CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208627Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 208627
Agency receipt date: December 8, 2017
Drug: Tecovirimat (TPOXX)
Sponsor: SIGA Technologies, Inc.
Indication: Treatment of Variola Virus

Reviewing Division: Division of Antiviral Products

The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of tecovirimat for the indication listed above under the Animal Rule. Clinical trials were limited to pharmacokinetics and safety studies in healthy volunteers. Demonstration of efficacy was based on studies in animal models of smallpox (monkeypox in monkeys and rabbitpox in rabbits). The nonhuman primate and rabbit models demonstrated that tecovirimat conferred a significant survival to both primates and rabbits.

Tecovirimat is a novel tricyclonene compound proposed to inhibit orthopoxvirus VP37 protein. Treatment is proposed to (b) (4) the infection by preventing release of virions cells via inhibition of VP37. The proposed clinical dose of tecovirimat is 600 mg BID.

The nonclinical toxicology program primarily consists of repeat-dose toxicity studies in rats, mice, rats, dogs and monkeys. The only general toxicities identified were reduced body weight and mild liver toxicity in a 12-day study in rats and neurotoxicity findings in 7-day studies in dogs. No adverse effect doses in 3-month mouse and monkey studies were associated with clinical exposure multiples of approximately 24 and 3, respectively.

Tecovirimat was negative in a battery of genotoxicity studies. Tecovirimat was not associated with fertility effects in female mice or developmental effects in studies in mice and rabbits at clinical exposure multiples approximating 23. However, male reproductive toxicities (increased abnormal sperm counts and decreased sperm motility) and decreased fertility and fecundity indices were observed.

Conclusion:
I agree with the Division pharmacology/toxicology conclusion that Tecovirimat can be approved from the nonclinical perspective. I have reviewed the proposed wording for the nonclinical sections of the product label and agree with the Division recommendations.

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/s/

TIMOTHY J MCGOVERN
06/08/2018

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 208627
Supporting document/s:	SN 001, SN 003
Applicant's letter date:	June 14th, 2017 (rolling review), December 8th, 2017
CDER stamp date:	December 8th, 2017
Product:	ST-246, Tecovirimat, Tpoxx®
Indication:	Treatment of human smallpox disease in adults and pediatric patients weighing at least 13 kg
Applicant:	SIGA Technologies, Inc.
Review Division:	Division of Antiviral Products
Reviewers:	L. Peyton Myers, PhD David McMillan, PhD
Supervisor/Team Leader:	Hanan Ghantous, PhD, DABT
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Reference ID: 4258909

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1 Executive Summary

1.1 Introduction

Tecovirimat (ST-246, Tpoxx®) is a novel tricyclononene compound developed as an oral drug for the treatment of human smallpox disease. Tecovirimat is proposed to inhibit orthopoxvirus VP37 protein, which evidence suggests may be involved in the exit of enveloped virions from an infected cell. Treatment with tecovirimat does not prevent the accumulation of virions within an infected cell, but is proposed to (b) (4) by preventing release of virions from infected cells via inhibition of VP37.

This application for tecovirimat as a treatment for human smallpox disease was submitted under the Animal Rule (21 CFR 314.610), and thus clinical trials were limited to pharmacokinetics and safety studies in healthy volunteers. Demonstration of efficacy was based on studies in animal models of smallpox (monkeypox in monkeys and rabbitpox in rabbits).

1.2 Brief Discussion of Nonclinical Findings

Efficacy/Primary Pharmacology Summary

Two primary animal models were used to demonstrate efficacy in a lethal model of orthopoxvirus disease, a monkeypox/NHP (nonhuman primate) model and a rabbitpox/rabbit model. The two animal models of orthopoxvirus disease were developed as surrogate models for human smallpox disease due to difficulties using variola virus as well as considerable limitations on the NHP/variola model of disease for human smallpox. *(See Section 2.7 - Regulatory Background).*

A lethal monkeypox (MPXV) challenge in NHPs used to demonstrate that tecovirimat provided enhanced survival in multiple studies. In a range-finding study up to 10 mg/kg for 14 days with drug intervention starting after disease onset on Day 4, the survival rate was 80% over placebo. In the pivotal PK study, 100% efficacy was demonstrated at doses of 3, 10, and 20 mg/kg for 14 days from Day 4-18 post infection. In a delayed treatment study dosing for 14 days, starting on post-infection Days 4, 5, or 6, survival was still significant at all days at 83%, 83%, and 50%, respectively. Lastly, in a treatment duration study, dosing 10 mg/kg daily from Day 4 for 3, 5, 7, or 10 total days, NHPs survived 50%, 100%, 100%, and 80% which was a significant increase over placebo. In conclusion, in a lethal NHP/MPXV model, dosing of at least 5 days of tecovirimat at 10 mg/kg starting on Day 4 post-infection conferred a significant survival advantage over placebo.

A lethal rabbitpox virus (RPXV) challenge in rabbits was used to demonstrate that tecovirimat provided enhanced survival in multiple studies. Two primary studies were utilized to demonstrate efficacy in the rabbit model: a PK study and a primary efficacy study. In the PK study, rabbits dosed at Day 4 (associated with severe rabbitpox disease) with 40, 80, or 120 mg/kg conferred 90-100% survival in all groups. There was no placebo in this study since it was primarily a PK study. However, in the pivotal PK

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study, rabbits dosed with 20, 40, 80, or 120 mg/kg of tecovirimat for 14 days conferred a 80-90% survival over placebo, which all succumbed to RPXV disease.

Taken together, the NHP and rabbit model data demonstrate that tecovirimat, when dosed during severe orthopoxvirus disease, conferred a significant survival to both NHPs and rabbits.

Toxicology Summary

Repeat-dose general toxicology studies with tecovirimat were conducted in mice, rats, dogs and monkeys. No adverse drug-related findings were observed in the pivotal 3-month studies in mice or monkeys up to the highest doses tested, and the findings in a 12-day rat study were limited to decreased body weight and food consumption, and mild liver toxicity. Exposure multiples at the NOAELs in the 3-month mouse and monkey studies were 24.3- and 2.7-fold, respectively, relative to the proposed clinical dose.

Two non-GLP 7-day toxicology studies were also conducted in Beagle dogs. Neurotoxicity consisting of convulsions (tonic and clonic), tremors, ataxia, stereotypic walk, excessive blinking, face-twitching and jerky head movements was observed in these studies. The exposure multiple at the NOAEL for convulsions in these studies was 2.6 based on C_{max}. Drug was detectable in the brain and cerebrospinal fluid at all dose levels, indicating that tecovirimat crosses the blood-brain barrier in this species.

No adverse findings were observed in females in a mouse fertility and early embryonic development study (exposure multiple = 23.8). However, male reproductive toxicities (increased abnormal sperm counts, decreased sperm motility), and associated decreases in fertility and fecundity indices, were observed in this study. In an embryofetal development study in rabbits, no fetal toxicities were observed, but maternal toxicities consisting of mortality, seizures, weight loss, premature delivery, total implant loss, increased resorptions, and decreased live fetuses and gravid uterine weights were reported (exposure multiples = 0.1 and 0.4 for maternal and fetal toxicity, respectively). No adverse findings were observed in either an embryofetal development study in mice (exposure multiple = 23.0 for both maternal and fetal toxicity), or in a pre/postnatal development study in mice (exposure multiple = 23.8 for both maternal and fetal toxicity).

All genotoxicology, phototoxicity and dedicated safety pharmacology studies were negative. Carcinogenicity studies were not conducted given a treatment duration less than 6 months.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of tecovirimat (600 mg BID) under the Animal Rule.

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1.3.2 Additional Nonclinical Recommendations

No studies are suggested at this time.

1.3.3 Labeling

Final labeling will be reflected in the approval package.

2 Drug Information

2.1 Drug

CAS Registry Number: 1162664-19-8

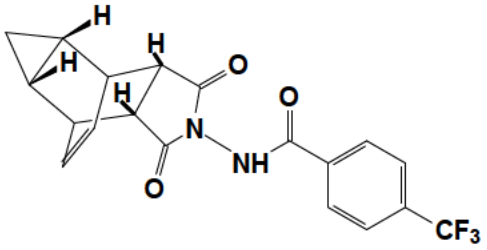
Generic Name: Tecovirimat, Tecovirimat Monohydrate

Code Name: SIGA-246, ST-246

Chemical Name: 4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide

Molecular Formula/Molecular Weight: $C_{19}H_{15}F_3N_2O_3 \cdot H_2O$ (376.33 g/mol)

Structure or Biochemical Description:



Pharmacologic Class: Orthopoxvirus VP37 envelope wrapping protein inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 69019 – primary IND for SIGA smallpox development program.

2.3 Drug Formulation

Tecovirimat drug product is an immediate release orange/black opaque hard gelatin capsule, size “0”, containing 200 mg of tecovirimat as white to off-white powder.

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Ingredient	Function	Quality Standard	200 mg Strength	
			mg/Capsule	%w/w
Tecovirimat monohydrate[a] (b) (4)	Active ingredient	Internal	(b) (4)	
Microcrystalline cellulose	(b) (4)	NF, EP, JP		
Lactose monohydrate[b]		NF		
Croscarmellose sodium		NF, EP, JP		
Colloidal silicon dioxide		NF		
Hypromellose (b) (4)		USP		
Sodium lauryl sulfate		NF		
Magnesium stearate		NF, EP, JP		
(b) (4)		USP		
Hard gelatin capsule shell, opaque black/orange, size 0		Internal		
Total net formulation		NA		
Total Drug Product		NA	485.99	NA

(b) (4)

KEY: NA = Not Applicable

2.4 Comments on Novel Excipients

No excipients of concern.

2.5 Comments on Impurities/Degradants of Concern

No impurities of concern.

2.6 Proposed Clinical Population and Dosing Regimen

600 mg BID orally for 14 days for the treatment of human smallpox disease in adults and pediatric patients weighing at least 13 kg.

2.7 Regulatory Background

Tecovirimat was developed as a medical countermeasure for the treatment of human smallpox disease under the Animal Rule (21 CFR 314.610) with efficacy studies performed in animal models of disease. The product was discussed as part of the development program for smallpox treatments at an FDA Antiviral Drugs Advisory Committee (AC) on December 14th and 15th, 2011. During the 2011 Antiviral Drugs Advisory Committee meeting, the advisory committee agreed with the FDA's assessment that current lethal non-human primate (NHP) models using variola virus are not consistently reproducible, do not mimic what is known about human smallpox disease, and present numerous feasibility challenges due to the worldwide restriction of variola

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virus research to two maximum containment laboratories. Because scientific limitations of the available variola virus/NHP model preclude definitive efficacy assessments, and uncertainty exists whether an adequate variola virus model can be developed, the FDA and the AC agreed that data from a combination of other lethal animal models using surrogate orthopoxviruses (e.g. non-human primate studies with monkeypox virus, rabbit studies with rabbitpox virus, mouse studies with ectromelia virus) could be used as evidence along with, or potentially instead of, animal studies using variola virus.

As a result of the discussion at the AC, the Applicant pursued two models for a treatment indication of human smallpox disease: monkeypox in the nonhuman primate (NHP) and rabbitpox in rabbits. Although several different routes of infection have been proposed, the Agency agreed that the IV model in the NHP and the subcutaneous model in the rabbit provided reliable, reproducible orthopoxvirus disease models. Therefore, these two models were accepted as the pivotal models for human smallpox disease for the development of tecovirimat.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacodynamics / Animal Efficacy

Study Title	Study Number
Pivotal In Vivo Studies in NHPs Infected With Monkeypox Virus	
Determining the minimum effective dose of tecovirimat for treatment of MPXV	AP-09-026G
Determining the minimum effective dose of tecovirimat for treatment of MPXV and assessment of PK at each dose	FY10-087
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	SR10-037F
Identifying the minimum dose-duration post-infection at which tecovirimat is efficacious	SR10-038F
Pivotal In Vivo Studies in Rabbits Infected With Rabbitpox Virus	
Investigating the efficacy of various doses of tecovirimat in RPXV-infected rabbits	SR14-008F
Investigating the efficacy of tecovirimat in RPXV-infected rabbits and development of a population PK model	SR13-025F
Supportive In Vivo Studies	
Natural history of MPXV disease in NHPs infected by the intravenous route	V04-16-MPX
Confirmation of the Potency of Rabbitpox Virus (Utrecht Strain) by Challenge of New Zealand White Rabbits via the Intradermal Route	SR14-015F

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Secondary Pharmacodynamics

Study Title	Study Number
Evaluating smallpox vaccine safety and protective efficacy following co-administration of tecovirimat and ACAM2000® in immune-suppressed monkeys	1218-100004544
Evaluating vaccine safety and protective efficacy following co-administration of tecovirimat and ACAM2000® in immune-compromised monkeys	SR11-011F
Optimal dose duration study in immune-deficient mice infected with VACV	246-PC-009
Efficacy study in immune-deficient mice infected with VACV	246-PC-012
Evaluating the efficacy of 2 topical formulations of tecovirimat in preventing or treating poxvirus lesions compared to an oral formulation of tecovirimat	ASM224

Pharmacokinetics

Study Title	Study Number
ST-246: A 14-Day Repeat Dose Pharmacokinetics Study in New Zealand White Rabbits	246-PK-001
ST246: Absolute Bioavailability in Monkeys	246-TX-011
ST246: Bioavailability and a 7-Day Repeat Dose Study in Rabbits	246-TX-012
ST246: Relative Bioavailability of Two Formulations in Monkeys	246-TX-013
ST-246: A Single Dose Oral Pharmacokinetic Study in Monkeys	(b) (4) 1151-045
Binding of Metabolites to Human, Mouse, and Monkey Plasma Proteins	15SIGAP2
Binding to Rabbit Plasma Proteins	17SIGAP1
Plasma Protein Binding in Mouse (BALB/c), Rat (Sprague-Dawley), Dog (Beagle), Monkey (Cynomolgus) and Human Plasma for [^{14}C]-ST-246	246-AD-001
Pilot Mass Balance and Tissue Distribution of [^{14}C]ST246 After Oral Administration to Mice	246-AD-005
Tissue Distribution of [^{14}C]-Tecovirimat Following Oral Administration to Mice	246-AD-006
ST-246®: Placental and Milk Transfer Study in Mice	(b) (4) -2083-003-001-SN9
ADME-Tox Study of ST-246	100010334
Metabolic Stability and Metabolite Characterization in Mouse (CD-1), Rat (Sprague-Dawley), Dog (Beagle), Monkey (Cynomolgus), and Human Microsomes for [^{14}C]-ST-246	246-AD-002
Multiple Dose Pharmacokinetic Study to Detect Parent Tecovirimat and Three Metabolites, M4, M5, and	8320257

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TFMBA in Monkeys Following Oral Administration of Tecovirimat	
Multiple Dose Pharmacokinetic Study to Detect Parent Tecovirimat and Three Metabolites, M4, M5, and TFMBA in Mice Following Oral Administration of Tecovirimat	8320258

Safety Pharmacology

Study Title	Study Number
Evaluation of the Effects of ST246 on Cloned hERG Channels Expressed in Human Embryonic Kidney (HEK293) Cells	246-PH-001
Pulmonary Effects of Orally Administered ST246 in Mice	246-PH-002
Neurobehavioral Evaluation of ST246 in Mice	246-PH-003

Single-Dose Toxicology

Study Title	Study Number
ST246: Acute Oral Toxicity Study in Mice	246-TX-002
ST246: Acute Oral Toxicity Study in Monkeys	246-TX-005

Repeat-Dose Toxicology

Study Title	Study Number
ST246: Bioavailability and a 7-Day Oral Repeat Dose Toxicity Study in Mice	246-TX-001
ST246: A 28-Day Oral Toxicity Study in Mice	246-TX-006
3-Month Oral (Gavage) Toxicity Study in Mice	2083-003-001-SN3
ST246: Bioavailability and A 12-Day Oral Repeat Dose Study in Rats	246-TX-003
ST246: A 7-Day Toxicokinetic Study in Dogs (Non-GLP)	246-TX-014
ST246: A Maximum Tolerated Dose Oral Toxicity Study in Dogs	246-TX-015
ST246: A 12-Day Oral Toxicity Study in Monkeys	246-TX-016
ST246: A 28-Day Oral Toxicity Study in Monkeys	246-TX-007
3-Month Oral (Gavage) Toxicity Study in Monkeys	2083-003-001-SN6
A 14-Day Toxicity, Toxicokinetics, Local Tolerability and Safety Pharmacology of ST-246 by Intravenous Infusion in Cynomolgus Monkeys with a 2-Week Recovery Period	246-TX-021

Genotoxicology

Study Title	Study Number
Bacterial Reverse Mutation Assay	246-TX-008
In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)	246-TX-009
Mammalian Erythrocyte Micronucleus Test	246-TX-010

Reference ID: 4258909

Reproductive Toxicology

Study Title	Study Number
ST-246®: Study of Fertility and Early Embryonic Development to Implantation in Mice (Segment I)	(b) (4) -2083-003-001-SN7
ST-246: A Pilot Embryo-Fetal Developmental Toxicity Study in Mice	-2083-003-001-SN1
ST-246®: An Embryo-Fetal Developmental Toxicity Study in Mice	-2083-003-001-SN4
ST-246: A Pilot Embryo-Fetal Development Study in Rabbits via Oral Administration	1151-024
ST-246: A Pilot Embryo-Fetal Developmental Toxicity Study in Rabbits	-2083-003-001-SN2
ST-246®: An Embryo-Fetal Developmental Toxicity Study in Rabbits	-2083-003-001-SN5
ST-246®: Study for Toxic Effects on Pre- and Postnatal Development, Including Maternal Function, in Mice (Segment III)	-2083-003-001-SN8

Special Toxicology Studies

Study Title	Study Number
Single Dose Oral (Gavage) Tolerance and Phototoxicity Evaluation of ST-246 (Polymorph Form I) in Hairless Mice	246-TX-017

3.2 Studies Not Reviewed

The following studies were reviewed during the IND to support development but were not considered pivotal to the approval of this NDA.

Study Title	Study Number
Investigating the efficacy of tecovirimat in MPXV-infected monkeys	FY06-035
Determining the effectiveness of a 10 mg/kg dose of tecovirimat for treatment of MPXV	AP-06-021E6
Determining the minimum effective dose of tecovirimat for treatment of MPXV and effect of treatment delay	AP-06-21-MPX
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	AP-06-021G
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	FY11-096
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	FY11-096A
Natural history of VARV disease in NHPs infected by the intravenous route	1400JAHMONC

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Investigating the efficacy of tecovirimat in VARV-infected monkeys	1470HUGMONC
Evaluating post-exposure prophylactic and therapeutic efficacy of tecovirimat in preventing VARV disease in monkeys	2238GOFMONC
Evaluating post-exposure therapeutic efficacy of tecovirimat in preventing VARV disease in monkeys	ST246-1745
Investigating the efficacy of tecovirimat in RPXV-infected rabbits and optimal dose for treatment	SR09-005F
Investigating the optimal dose of tecovirimat for treatment of orthopoxvirus infection	246-PC-028
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	SR13-007F
Identifying the maximum delay post-infection at which tecovirimat is effective at preventing mortality	246-PC-029
Investigating the impact of dose and time of intervention for effective treatment of orthopox infection	SR09-014F
Determining effect of deleting F13L on pathogenesis of infection	246-PC-020
Characterizing VACV variants with reduced susceptibility to tecovirimat	246-PC-035
Determining the pathogenesis and relative replication efficiencies of VACV variants with reduced susceptibility to tecovirimat	246-PC-036
Efficacy study in mice infected with ECTV	246-PC-010
Efficacy study in mice infected with ECTV	246-PC-004
Efficacy study in mice infected with VACV (comparison to CDV)	246-PC-002
Efficacy study in mice infected with VACV (comparison to CDV)	246-PC-001
Efficacy study in mice infected with VACV (comparison to CDV)	246-PC-019
Efficacy study in mice infected with CPXV (comparison to CDV)	246-PC-023
Efficacy study in mice infected with ECTV-11KM-IL-4	246-PC-030
Optimal dose duration study in mice infected with ECTV	246-PC-025
Optimal dose duration study in mice infected with VACV	246-PC-014
Optimal dose duration study in mice infected with VACV	246-PC-003
Optimal dose duration study in mice infected with CPXV	246-PC-016
Optimal dose duration study in mice infected with VACV	246-PC-017
Evaluating the impact of treatment initiation delay study in mice infected with CPXV	246-PC-018
Evaluating the impact of treatment initiation delay study in mice infected with ECTV	246-PC-026
Efficacy study in prairie dogs infected with MPXV	246-PC-027

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Efficacy study in prairie dogs infected with MPXV	246-PC-018
Efficacy study in ground squirrels infected with MPXV	246-PC-022
ST-246: A Bioequivalence Study of Form I and III Capsules with Form V Phase 1 CT Capsules in Monkeys	(b) (4) 1151-046
A Bioequivalence Study of ST-246 Polymorphs Form I, III and V as an Oral Suspension in Monkeys	(b) (4) 1151-051
ST-246: A 14-Day Oral Pharmacokinetic Study of Form I and Form V in Monkeys	1151-056
ST-246: A 14-Day Oral Pharmacokinetic Study in Monkeys	1151-065
A Crossover Study to Determine Bioequivalence of Three Different Particle Size Suspensions of ST-246 Polymorph-I in Cynomolgus Monkeys after a Single Oral Administration	1151-071
Pharmacokinetic and Tolerability Evaluation of ST-246® (Polymorph Form I) in SKH1-hr Mice	ASM270
ST246: Acute Oral Dose Range-Finding Study in Monkeys	246-TX-004

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies encompass multiple proof-of-concept studies in multiple species. The primary species used to support variola virus efficacy in humans are the nonhuman primate (NHP) infected with monkeypox and the rabbit infected with rabbitpox. Although multiple studies were submitted, only the pivotal studies used to support efficacy were reviewed here.

Pivotal monkeypox/NHP and rabbitpox/rabbit studies were inspected by OSIS during the IND process. None of the inspected studies had findings that would significantly affect the data quality or integrity or the interpretation of the studies.

Study title: Confirmation of the Potency of Rabbitpox Virus (Utrecht Strain) by Challenge of New Zealand White Rabbits via the Intradermal Route

Study no.:	**SR14-015F**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 14th, 2013 (Protocol signature)
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	None. No drug was used. Viral challenge

Reference ID: 4258909

	was the only intervention in this study.
Deviations:	Non-GLP study. None that would affect study outcome.

Key Study Findings

This natural history study demonstrates that the stock was lethal and animals succumb as expected to rabbitpox virus (RPXV) disease. Furthermore, only two of the endpoints (temperature and clinical scores) appeared to be reliable markers for RPXV disease progression.

Summary findings:
This non-GLP study was a "natural history" study for RPXV. No drug was given in order to demonstrate the progression of RPXV disease in a rabbit model. Furthermore, the study confirmed the lethal potency of a new clonal challenge virus stock of rabbitpox virus which was derived from the ATCC (American Tissue Culture Collection) stock.

Rabbits (16-week old New Zealand White rabbits) were challenged with an intradermal dose (1000 PFU target dose) of RPXV that was proposed to be lethal. Following challenge, animals were monitored for clinical signs of disease, survival, viremia, and changes in temperature/weight for 14 days.

All RPXV-challenged animals had elevated temperatures (meeting the definition of fever following infection): 88% of infected animals (7/8) exhibited fever onset by post-challenge Day 3 and 100% (8/8) by Day 5. RPXV-infected rabbits also demonstrated an increase in the cumulative clinical scores and viremia which were indicative of RPXV infection.

Two other endpoints did increase across some (but not all animals): viremia and lesions (pox). The viremia was not as clearly associated with disease progression, as at least one animal did not exhibit a reliable increase in tissue or serum virus (by PCR). Lesions were not a reliable marker for RPXV infection (especially the ears, which seldom showed reliable pox lesions). Furthermore, one animal did not demonstrate secondary lesions prior to succumbing to disease. The lack of consistency for these two endpoints suggested they were not reliable for predicting disease progression.

Methods

Species/Strain:	NZW rabbits, from (b) (4)
Number/Sex/Group:	See below
Age:	15 weeks
Weight:	2.2-2.6 kg during randomization.
Blinding:	No

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Methods (pathogen challenge)

Challenge Agent info:	Rabbitpox virus (RPXV)
Challenge agent lot:	#090314-RPXV (clonally derived at (b) (4) from RPXV (b) (4) 01 from RPXV master stock from ATCC - American Tissue Culture Collection)
Doses:	1000 PFU/200 µL
Frequency of dosing:	Once on Day 0
Route of administration:	Bilateral intradermal injections (100 µL each) in shaved thigh
Back Titrated Final Dose:	1500 PFU (Plaque Forming Units -- estimated)

Group*	1	2
Number of rabbits/group	8 (4F+4M)	4 (2F+2M)
Challenge dose/animal (PFU)[1]	1,000	NA[2]
Challenge dose concentration (PFU/mL)	5,000	NA
Challenge route	i.d.	i.d.
Challenge volume (uL)[1]	200	200
Animal ID Number	871, 872, 874, 876, 879, 882, 883, 885	875, 878, 880, 886
Assigned room number	132, 155	133

*Since the study was conducted blinded, the designation of animal group numbers in the table is for illustration purpose only.
[1]Each 200 uL dose / animal was split into two syringes of approximately 100 uL doses (one/thigh area).
[2]Not Applicable. Group 2 was mock-challenged with the virus diluent (DPBS) only and served as the uninfected control group.

- *Group 1 – rabbitpox virus (RPXV) challenge*
- *Group 2 – Mock (untreated) control*

Protocol Related Discussion Points

- Anesthesia: IM injection with ketamine 20-60 mg/kg and xylazine 1-10 mg/kg for sites of injection preparation (for viral challenge).
- Veterinary/supportive care: The animals will be given supplemental edible enrichment daily to help reduce rapid and excessive weight loss. Anesthetics and/or analgesics were not administered to alleviate pain/distress due to the potential for the drugs to mask or alter natural disease progression.
- Blinding: The challenge inoculum for each group.

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

The euthanasia criteria (which was pivotal for the interpretation of the final endpoint – survival or morbidity to disease) were documented on a Euthanasia form, which was used to provide justification and authorization for euthanasia. Animals meeting any of the following 5 criteria were considered candidates for euthanasia:

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1. *Body temperature less than 99°F if confirmed by a second reading 2 hours (±15 min) later.*
2. *Body weight loss greater than 15% from baseline (pre-challenge weight on Day 0).*
3. *Open mouth breathing.*
4. *Persistent prostration and unresponsive to gentle prodding*
5. *At least 2 of the following:*
    - *A temperature decrease of >1.6°F from a prior fever temperature reading within a 48-hour period.*
    - *Severe lung sounds such as rales or wheezing.*
    - *Weight loss of 12.5% or greater.*
    - *No food or water intake in past 24 hours or from the last morning clinical observations*.

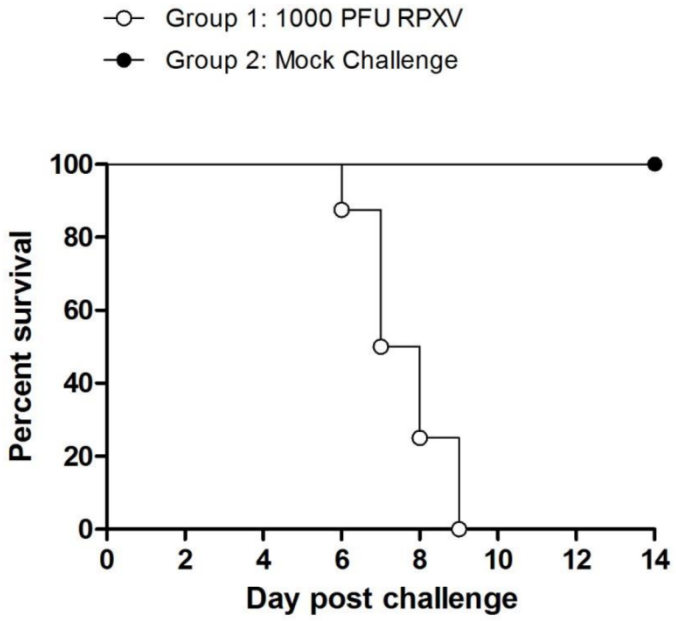
All euthanasia decisions appeared to be valid based on the protocol-defined euthanasia criteria.

