelia Virus-Recombinant Expressing IL-4, 260 PFU i.d.	A/NCR Mice (7-9wk)	56	Tecovirimat alone 100 mg/kg QD oral for 14 days starting on Day 0, cidofovir alone 100 mg/kg i.p. 5 doses on Day -1 to Day 15, tecovirimat + cidofovir, or placebo. Survivors re-challenged with wt ECTV.	Combination of tecovirimat + cidofovir protected mice from lethal disease. Neither drug alone provided significant protection. Insufficient details on re-challenge design and results.
Variola Virus/NHP Study (Pilot)						
1470HUGMONC	Evaluation of Oral ST-246 in the Lesional Variola Cynomolgus Monkey Model (Pilot Study)	Variola Virus-Harper, 1x10^8 PFU i.v.	Cynomolgus Monkeys	8	Tecovirimat 300 mg/kg/day orally starting on Day 0 or Day 1 Post-Challenge, or Vehicle, for 14-15 days	Vehicle-treated animals required early euthanasia. Tecovirimat-treated animals survived (22 days). Viral DNA levels lower in tecovirimat-treated animals, with trend of lowest viral DNA level in animals that started treatment on Day 0 Post-Challenge. Lesions developed in vehicle- but not tecovirimat-treated animals.

*Age of animals as reported for when study started, not necessarily time of challenge or treatment initiation (e.g., following an acclimation period).
#Studies were not blinded or blinding is unclear, unless a blinded design (i.e., study personnel blinded to treatment assignments) is specifically noted.
(?) Indicates unclear or inconsistent descriptions in study report.
Abbreviations for challenge routes: i.d., intradermal; i.n., intranasal; i.v., intravenous; s.c., subcutaneous

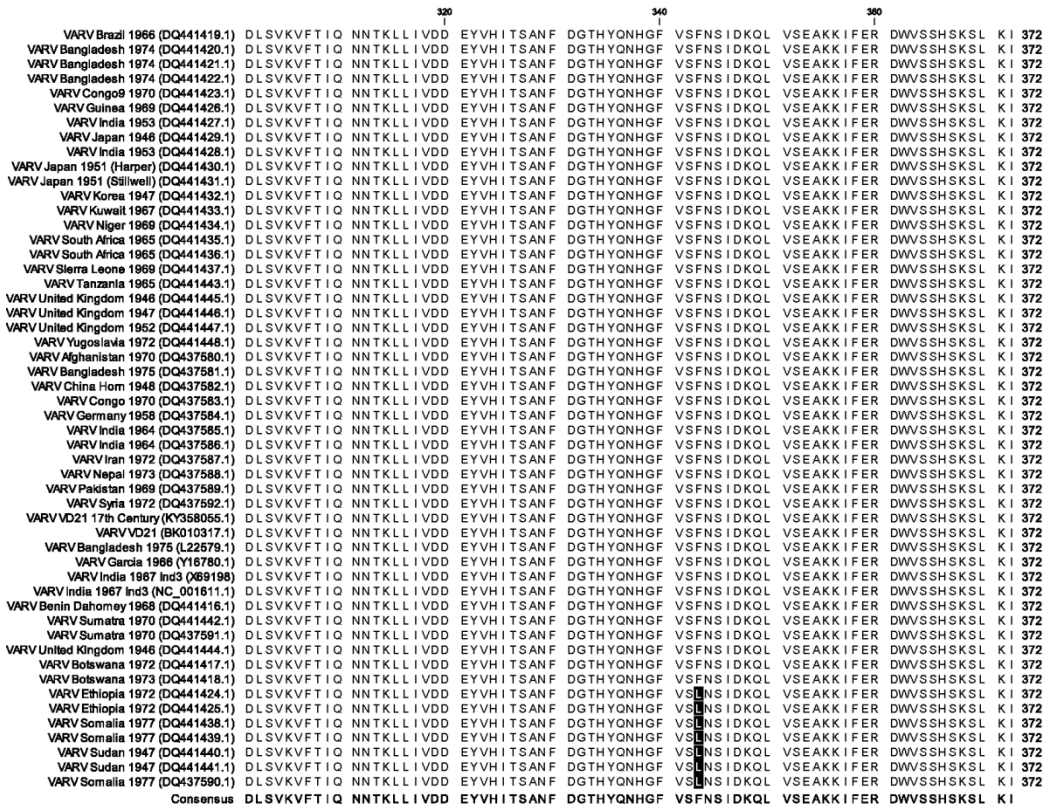
Appendix B. Alignment of 52 Variola Virus (VARV) VP37 Amino Acid Sequences from NCBI/Genbank.



Appendix B continued. Alignment of 52 Variola Virus (VARV) VP37 Amino Acid Sequences from NCBI/Genbank.



Appendix B continued. Alignment of 52 Variola Virus (VARV) VP37 Amino Acid Sequences from NCBI/Genbank.



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