Observations and Results

Mortality

Measured daily. RPXV at 1500 PFU (actual dose) resulted in 100% mortality in challenged animals. All mock (untreated) animals survived till the end of study.

Figure 1 - Rabbit/RPXV Mortality



Reference ID: 4258909

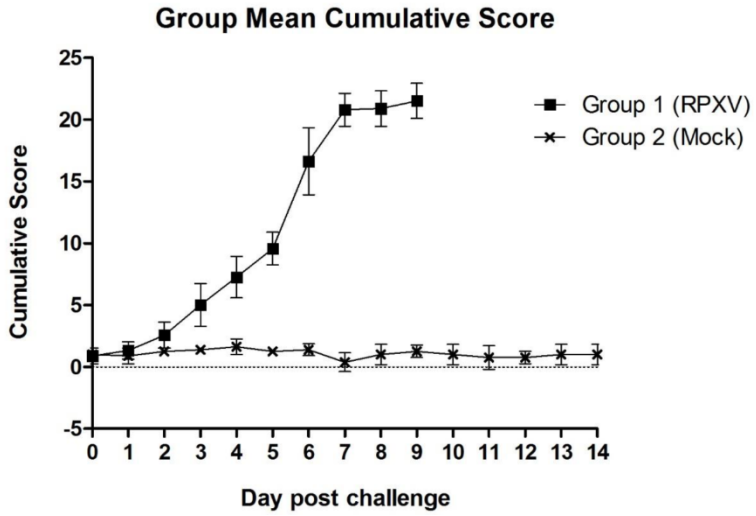
Clinical Signs

Rabbits were observed for the development of RPXV-specific clinical signs and scored once daily in the morning and at euthanasia when warranted. Clinical signs were based on a scoring system per SOP.

Clinical signs significantly increased with disease progression. Mock (untreated) animals did not exhibit significant changes in clinical signs.

Based on these data, the clinical scores appear to correlate with disease progression in the rabbit/RPXV model.

Figure 2 - Rabbit/RPXV Cumulative Scores



Body Weights

Daily via transponder chip. Body weight decreased with disease progression (evident by Day 3). Mock (untreated) animals exhibited a normal increase in body weight gain.

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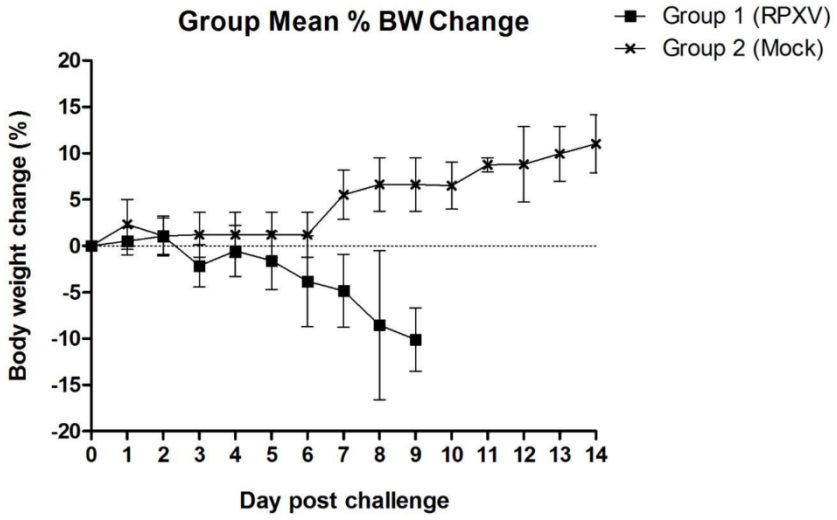
Figure 3 - Rabbit/RPXV Body Weight Change

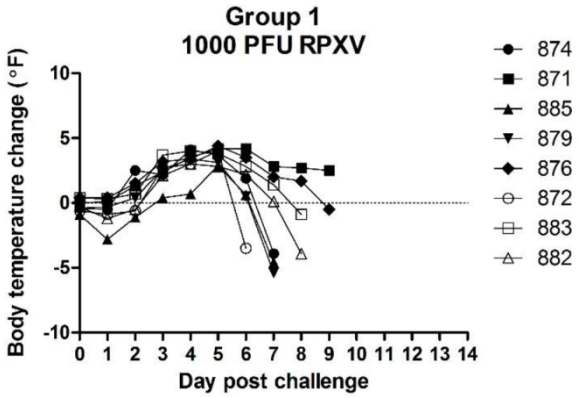


Temperature

Daily via transponder chip. Mock animals did not exhibit significant temperature changes from baseline. Animals challenged with RPXV demonstrated an initial increase of temperature (around Day 4) followed by a decline (hypothermia or a trend downward) prior to euthanasia (see figure below).

Based on these data, it suggested that temperature was indicative of disease progression (a temperature increase) and possible moribund state (a temperature decrease prior to euthanasia).

Figure 4 - Rabbit/RPXV Temperatures



Lesion Score

The sites of injection of RPXV challenge inoculum were monitored daily on all animals. The site of injection reactogenicity or “take” was assessed for the presence of redness, swelling and/or necrosis in either thigh. All animals that received intradermal injections with RPXV exhibited development of reactogenicity at the sites of injections.

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The development of secondary lesions on the ears and the shaved back skin surface following RPXV challenge were monitored and counted every day. Additionally, other areas of the body, such as the mouth, nose, eyes, and ano-genital region were observed for the presence of lesions.

None of the mock animals had secondary lesions. Most challenge animals, developed secondary lesions on at least one body site on Day 5 or 6 post-challenge. One RPXV-challenged animal did not develop any secondary lesions prior to succumbing to RPXV disease on Day 6.

The number of lesions in the remaining animals increased with a maximum of 3 body sites (back, nose/muzzle, eyes). No animals demonstrated lesions on their ears prior to succumbing to RPXV disease.

Based on these data, lesion counts often (but did not always) correlate with disease progression. Based on the lack of secondary lesions in the one animal, lesion counts should not be used as a predictor of RPXV disease progression.

Blood/Tissue Viral Load (by PCR)

Lung, spleen, liver, and kidney were collected at the time of euthanasia to determine viral load.

Most RPXV-infected animals exhibited high qPCR viral loads in all four tissues with lung and spleen having the highest viral load. The only exceptions are animals 871 (Group 1 male) which showed low or below LLOQ viral load in the spleen and liver, respectively, and animal 876 (Group 3 male) that showed very low levels in the liver.

The mock-challenged animals from Group 2 primarily exhibited viral loads in tissues below the LLOQ except for a very low amount of viral DNA detected in the lung and liver of one animal.

Based on these data, viral loads can (but do not always) correlate with disease progression. However, based on the low (or undetectable) viral loads in one animals, RPXV viral load (by PCR) should not be used as a predictor of RPXV disease progression.

Reference ID: 4258909

Study title: 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

Study no.:	**SR14-008F**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 14th, 2013 (Protocol signature)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246, Lot #SG-14G32-M, manufactured by (b) (4)

Key Study Findings

Based on the survival and recovery profiles in this study, tecovirimat (at doses between 20 mg/kg – 120 mg/kg) can protect rabbits from a lethal dose of RPXV if dosing is initiated during the peak of disease (Day 4) for 14 days.

Summary findings:
This was a challenge model with both RPXV and drug (tecovirimat) in a NZW rabbit model. The rabbits were dosed with tecovirimat 0, 20, 40, 80, or 120 mg/kg/day) by oral gavage for 14 consecutive daily doses (4 days after RPXV infection)

Overall survival for these animals was 80-90% with tecovirimat dosing. All placebo (RPXV only) animals were euthanized moribund with RPXV disease.

Of the pivotal endpoints for measuring RPXV disease, it appeared that temperature (and body weights, although not a pivotal measure of disease) recovered significantly after initiation of drug. Furthermore, clinical signs recovered in the tecovirimat-treated animals. The clinical signs never returned to baseline, but it appeared that the animals were recovering well from RPXV disease.

Viral loads trended lower after tecovirimat treatment. Viral DNA in the blood and tissues was reduced compared to RPXV-challenged, placebo-treated animals and returned to baseline by the end of study. Lastly, lesion counts increased and secondary lesions peaked by Day 7 in all animals.

Methods (drug)

Doses:	0, 20, 40, 80, 120 (pre-aliquoted)
Frequency of dosing:	Daily for 14 days starting on Day 4
Route of administration:	Oral gavage
Dose volume:	1 mL
Formulation/Vehicle:	1% w/w hydroxypropyl methylcellulose (HPMC) and 0.5% Tween 80 (placebo had 4% Avicel-Ph-101 as a masking agent)

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Species/Strain: NZW rabbits, from [redacted] (b) (4)
Number/Sex/Group: See below
Age: 16 weeks
Weight: 2.3-2.4 kg during randomization.
Blinding: Double blind
Deviations: No significant deviations that would affect study interpretation.

Methods (pathogen challenge)

Challenge Agent info: Rabbitpox virus (RPXV)
Challenge agent lot: #090314-RPXV (clonally derived at [redacted] (b) (4) from RPXV [redacted] (b) (4) -01 from RPXV master stock from ATCC)
Doses: 1000 PFU/200 µL
Frequency of dosing: Once on Day 0
Route of administration: Bilateral intradermal injections (100 µL each) in shaved thigh
Back Titrated Final Dose: 1160 PFU (estimated)

Group	Treatment[1] Group	Number of Animals/group (Male/Female)	RPXV ID Challenge Dose[2]
1	Placebo	10 (5/5)	1,000 PFU
2	Tecovirimat 20 mg/kg/day	10 (5/5)	1,000 PFU
3	Tecovirimat 40 mg/kg/day	10 (5/5)	1,000 PFU
4	Tecovirimat 80 mg/kg/day	10 (5/5)	1,000 PFU
5	Tecovirimat 120 mg/kg/day	10 (5/5)	1,000 PFU

[1]All animals were treated with the specified dose once daily for 14-consecutive days from Day 4 to Day 17 post-challenge.
[2]The target challenge dose for each animal was 1,000 PFU/200 uL given by bilateral intradermal injections, one injection of 100 uL in each shaved thigh using 2 syringes of 100 uL (500 PFU) each.

Protocol Related Discussion Points

- Anesthesia: The animals were anesthetized by 1.2 mL intramuscular injection of anesthesia cocktail consisting of 20 to 60 mg/kg ketamine and 1 to 10 mg/kg xylazine prior to Viral challenge. The exact anesthetic dose given was recorded in Provantis or on the appropriate data collection sheet.
- Veterinary/supportive care: Animals needing support were given supplemental edible enrichment daily to reduce rapid/excessive weight loss. Anesthetics and/or analgesics were not administered to alleviate pain/distress due to the potential for the drugs to mask or alter natural disease progression.
- Blinding: Conducted in a double-blinded fashion such that the Study Director and technical staff involved in the conduct of the study, including dosing and clinical monitoring (e.g., those making euthanasia decisions for moribund animals), did not know what treatment (tecovirimat or placebo) the animals received.

Reference ID: 4258909

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

The euthanasia criteria were documented on a Euthanasia form, which was used to provide justification and authorization for euthanasia. Animals meeting any of the following 4 criteria were considered candidates for euthanasia:

1. *Open mouth breathing.*
2. *Moribund/Persistent prostration and unresponsive to gentle prodding or noxious stimuli.*
3. *Weight loss greater than 20% from pre-challenge weight.*
4. *At least 2 of the following:*
    - *A respiration rate 75% lower or higher than the average rate observed during the pre-study acclimation period (confirmed by a second observation within 1 hour).*
    - *Body temperature less than 37.2^{o}C (99°F) (confirmed by a second reading hour (±10 min.) later).*
    - *Weight loss of greater than 15% from pre-challenge weight.*

All euthanasia decisions appeared to be valid based on the protocol-defined euthanasia criteria.

Observations and Results

Mortality

Monitored at least 1x daily. After temperature trigger, animals on study were monitored around the clock at 4 hour intervals until meeting requirements for euthanasia or found dead or until clinical symptoms improved and death/euthanasia was unlikely.

Mortality was significantly higher in the placebo than treatment groups, indicating that treatment increased survival. The majority of the rabbits treated with tecovirimat on day 4 post RPXV challenge survived RPXV infection at all doses.

Two rabbits (one in Group 2 and one in Group 3) were euthanized on Days 14 and 12, respectively, due to severe RPXV disease. Group 4 and 5 rabbits received 80 and 120 mg/kg of tecovirimat, respectively, and 80% of animals were protected from mortality. In Group 5, one rabbit succumbed on Day 9 and another on Day 12 due to severe RPXV disease.

Group	Survival (including all causes of death)
Vehicle	0%
20 mg/kg	90%
40 mg/kg	90%
80 mg/kg	80%
120 mg/kg	80%

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Two rabbits were proposed to be excluded from analysis. Rabbit 990 (female, placebo) died on Day 5 from complications from blood collection. Rabbit 989 (female, 80 mg/kg group), died with a low clinical score and appeared healthy when found dead. Death was not determined, but likely not related to RPXV. Removal of the two animals did not affect the p-values from statistical analysis (see **Figures 5 and 6**).

Figure 5 - Rabbit/RPXV Survival (all animals)

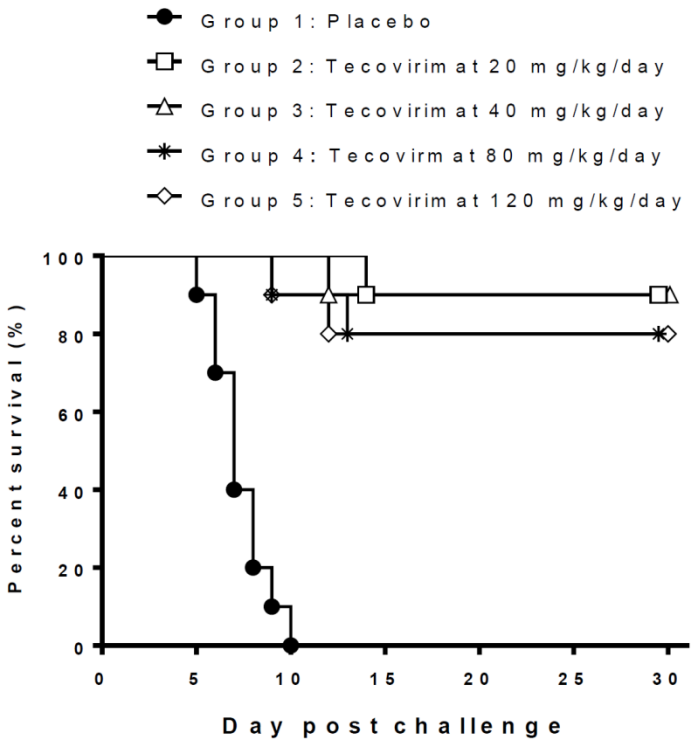

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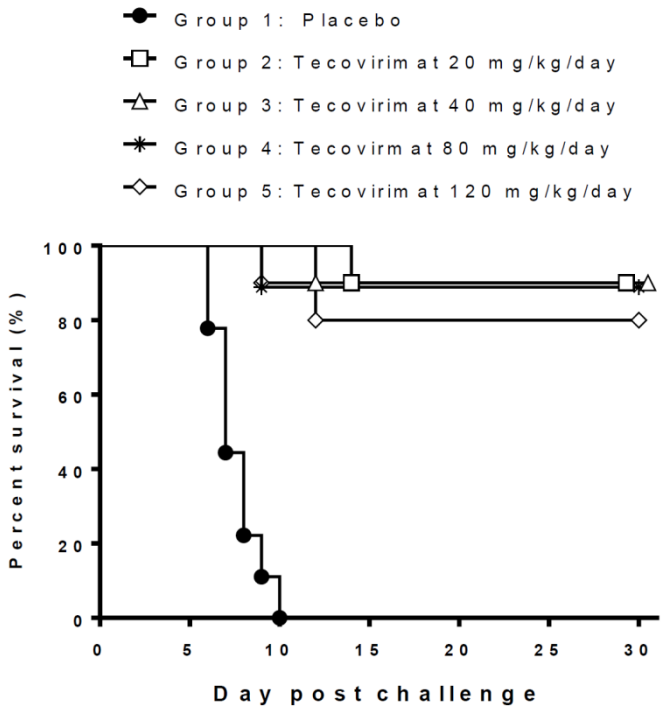
Figure 6 - Rabbit/RPXV Survival (excluding animals #990 and #989)



Clinical Signs

Rabbits were monitored for the development of RPXV-specific clinical signs, and scored once daily in the morning and at euthanasia when warranted.

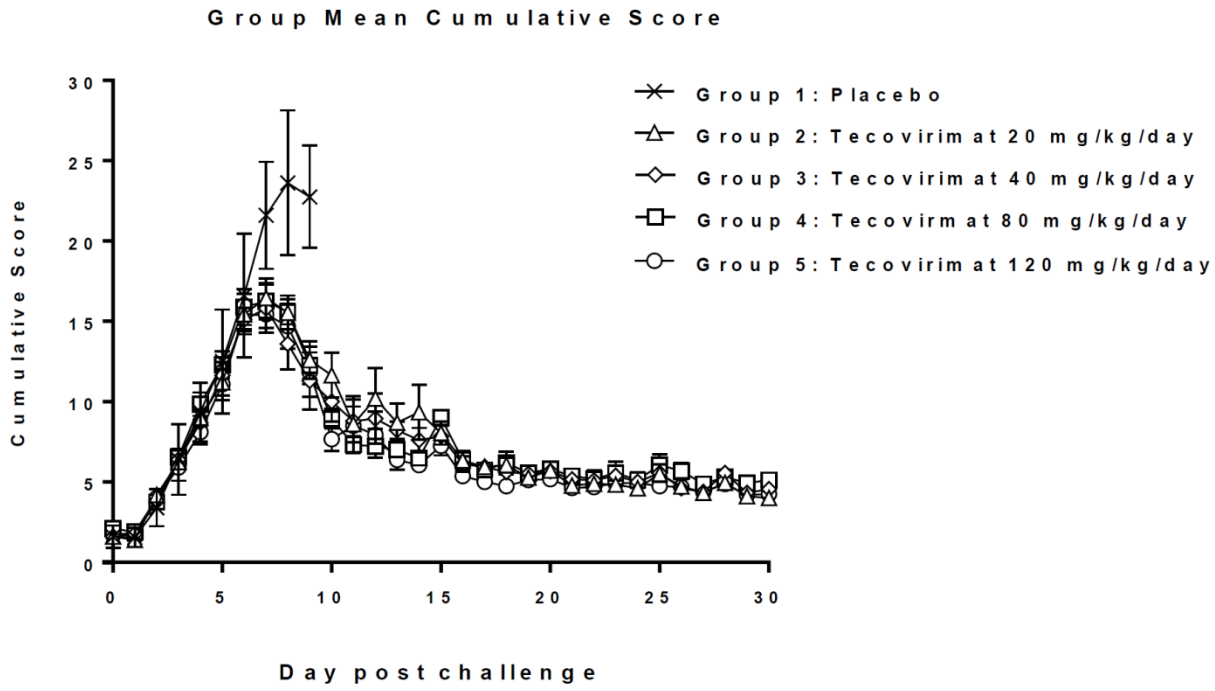
Clinical signs were based on a scoring system per SOP. The clinical signs were scored on a 0-2 scoring system (normal to adverse) for each of the following clinical signs: depression/weakness, posture, drinking, eating, feces, dehydration, primary lesion (site of injection), ear lesion, back/torso skin lesions, nose/muzzle, eyes, secondary lesions, lung sounds/dyspnea, body temperature, body weight.

Cumulative clinical sign scores increased with disease progression. This was expected since the scoring was focused on expected endpoints for RPXV disease.

Overall the scoring was mostly overlapping for the dose groups. However, there was a slight dose response (inversely proportional to dose). The placebo animals (no drug) had the highest score.

Reference ID: 4258909

Figure 7 - Rabbit/RPXV Cumulative Score



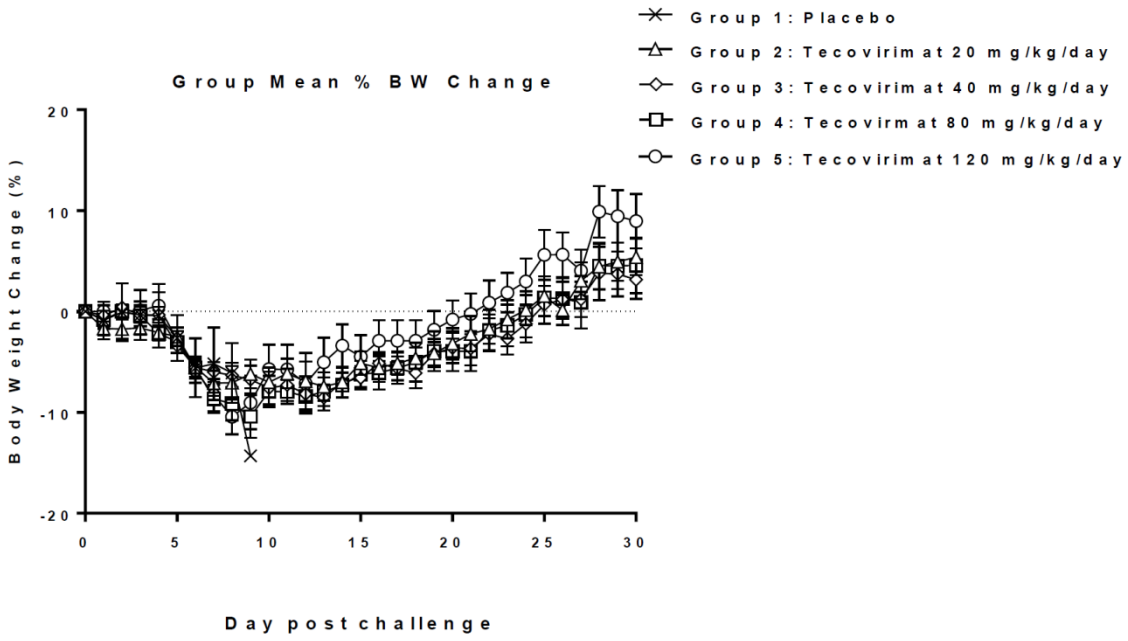
Body Weights

Monitored 1x daily by microchip transponder. Body weights in placebo animals (no drug) were maintained until Day 6 post-RPXV challenge, when weight loss became apparent. These placebo animals were all euthanized between Days 6-9.

Tecovirimat treatment animals demonstrated RPXV-related weight loss around Day 5/6 with recovery over time. Weight loss was inversely related to tecovirimat dose (the greater the dose, the less the overall trend in weight loss). The highest dose (120 mg/kg) of tecovirimat demonstrated the least amount of weight loss with the highest average values from Days 13 onward till the end of study.

Reference ID: 4258909

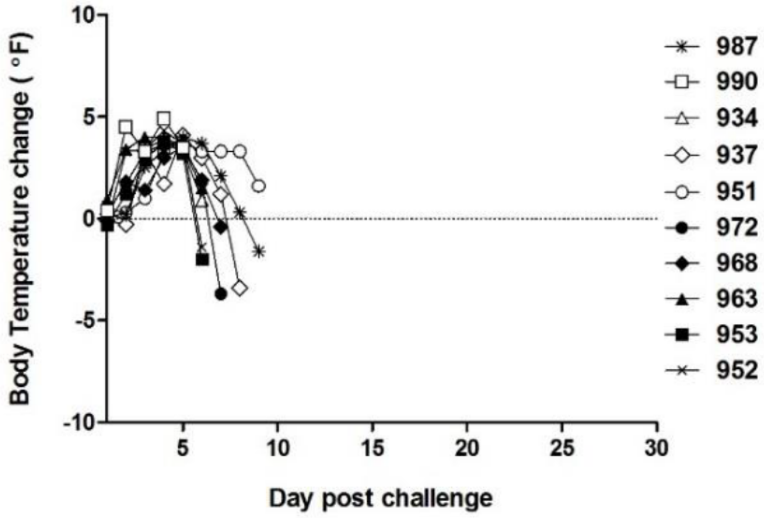
Figure 8 - Rabbit/RPXV Body Weight



Temperature

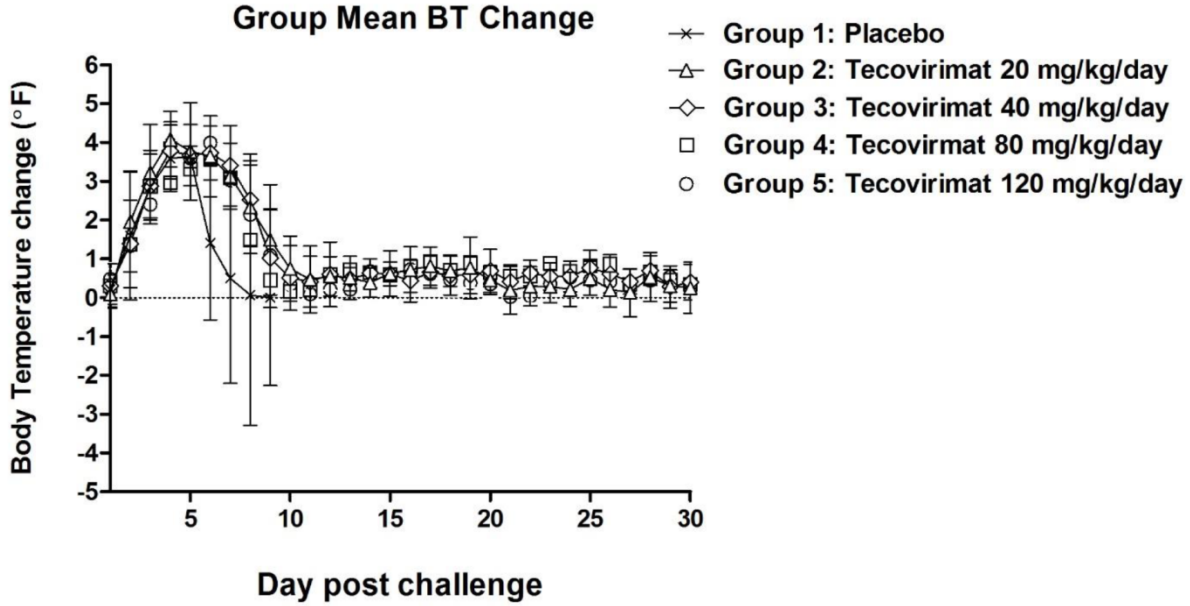
Monitored at least 1x daily by microchip transponder. More frequently if warranted. Temperature dropped below baseline (or was trending downward) in the placebo animals prior to sacrifice (see Figure 9 below). In the treatment animals, the temperature increased with infection and gradually returned to baseline in surviving animals (see figure on next page).

Figure 9 - Rabbit/RPXV Temperature (placebo animals)



Reference ID: 4258909

Figure 10 - Rabbit/RPXV Temperature



Lesion Score

The sites of injection of RPXV challenge inoculum were monitored daily on all animals. The site of injection reactogenicity or "take" was assessed for the presence of redness, swelling and/or necrosis in either thigh. All animals in Groups 1-5 that received intradermal injections with RPXV exhibited development of reactogenicity at the sites of injections.

The development of secondary lesions on the ears and the shaved back skin surface following RPXV challenge were monitored and counted every day. Additionally, other areas of the body, such as the mouth, nose, eyes, and ano-genital region were observed for the presence of lesions.

For Groups 1-5, the first appearance of secondary lesions on at least one body site occurred on Day 4 post-challenge and increased in the number of body sites with lesion as infection progressed. Peak lesion counts were on Day 7 for all groups.

Blood/Tissue Viral Load (by PCR)

Blood was collected over time to determine viral load. Tissue (lung, spleen) was also collected at euthanasia. PCR was performed on lung, spleen, and blood to detect viral load. Overall, the viral load was higher in untreated animals (placebo) compared to treated animals. There was not a large difference between the different treatments on viral load (by PCR – see **Figure 11**).

Lung/spleen PCR for placebo animals was much higher with ~10^7 copies/500 ng vs. ~10^2-10^3 for animals receiving drug.

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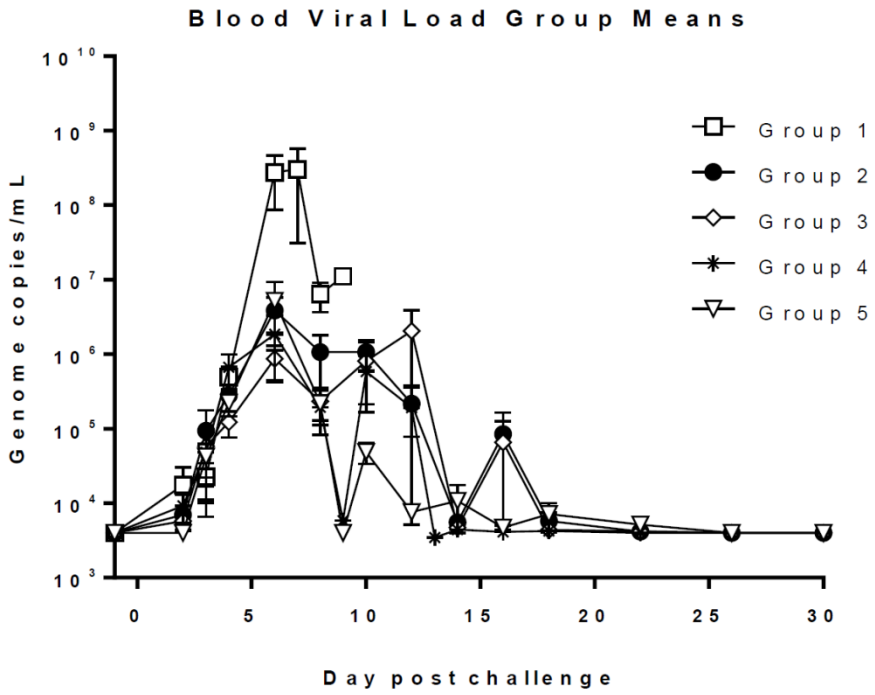
Blood viral load decreased over time in surviving treated animals to baseline.

Figure 11 - Rabbit/RPXV Blood Viral Load (via PCR)



Toxicokinetics

Tecovirimat was analyzed in rabbit lithium heparin plasma samples. Plasma tecovirimat concentrations were only measured at 2 and 24 hours post-dosing. At the limited timepoints, it appeared that there was a correlation between Day 4 tecovirimat levels and survival (P = 0.01) from the 2 hour post-dose value. The 95% probability of survival was estimated to be at plasma levels of 746.00 ng/mL. See **Figure 12.**

Reference ID: 4258909

Figure 12 – Rabbit/RPXV Plasma Tecovirimat Levels

				Tecovirimat Plasma Exposure (ng/ml)					
				2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
Animal ID	Group	Challenge	Treatment Regimen	Day 4	Day 5	Day 10	Day 11	Day 17	Day 18
934	1	RPXV	Placebo	LLOQ	LLOQ	-	-	-	-
937				70.5	5.03	-	-	-	-
951				LLOQ	LLOQ	-	-	-	-
952				LLOQ	LLOQ	-	-	-	-
953				LLOQ	LLOQ	-	-	-	-
963				LLOQ	LLOQ	-	-	-	-
968				LLOQ	LLOQ	-	-	-	-
972				LLOQ	LLOQ	-	-	-	-
987				LLOQ	LLOQ	-	-	-	-
990				LLOQ	-	-	-	-	-
938	2	RPXV	Tecovirimat (20 mg/kg/day)	96.1	77.8	296	12.1	173	34.2
941				166	37.8	231	27.3	251	14
957				149	8.97	268	137	-	-
960				257	115	390	53	216	21.1
962				293	LLOQ	162	28.5	135	LLOQ
966				391	88.1	364	27.6	327	10.2
973				217	8.47	200	14.5	355	8.97
975				173	95.8	440	18.3	453	9.77
979				119	11.8	131	17.2	72.4	LLOQ
986				123	36.3	425	57.2	211	10.3
935	3	RPXV	Tecovirimat (40 mg/kg/day)	320	234	260	225	452	130
936				77.2	299	593	444	288	232
940				154	345	648	390	226	26
944				408	122	501	94.4	303	44.9
959				138	18.2	489	190	-	-
970				433	140	360	14.4	372	53.7
971				375	81.8	427	49.5	423	45.2
974				180	62	96.9	10.3	172	16
982				357	281	658	316	546	112
983				270	193	563	78.2	412	14.1

- = animal euthanized early or found dead
LLOQ = <5 ng/ml

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				Tecovirimat Plasma Exposure (ng/ml)					
				2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
Animal ID	**Group**	**Challenge**	**Treatment Regimen**	**Day 4**	**Day 5**	**Day 10**	**Day 11**	**Day 17**	**Day 18**
943	**4**	**RPXV**	**Tecovirimat (80 mg/kg/day)**	179	246	534	244	535	54.2
946				198	119	358	105	163	31.6
947				509	505	977	717	415	181
949				269	405	999	531	307	43.8
950				557	618	692	357	667	552
964				506	179	352	342	245	13.3
976				676	652	-	-	-	-
978				461	667	771	230	650	151
985				301	194	863	166	316	84.3
989				422	551	1630	414	-	-
939	**5**	**RPXV**	**Tecovirimat (120 mg/kg/day)**	494	684	959	887	371	42.2
942				431	262	516	399	359	212
945				211	496	1320	884	-	-
956				859	939	1120	297	503	170
961				891	660	452	760	562	143
967				713	362	921	366	372	385
969				426	510	358	152	591	74.5
977				803	528	649	108	589	206
981				364	466	532	314	436	181
984				810	507	-	-	-	-

- = animal euthanized early or found dead

Reference ID: 4258909

Study title: Evaluation of the impact of rabbitpox virus infection on oral pharmacokinetics of tecovirimat in male and female New Zealand White rabbits

Study no.:	**SR13-025F**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 14th, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	St-246, Lot #SG-14G32-M, pre-aliquotted by (b) (4).

Key Study Findings

This PK (pharmacokinetic) study (although without a placebo) demonstrated that tecovirimat (at 40, 80, and 120 mg/kg) can protect rabbits from a lethal RPXV infection if dosed by Day 4 (during peak of disease). PK values from this study were used by clinical pharmacology for the calculation of the estimated efficacious human dose.

Predose (Day -7), Day 4, and Day 10 PK values were similar. However, exposures at the end of the study (i.e., Day 14) tended to be lower than on Day 4 for both female and male RPXV infected rabbits. The cause for the lower AUC on Day 14 was not determined.

Reference ID: 4258909

Summary findings:

This study was designed to evaluate the PK of tecovirimat in infected rabbits. The rabbits received a dose of tecovirimat on Day -7 and then were washed out for 7 days before viral challenge. On Day 0, animals were challenged with RPXV. On Day 4, animals received study drug at increasing doses. No control (placebo) was given since this was primarily a PK study.

Most animals survived to the end of study. Of the 2 animals that died, both appeared to die of a procedural error and not of RPXV infection.

Temperature and cumulative scores peaked and then returned toward baseline as the rabbits recovered from disease. Temperature returned to baseline whereas the cumulative scores never returned to pre-RPXV levels by end of study. Body weight decreased with RPXV disease and then trended toward baseline during recovery but never returned to pre-RPXV levels by end of study.

PK (pharmacokinetic) levels appeared adequately assessed. Overall, the PK of tecovirimat in uninfected and RPXV infected rabbits were comparable after single doses of tecovirimat by oral gavage of three dose levels of tecovirimat (40 mg/kg, 80 mg/kg and 120 mg/kg). The rate and extent of exposure to tecovirimat, as measured by AUC_{0-t} and C_{max}, generally increased in a dose-proportional manner when tecovirimat doses were increased from 40 to 120 mg/kg in both naive and infected rabbits.

Predose (Day -7), Day 4, and Day 10 PK values were similar. However, exposures at the end of the study (i.e., Day 14) tended to be lower than on Day 4 for both female and male RPXV infected rabbits.

The PK evaluation and comparison to human levels were addressed by the Clinical Pharmacology reviewer (see review by Dr. Su-Young Choi).

Although not the primary goal of the study, this study (although without a placebo) further demonstrated that tecovirimat (at 40, 80, and 120 mg/kg) can protect rabbits from a lethal RPXV infection if dosed by Day 4 (during peak of disease).

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Methods (drug)

Doses:	40, 80, 120 (pre-aliquoted) (no 0 control)
Frequency of dosing:	Daily for 14 days starting on Day 4
Route of administration:	Oral Gavage
Dose volume:	1 ml
Formulation/Vehicle:	1% w/w hydroxypropyl methylcellulose (HPMC) and 0.5% Tween 80
Species/Strain:	NZW, (b) (4)
Number/Sex/Group:	See below.
Age:	16 weeks
Weight:	2.4 kg (average) at time of randomization
Blinding:	Double blind
Deviations:	No significant deviations that would affect study interpretation

Methods (pathogen challenge)

Challenge Agent info:	Rabbitpox virus (RPXV)
Challenge agent lot:	#090314-RPXV (clonally derived at (b) (4) from RPXV (b) (4)-01 from RPXV master stock from ATCC)
Doses:	1000 PFU/200 µL
Frequency of dosing:	Once on Day 0
Route of administration:	Bilateral intradermal injections (100 µL each) in shaved thigh
Back Titrated Final Dose:	940 PFU (estimated)

Group	Treatment[1] Group	RPXV Intradermal Challenge Dose[2]	Animal ID Number
1	Tecovirimat 40 mg/kg/day	1000 PFU	992, 994, 1001, 1005, 1011, 1012, 1017, 1022
2	Tecovirimat 80 mg/kg/day	1000 PFU	993, 1000, 1002, 1006, 1014, 1019, 1020, 1023
3	Tecovirimat 120 mg/kg/day	1000 PFU	995, 996, 999, 1003, 1015, 1016, 1018, 1021

[1]All animals were treated with the specified dose on Day -7, and once daily for 14-consecutive days from Day 4 to Day 17 post-challenge.
[2]The target challenge dose for each animal was 1,000 PFU/200 uL given by bilateral intradermal injections, one injection of 100 uL in each shaved thigh using 2 syringes of 100 uL (500 PFU) each.

Protocol Related Discussion Points

- Anesthesia: The animals were anesthetized by 1.2 mL intramuscular injection of anesthesia cocktail consisting of 20 to 60 mg/kg ketamine and 1 to 10 mg/kg xylazine prior to Viral challenge. The exact anesthetic dose given was recorded in Provantis or on the appropriate data collection sheet.
- Veterinary/supportive care: Animals needing support were given supplemental edible enrichment daily to reduce rapid/excessive weight loss. Anesthetics and/or

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analgesics were not administered to alleviate pain/distress due to the potential for the drugs to mask or alter natural disease progression.

- Blinding: Conducted in a double-blinded fashion such that the Study Director and technical staff involved in the conduct of the study, including dosing and clinical monitoring (e.g., those making euthanasia decisions for moribund animals), did not know what treatment (tecovirimat or placebo) the animals received.

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

The euthanasia criteria were documented on a Euthanasia form, which was used to provide justification and authorization for euthanasia. Animals meeting any of the following 3 criteria were euthanized:

1. *Open mouth breathing.*
2. *Moribund/Persistent prostration and unresponsive to gentle prodding or noxious stimuli.*
3. *At least 2 of the following:*
    - *A respiration rate 75% lower or higher than the average rate observed during the pre-study acclimation period (confirmed by a second observation within 1 hour).*
    - *Body temperature less than 37.2^{o}C (99°F) (confirmed by a second reading hour (±10 min.) later).*
    - *Weight loss of greater than 15% from pre-challenge weight.*

All euthanasia decisions appeared to be valid based on the protocol-defined euthanasia criteria.

Observations and Results

Mortality

Monitored at least 1x daily. Day 6, all animals on study were monitored around the clock at 4 hour intervals until meeting requirements for euthanasia or found dead or until clinical symptoms improved and death/euthanasia was unlikely.

Two rabbits (one in Group 1 and Group 2) were found dead on Days 16 and 17, respectively. Both animals were found dead immediately after dosing. The timing of the deaths and the fact that the circulating level of virus was decreasing suggested that the deaths were not related to RPXV disease, but likely dosing procedure (gavage error).

All other animals survived to scheduled sacrifice.

Survival (including deaths not related to RPXV infection):

Group	Survival (all causes of death)
40 mg/kg	90%
80 mg/kg	90%
120 mg/kg	100%

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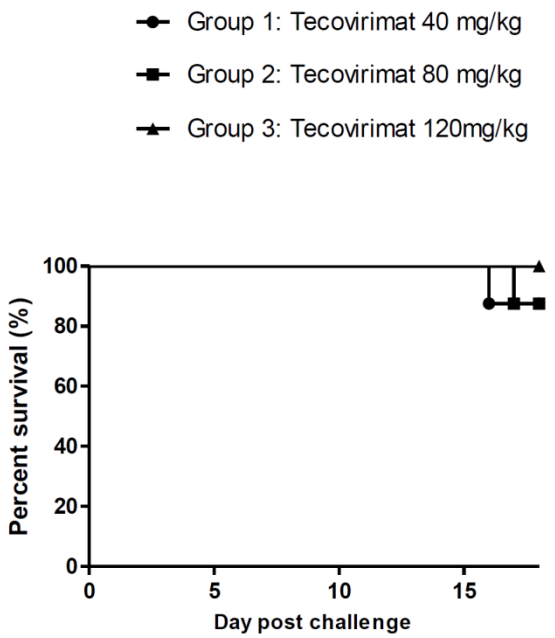
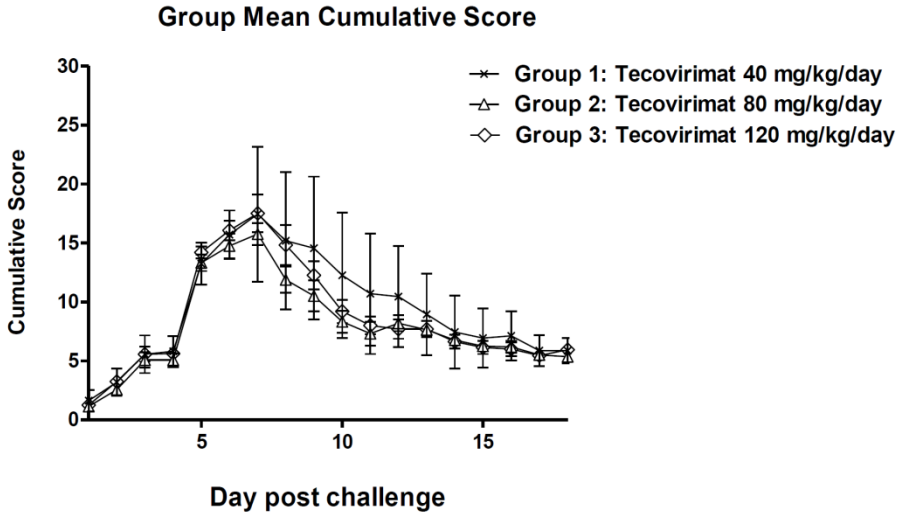
Figure 13 - Rabbit/RPXV Survival Curve



Clinical Signs

Rabbits were observed for the development of RPXV-specific clinical signs, and were scored once daily in the morning and at euthanasia when warranted. Clinical signs were based on a scoring system SOP. The clinical signs were scored on a 0-2 scoring system (normal to adverse) for each of the following clinical signs: depression/weakness, posture, drinking, eating, feces, dehydration, primary lesion (site of injection), ear lesion, back/torso skin lesions, nose/muzzle, eyes, secondary lesions, lung sounds/dyspnea, body temperature, body weight.

Overall the scoring was mostly overlapping for the dose groups and corresponded to disease progression.



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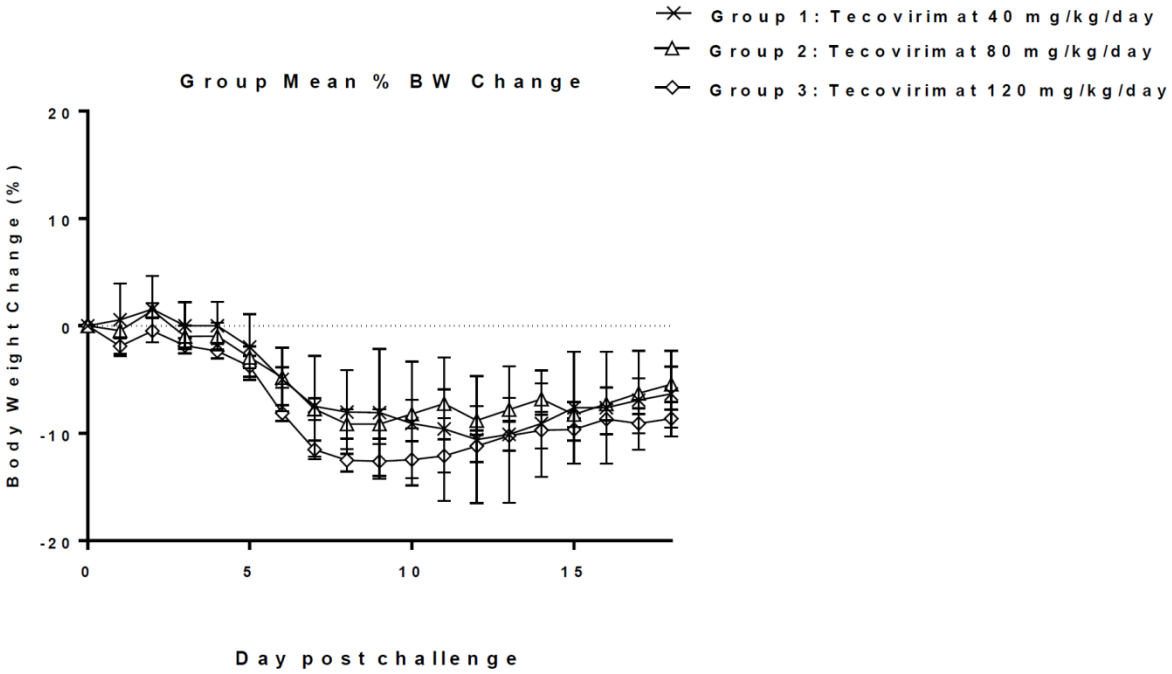
Body Weights

Collected daily by microchip. Body weight decreased across all groups (no differences noted statistically) with disease progression and recovered over time (although never reaching original baseline).



Temperature

Monitored at least 1x daily by microchip. More frequently if warranted.

Temperature increased equally in all dose groups with disease progression and decreased after dosing with tecovirimat in all groups. Temperature returned to baseline slightly faster (on average) in the highest dose groups compared to the 40 mg/kg, suggesting a dose response of the drug effect.

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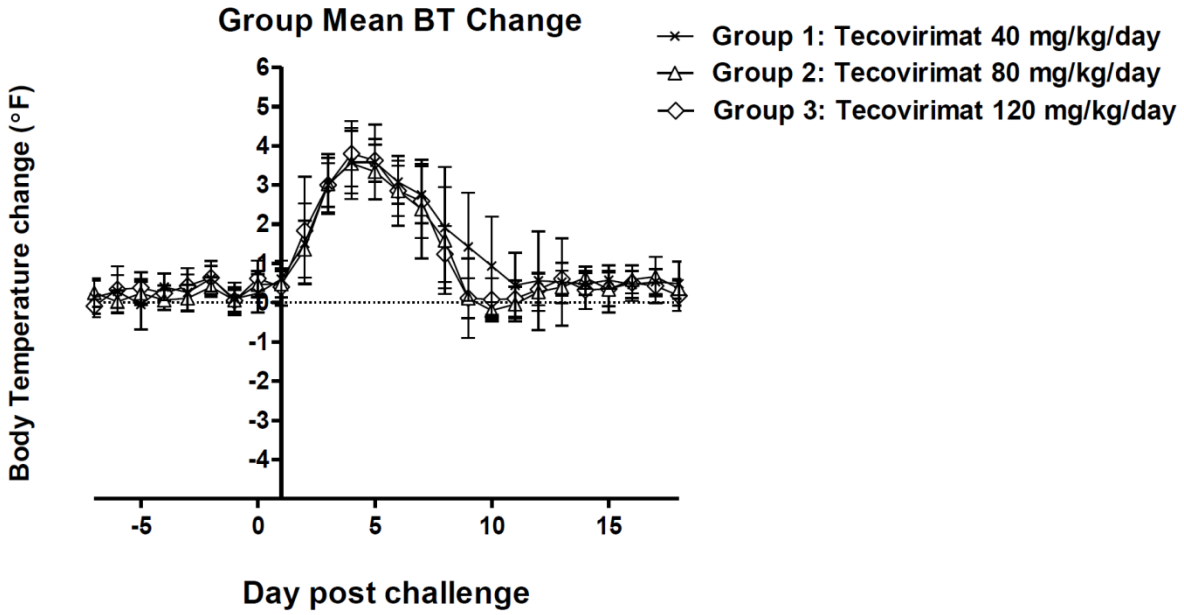
Figure 14 - Rabbit/RPXV Temperature



Lesion Score

The sites of injection of RPXV challenge inoculum were monitored daily on all animals. The site of injection reactogenicity, or "take", was assessed and scored for the presence of redness (score=0.5), swelling (score=1) and/or necrosis (score=2) in either thigh.

All animals in Groups 1-3 that received intradermal injections with RPXV exhibited development of reactogenicity at the sites of injections.

Onset, progression and severity/frequency of secondary lesion were comparable between Group 1-3 animals. The first appearance of secondary lesions on at least one body site occurred on Day 5 post-challenge and was detected for all 3 groups. The number of body sites with lesions increased as infection progressed. The number of body sites with pox lesions peaked on Day 7 post-challenge for all 3 groups.

Blood/Tissue Viral Load (by PCR)

Blood was collected over time to monitor serum viral load. Lung, spleen, and brain were collected at the time of euthanasia to determine the viral load.

Viral serum levels increased over time, peaking around Day 4.

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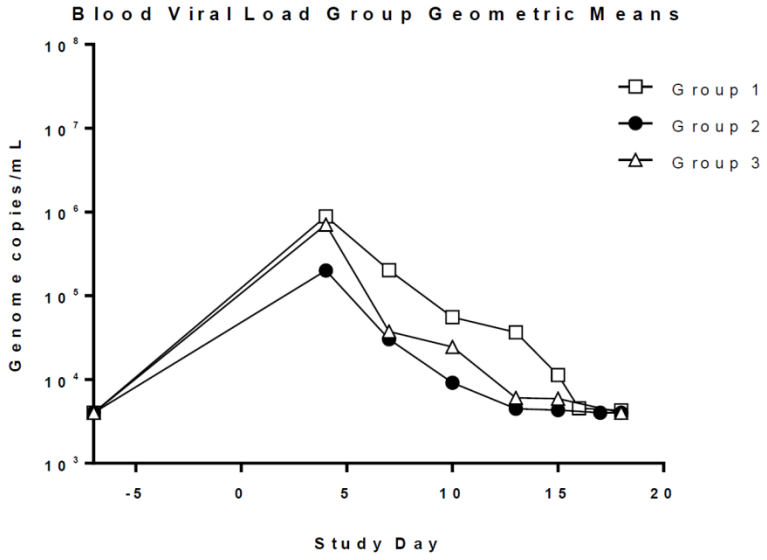
Figure 15 - Rabbit/RPXV Blood Viral Load (by PCR)



Tissue PCR did not consistently demonstrate viral load. Lung was most frequently detected regardless of treatment group. Brain moderately demonstrated viral DNA (in 3 of 8 and 1 of 8 animals in Groups 1 and 3, respectively). Spleen did not product quantifiable viral DNA in any group. There were no significant correlations between tissue viral load and survival.

Toxicokinetics

Tecovirimat was analyzed in rabbit lithium heparin plasma samples. PK (pharmacokinetic) assessment was adequate. *(See Clinical Pharmacology review by Dr. Su-Young Choi for the calculation of the human dose).*

Overall, the PK of tecovirimat in uninfected and RPXV infected rabbits were comparable after single dose of tecovirimat by oral gavage of three dose levels of tecovirimat (40 mg/kg, 80 mg/kg and 120 mg/kg). The rate and extent of exposure to tecovirimat, as measured by AUC_{0-t} and C_{max}, generally increased in a dose-proportional manner when tecovirimat doses were increased from 40 to 120 mg/kg in both naive and infected rabbits.

Predose (Day -7), Day 4, and Day 10 PK values were similar. However, exposures at the end of the study (i.e., 14th dose post-infection) tended to be lower than on Day 4 (i.e., 1st dose post-infection) for both female and male RPXV infected rabbits. It is unclear why the PK on the last day was lower. The Applicant hypothesized that absorption may have restricted exposure increases at higher doses or that repeated dosing results in autoinduction of tecovirimat metabolism.

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Table 1 - Rabbit/RPXV PK Summary Table

	Mean (CV%)					
	Day -7 (Pre-Infection)			Day 4 (Post-Infection)		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
PK Parameters	40 mg/kg Tecovirimat	80 mg/kg Tecovirimat	120 mg/kg Tecovirimat	40 mg/kg Tecovirimat	80 mg/kg Tecovirimat	120 mg/kg Tecovirimat
N	8	8	8	8	8	8
AUC_{0-t} (ng•h/mL)	8547.1(53.0)	12750.9 (46.8)	16951.4 (37.1)	7904.5(53.0)	13412.7 (47.0)	18225.2 (40.3)
C_{max} (ng/mL)	698.5 (50.8)	1059.9 (43.2)	1213.5 (30.3)	602.9 (56.9)	899.0 (46.7)	1199.0 (37.8)
t_{max}[a] (h)	7.88 (3.87, 11.90)	7.84 (3.85, 8.00)	7.87 (7.80, 7.97)	7.88 (3.90, 12.07)	7.88 (3.77, 11.87)	7.77 (3.85, 12.00)
C_{min} (ng/mL)	151.39 (64.4)	254.24 (65.8)	455.63 (56.8)	177.68 (55.1)	473.13 (53.4)	578.25 (48.5)
	Day 10 (7th Dose Post-Infection)			Day 17 (14th Dose Post-Infection)		
N	8	8	8	7	7	8
AUC_{0-t} (ng•h/mL)	8973.9 (54.6)	13943.2 (62.4)	24614.6 (35.4)	3993.6 (63.4)	5516.2 (52.7)	10198.3 (53.5)
C_{max} (ng/mL)	652.9 (49.3)	936.0 (54.2)	1382.4 (28.0)	428.4 (51.4)	568.4 (51.5)	807.9 (35.1)
t_{max}[a] (h)	3.87 (2.05, 7.78)	7.75 (3.73, 11.98)	7.75 (3.77, 12.10)	4.07 (3.15, 7.92)	4.12 (4.00, 4.27)	4.05 (3.12, 11.80)
C_{min} (ng/mL)	161.88 (71.4)	271.85 (63.1)	669.88 (50.7)	40.4 (101.4)	46.60 (74.0)	135.73 (101.0)

[a] Median (Min - Max)

Study title: Evaluation of the Progression of Pathogenic Events in the Cynomolgus Macaque Monkeypox Model as a Surrogate Virus to Support the Development of Smallpox Countermeasures

Study no.:	**V04-16-MPX**
Study report location:	EDR
Conducting laboratory and location:	USAMRIID, Fort Detrick, Frederick, MD
Date of study initiation:	May 24th, 2004 (protocol date) *(note: multiple dates on the signature sheet, and many signatures months after the PI signed the form)*
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	No drug. Only pathogen challenge (Natural History study).
Deviations:	Appendix B states that there are no protocol deviations. Non-GLP study.

Key Study Findings

The report is a natural history study of monkeypox virus (MPXV) in a time course over 13 days in monkeys. Overall, pox lesions (especially on the legs), serum DNA, and tissue DNA increased as disease progressed. Clinical signs also increased with disease progression, with the more severe disease signs showing around Days 7 to 13.

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Methods

Species/Strain:	Cynomolgus Monkey, USAMRIID Colony
Number/Sex/Group:	21 animals, all male (serum tested to be negative for antibodies for orthopox viruses) 6 groups of 3 animals: 3 animals were randomly post-MPXV infection on Days 1, 3, 5, 7, 9, and 11 to be euthanized for necropsy. This report was a merging of data between two studies.
Age:	Average of 6.3 years. No individual animal data included.
Weight:	6-11 kg
Blinding:	No
Deviations:	Not a GLP study. Followed SOPs from USAMRIID. The study was designed as protocol V04-16 with additional data from protocol PA-06-21 added into the study report.

Methods (pathogen challenge)

Challenge Agent info:	Monkeypox virus
Challenge agent lot:	Strain ZAI 1979-005 (isolated from Zaire in 1979 – also called Zaire 79 strain) No info on storage or other strain info.
Doses:	5×10^7 PFU/mL
Frequency of dosing:	Once
Route of administration:	IV
Back Titrated Final Dose:	No data on back titration.

Protocol Related Discussion Points

- The study was designed as protocol V04-16 with additional data from protocol PA-06-21 added into the study report. The data presented are a merging of the two studies.
- Anesthesia: Unclear/undocumented. It was assumed that the anesthesia was given to animals (for handling of infected animals), which was the SOP at the time of study.
- Veterinary/supportive care: If the animals became dehydrated, Lactated Ringer's solution was provided. The fluids were given by slow subcutaneous inoculation in up to 4 sites over the dorsum of the body and lateral aspects of the thorax and abdomen, with approximately 20 mL being injected in each site per USAMRIID SOP.
- Blinding: No.
- Scoring: GDHES (General Distress and Humane Endpoint Scoring Sheet for non-human primates) and OPCOS (Orthopoxvirus Primate Clinical Observation Score

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Sheet) were used to document the animal status. *The GDHES scoring system is presented below.* The GDHES scoring was then used for the OPCOS sheets to give a representative "score" for overall status of the animal. The higher the OPCOS score, the more severe state for the animal. The OPCOS scoring sheet is not presented since each animal would have a scoring sheet for each day. The average group OPCOS scoring is represented in the results section under ***GDHES scores***.

Parameter	Degree of Parameter	Weighted Score
Dyspnea	No Symptoms	0
	Mild	1
	Moderate	2
	Severe	3
Recumbency	No Symptoms	0
	Occasionally lies down	1
	Lies down; will get up when approached	3
	Lies down; will not get up when approached	5
Unresponsiveness	No symptoms	0
	Mild	1
	Moderate; response requires prodding	3
	Severe; no toe-pinch reflex	5
Total		0-13

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

- "To minimize pain and distress, animals were euthanized when a score of ≥10 was reached on the GDHES. Animals were euthanized in agreement with the 2000 Report of the AMVA Panel on Euthanasia and the Veterinary Medicine Division SOP by exsanguination following deep anesthesia (9 mg/kg Telazol). Euthanasia was performed by a qualified individual from the Veterinary Medicine Division."

Observations and Results

Mortality

Animals were euthanized on schedule per the two protocols. Out of 21 infected monkeys, four animals (or 19%) were either found dead or euthanized moribund. Sixteen of the 21 animals were euthanized as planned (per protocol).

- 1 animal was euthanized one day early on Day 8 post-infection
- 3 animals were euthanized one day early on Day 10 post-infection
- 1 animal was found dead on Day 10 post-infection
- 2 animals were euthanized on Days 12 and 13 post-infection

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Table 3: Disposition of Infected Animals

	Protocol V04-16	Protocol AP-06-21
Euthanized	N	N
Day 01 Post-infection	3	-
Day 03 Post-infection	3	-
Day 05 Post-infection	3	-
Day 07 Post-infection	3	-
Day 08 Post-infection	1	-
Day 09 Post-infection	2	-
Day 10 Post-infection	3	-
Day 12 Post-infection	-	1
Day 13 Post-infection	-	1
Died		
Day 10 Post-infection	-	1

Clinical Signs

Temperature slightly increased from Days 1-8 compared to baseline and returned to baseline by Days 9-10. Body weights decreased from baseline at all time points measured. Pulse (bpm) was generally increased at most time points along with decreases of pulse oximetry at all timepoints.

Clinical Chemistry

Data were collected via serial sampling. In order to simplify the multiple data points, the first time that the effect is described in the study report, it will be noted in the list below. Overall, minor effects were noted at early time points and accumulated over time.

Day 1-2
- First noted decreases for albumin and increases for SGOT, creatinine, and bilirubin

Days 3-4
- First noted decreases in calcium, cholesterol, and potassium and increases in ALP and CO_2

Days 5-6
- First noted decreases for total protein

Lesions

Pox lesions increased over time in the MPXV model with initial pox beginning on days 3-4, increasing at Days 5-6, and then maximal levels reached around Days 7-8 (see below).

Pox lesions were on multiple body parts but were more significantly presented on the legs compared to other areas (see below).

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Figure 16 – NHP/MPXV Average Total Body Pox Lesion Count (by Day)

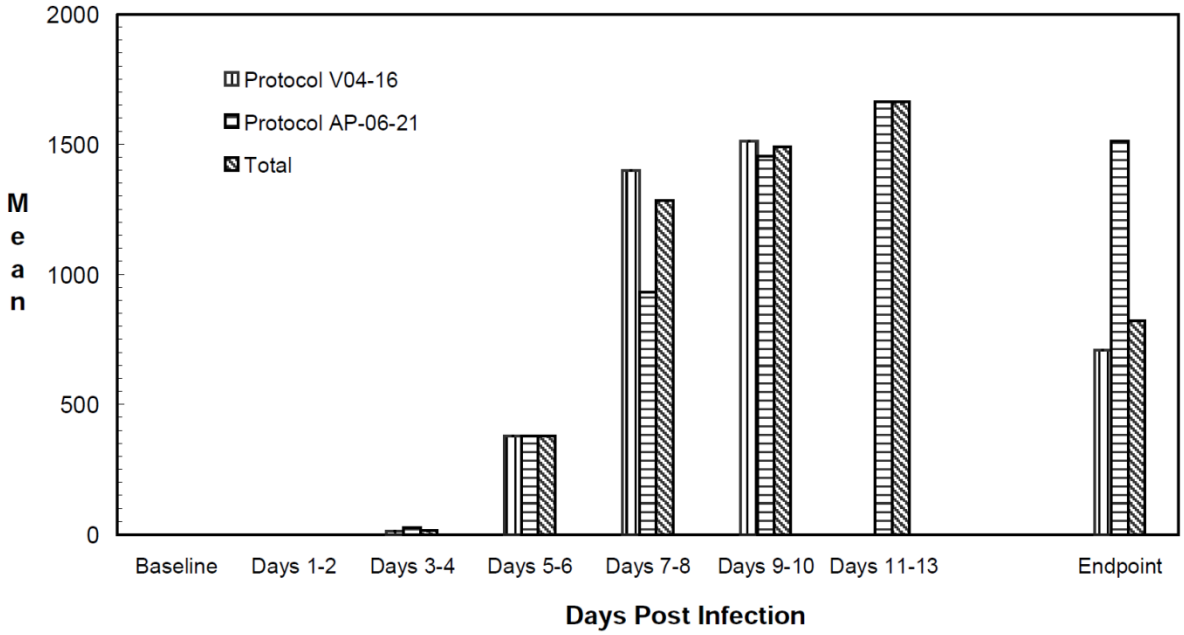
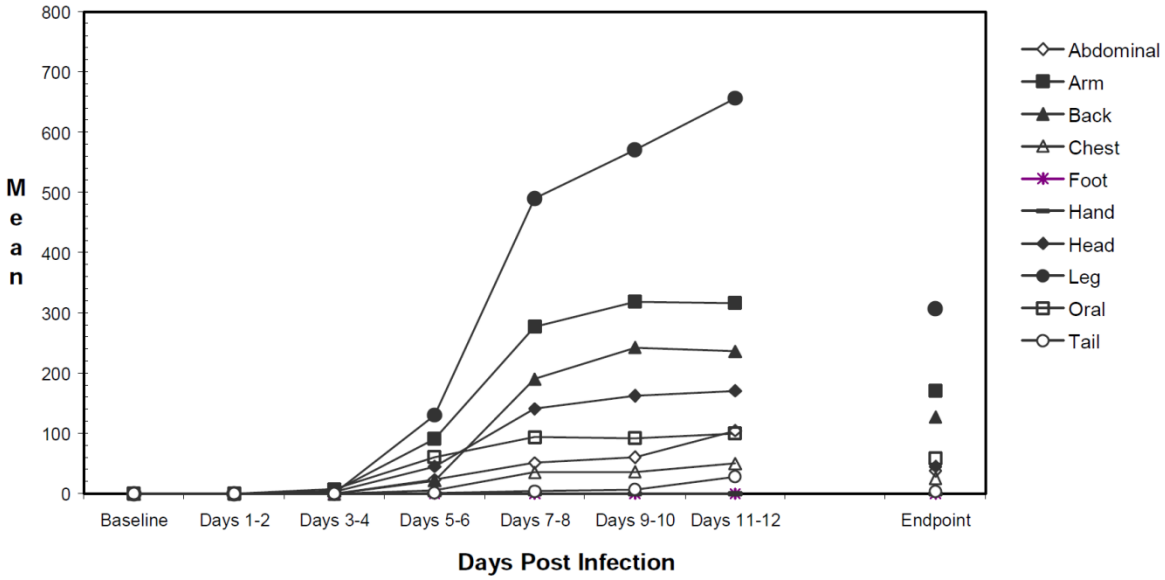


Figure 17 – NHP/MPXV Pox Lesion Counts by Body Region



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Blood/Tissue Viral Load (by PCR)

Blood viral DNA and tissue DNA increased post-infection.

Figure 18 – NHP/MPXV Serum MPXV DNA Levels

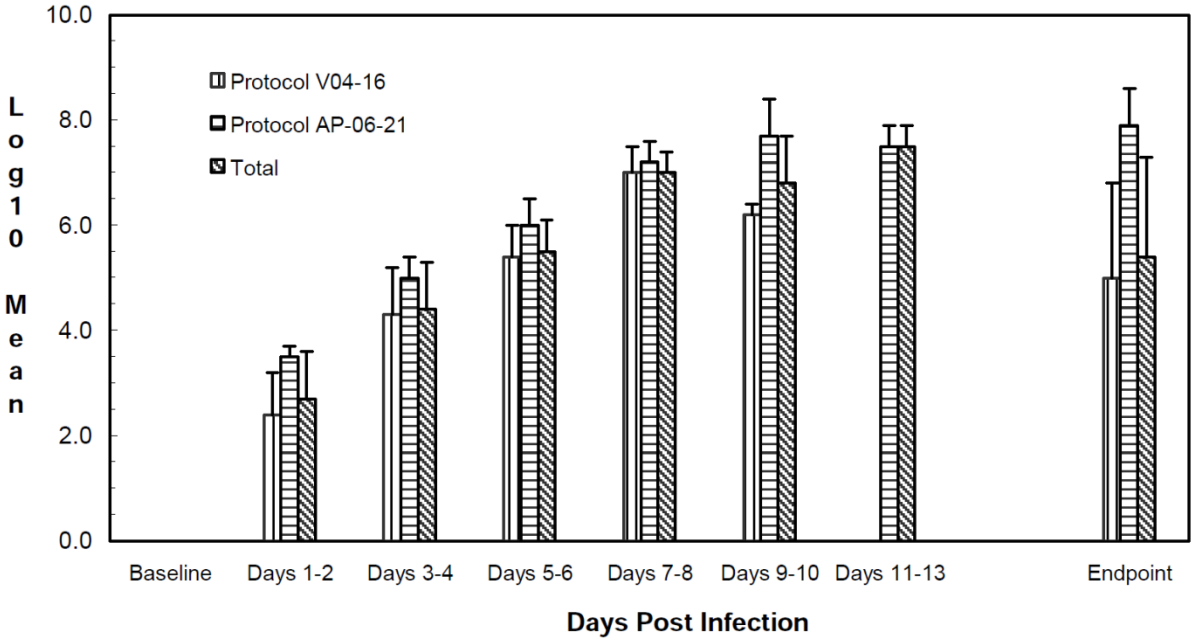
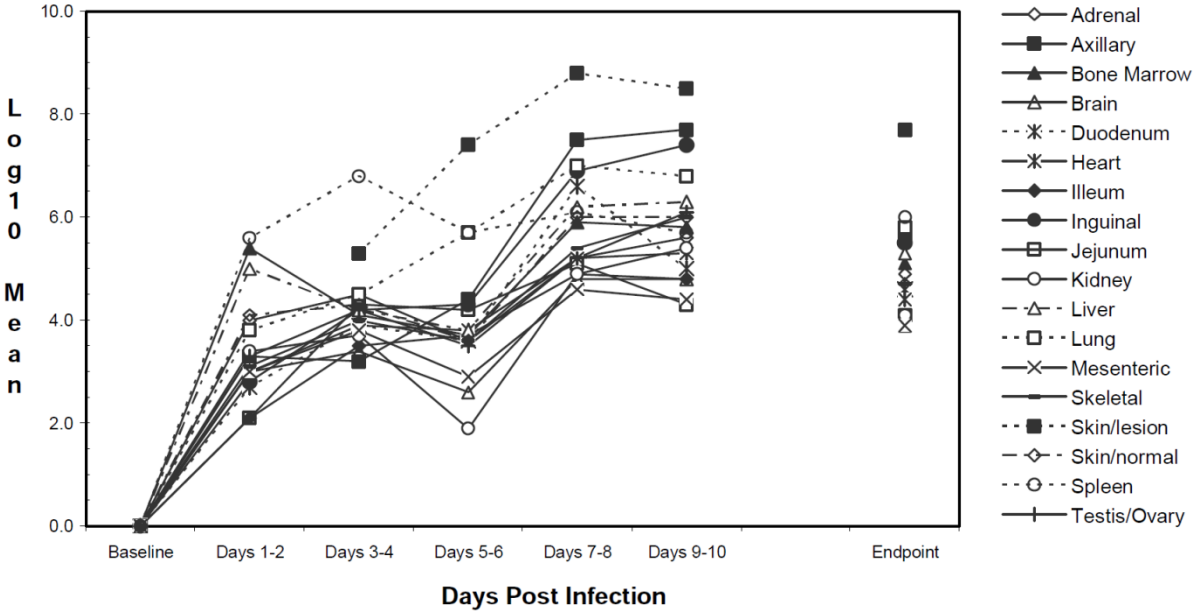


Figure 19 - NHP/MPXV Tissue MPXV DNA Levels



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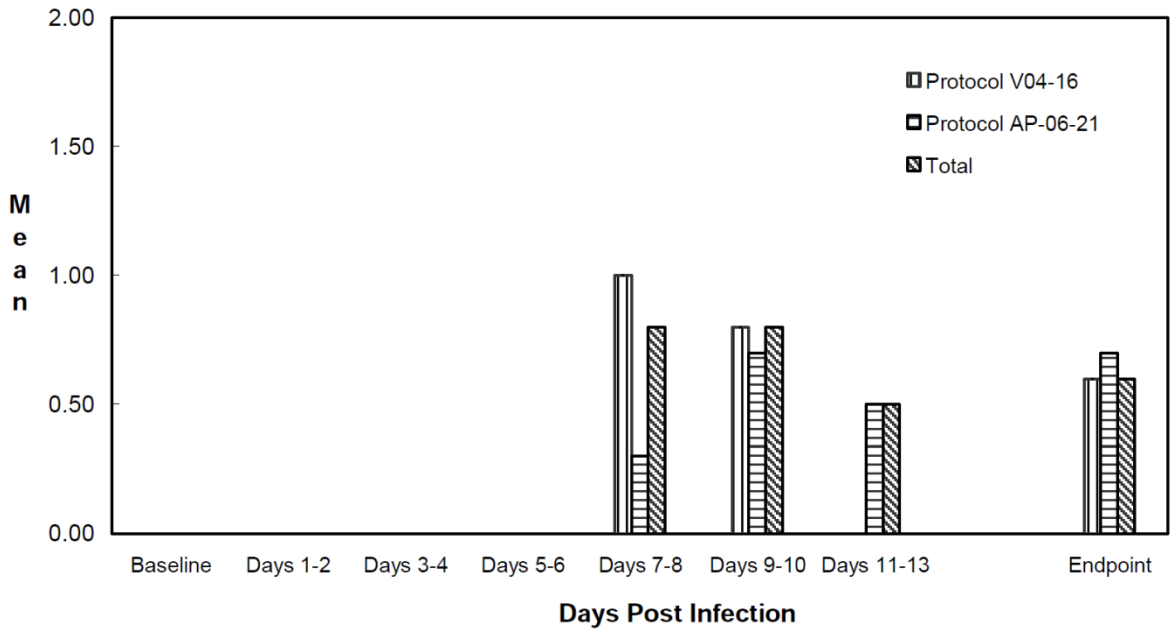
GDHES (Disease) Scores

GDHES scores (the scoring system which indicated severity of disease) peaked on Day 7-8 and maintained to the end of study.

Figure 20 - NHP/MPXV Disease Scores



Disease Course

Findings increased as a function of time with MPXV. Data are presented below on the Day that the effects were first reported. The effects were cumulative and increased in severity over time.

Day 1

- First reports of spleen and liver congestion and enlargement of peripheral lymph nodes in some monkeys

Day 3

- First reports of petechial rash in the axillary regions and in the skin on the tibias
- First reports of diffuse reticular pattern in the hepatic parenchyma, miliary white foci scattered throughout the spleen parenchyma, indicating necrosis, and proteinuria

Day 5

- First reports of petechial rash spread into the inguinal regions
- First reports of lesions involving the lips and oral cavity, pulmonary lesions, hemorrhage of testes, and enlarged peripheral lymph nodes

Day 7

- First reports of papules were evident in the axillary, tibial and inguinal areas, and face

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- First reports of vesicle/papules formation was also observed on the lips or within the oral cavity.
- First reports of friable livers, pulmonary lesions, gross lesions in the esophageal tract, hemorrhage of testis, and marked enlarged peripheral lymph nodes

Day 9-10

- First reports of ulcerated papules and necrotic cutaneous lesions on the arms, legs, axillae, and face with extensive, ulcerating lesions in the oral cavity.
- First reports of friable splenic lesions, hepatic congestion, pulmonary congestion and white foci, papules in the stomach and ileum and colon, and enlarged lymph nodes

Study title: Double-blind, randomized, placebo-controlled study to evaluate the minimum effective therapeutic dose of oral ST-246 Polyform I in cynomolgus monkeys infected with MPXV

Study no.:	**AP-09-026G**
Study report location:	EDR
Conducting laboratory and location:	USAMRIID, Fort Detrick, Frederick, MD
Date of study initiation:	November 24th, 2009
GLP compliance:	GLP study
QA statement:	Yes
Drug, lot #, and % purity:	ST-246, lot #SG-08K07-M, purity 99.5%

Key Study Findings

In an IV model of lethal monkeypox disease (5.5 x 10^7 PFU), daily oral administration of tecovirimat at 3 or 10 mg/kg after disease was established on day 4 were sufficient to confer survival to MPXV infected cynomolgus monkeys.

<u>Summary:</u>
The study was a double blind, randomized, placebo-controlled repeat dose efficacy study of tecovirimat in monkeypox infected cynomolgus monkeys.

Monkeys (male cynomolgus monkeys of Mauritius origin) were exposed to a lethal IV dose of monkeypox (5.5 x 10^7 PFU). Monkeys were divided into groups of 7 (placebo) or 5 (dosed groups) of animals. Tecovirimat (or placebo) was administered starting on day 4 post infection. Doses of tecovirimat were 0.3, 1, 3, and 10 mg/kg. The weight range was relatively large (4-8 kg) but it did not appear to be a confounding factor for infection or weight based dosing.

All monkeys in the 1 mg/kg group succumbed to disease (as well as the placebo group). The 0.3 mg/kg group had one surviving animal at scheduled sacrifice at end of study.

Pathological findings of the animals was typical of pox disease in cynomolgus monkeys. Furthermore, many of the animals that succumbed to disease had secondary infections upon death.

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Disease progression was monitored by overall health status and lesion counts. Temperature and viral load was not a clear surrogate for disease progression as the endpoint varied greatly among the test animals. Also, tissue viral load could not be evaluated since the timepoints (death) varied greatly among animals.

Euthanasia criteria were established. However, the study director used broad discretion to determine the morbidity and humane status of the animals throughout this study. Although this could add bias into the primary study endpoint (mortality), given the other supportive data on the health status of the animals, it was likely not a major confounding factor.

Methods (drug)

Doses:	0, 0.3, 1, 3, 10 mg/kg (dosing was subdivided into 2 cohorts/iterations)
Frequency of dosing:	1x daily starting when pox lesions first appeared (Day 4 post-infection) every 24±2 hours for 14 consecutive days (except for 1 treatment day where dosing was not performed within the 24±2 hour window) (*noted in deviations*)
Route of administration:	Oral
Dose volume:	2 mL/kg followed by a suspension of 5 (±0.5) mL/kg hydrated monkey chow.
Formulation/Vehicle:	1% (w/v) HPMC plus 0.5% (w/v) Tween 80 in deionized water. • Negative Control (placebo) contained 200g of Avicel (to assist in the suspension) • Final drug concentrations ranged from 88.9-99.8% (via formulation analysis)

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Species/Strain:	Cynomolgus monkeys, Mauritius origin (no other source information was provided)
Number/Sex/Group:	27 males (one per cage). See below for groups
Age:	2.5-4.0 years
Weight:	4.2-8.6 kg
Blinding:	PI and technicians were blinded to treatment groups during study conduct.
Deviations:	The virus was not stored or propagated in accordance with GLP standards. From the Applicant: *"The inoculum used as challenge material (lot EMM10152008) was prepared using the non-GLP lot; however, the concentration of that lot was verified by plaque assay in accordance with an approved SOP prior to study initiation. Additionally, the concentration of the inoculum was verified once again after animals were challenged."* Numerous pathological recording deviations were noted. However, since the pathological data were not a primary endpoint, it did not affect the overall primary endpoint. In summary, the noted deviations did not impact study interpretation.

Methods (pathogen challenge)

Challenge Agent info:	Monkeypox (Zaire 79 strain)
Challenge agent lot:	WTG03Oct05 (unverified – data on storage or handling of the pathogen was not supplied by the CRO or in the study report)
Doses:	5.0×10^7 PFU
Frequency of dosing:	Once on Day 0
Route of administration:	IV
Back Titrated Final Dose:	5.55×10^7 PFU

Reference ID: 4258909

Iteration 1					
Group	Number per Group[a] (male)	ST-246 Dose (mg/kg)	Volume (mL/kg)	Target Concentration [mg/mL (w/v)]	Actual Average Concentration[b] [mg/mL (w/v)]
1	3	10	2	5.0 ± 0.25	4.5401**
2	3	3	2	1.5 ± 0.08	1.3480**
3	3	1	2	0.5 ± 0.03	0.4566**
4	3	0.3	2	0.15 ± 0.008	0.1334**
5	4	0	2	0.0 ± 0.0	N/A*
Iteration 2					
Group	Number per Group[a] (male)	ST-246 Dose (mg/kg)	Volume (mL/kg)	Target Concentration [mg/mL (w/v)]	Actual Average Concentration[b] [mg/mL (w/v)]
1	2	10	2	5.0 ± 0.25	4.8325
2	2	3	2	1.5 ± 0.08	1.4963
3	2	1	2	0.5 ± 0.03	0.4858
4	2	0.3	2	0.15 ± 0.008	0.1319**
5	3	0	2	0.0 ± 0.0	0.00

Protocol Related Discussion Points

- Anesthesia: Animals were anesthetized with Telazol® (50 mg of tiletamine and 50 mg of zolazepam per mL) prior to handling (once daily in the AM). A dose of 3 mg/kg (up to 6 mg/kg) was injected intramuscularly (IM).
- Veterinary/supportive care: Pedialyte (or fruit flavored drink, e.g., Tang®) was offered ad libitum after onset of pox lesions. All animals received meloxicam (an NSAID, at 3 mg/kg) daily. Lactated Ringer's solution (LRS) was administered SC on day 4 post infection for dehydration 20 ml/kg. Animals exhibiting severe dehydration received a doubling of LRS (40 ml/kg) until hydration was achieved (at the discretion of the study director)

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

- Criteria for early euthanasia of moribund animals included, but were not limited to: 1) persistent prostration (4 hours or longer) and no response to gentle prodding through the cage, or 2) persistent prostration (4 hours or longer), but responsive to gentle prodding through the cage and a rectal temperature of <34ºC. Euthanasia criteria were assessed for each animal, as necessary, on the Orthopoxvirus Infected NHP Final Observation Sheet. Several animals did not meet the euthanasia criteria, but were euthanized at the discretion of the study director for humane reasons (noted in deviations)
- However, upon reviewing the reports regarding the findings at the time of euthanasia, the animals appeared to demonstrate signs of overt monkeypox disease (see summary tables in the Mortality section below)

Reference ID: 4258909

Observations and Results

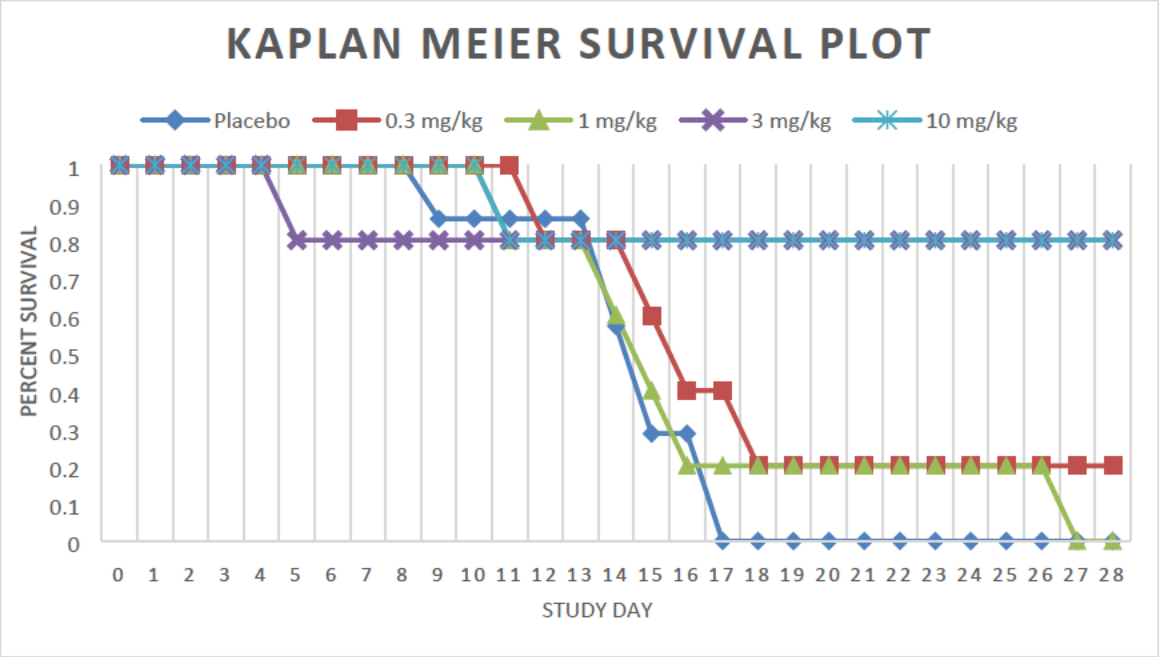
Mortality

Mortality was monitored 2x daily (physical observation in the AM, health check in the PM).

Survival increased with dose of tecovirimat. Animals on the higher doses of tecovirimat (3 and 10 mg/kg) had the highest survival (80%) with 0.3 and 1.0 mg/kg doses of tecovirimat conferring a slight increase in survival time, however, all animals in the 1 mg/kg group eventually succumbed to disease.

Group	Survival
Vehicle	0%
0.3 mg/kg	20%
1 mg/kg	0%
3 mg/kg	80%
10 mg/kg	80%

Therefore, doses of 3 or 10 mg/kg were likely sufficient to confer survival to cynomolgus monkeys dosed at day 4 after a lethal IV monkeypox infection.



ST-246 Dose (mg/kg)	Number Started Study	Number Completed Study	Day Post-Infection of Unscheduled Death[a]
Placebo	7	0	9, 14 (2), 15 (2), 17 (2)
0.3	5	1	12, 15, 16, 18
1	5	0	11, 14, 15, 16, 27
3	5	4	5
10	5	4	11

[a] Number in parentheses indicates the number of animals necropsied on that day.

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See tables below for the individual/tabulated findings at time of death.

Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
2	Placebo	Day 15	Yes	Yes	• Persistent prostration • Severe unresponsiveness (does not withdraw when prodded) • Decreased food consumption (0% of biscuits and fruit) • No feces • Typical disease apparent
3	Placebo	Day 15	No	Not applicable	• Lymphadenopathy (≥10 to19 mm max dimension) • Mild dehydration • Found dead on Day 15 • Typical disease apparent
11	Placebo	Day 14	No	Not applicable	• Moderate nasal discharge • Lymphadenopathy (≥20 mm max dimension) • Moderate dehydration • Eyes crusted shut • Animal did not recover from Telazol® administration • Rectal temperature 34.2°C after a.m. physical • Typical disease apparent
13	Placebo	Day 9	Yes	Yes	• Decreased food consumption (0% consumption of biscuits) • Moderate nasal discharge • Lymphadenopathy (≥10 to 19 mm max dimension) • Moderate dehydration • Persistent prostration (for a period of four hours or longer) in afternoon • Unresponsive to gentle prodding through the bottom of the cage in afternoon • Animal still bleeding from subcutaneous fluid injection site in afternoon • Typical disease apparent

Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
26	Placebo	Day 14	Yes	No	• Decreased food consumption (0% consumption of biscuits) • Feces not fully formed • Copious nasal discharge • Rectal temperature 33.0°C • Lymphadenopathy (≥20 mm max dimension) • Profuse bleeding in nares and oral cavity • Severe swelling • Typical disease apparent
18	Placebo	Day 17	Yes	No	• Moderate unresponsiveness (withdraws when prodded) • Decreased food consumption (10%) • Liquid feces • Copious nasal discharge • Mild edema • Lymphadenopathy (≥20 mm max dimension) • Moderate dehydration • Possible bacterial infection in roof of oral cavity • Severe unresponsiveness in afternoon • Profuse bleeding in afternoon • Rectal temperature 33.4°C in afternoon • Typical disease apparent
23	Placebo	Day 17	Yes	No	• Mild unresponsiveness • Feces not fully formed • Nasal discharge barely visible • Mild edema • Lymphadenopathy (≥20 mm max dimension) • Mild dehydration • Unresponsive in afternoon • Severe edema in afternoon • Rectal temperature 33.8°C in afternoon • Typical disease apparent

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Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
7	0.3 mg/kg	Day 15	Yes	Yes	• Persistent prostration • Severe unresponsiveness (does not withdraw when prodded) • Decreased food consumption (0% consumption of biscuits and fruit) • No feces • Rectal temperature not readable • Typical disease apparent
8	0.3 mg/kg	Day 18	Yes	Yes	• Persistent prostration • Unresponsiveness to gentle prodding through the bottom of the cage • Foam around nose and mouth • Animal in bottom of cage • Open mouth breathing (gasping) • Unresponsive to toe pinch • Typical disease apparent
9	0.3 mg/kg	Day 16	Yes	Yes	• Mild unresponsiveness (becomes active when approached) that developed into severe unresponsiveness (unresponsive to painful stimuli) • Decreased food consumption (0% consumption of biscuits) • Feces not fully formed • Lymphadenopathy (≥10 to 19 mm max dimension) • Mild dehydration • Persistent prostration in afternoon • Unresponsiveness to gentle prodding through the bottom of the cage in afternoon • Typical disease apparent

Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
22	0.3 mg/kg	Day 12	Yes	No	• Moderate unresponsiveness (withdraws when prodded) • Decreased food consumption (0% consumption of biscuits) • Feces not fully formed • Moderate nasal discharge • Lymphadenopathy (≥20 mm max dimension) • Severely swollen • Eyes swollen shut • Oral cavity is non functional • Typical disease apparent
4	1 mg/kg	Day 11	Yes	No	• Moderate unresponsiveness (withdraws when prodded) • Decreased food consumption (0% consumption of biscuits and fruit) • No feces • Copious nasal discharge • Copious bleeding (except venipuncture site) • Lymphadenopathy (≥20 mm max dimension) • Moderate dehydration • Entire oral cavity sloughed off • Bleeding from the face • Palms and feet sloughed • Eyes swollen shut • Trachea almost swollen shut • Typical disease apparent
6	1 mg/kg	Day 16	No	Not applicable	• Mild unresponsiveness • Decreased food consumption (>20 to 40% consumption of biscuits) • Moderate nasal discharge • Lymphadenopathy (≥20 mm max dimension) • Moderate dehydration • Found dead on Day 16 • Typical disease apparent

Reference ID: 4258909

Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
12	1 mg/kg	Day 14	No	Not applicable	• Decreased food consumption (>20 to 40% consumption of biscuits) • Moderate nasal discharge • Lymphadenopathy (≥10 to 19 mm max dimension) • Moderate dehydration • Animal did not recover from Telazol® administration • Typical disease apparent
17	1 mg/kg	Day 15	Yes	No	• Moderate unresponsiveness (withdraws when prodded) • Decreased food consumption (0% consumption of biscuits and fruit) • No feces • Copious nasal discharge • Severe edema • Barely visible bleeding (except venipuncture site) • Lymphadenopathy (≥20 mm max dimension) • Moderate dehydration • Sloughing of mouth • Typical disease apparent
19	1 mg/kg	Day 27	No	Not applicable	• Found dead on Day 27 • Weight 3.82 kg • Rectal temperature 32.1°C • Decreased food consumption (10+ biscuits in cage but ate some supplement) • No urine or feces • Some nasal discharge from left nostril • All pox lesions recorded as scabbing or desquamating • Typical disease NOT apparent

Animal Number	Dose	Day of Death (Post-infection)	Euthanized Moribund	Euthanasia Criteria Met	Findings on Day of Death (and/or day before death)
27	3 mg/kg	Day 5	No	Not applicable	• Decreased food consumption (20 to 40% consumption of biscuits) • Animal died under anesthesia prior to physical exam and dosing • Typical disease NOT apparent
21	10 mg/kg	Day 11	No	Not applicable	• Animal died under anesthesia prior to physical exam and dosing • Typical disease NOT apparent

Clinical Signs

Monkeys were scored on an Orthopoxvirus Infected NHP Clinical Observation Sheet (OPCOS). The OPCOS was a standardized form to record symptoms of illness including poxvirus lesion count by body system, recumbency, unresponsiveness, dyspnea, cough, fruit and biscuit consumption, nasal discharge, condition of stool, rash, edema, bleeding, lymphadenopathy (enlarged lymph nodes), and dehydration. Most of the symptoms were rated on a 4-point scale (0=not present; 1=mild; 2=moderate; and 3=severe). Poxvirus lesion was scored by poxvirus lesion type for each body system and total count for the entire body.

Overall clinical scores were higher in the placebo group and with decreased frequency, in a dose-related manner, in tecovirimat-treated animals. Clinical signs also increased over time, which correlated with MPXV disease. The earliest sign (lymphadenopathy) was related to the immune response to MPXV disease in all animals.

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Table 2 - NHP/MPXV Clinical Signs

Observation	No.	%	No.	%	No.	%	No.	%	No.	%
Post-Infection Day 4 (Treatment Day 1)										
	Placebo (n=7)		**0.3 mg/kg (n=5)**		**1 mg/kg (n=5)**		**3 mg/kg (n=5)**		**10 mg/kg (n=5)**	
Lymphadenopathy	6	86%	4	80%	4	80%	4	80%	5	100%
Post-Infection Day 6 (Treatment Day 3)										
	Placebo (n=7)		**0.3 mg/kg (n=5)**		**1 mg/kg (n=5)**		**3 mg/kg (n=4)**		**10 mg/kg (n=5)**	
Lymphadenopathy	7	100%	5	100%	5	100%	4	100%	5	100%
Feces (not fully formed/liquid/none)	2	29%	3	60%	2	40%	3	75%	0	0%
Dehydration	1	14%	0	0%	0	0%	1	25%	0	0%
Post-Infection Day 17 (Treatment Day 14)										
	Placebo (n=2)		**0.3 mg/kg (n=2)**		**1 mg/kg (n=1)**		**3 mg/kg (n=4)**		**10 mg/kg (n=4)**	
Lymphadenopathy	2	100%	2	100%	1	100%	3	75%	3	75%
Unresponsiveness	2	100%	1	50%	0	0%	0	0%	0	0%
Feces (not fully formed/liquid/none)	2	100%	1	50%	0	0%	0	0%	0	0%
Nasal Discharge	2	100%	0	0%	0	0%	0	0%	0	0%
Edema	2	100%	0	0%	0	0%	0	0%	0	0%
Dehydration	2	100%	1	50%	0	0%	0	0%	0	0%

Observation	No.	%	No.	%	No.	%	No.	%	No.	%
Post-Infection Day 28										
	Placebo (n=0)		**0.3 mg/kg (n=1)**		**1 mg/kg (n=0)**		**3 mg/kg (n=4)**		**10 mg/kg (n=4)**	
Lymphadenopathy	NA		0	0%	NA		1	25%	0	0%
Post-Infection Day 42										
	Placebo (n=0)		**0.3 mg/kg (n=1)**		**1 mg/kg (n=0)**		**3 mg/kg (n=4)**		**10 mg/kg (n=4)**	
Lymphadenopathy	NA		0	0%	NA		0	0%	0	0%

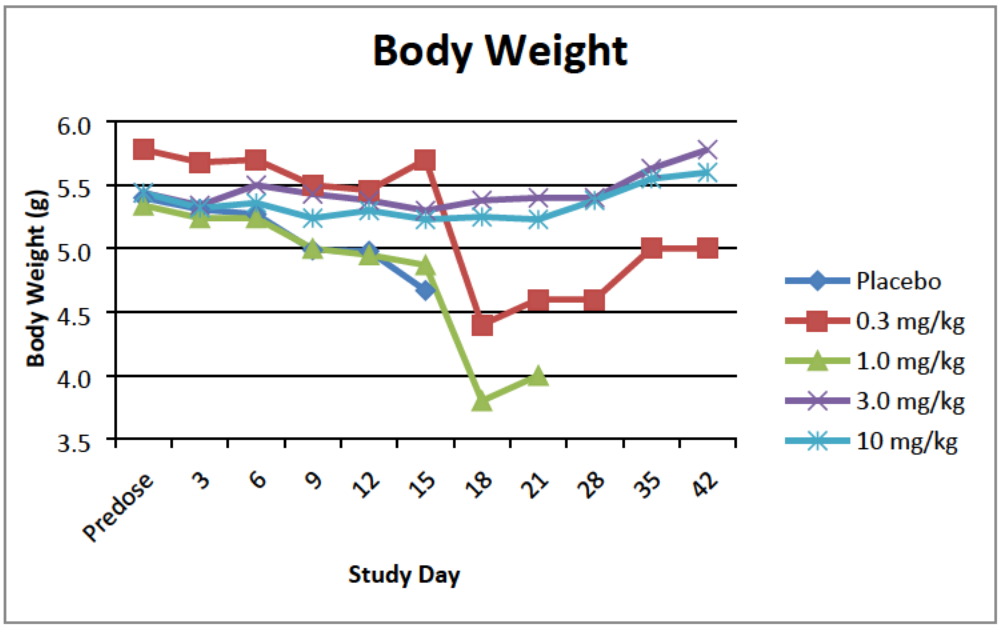
NA: not applicable

Body Weights

Recorded daily during physical examination.

Body weights decreased after infection with recovery in body weight correlating with increasing drug dose. Animals in the placebo and 1.0 mg/kg were all sacrificed moribund. Only 1 animal in group 0.3 mg/kg survived to Day 42.

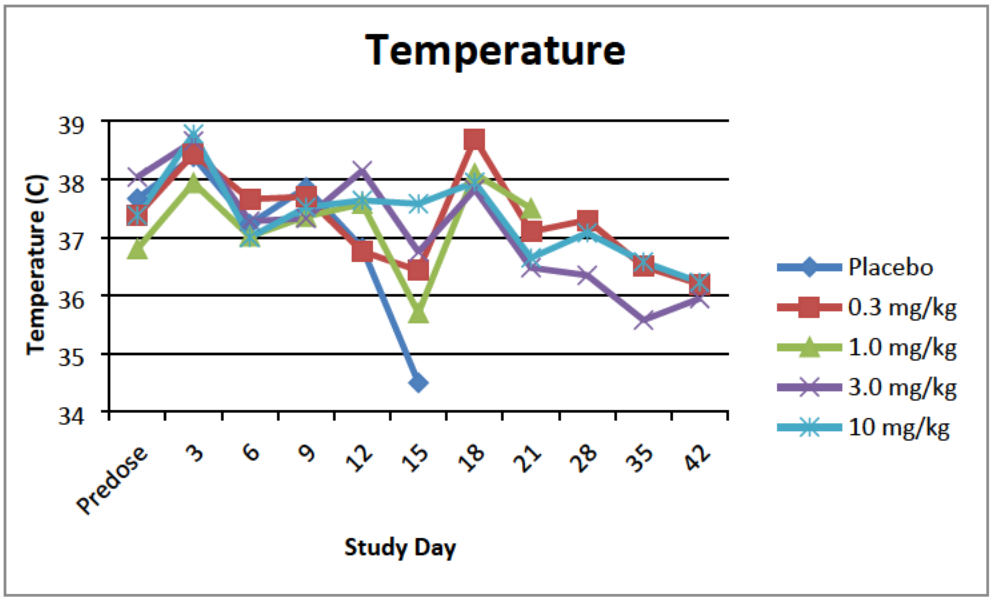
Reference ID: 4258909



Temperature

Measured (rectally) daily. Rectal temperatures were variable over the course of the study, but in general were ± 2ºC of the baseline values with several exceptions.

Temperatures increased after infection (Day 3) with average increases on Days 3, 6, and 9 to be (≤ 2.8ºC) across the groups. Body temperature decreases were noted later in infection. Median decreases were slightly greater in the animals in the placebo and 0.3 mg/kg groups than in the 1, 3 and 10 mg/kg groups. A significant decrease was also noted in one placebo animal (-7.6ºC) on Day 15 prior to euthanasia and in several 3 mg/kg animals on Days 21 through 42 post-infection. All surviving animals had lower body temperatures by Day 42 post infection.



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Hematology

The most significant findings were the increase in WBC's (mean increase of 142% over baseline by post infection Day 7) that was related to infection/inflammation by MPXV.

Changes in RBC's (decreases in red blood cell count, hematocrit, and hemoglobin concentration) were associated with blood collection/sampling and not disease progression.

Clinical Chemistry

There were widespread, adverse increases in BUN among moribund and spontaneous death animals; surviving animals had non-dose related BUN concentrations within the range of expected variability. Non-dose related decreases in total protein, albumin (a negative acute-phase reactant), and the decreases in calcium (approximately 40% being albumin-bound) occurred in all groups and correlated with gross findings of subcutaneous edema and/or thoracic/abdominal effusions and inflammation during peak of disease.

Pathology

Necropsies were conducted by a board-certified veterinary pathologist under BSL-3 practices on study animals that died or had been euthanized.

Animals that succumbed to disease or were euthanized had lesions typical of monkeypox infection. Bacterial co-infection was present in 8 animals on study and likely contributed to morbidity and mortality.

There were substantially fewer signs of lesions among surviving animals.
All nine of the surviving ST-246-treated animals had grossly evident cutaneous desquamated and/or scar lesions.

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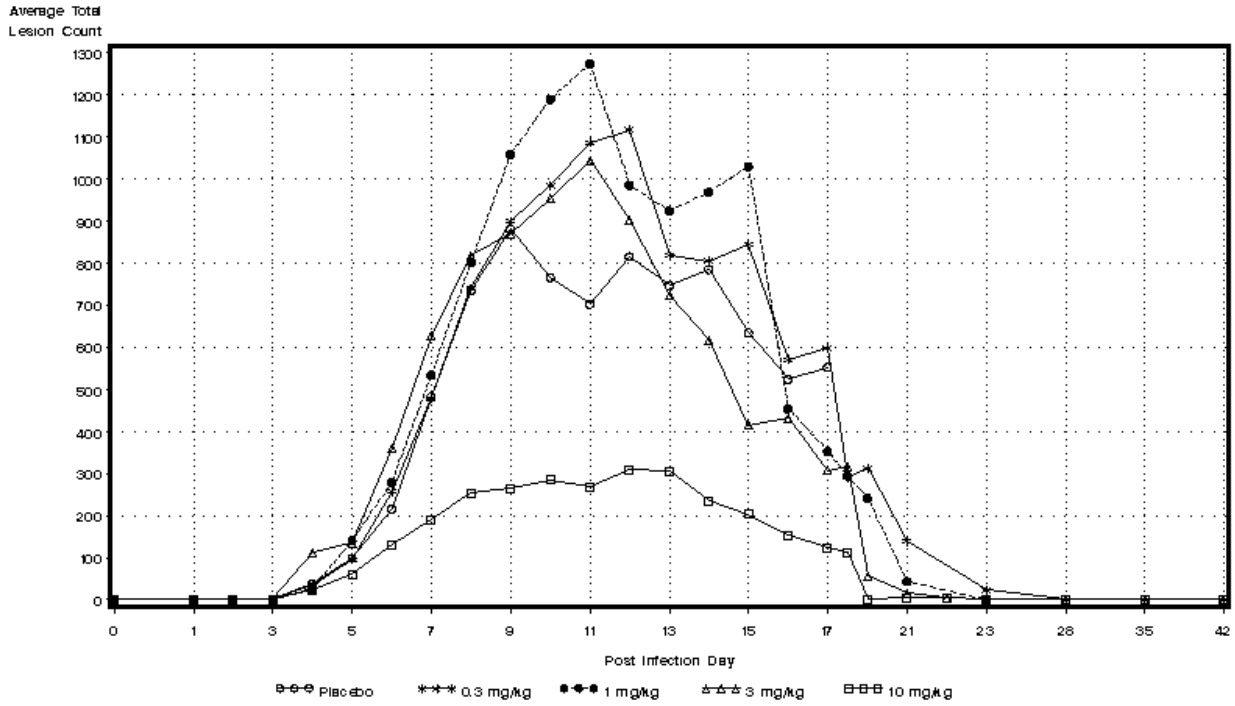
Figure 21 - NHP/MPXV Total Pox Lesions

(Note: The poor resolution of the figure is from the Applicant's Application)



Blood/Tissue Viral Load (by PCR)

Whole Blood PCR indicated that viral load was lower in the higher survival groups (3 mg/kg and 10 mg/kg).

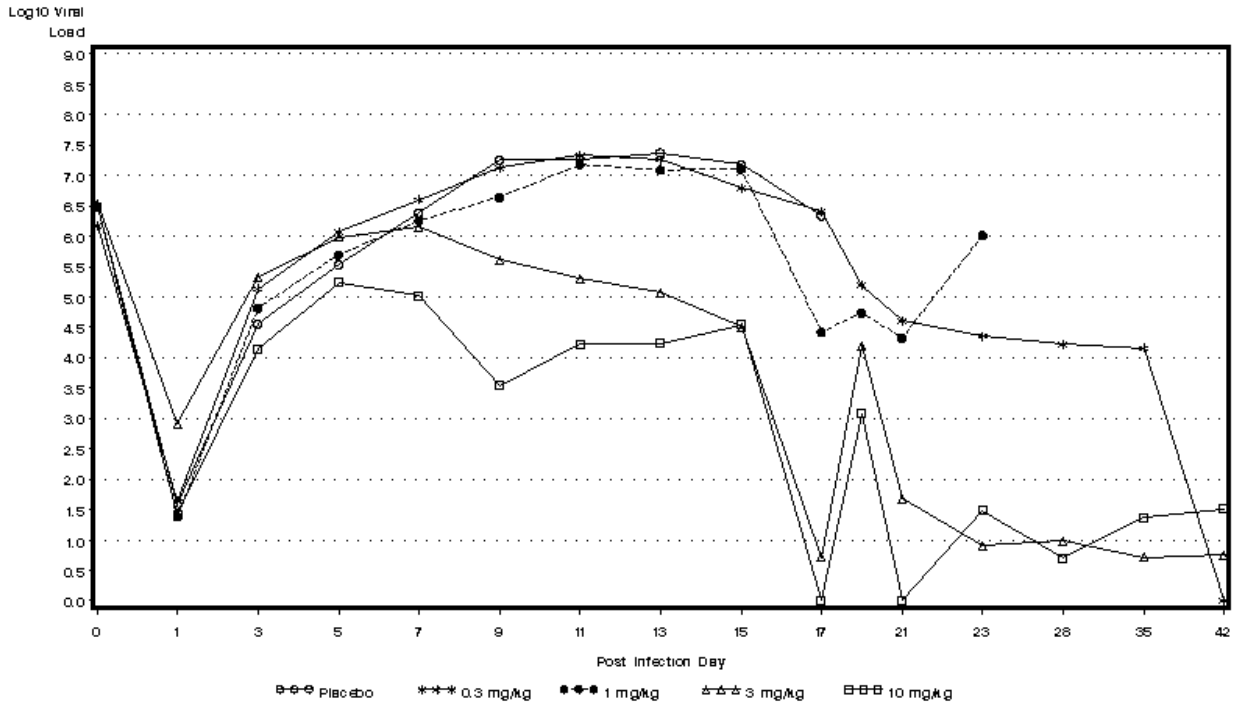
Generally, viral load was higher in animals that succumbed to disease or were euthanized moribund (compared to survivors). However, this finding could be an artifact of temporal sampling since tissue from survivors was taken days/weeks later than the animals sacrificed prior to study end.

Therefore, no conclusions can be drawn from these data since the sampling timepoints are drastically different among the animals.

Reference ID: 4258909

Figure 22 - NHP/MPXV Whole Blood Viral Load (log 10)

(Note: The poor resolution of the figure is from the Applicant's Application)



Toxicokinetics

Tecovirimat concentrations were determined prior to and 4 hours after dosing on Study Days 4, 10, and 17. Plasma concentrations of tecovirimat increased in a dose-proportional manner. There are no calculated TK values presented in the study report. Values on Day 4 and 14 are presented below.

- Day 4 (treatment Day 1): mean plasma concentrations were 25.4, 77.9, 259, and 504 ng/mL in the 0.3, 1, 3, and 10 mg/kg groups, respectively.
- Day 14: mean plasma concentrations were 50.6, 54.2, 246, and 868 ng/mL in the 0.3, 1, 3, and 10 mg/kg groups, respectively.

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Study title: Evaluation of the Pharmacokinetics of ST-246 in Cynomolgus Macaques Infected Intravenously with Monkeypox Virus

Study no.:	**FY10-087**
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	August 6th, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246, OTW Lot #3004295, Supplier Lot #SG-10A11-M

Key Study Findings

This study was designed to evaluate the PK and survival of cynomolgus monkeys infected with a lethal IV dose of monkeypox virus (MPXV) and tecovirimat. Overall, the study drug gave a survival advantage to monkeys challenged with monkeypox.

- 100% of animals dosed with 3, 10, or 20 mg/kg of tecovirimat starting on Day 4 post-infection survived a lethal MPXV infection
- 0% of placebo (no drug) animals survived a lethal MPXV infection

PK was collected adequately. *See Clinical Pharmacology review by Dr. Su-Young Choi for PK modeling to the human dose.*

Summary

Cynomolgus monkeys were dosed with a lethal IV dose of monkeypox (5×10^7 PFU) and then dosed with study drug (0, 3, 10, or 20 mg/kg) starting on Day 4 post-infection until Day 17. Animals dosed with drug survived to sacrifice whereas placebo were all euthanized moribund by Day 16.

The actual monkeypox dose was significantly lower than expected (30.5% of target for placebo and 3 mg/kg and 39.6% of target for 10 and 20 mg/kg). Therefore, disease progression may have shifted to hours/several days later than the expected peak on Day 4. It was possible that the dosing of the drug in this study on Day 4 may have started prior to establishment of severe disease due to the lower viral challenge.

At the time of the study, there were no clear euthanasia criteria developed for this protocol. Therefore, the moribund sheet was used with the professional opinion of the study director for the euthanasia of moribund study animals.

Body weights initially decreased for all animals but increased after study drug. Body weights of placebo never recovered by the time of euthanasia. Temperature was notably unremarkable for most animals (due to the high variability within groups) and was not a reliable marker for disease progression. Clinical signs and pox lesions

Reference ID: 4258909

progressed with monkeypox infection. PCR levels of serum virus increased over time in the vehicle animals but decreased after study drug in all dosed groups. Lastly, the TK analysis indicated that there was no accumulation or significant change in the TK/PK parameters between the beginning and end of study.

Overall, it appeared that study drug (at all doses, as low as 3 mg/kg) salvaged the animals from a lethal IV dose of monkeypox virus.

Methods (drug)

Doses:	0, 3, 10, 20 mg/kg (listed as 1000, 2000, 3000, and 4000 series for some graphs)
Frequency of dosing:	- 1x on Day -10 then a washout prior to virus challenge. - 1x daily from Days 4-17
Route of administration:	Oral
Dose volume:	- On the first attempt, tecovirimat or vehicle was delivered first followed by hydrated monkey chow [5 (± 0.5) mL/kg] and meloxicam [0.3 (± 0.03) mg/kg]. - If an animal regurgitated or vomited during or after administration (within 10 minutes of dosing), the animal was redosed with tecovirimat or vehicle, 50% of the hydrated monkey chow dose, and meloxicam as soon as practical for the study. - If the animal regurgitated a second time, no redosing occurred on that study day.
Formulation/Vehicle:	(0.5% Tween 80 [Lot #108K0111] and 1% hydroxypropylmethylcellulose [Lots H25U014 and F16W040]. Final drug concentrations were within 15% of the target value (8.36% difference from target was the highest noted variation)
Species/Strain:	Cynomolgus monkeys ((b) (4)
Number/Sex/Group:	24 (equal number of males and females -- one per cage). See below for groups. Identified by tattoo and microchip.
Age:	3-5 years
Weight:	2-4 kg
Blinding:	Not described in the protocol or report.
Deviations:	No deviations from the study protocol, subsequent amendments, or study specific procedures affected the quality or integrity of the study.

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Methods (pathogen challenge)

Challenge Agent info:	Monkeypox virus. Strain: Zaire 79, CDC V79-I-005. Source: BEI Resources (Manassas VA)
Challenge agent lot:	NR-2324
Doses:	5.0 x 10^7 PFU
Frequency of dosing:	Once on Day 0
Route of administration:	IV
Back Titrated Final Dose:	1.63 x 10^7 or 2.18 x 10^7 PFU

Group	#NHP	MPXV
1 – Vehicle	6 (3 male/3 female)	5x10^7 pfu IV
2 – ST-246 [a] 3 mg/kg	6 (3 male/3 female)	5x10^7 pfu IV
3 – ST-246 [a] 10 mg/kg	6 (3 male/3 female)	5x10^7 pfu IV
4 – ST-246 [a] 20 mg/kg	6 (3 male/3 female)	5x10^7 pfu IV

[a]ST-246 treatment was initiated at 96 hours (Day 4) post-infection via oral delivery (gavage) and was continued as once-a-day dosing for 14 total doses (up to and including Day 17)

Protocol Related Discussion Points

- Anesthesia: Ketamine, 5 mg/kg, IM
- Veterinary/supportive care: In addition to biscuits, moistened food was provided to the animals. Animals had unlimited access to municipal water except during conditioning, dose administration, blood collections, lesion documentation, and observations. Animals were also given meloxicam meloxicam 0.3 mg/kg formulated as a 1.5 mg/mL oral suspension.

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

- Euthanasia was performed based on morbidity scores (using an SOP and a scoring sheet). From the protocol: "Moribund animals can be defined as those (1) demonstrating seizures, severe depression, or coma (2) respiratory distress or severe dyspnea (3) persistent recumbency and weakness (4) unresponsiveness to touch or external stimuli (5) body weight loss >20% over a seven day period or (6) any combination of these observations. The Study Director will make decisions regarding the euthanasia of moribund animals."

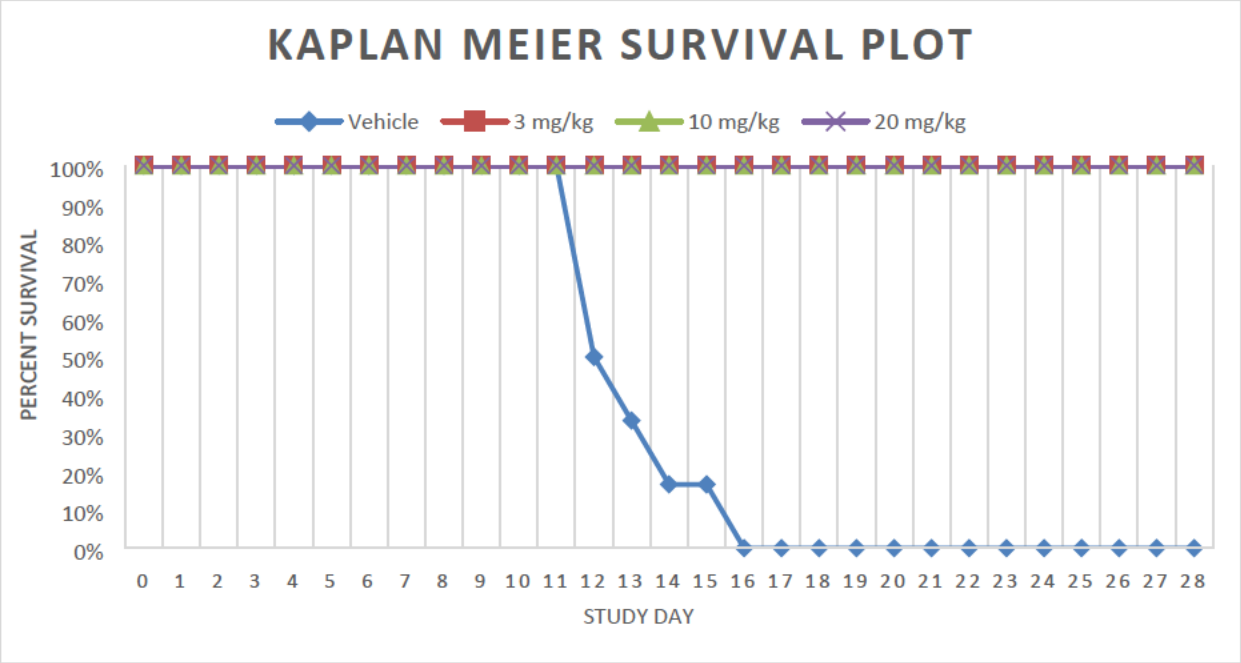
Observations and Results

Mortality

Mortality was monitored 2x daily (physical observation in the AM, health check in the PM). Control animals were euthanized on Days 12, 13, 14, and 16. All other animals survived to scheduled sacrifice.

Reference ID: 4258909

Group	Survival
Vehicle	0%
3 mg/kg	100%
10 mg/kg	100%
20 mg/kg	100%



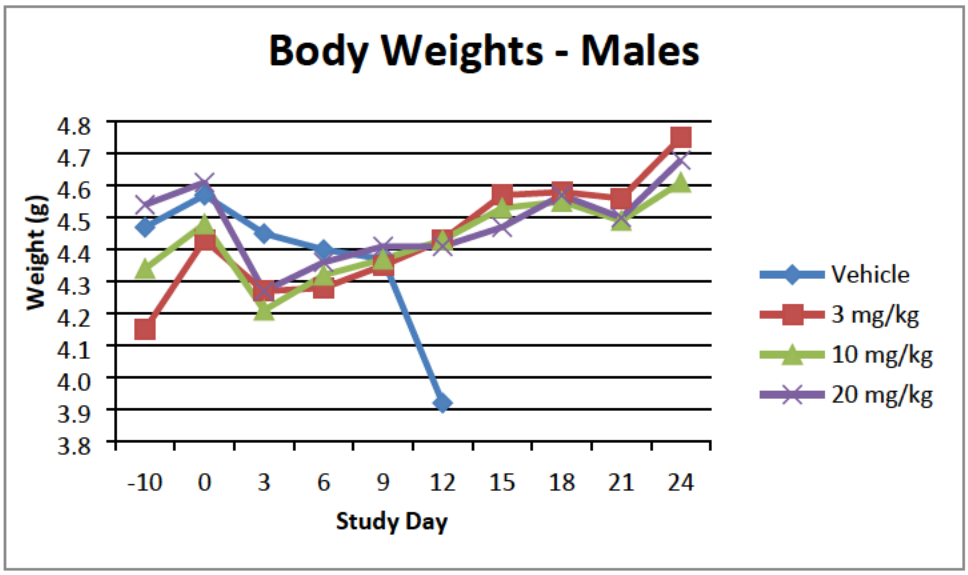
Clinical Signs

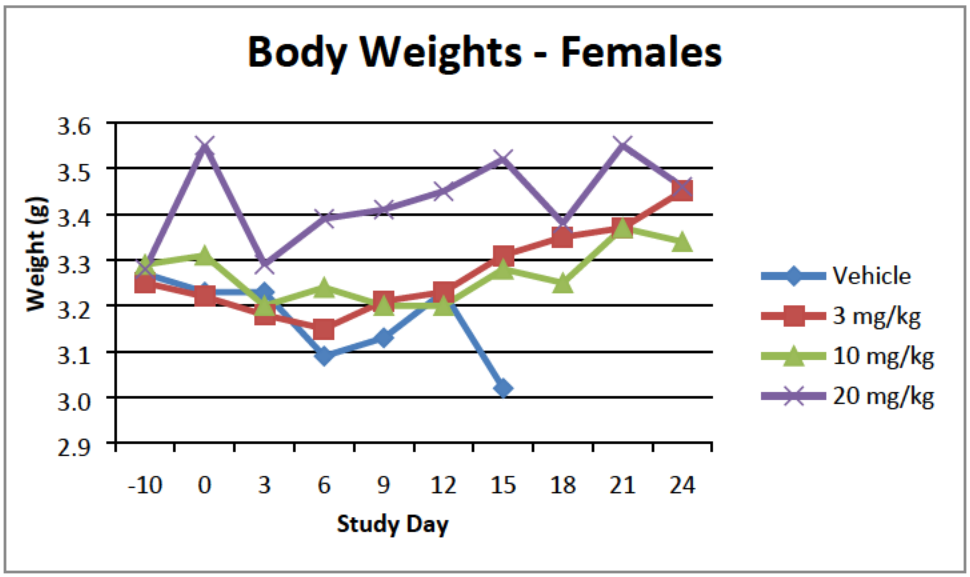
Monitored Daily. Observations noted throughout the study were associated with monkeypox infection or serial pharmacokinetic blood collections. These include: thin, dehydrated, decline in posture, shaking, piloerection, nasal discharge, ocular discharge, skin reddening/swelling, lesions, change in appetite, changes in stool and urine output, diaphragmatic respirations, increased temperature, and bruising.

Body Weights

Recorded daily during physical examination. Body weight decreased with infection, but in treated animals, the body weight recovered. The body weight of the placebo animals did not recover prior to euthanasia.

Reference ID: 4258909





Temperature

Measured electronically from microchips. Data are presented below. Data in the graphs correspond to: Vehicle (1000 series), 3 mg/kg (2000 series), 10 mg/kg (3000 series), 20 mg/kg (4000 series).

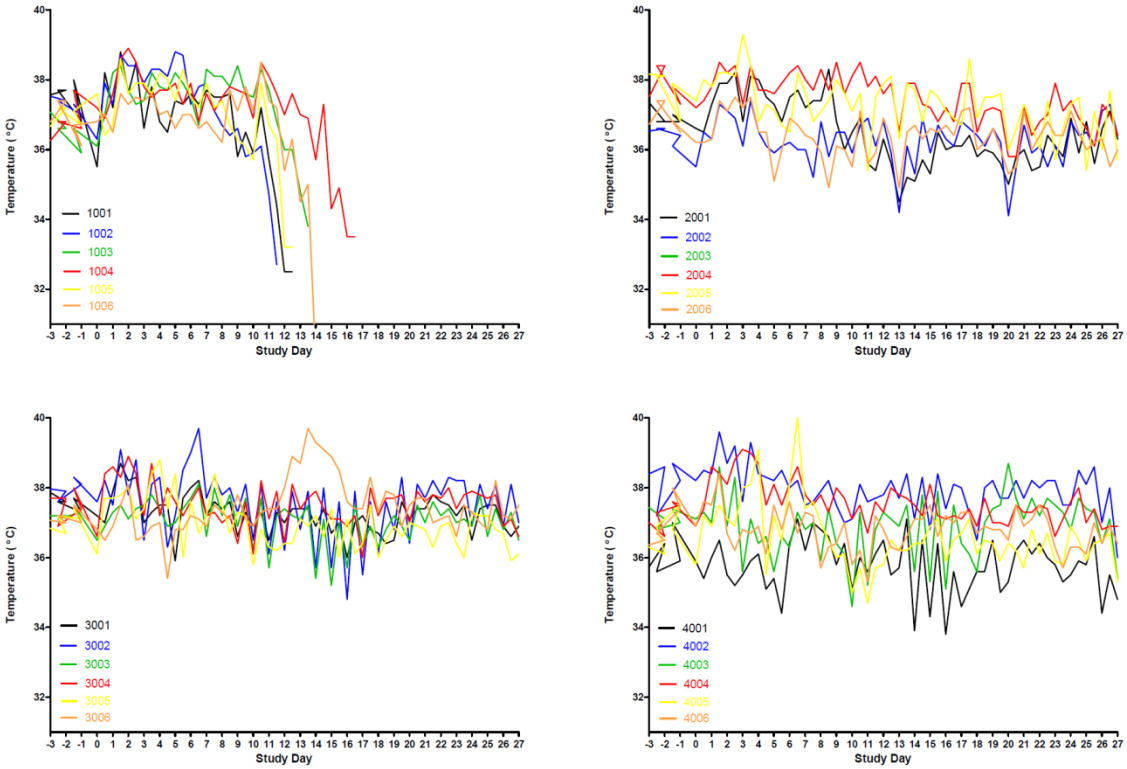
Pre-infection and post infection temperatures were variable making it difficult to use temperature as a marker for disease progression. Baselines temperatures (Day -3 through Day 0, prior to infection) ranged from 35-39°C.However, for placebo animals (the 1000 series below), the temperatures dropped significantly prior to death with temperatures as low as 32°C prior to euthanasia.

Reference ID: 4258909

One animal (2003, top right chart, green line) did not have data due to a chip malfunction during the study.

Figure 23 - NHP/MPXV Body Temperatures

Note: Figure from Applicant's Application



Pox Lesions

By Day 3 lesions were noted in animals from all study groups (macule, papule, pustule, or crust) with some animals exhibiting pox prior to Day 3.

By Day 18, lesions were described as umbilicated, crusted, or desquamated with further progression by Day 21 to crusted or desquamated.

Animals were not anesthetized in order to lower that confounding variable for the pharmacokinetics. Therefore, the process of counting lesions (on non-anesthetized / fully awake NHPs) was more difficult than in other studies that used anesthesia. Due to the lack of anesthesia, the lesions counts were highly variable in this study.

Blood/Tissue Viral Load (by PCR)

Viral infection was monitored by quantitative real-time polymerase chain reaction (qPCR). All animals lacked detectable circulating MPXV (PCR for the HA gene) prior to challenge. After challenge, all groups demonstrated high concentrations of MPXV in the blood. Overall animals treated with tecovirimat trended downward until sacrifice,

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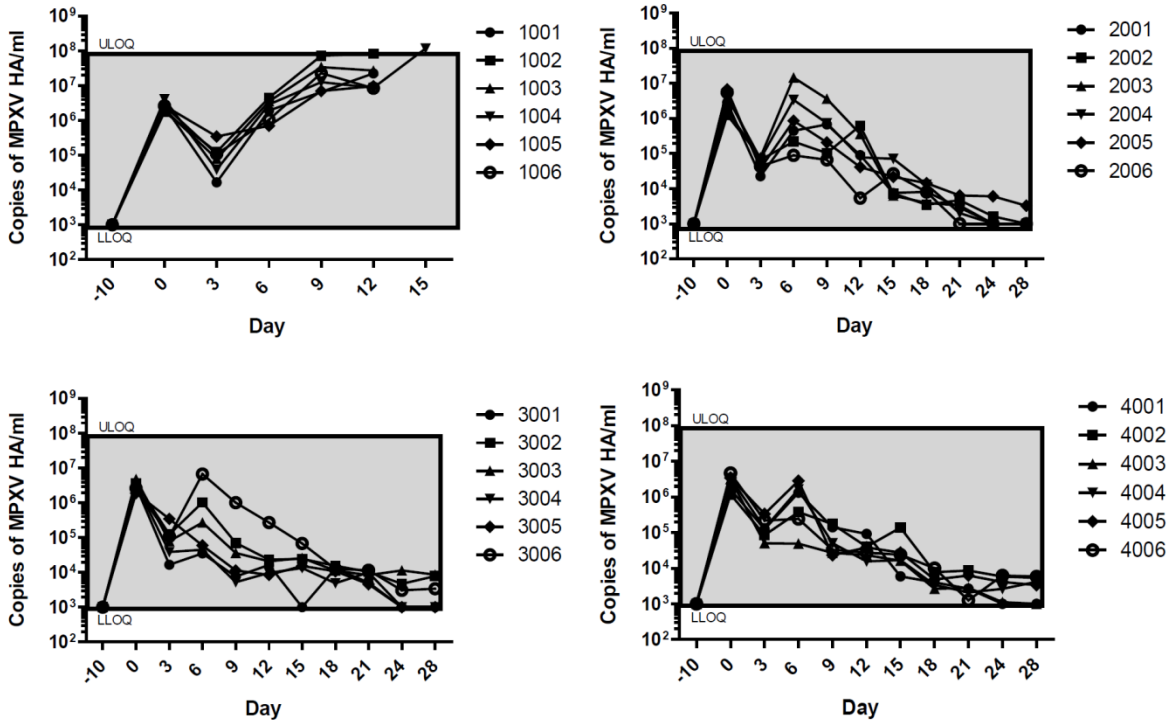
whereas vehicle/placebo animals trended downward initially, and then increasing in serum virus until euthanasia. Graphs are below.

Figure 24 - NHP/MPXV Quantified Copies of MPXV HA Gene in Whole Blood



Toxicokinetics

TK/PK was measured predose (Day -10), Day 4 (first day of drug), Day 10, and Day 17 (last day of drug). Although there was a slight drop in tecovirimat noted on Day 4 (in C_{max} and $AUC_{0\text{-last}}$), the predose and final doses were not significantly different. Also, there was a dose response with no plateau noted.

Table 3 - NHP/MPXV Tecovirimat $AUC_{0\text{-last}}$

Exposure Group	Day -10	Day 4	Day 10	Day 17
		Males[a]		
3 mg/kg	3360 (1800)	3560 (804)	3560 (253)	2320 (640)
10 mg/kg	20000 (7000)	10300 (3150)	15300 (1960)	20400 (4490)
20 mg/kg	44600 (22300)	19600 (11100)	39100 (17000)	40700 (19200)
		Females[a]		
3 mg/kg	3320 (1490)	3420 (1570)	4330 (1180)	4380 (2390)
10 mg/kg	11000 (10000)	6210 (1750)	13100 (7370)	14600 (5160)
20 mg/kg	25900 (9450)	14900 (5810)	26800 (12200)	24700 (10300)

[a]all data was rounded to 3 significant figures; shown as average (standard deviation)

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Table 4 - NHP/MPXV Tecovirimat C_{max}

Exposure Group	Day -10	Day 4	Day 10	Day 17
		Males[a]		
3 mg/kg	406 (248)	365 (113)	467 (80)	335 (99)
10 mg/kg	1250 (352)	1050 (284)	1620 (86)	1440 (242)
20 mg/kg	2420 (1500)	1410 (707)	3340 (1430)	2740 (1100)
		Females[a]		
3 mg/kg	332 (34)	562 (349)	453 (87)	563 (193)
10 mg/kg	924 (526)	570 (179)	1290 (713)	1450 (539)
20 mg/kg	1630 (684)	1100 (397)	2670 (1450)	2160 (1026)

[a]all data was rounded to 3 significant figures, shown as average (standard deviation)

Study title: Double-Blind Placebo Controlled Study to Evaluate Effect of Delayed ST-246 Treatment on Efficacy Following Lethal Monkeypox Virus Challenge in Cynomolgus Macaques

Study no.: **SR10-037F**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: July 22th, 2010
GLP compliance: No, but did follow SOPs
QA statement: Yes
Drug, lot #, and % purity: ST-246, lot #SG-08K07-M, 99.5% pure

Key Study Findings

Animals receiving drug starting on Day 4 or 5 post infection had a high rate of survival (83%) to a lethal dose of MPXV. Animals receiving drug on Day 6 had a decreased rate of survival (50%) compared to the Day 4 or Day 5 drug groups, but still significantly more than placebo (0%).

Overall, starting drug on Day 4 or 5 post MPXV infection leads to a high rate of survival. Starting drug on Day 6 leads to a significant, but less robust rate of survival.

Summary:
This was a delayed treatment study with animals receiving tecovirimat (10 mg/kg) on Day 4, Day 5, or Day 6 after inoculation with a lethal dose of monkeypox (with 5 x 10^7 PFU monkeypox virus).

This study was designed to identify the maximum delay post-challenge at which tecovirimat (10 mg/kg) is effective at preventing mortality in nonhuman primates. The secondary objective was to assess the impact of tecovirimat on viremia and pox lesion counts.

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The findings in the study were related to MPXV infection. Animals which survived to scheduled sacrifice had significantly less findings compared to the animals that died prior to scheduled sacrifice. Viremia was not a useful correlate in this study since tissue viremia in animals sacrificed prior to the end of study would normally have higher rates of virus compared to animals that were allowed to recover from MPXV disease. Due to the significant difference in times between the sacrifices, it is not possible to compare these animals. Serum virus peaked during MPXV infection around Day 3-9 and decreased in the surviving animals until scheduled sacrifice.

Animals did not have back titrations of virus to evaluate the actual virus dose. However, lethality in the placebo indicated that the animals received a lethal dose of MPXV and study drug did perform as expected (dosing on Day 4 or 5 correlated to a higher rate of survival compared to a delay to Day 6 post MPXV infection).

Methods (drug)

Doses:	10 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	2.0 mg/mL
Formulation/Vehicle:	1% hydroxypropyl methylcellulose (HPMC)/0.5% Tween® 80
Species/Strain:	Cynomolgus monkeys (Chinese origin), (b) (4) *The animals were pre-screened for antibodies against vaccinia virus (cross-reactivity with monkeypox and other pox viruses). Only those with negative result were used on study.*
Number/Sex/Group:	21 (dose groups below), males and females
Age:	3.27-3.50 years
Weight:	2.64-3.66 kg
Blinding:	Double blind
Deviations:	None that would interfere with study interpretation.

Methods (pathogen challenge)

Challenge Agent info:	Monkeypox Virus
Challenge agent lot:	V79-I-005, NR-523 (also called the Zaire 79 strain)
Doses:	5.0×10^7 PFU
Frequency of dosing:	One dose
Route of administration:	IV
Back Titrated Final Dose:	No back titration noted. Virus assumed to be as stated.

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Group[a]	No. of Animals		MPXV Challenge Dose (Day 0)	ST-246 Dose Level	Dosage Schedule[b]	
	M	F			ST-246	Placebo
1 – Placebo[c]	1	2	5×10^7 PFU IV	0 mg/kg	N/A	Days 4-19
2 – ST-246 Day 4	3	3	5×10^7 PFU IV	10 mg/kg	Days 4-17	Days 18 and 19
3 – ST-246 Day 5	3	3	5×10^7 PFU IV	10 mg/kg	Days 5-18	Days 4 and 19
4 – ST-246 Day 6	3	3	5×10^7 PFU IV	10 mg/kg	Days 6-19	Days 4 and 5

[a]Group numbers were assigned to aid in clarity in the protocol. The animals were arranged by animal number, not by group.
[b]In addition to being dosed with ST-246, the animals in Groups 2-4 received placebo on the days indicated to accommodate blinding.
[c]Animals in Group 1 were administered the drug vehicle by oral gavage and served as placebo controls.
KEY: PFU = plaque-forming units; N/A = not applicable; IV = intravenous

Protocol Related Discussion Points

- Anesthesia: Ketamine (100 mg/mL) prior to handling for study procedures (daily). A dose of 10 to 30 mg/kg (volume < 1 mL/injection) was injected intramuscularly in the caudal thigh.
- Veterinary/supportive care: Meloxicam, a long acting non-steroidal anti-inflammatory drug, was administered orally to all monkeys at 0.3 ± 0.03 mg/kg prior to dose administration on Days 4-19. Hydration status was evaluated each time the monkeys were anesthetized for study procedures. Monkeys that appeared clinically dehydrated were given 60 mL of Lactated Ringer's solution subcutaneously.
- Blinding: Double blind (study director and technicians were both blind to the dose groups)

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

- No specific euthanasia criteria were used for this study. However, daily observations were made and moribund animals were sacrificed based on a combination of observations of moribund status and monkeypox disease progression.
- *From the Applicant: "Animals found in moribund condition prior to scheduled termination (Day 56) were immobilized via intramuscular injection of ketamine and then humanely euthanized via intravenous injection of sodium pentobarbital. The decision to euthanize a particular animal prior to scheduled termination was determined through consultation between the Study Director and the veterinary staff and was based on the severity of the clinical observations in combination with significant weight loss, severity of pock [pox] lesions, elevated body temperature, and anorexia. Daily clinical observations for each animal were recorded on the Orthopoxvirus Clinical Observation Form."*

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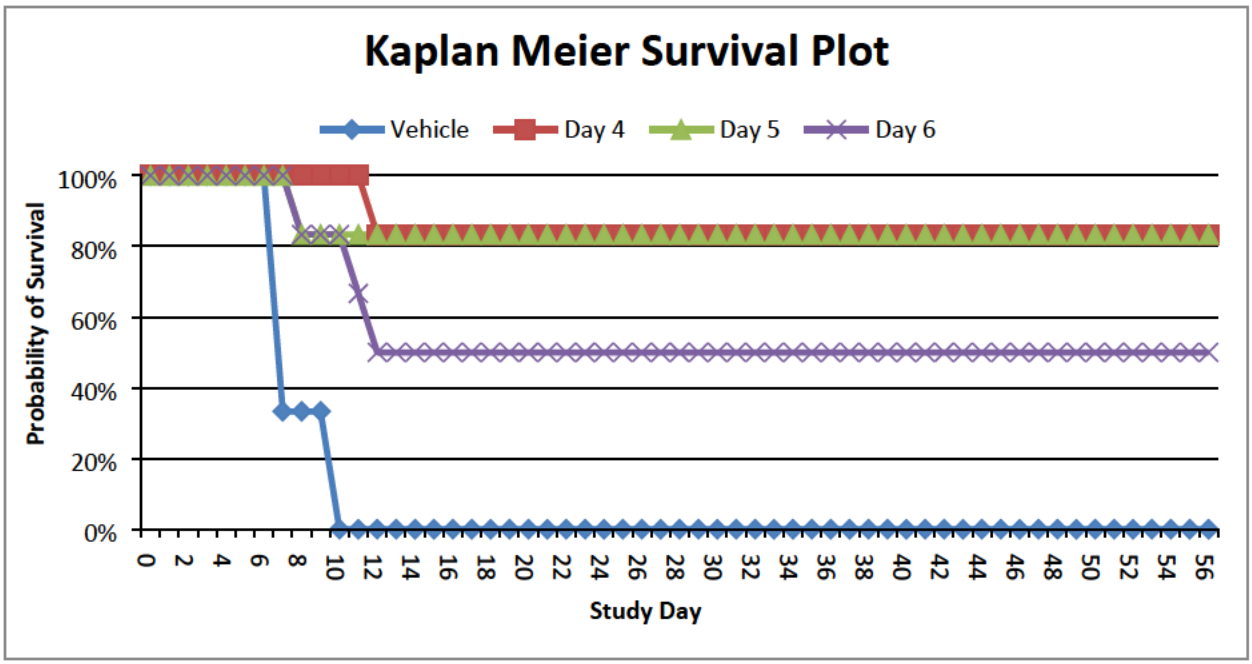
Observations and Results

Mortality

Mortality was associated with time of dosing. The animals dosed with drug on Day 4 or 5 had a 83% rate of survival, whereas the animals dosed on Day 6 had only a 50% chance of survival. All placebo animals died by Day 10. Animals in the dose groups were sacrificed moribund on days 8 (Day 4 animal), 11 (Day 5 and Day 6 animals), and 12 (Day 4 and 6 animals). All other animals survived to scheduled sacrifice.

Therefore, early intervention on Day 4 correlated with a high rate of survival from established, lethal monkeypox disease.

Group	Survival
Vehicle	0%
Day 4	83%
Day 5	83%
Day 6	50%



Clinical Signs

Monitored Daily. Observations noted throughout the study were associated with monkeypox infection. The most common clinical observations included dehydration, depression, dyspnea, ocular discharge, nasal discharge, weakness, and reduced food consumption.

Body Weights

Body weights were measured every three days and was changed to daily on dosing days to assist with dose calculations. Randomization average body weights were

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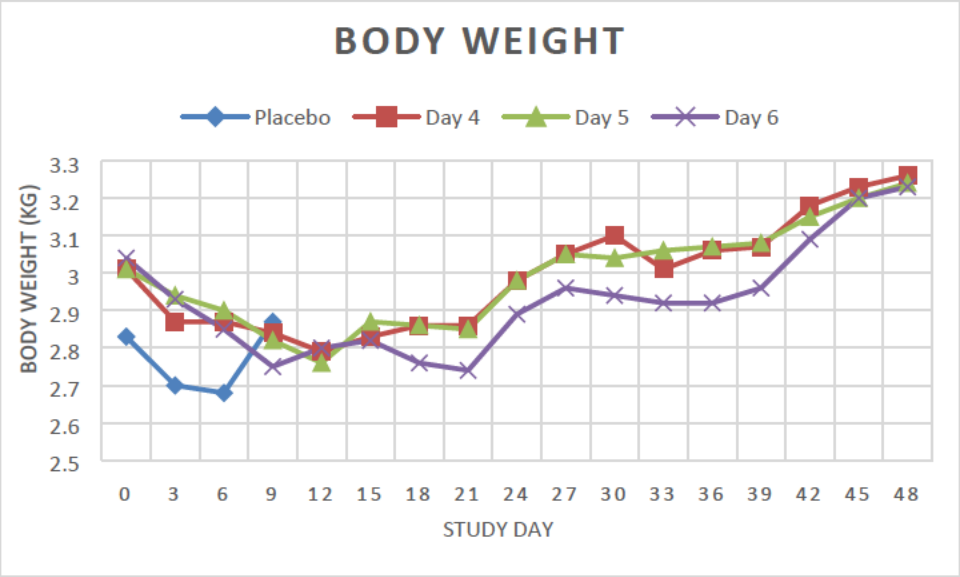
relatively lower for the placebo group compared to the dosed groups. However, the distribution overlapped between groups.

Body weights initially dropped with monkeypox infection and then recovered in animals that did not succumb to disease. Body weights continued to increase until scheduled sacrifice. It is notable that the Day 6 animals did have a lag in body weight gain compared to the other treatment groups from Days 18-48. However, the low number of animals in Group 6 (3 survivors) at those time points prevents any statistical comparison. However, the trend is notable.



Temperature

Body temperatures (rectal) were measured every 3 days. Temperatures were mostly overlapping between all groups. The trend was that temperatures initially increased with infection around Day 3-6 (indicating an early fever with viremia). However, the temperatures then decreased in all animals that survived infection (around Day 9) and stayed around baseline until scheduled sacrifice.

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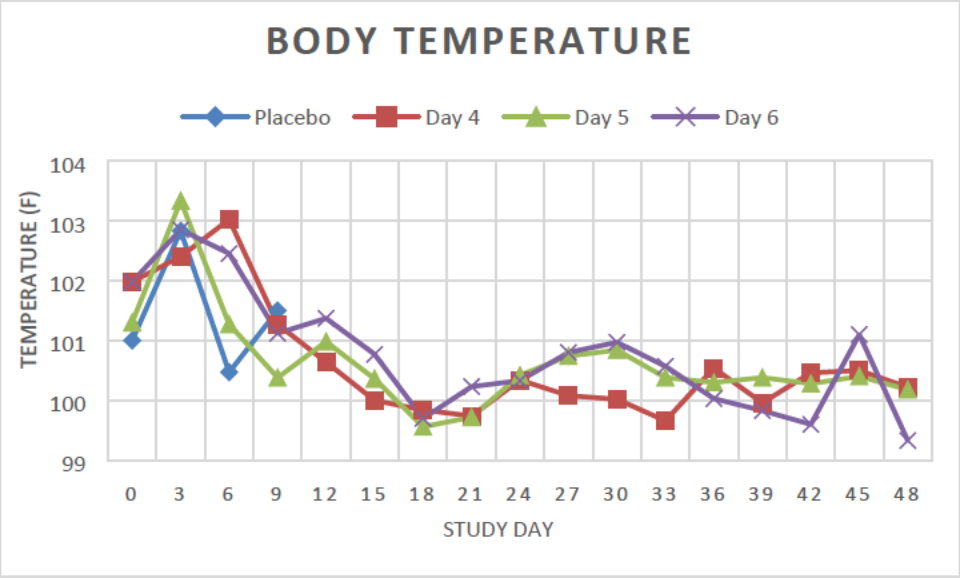

Food Consumption

Food consumption was relatively normal (or mildly decreased) until infection Day 3. From post-infection Days 4-6, mild to moderate reductions in food consumption were observed in all groups, except for one placebo animal and two monkeys in each of the tecovirimat treatment groups. These monkeys had severely reduced food consumption on Days 5 and/or 6. These time points correlate to the severe stage of MPXV disease.

In general, animals that survived to scheduled sacrifice had transient food consumption decreases during the peak of disease which increased by the end of study. Animals that were sacrificed moribund generally had decreased food consumption. This decrease correlates to moribund conditions during MPXV infection.

Clinical Observations

Clinical observations were made daily. The most common clinical observations included dehydration, depression, dyspnea, ocular discharge, nasal discharge, weakness, and reduced food consumption. These observations were consistent with MPXV infection. Clinical observations returned to normal on Study Day 12 in surviving animals starting dosing on Day 4. However, clinical observations in animals starting drug on Days 5 and 6 resolved clinical observations by Day 14.

There was a slight increase in findings in the animals starting drug on Day 5 and especially Day 6 compared to dosing Day 4. This strongly suggests that a delay in treatment after peak of disease will diminish the drug effects to increase survival in MPXV treated NHPs.

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Table 5 - NHP/MPXV Clinical Observations

Adverse Event	Placebo (n=3)		Day 4 Dosing (n=6)		Day 5 Dosing (n=6)		Day 6 Dosing (n=6)	
	No.	%	No.	%	No.	%	No.	%
Treatment Day 1 (Post-Infection Day 4)								
Dehydration	1	33%	2	33%	1	17%	1	17%
Treatment Day 4 (Post-Infection Day 7) [n=2 (Placebo), n=6 (Day 4, Day 5, and Day 6)]								
Depression	2	100%	1	17%	1	17%	2	33%
Weakness	1	50%	0	0%	1	17%	2	33%
Dehydration	2	100%	6	100%	6	100%	6	100%
Dyspnea	1	50%	1	17%	3	50%	2	33%
Ocular Discharge	0	0%	1	17%	0	0%	2	33%
Treatment Day 16 (Last Day of Treatment; Post-Infection Day 19) [n=0 (Placebo), n=5 (Day 4, Day 5), n=3 (Day 6)]								
No abnormal signs noted								
Post-Infection Day 56 [n=0 (Placebo), n=5 (Day 4, Day 5), n=3 (Day 6)]								
No abnormal signs noted								

Hematology

RBC and platelets were not remarkable (mean changes were all within ±30% of baseline) in most animals. WBCs, however, increased with MPXV disease. WBC count increased above baseline (at least 30%) in all animals except one between post-infection Days 6 and 28. The timing of WBC count increases was sporadic, but was seen in most animals at least between Days 6 and 28 post-infection.

Creatinine exhibited notable, but inconsistent increases. Potassium showed slightly greater elevations in non-survivors than in survivors. These changes could suggest impairment in liver and/or kidney function in the non-surviving monkeys prior to early death or euthanasia.

Calcium, sodium, total protein, and globulin levels were not altered significantly in any animals in the study.

Prior to death:
In most monkeys that did not survive to scheduled termination, platelet counts were notably reduced in the days preceding death (-67.6% to -40.3%). However, in 2 monkeys, platelet counts were increased (36.9% and 75.4%) prior to death.

WBC count significantly increased in most animals (86.9% to 443% above baseline) on the day prior to death.

Prior to unscheduled death, animals that were found dead or were euthanized early consistently had markedly elevated alkaline phosphatase, ALT, total bilirubin, and BUN concentrations, and slightly reduced amylase levels compared with monkeys that survived to scheduled termination.

Pathology

The most common findings during necropsy were related to the immune response to MPXV (enlarged or slightly enlarged darkened lymph nodes, enlarged or slightly enlarged spleen with discolorations).

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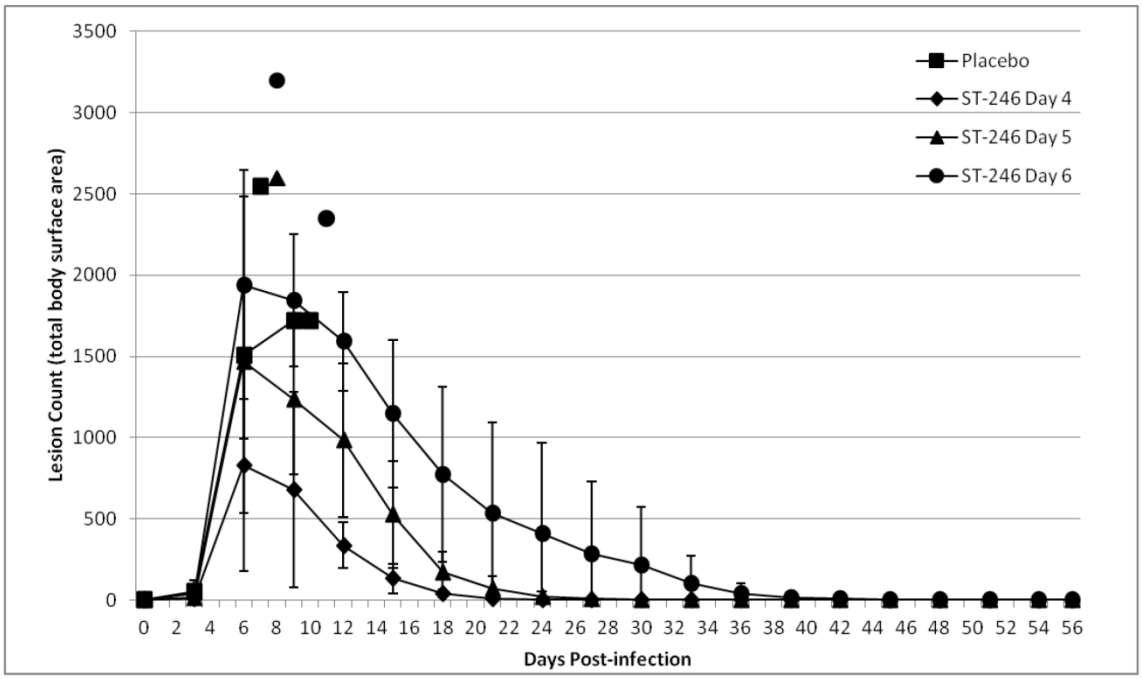
Other findings in non-survivors: yellow/pale with a lesion (pancreas), focal lesions (lung, stomach), red lesions (lung, stomach – with blood clots, heart), pale (lung, liver, kidney, heart), gelatinous (lung, liver), thickening (bladder, colon, stomach, esophagus), bloody urine, sloughing skin on tongue, gas filled colon, leathery kidney, emphysema lesion in the lung.

Overall these findings were consistent with advanced MPXV disease.

Lesions

There were no statistically significant differences in the change in total lesion count from treatment baseline for any treatment group compared to the placebo group on any of treatment Days 1-16. However, the trends were lower for the earlier drug intervention.

Figure 25 - NHP/MPXV Total Lesion Count (Excluding Injection Site)



NOTES: Symbols connected by lines indicate the mean of each group at each scheduled counting interval, with error bars indicating standard deviations. Stand-alone symbols not connected by lines indicate individual animal lesion counts performed off-schedule at the time of euthanasia. Symbols for individual animals are the same as the treatment group to which they belong.

Blood/Tissue Viral Load (by PCR)

The PCR analysis in tissue is not easily interpretable - due to the inconsistent timing of the sampling. Samples were taken at necropsy (which was not on the same day across the study animals). Therefore, the animals that died early had very high virus levels compared to PCR levels at scheduled sacrifice of animals that recovered from disease.

Across all animals, the highest viral DNA levels were observed in lesional skin, normal skin, liver, tongue, trachea, bone marrow, inguinal lymph node, lung, spleen, and axillary lymph node.

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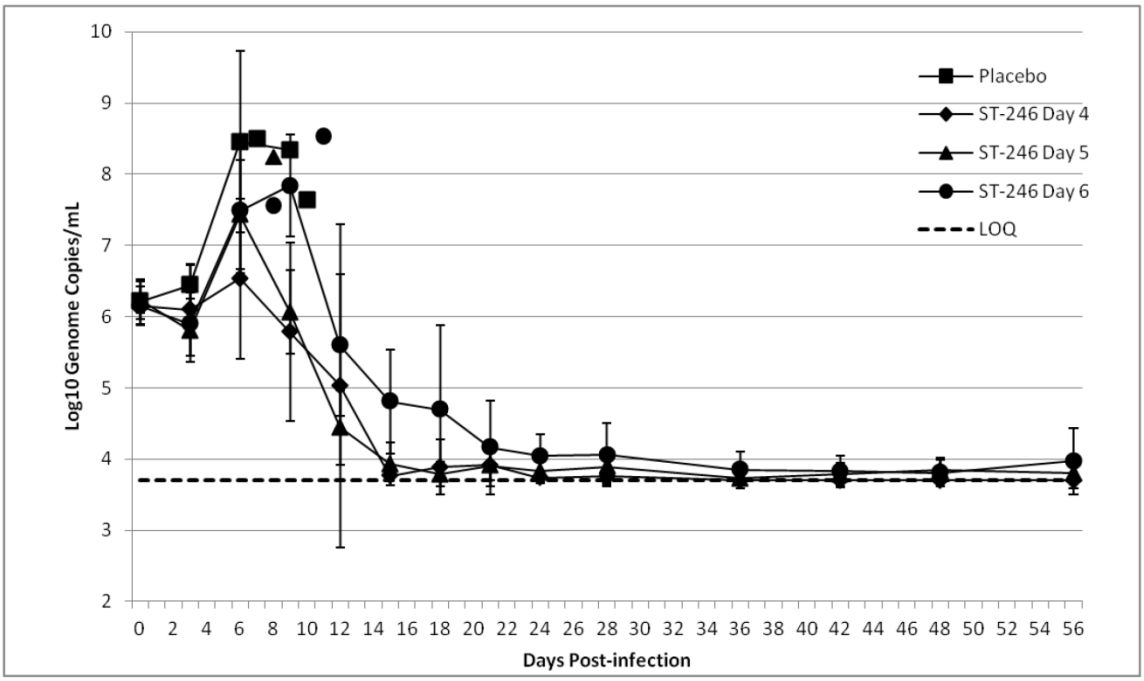
Serums levels of MPXV corresponded to MPXV disease. Peak levels were early (around Day 3-9) and decreased over time in the animals that survived to scheduled sacrifice. Serum virus levels tended to be lower in the animals that started drug early. However, due to the limited number of animals at scheduled sacrifice, only a trend can be suggested from the data.

Figure 26 - NHP/MPXV Mean Whole Blood Viral Load



KEY: LOQ = limit of quantitation (dashed line)
NOTES: Symbols connected by lines indicate the mean of each group at each scheduled sampling, with error bars indicating standard deviations. Stand-alone symbols not connected by lines indicate individual animal data collected off-schedule at the time of euthanasia. Symbols for individual animals are the same as the treatment group to which they belong.

Toxicokinetics

Not performed for this study.

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

Study no.:	**SR10-038F**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 8th, 2010
GLP compliance:	No, but did follow SOPs
QA statement:	Yes
Drug, lot #, and % purity:	ST-246, lot #SG-08K07-M, 99.5%

Key Study Findings

Tecovirimat treatment of 5 days or greater associated with improved survival.

Group	Survival
Vehicle	20%
3 Days of drug	50%
5 Days of drug	100%
7 Days of drug	100%
10 Days of drug	80%

Summary

This study was designed to evaluate whether the duration of drug exposure (starting on Day 4 post MPXV infection) would correlate to survival. Animals were dosed daily for 3, 5, 7, or 10 days post infection.

Treatment with 5, 7, or 10 doses of tecovirimat was associated with increased survival (100% with 5 or 7 doses, 80% with 10 doses) compared to animals given placebo (25%) or 3 doses of tecovirimat (50%). Treatment with 5, 7, or 10 doses of tecovirimat: decreased viral DNA in blood, lower trends for skin lesion counts, milder clinical manifestations, and less severe changes in clinical chemistry parameters (compared to 3 doses or placebo).

Methods (drug)

Doses:	10 mg/kg
Frequency of dosing:	Daily (starting on Day 4 for various lengths of time)

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Route of administration:	Oral Gavage
Dose volume:	2.0 mg/mL
Formulation/Vehicle:	1% hydroxypropyl methylcellulose (HPMC)/0.5% Tween® 80
Species/Strain:	Macaca fascicularis (cynomolgus monkey); Chinese origin, [redacted] (b) (4) *The animals were pre-screened for antibodies against vaccinia virus (cross-reactivity with monkeypox and other pox viruses). Only those with negative result were used on study.*
Number/Sex/Group:	25 (see below)
Age:	3.81-5.05 years
Weight:	2.56 to 4.52 kg
Blinding:	Dosing of drug was blinded to the technicians and the study director
Deviations:	None that would interfere with study interpretation.

Methods (pathogen challenge)

Challenge Agent info:	Monkeypox Virus
Challenge agent lot:	V79-I-005, NR-523 (also called the Zaire 79 strain)
Doses:	5.0×10^7 PFU
Frequency of dosing:	Single dose
Route of administration:	IV
Back Titrated Final Dose:	No back titration noted. Virus assumed to be as stated.

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Group[a]	No. of Animals		MPXV Challenge Dose (Day 0)	ST-246 Dose Level	Dosage Schedule[b]	
	M	F			ST-246	Placebo
1 – Placebo[c]	2	2	5 x 10^7 PFU IV	0 mg/kg	N/A	Days 4-13
2 – ST-246 3 doses	2	2	5 x 10^7 PFU IV	10 mg/kg	Days 4-6	Days 7-13
3 – ST-246 5 doses	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-8	Days 9-13
4 – ST-246 7 doses	3	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-10	Days 11-13
5 – ST-246 10 doses	2	3	5 x 10^7 PFU IV	10 mg/kg	Days 4-13	N/A

[a]Group numbers were assigned to aid in clarity in the protocol. The animals were arranged by animal number, not by group.
[b]In addition to being dosed with ST-246, the animals in Groups 2-4 received placebo on the days indicated to accommodate blinding.
NOTE: Animals in Group 1 were administered the drug vehicle plus Avicel® by oral gavage and served as placebo.
KEY: PFU = plaque-forming units; N/A = not applicable; IV = intravenous

Protocol Related Discussion Points

- Anesthesia: Ketamine (100 mg/mL) prior to handling for study procedures (daily). A dose of 10 to 30 mg/kg (volume < 1 mL/injection) was injected intramuscularly in the caudal thigh.
- Veterinary/supportive care: Meloxicam, a long acting non-steroidal anti-inflammatory drug, was administered orally to all monkeys at 0.3 ± 0.03 mg/kg prior to dose administration on Days 4-19. Hydration status was evaluated each time the monkeys were anesthetized for study procedures. Monkeys that appeared clinically dehydrated were given 60 mL of Lactated Ringer's solution subcutaneously.
- Blinding: Double blind (study director and technicians were both blind to the dose administration)

Protocol: Euthanasia Criteria Documentation (to verify Mortality)

- No specific euthanasia criteria were used for this study. However, daily observations were made and moribund animals were sacrificed based on a combination of observations of moribund status and monkeypox disease progression.
- *From the Applicant: "Animals found in moribund condition prior to scheduled termination (Day 56) were immobilized via intramuscular injection of ketamine and then humanely euthanized via intravenous injection of sodium pentobarbital. The decision to euthanize a particular animal prior to scheduled termination was determined through consultation between the Study Director and the veterinary staff and was based on the severity of the clinical observations in combination with significant weight loss, severity of pock [pox] lesions, elevated body*

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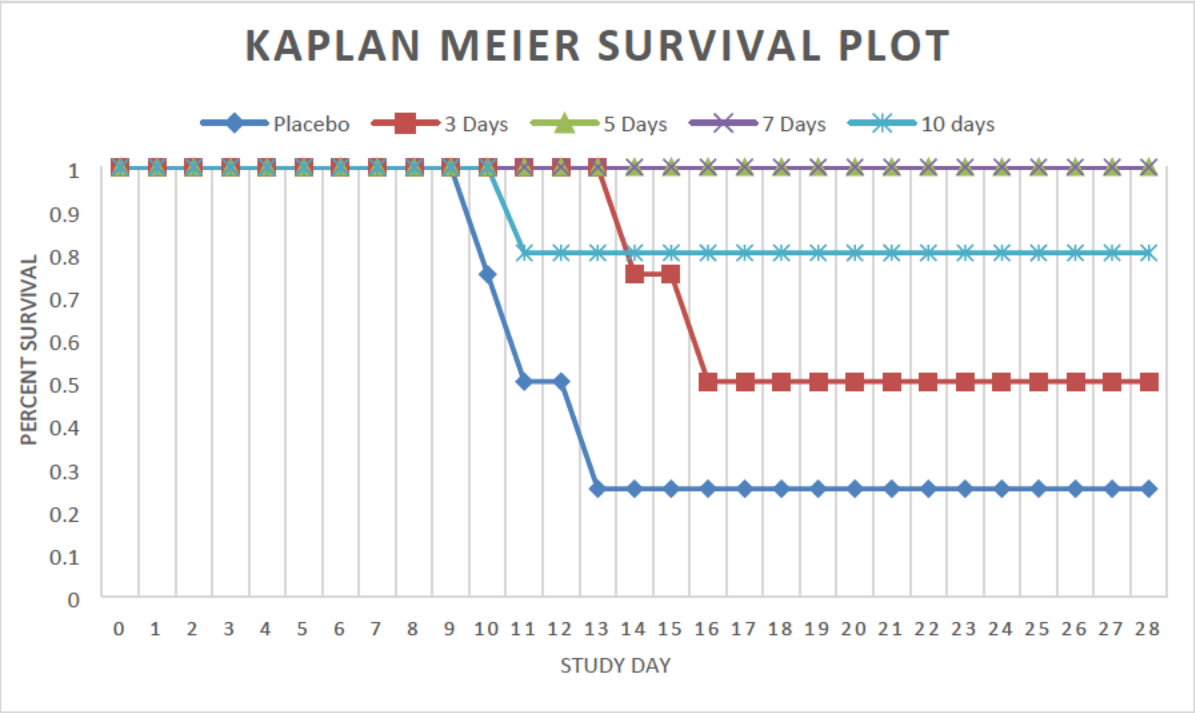
temperature, and anorexia. Daily clinical observations for each animal were recorded on the Orthopoxvirus Clinical Observation Form."

Observations and Results

Mortality

Unlike other studies, the placebo was not 100% lethal. However, there was still a significant survival enhancement with tecovirimat administration. Dosing of drug for 3 days only slightly increased survival, whereas dosing for 5, 7, or 10 days with drug increased survival significantly (compared to placebo). All animals that were sacrificed prior to scheduled euthanasia had signs of overt monkeypox virus infection (including the 1 animal in the 10 day group that was euthanized moribund on Day 11).

Group	Survival
Vehicle	20%
3 Days of drug	50%
5 Days of drug	100%
7 Days of drug	100%
10 Days of drug	80%



Clinical Signs

Monitored Daily. Observations noted throughout the study were associated with monkeypox infection. The most common clinical observations were dehydration,

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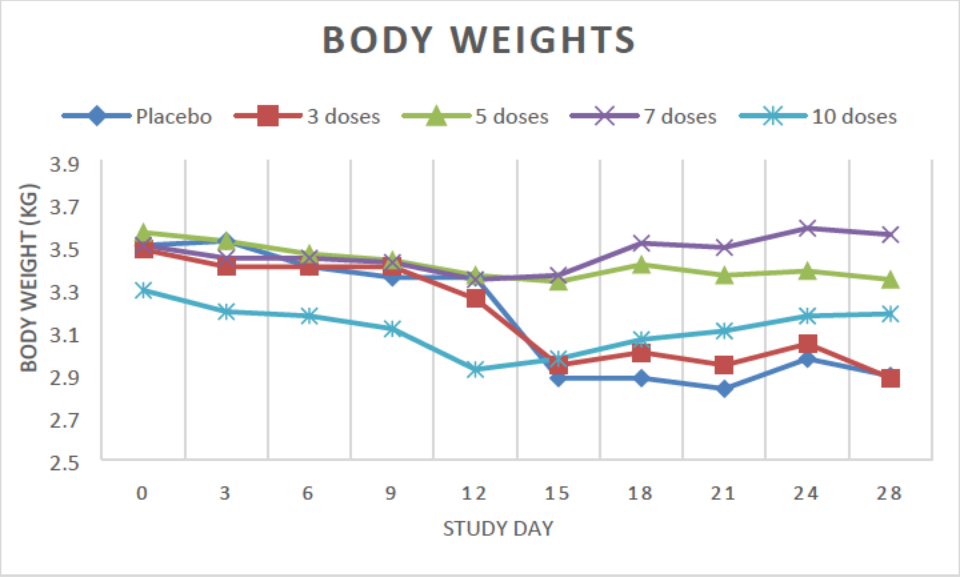
dyspnea, depression, weakness, edema, nasal discharge, and reduced food consumption.

Table 6 - NHP/MPXV Clinical Signs

Group	Severity Score	Clinical Sign							
		Depression	Weakness	Dehydration	Dyspnea	Reduced Food Consumption	Nasal Discharge	Ocular Discharge	Edema
Placebo	Not Present	1/4	1/4	0/4	0/4	0/4	4/4	4/4	3/4
	Mild	0/4	2/4	0/4	4/4	0/4	0/4	0/4	1/4
	Moderate	3/4	1/4	3/4	0/4	1/4	0/4	0/4	0/4
	Severe	0/4	0/4	1/4	0/4	3/4	0/4	0/4	0/4
ST-246, 3 Doses	Not Present	0/4	1/4	0/4	0/4	0/4	0/4	4/4	2/4
	Mild	3/4	1/4	0/4	3/4	1/4	4/4	0/4	2/4
	Moderate	1/4	2/4	3/4	1/4	0/4	0/4	0/4	0/4
	Severe	0/4	0/4	1/4	0/4	3/4	0/4	0/4	0/4
ST-246, 5 Doses	Not Present	4/6	6/6	0/6	0/6	0/6	6/6	6/6	4/6
	Mild	2/6	0/6	0/6	6/6	4/6	0/6	0/6	1/6
	Moderate	0/6	0/6	6/6	0/6	0/6	0/6	0/6	1/6
	Severe	0/6	0/6	0/6	0/6	2/6	0/6	0/6	0/6
ST-246, 7 Doses	Not Present	5/6	6/6	0/6	1/6	0/6	6/6	6/6	6/6
	Mild	1/6	0/6	0/6	5/6	2/6	0/6	0/6	0/6
	Moderate	0/6	0/6	6/6	0/6	4/6	0/6	0/6	0/6
	Severe	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
ST-246, 10 Doses	Not Present	4/5	4/5	0/5	0/5	0/5	4/5	5/5	5/5
	Mild	1/5	1/5	0/5	5/5	1/5	1/5	0/5	0/5
	Moderate	0/5	0/5	5/5	0/5	1/5	0/5	0/5	0/5
	Severe	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5

Body Weights

There were no significant changes between groups that were noted. There was a slight trend downward in the placebo and 3 dose groups compared to the groups given drug for 7, 5, or 10 days.



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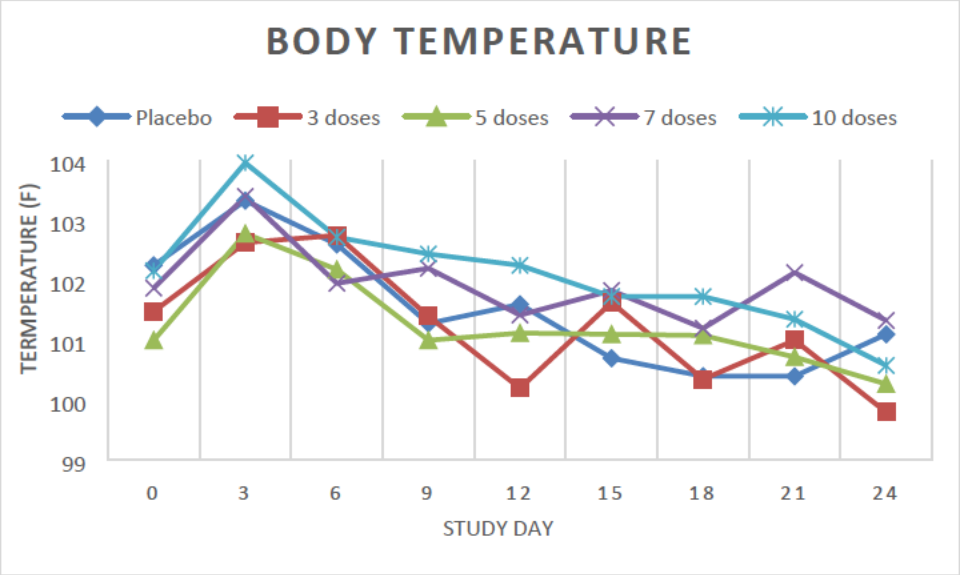
Temperature

Measured every 3 Days (rectal temperature). There was a general increase in body temperature in all groups around Day 3 (start of the peak of disease). All groups (placebo and treatment) all decreased over time after the peak on Day 3. No significant differences were noted.



Lesion Count

Lesions were not counted on the initial day of drug administration (Day 4), but all animals were confirmed qualitatively to have lesions on this day.

To facilitate statistical analysis, if pox lesions were recorded as TNTC (too numerous to count) in an anatomic region, a count of 200 was assigned to that region in establishing the monkey's total body lesion count.
There was considerable variability in the lesions across all groups. Mean lesion counts in the 5, 7, and 10 day dosing was comparable – which were all lower than the placebo or 3 day dosing groups.

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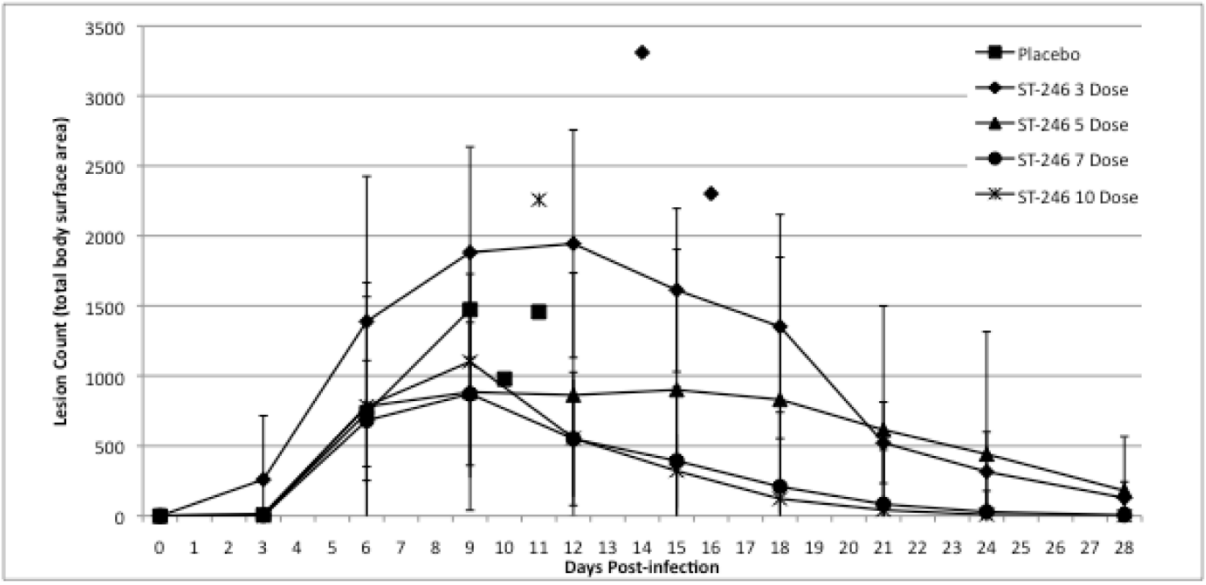
Figure 27 - NHP/MPXV Lesion Counts



NOTES: Symbols connected by lines indicate the mean of each group at each scheduled counting interval, with error bars indicating standard deviations. Stand-alone symbols not connected by lines indicate individual animal lesion counts performed off-schedule at the time of euthanasia. Symbols for individual animals are the same as the treatment group to which they belong.

Hematology

Measured at termination. RBC/Erythrocyte-related parameters (RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, and RDW) were relatively unchanged during the study. Initially, platelets declined during infection. However, platelet count then increased above baseline, markedly in some cases, through Day 21 and decreased thereafter. MPV values were slightly reduced.

As expected during MPXV infection, WBCs were elevated in all groups above baseline throughout most of the study. The largest changes in WBC counts were in the placebo, 3-Dose, and 5-Dose groups.

There no notable differences in hematology parameters between monkeys that survived to scheduled termination and those that did not.

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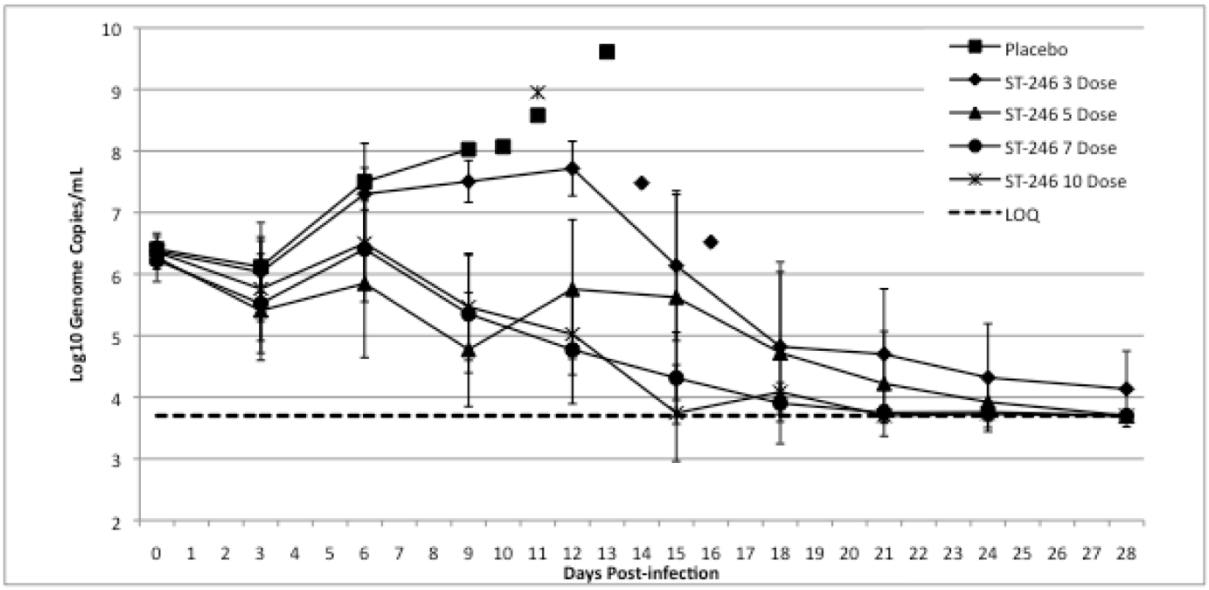
Clinical Chemistry

Mean albumin and bilirubin were modestly decreased in the study. Alanine aminotransferase (ALT) and globulin were elevated across all groups. ALT did not have any consistent pattern. However, ALT tended to be greater in NHPs that were euthanized moribund. Globulin levels were not different between surviving or moribund/euthanized animals. Mean amylase, blood urea nitrogen (BUN), and phosphorous generally declined below baseline at early sampling times and then increased over the remainder of the time course. Amylase levels were generally lower and BUN levels generally higher in animals that did not survive to scheduled termination than in those that survived. Levels of alkaline phosphatase, glucose, and potassium varied across the time of the study (generally increasing early and then declining). There were no consistent or dramatic changes in creatinine during the study. Calcium, sodium, and total protein levels were not altered significantly in any animals in the study.

Blood Viral Load (by PCR) – Mean Time-Weighted Average of log10

The mean time-weighted average of log10 viral DNA levels in blood reflects the overall viral burden over the course of the study. Levels were similar in the 5-Dose, 7-Dose, and 10-Dose groups – and were lower than those in the placebo and 3-Dose groups. This suggested that the viral load decreased in the serum due to prolonged study drug exposure.

Figure 28 - NHP/MPXV Serum Viral Load (via PCR)



NOTES: Symbols connected by lines indicate the mean of each group at each scheduled sampling, with error bars indicating standard deviations. Stand-alone symbols not connected by lines indicate individual animal data collected off-schedule at the time of euthanasia. Symbols for individual animals are the same as the treatment group to which they belong.
KEY: LOQ = limit of quantitation (dashed line)

The Applicant further did an exploratory analysis combining the animals that were euthanized early v/s the survivors. Overall it appears that the animals that were

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euthanized early had higher trends of virus than the survivors. However, the data are not presented here since the data are highly exploratory and an unverified post-hoc analysis which combined and excluded data across groups.

Exploratory Analysis of Anti-MPXV Antibody Titers (via ELISA)

Anti-monkeypox virus antibody titers were measured by ELISA.

Antibody titers were below the LLOQ (< 1:100) in all animals on Day -7 (the pre-infection control) and were at or below the LLOQ on study Day 6.

Moderate to high titers (1:6400 to 1:25,600) were observed on the day of euthanasia in all macaques that did not survive to scheduled termination except for the single monkey in the 10-day dose group that did not mount a measurable antibody response on any day.

Significant antibody responses were observed in all animals that survived to the end of the study.

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Table 7 - NHP/MPXV Exploratory Analysis of Anti-MPXV Antibody Titers

Monkey Number	Study Day								
	-7	6	10	11	12	13	14	16	28
Group 1 (Placebo)									
4803	<100	100	ND	ND	25600	25600	ND[a]		
4814	<100	<100	ND	ND	12800	ND	ND	ND	>51200
4815	<100	<100	6400	ND[b]					
4821	<100	<100	ND	12800	ND[c]				
Group 2 (3 Doses)									
4801	<100	<100	ND	ND	3200	ND	ND	ND	25600
4808	<100	<100	ND	ND	3200	ND	ND	12800	ND[d]
4812	<100	<100	ND	ND	12800	ND	ND	ND	25600
4818	<100	<100	ND	ND	6400	ND	12800	ND[e]	
Group 3 (5 Doses)									
4799	<100	<100	ND	ND	6400	ND	ND	ND	25600
4805	<100	<100	ND	ND	12800	ND	ND	ND	25600
4809	<100	<100	ND	ND	3200	ND	ND	ND	12800
4816	<100	100	ND	ND	6400	ND	ND	ND	>51200
4817	<100	<100	ND	ND	6400	ND	ND	ND	>102400
4823	<100	<100	ND	ND	25600	ND	ND	ND	25600
Group 4 (7 Doses)									
4800	<100	<100	ND	ND	12800	ND	ND	ND	51200
4802	<100	<100	ND	ND	25600	ND	ND	ND	51200
4806	<100	<100	ND	ND	3200	ND	ND	ND	12800
4807	<100	<100	ND	ND	3200	ND	ND	ND	51200
4811	<100	100	ND	ND	6400	ND	ND	ND	25600
4822	<100	<100	ND	ND	12800	ND	ND	ND	25600
Group 5 (10 Doses)									
4804	<100	<100	ND	ND	12800	ND	ND	ND	25600
4810	<100	100	ND	ND	6400	ND	ND	ND	6400
4813	<100	<100	ND	ND	25600	ND	ND	ND	6400
4819	<100	<100	ND	<100	ND[c]				
4820	<100	100	ND	ND	12800	ND	ND	ND	25600

[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 = 100; ND = Not determined

Toxicokinetics

Not performed for this study.

4.2 Secondary Pharmacology

Exploratory secondary pharmacology studies with tecovirimat were conducted in immunocompromised NHPs and mice.

Reference ID: 4258909

These studies were conducted in order:

- to investigate the ability of tecovirimat (oral and dermal formulations) to ameliorate the adverse effects of smallpox vaccines in immunocompromised hosts and
- to identify if tecovirimat may interfere with the current smallpox vaccine

Studies in Monkeys

Study title: **Effect of ST-246, an Anti-Poxvirus Therapeutic, in Preventing Vaccinia Virus Disease in FK-506-immunosuppressed Non-Human Primates**
Study number: **1218-100004544**

Study Design:

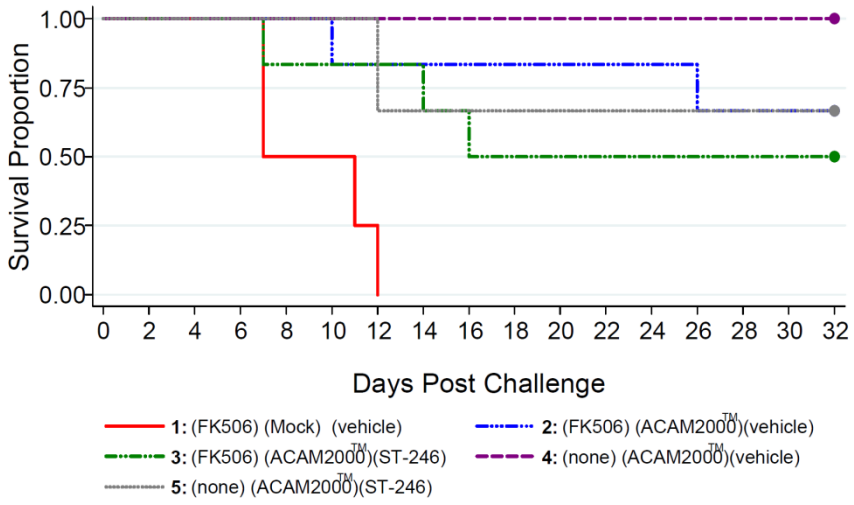
- Three groups (Groups 1, 2, and 3) were placed on an immunosuppression regimen with twice-daily intramuscular (IM) injections of Tacrolimus (FK-506) at a dose of 0.05 mg/kg; and
- Two groups (Groups 4 and 5) did not receive FK-506 treatment (immunocompetent groups).

Group	# Animals	M/F	FK506 (2X daily)	Vaccine	Treatment (10mg/kg)	MPXV	Clin Obs	Blood	Temp	Body Weights	Lesion Counts	Photos	Necropsy
1	4	2M/2F	0.05mg/kg	Mock	vehicle	$5x10^7$ PFU/mL	2x daily	see Tables 3 and 4	daily am	every 3rd day			upon death or end of study
2	6	3M/3F	0.05mg/kg	ACAM2000™	vehicle	$5x10^7$ PFU/mL	2x daily						
3	6	3M/3F	0.05mg/kg	ACAM2000™	ST-246	$5x10^7$ PFU/mL	2x daily						
4	3	2M/1F	none	ACAM2000™	vehicle	$5x10^7$ PFU/mL	2x daily						
5	3	2F/1M	none	ACAM2000™	ST-246	$5x10^7$ PFU/mL	2x daily						

Study Day 0 = vaccination day
MPXV = monkeypox virus (Zaire strain V79-I-005; NR-21738); IV delivered 1 mL
Blood draws are based on bi-weekly limit of 15% total blood volume and are estimated to be approximately 10 mL per kg
At least one baseline blood draw will be obtained prior to vaccination and challenge
PFU = plaque-forming units

Key study findings:
Survival was increased in all animals given vaccine or study drug. See **Figure 29** below.

Figure 29 - Kaplan-Meier Curves



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Tecovirimat treatment for 14 days post-smallpox vaccination with and without immunosuppression decreased the adverse effects of the ACAM2000. The decrease in the vaccine effects at first glance, did not adversely impact the immunogenicity of the vaccine in the majority of animals as evidenced by similar survival rates in all vaccinated groups relative to the mock-vaccinated control.

However, further analysis by Dr. Pat Harrington noted a possible effect on the vaccine by tecovirimat. *Please see Clinical Virology review for further information.*

Study title: Effect of ST-246 Co-administration on ACAM2000® Reactogenicity and Immunogenicity in SHIV-Immunocompromised Nonhuman Primates

Study number: SR11-011F

Study Design: Monkeys were exposed to SHIV (an animal model of HIV infection in humans). The overall design was in 5 groups to see the effects of drug on the vaccination (ACAM2000®) in healthy or SHIV-immunocompromised animals – in a Monkeypox model.

Group	Vaccination	Tecovirimat	SHIV	MPXV challenge
1) Placebo	No	No	No	Yes
2) Vaccine + Placebo + SHIV	Yes	No	Yes	Yes
3) Vaccine + Drug + SHIV	Yes	Yes	Yes	Yes
4) Vaccine + Placebo	Yes	No	No	Yes
5) Vaccine + Drug	Yes	Yes	No	Yes

Key study findings: ACAM2000 vaccination was shown to be safe in immunocompromised NHPs infected with SHIV-89.6P. However, the vaccination failed to elicit a robust protective immunity, irrespective of tecovirimat treatment. The reduction in protection was attributed to the loss of cellular responses and consequently the loss of protective immunity elicited by humoral and cellular responses. Thus, the potential use of ACAM2000 in HIV-infected individuals may be limited.

Studies in Mice

Study title: Antiviral efficacy of ST-246 in a model of progressive vaccinia virus infection in athymic (nu/nu) mice

Study number: 246-PC-009

Study Design: The purpose of the study was to determine the antiviral efficacy of tecovirimat in immunocompromised mice following inoculation of vaccinia virus by lumbosacral scarification.

Reference ID: 4258909

Model information: Nude (immunocompromised) mice have been used with vaccinia in a prior proof of concept model for progressive disseminated vaccina. Vaccinia virus Lister strain (used in the WHO smallpox eradication campaign) at a dose of $5x10^5$ pfu per mouse has previously been used in athymic nude mice to induce progressive disseminated vaccinia lesions.

Virus challenge: Mice were challenged by lumbosacral scarification with $5x10^5$ pfu of vaccinia virus Lister, in 50 µL volume, in order to induce progressive disseminated vaccinia lesions.

Drug intervention: Mice were treated with placebo (vehicle; oral gavage) or tecovirimat administered by oral gavage at 50 mg/kg (150 mg/m^2) BID. The treatment duration was for 5, 10, or 15 days starting at 2h post-challenge. Compound was administered for 5 day intervals with a 2-day recovery period between dosing schedules. CDV (cidofovir) was administered by i.p. injection at 50 mg/kg for a total of 10 days.

Key study findings: Oral administration of tecovirimat decreased disease severity and extended the mean time to death in a progressive vaccinia virus infection in athymic (nu/nu) mice. Lesion number and size were decreased significantly in all tecovirimat dosage regimens (5, 10, or 15 days), whereas mean time to death (MTD) was decreased significantly with 15-day dosing.

		Mortality					Lesion Score at Day 22		
Group	Condition	Days of treatment	MTD	S.D.	*p value*	Survivors	Mean	S.D.	*p values*
1	Placebo	0-4, 7-11,14-18	57.4	11.8		0	4.0	1.4	
2	ST-246, 50 mg/kg	0-4, 7-11, 14-18	74.0	6.1	*0.04*	2	1.1	0.8	*0.006*
3	ST-246, 50 mg/kg	0-4, 7-11	69.0	17.4	*0.38*	2	0.6	0.4	*0.004*
4	ST-246, 50 mg/kg	0-4	52.0	4.8	*0.38*	0	1.6	1.8	*0.048*
5	CDV, 50 mg/kg	0-4, 7-11	76.0	13.4	*0.05*	0	0.7	0.8	*0.003*

Note: Animals that survived infection were not included in the p value calculation.

Study title: Antiviral efficacy of topical application of ST-246 in a model of progressive vaccinia virus infection in athymic (nu/nu) mice.

Study number: 246-PC-012

Study Design: The purpose of the study was to determine the antiviral efficacy of topical administration of tecovirimat in preventing intradermal vaccinia infection in athymic (nu/nu) mice. Similar to the prior study, this is the athymic nu/nu (nude) mouse model for disseminated vaccinia.

Virus challenge: Mice were challenged by lumbosacral scarification with 5×10^5 pfu of vaccinia virus Lister, in 50 µL volume, in order to induce progressive disseminated vaccinia lesions.

Drug intervention: Mice were treated with placebo (vehicle; Topical DMSO) or a 1% or 5% tecovirimat solution in DMSO administered by topical application twice daily. The treatment duration was for 10 days.

Reference ID: 4258909

Vaccinia virus-induced lesions were photographed at day 11 post-infection.

Key study findings: Mice treated topically with 1% or 5% tecovirimat showed a reduction in lesion progression relative to vehicle treatment.

Photography (of treated vs placebo) was included in the study report, but no statistical analysis. The data from the photographic data are summarized below in the table.

Table 8 - Lesion Analysis of Photographic Data in Athymic Nude (nu/nu) Mice with Disseminated Vaccinia

Group	% with lesions (of 5 mice)	Size of lesion
Placebo	5 of 5 – 100%	Large. Appears to be a significant portion of the back area covered in lesions.
1% tecovirimat (dermal)	1 of 5 – 20%	A single minor lesion on mouse B3. Other mice appear without lesions (although B5 may have a discoloration that may be a tiny lesion, but hard to determine with the granular photo)
5% tecovirimat (dermal)	0 of 5 – 0%	No lesions evident.

Study title: **Efficacy Evaluation of Two Topical Formulations of ST-246 for the Treatment of Poxvirus Skin Lesions**

Study number: **ASM224**

Study Design: The purpose of the study was to evaluate the efficacy of two topical formulations of tecovirimat, in comparison to oral tecovirimat, in preventing or treating poxvirus lesions resulting from dermal scarification with vaccinia virus in SKH1 mice.

Unlike the prior two mouse studies, this strain is not the nu/nu (athymic/nude and immunocompromised) mouse model but is an SKH1 (hairless, immunocompetent, outbred mouse strain used to model skin diseases).

Virus challenge: Mice were challenged with Vaccinia virus strain WR (VR-1354) from ATCC and passaged three times by low multiplicity infection in BSC40 cells. The strain is referred to as VV-WR in the table below. The dose of virus was 1 x 10^6 PFU by dermal scarification.

Drug intervention: See “treatment” column in the following table.

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Group	# Mice	VV-WR infection	Treatment	Treatment Days
A	2	Mock	Vehicle BID	0-14
B	3	$1x10^6$ PFU	Vehicle BID	0-14
C	3	$1x10^6$ PFU	Topical Form. 1 BID	0-14
D	3	$1x10^6$ PFU	Topical Form. 1 BID	3-17
E	3	$1x10^6$ PFU	Topical Form. 2 BID	0-14
F	3	$1x10^6$ PFU	Topical Form. 2 BID	3-17
G	3	$1x10^6$ PFU	Oral Form. SID	0-14
H	3	$1x10^6$ PFU	Oral Form. SID	3-17

Key study findings:
All animals survived infection to scheduled sacrifice. This model is a dermal lesion model and is not lethal.

Based on visual evaluation of the photographs, formulation 1 (a 1% tecovirimat solution) was the most effective in the prevention and treatment of dermal lesions following dermal infection. This was surprising since Formulation 1 was a 1% solution whereas Formulation 2 was a 5% tecovirimat suspension.

The Applicant theorized the lower drug concentration in Formulation 1 (1% solution) may have been better delivered transdermally in solution than suspension (Formulation 2 – 5% suspension), which may explain why Formulation 1 caused a more dramatic drug effect than Formulation 2.

4.3 Safety Pharmacology

Study Title: Evaluation of the Effects of ST246 on Cloned hERG Channels Expressed in Human Embryonic Kidney (HEK293) Cells (246-PH-001)
hERG potassium channel current was evaluated in stably-transfected HEK293 cells at concentrations of 0.03, 0.1, 1.0, 10 and 30 µM of tecovirimat. Little to no inhibition of hERG current was observed up to the highest concentration tested (7.1% inhibition at 30 µM only; IC_{50} not calculated). The assay was properly validated.

Study Title: Pulmonary Effects of Orally Administered ST246 in Mice (246-PH-002)
Parameters of respiration (respiratory rate, tidal volume and minute volume) were evaluated in BALB/c mice following single oral gavage doses of tecovirimat (0, 500, 1000 and 2000 mg/kg; 8/sex/group). Minor decreases in respiratory rate, tidal volume and minute volume were observed throughout the 4-hour monitoring period, though the parameters were not clearly dose-dependent and were within historical control ranges. NOAEL = 2000 mg/kg. The study was properly validated.

Study Title: Neurobehavioral Evaluation of ST246 in Mice (246-PH-003)
A functional observational battery, including assessments of activity/arousal and neuromuscular, sensorimotor, autonomic and physiological function, was conducted in BALB/c mice following single oral gavage doses of tecovirimat (0, 500, 1000 and 2000

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mg/kg; 8/sex/group). Decreases in general arousal, defecation counts, and body temperature were observed in high-dose females. NOAEL = 1000 mg/kg. The study was properly validated.

Note: See the single- and repeat-dose studies in dogs and monkeys for additional cardiovascular and neurological safety pharmacology information.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study Title: ST-246: A 14-Day Repeat Dose Pharmacokinetics Study in New Zealand White Rabbits (246-PK-001)
The pharmacokinetics of tecovirimat were evaluated in New Zealand White rabbits following daily oral gavage doses of drug (10, 40 or 100 mg/kg/dose; 3/sex/group) for 14 days. Pharmacokinetic parameters are presented in **Table 9**.

Table 9 - PK Parameters From the 14-Day Rabbit Study

Day	Dose	C_{max}	C_{max}/Dose	AUC_{last}	AUC_{last}/Dose
	(mg/kg)	ng/mL	ng/mL/mg/kg	ng*hr/mL	ng*hr/mL/mg/kg
1	10	502	50	2422	242
	40	562	14	4816	120
	100	1277	13	15915	159
7	10	292	29	2670	267
	40	493	12	4703	118
	100	834	8	8812	88
14	10	376	38	2586	259
	40	617	15	3974	99
	100	830	8	9755	98

Study Title: ST246: Absolute Bioavailability in Monkeys (246-TX-011)
The bioavailability of tecovirimat was determined in either fed or fasted cynomolgus monkeys following either single IV or oral gavage doses of drug (1 mg/kg IV or 30 mg/kg oral; 3/sex/group) using two formulations (HPMC or PEG). Oral bioavailability was 50% in fed monkeys and 28% in fasted monkeys with HPMC, and was 45% in fed monkeys and 44% in fasted monkeys with PEG. Oral absorption was also faster with HPMC than with PEG (T_{max} = 1.2 and 1.7 hours, respectively).

Study Title: ST246: Bioavailability and a 7-Day Repeat Dose Study in Rabbits (246-TX-012)
The pharmacokinetics and bioavailability of tecovirimat were evaluated in New Zealand White rabbits following either a single IV dose or daily oral gavage doses for 7 days (1 mg/kg IV or 100 mg/kg oral; 3/sex/group). Bioavailability was 29% in males and 7.5% in females on Day 1, and was 15.5% in males and 14.1% in females on Day 7.

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Study Title: ST246: Relative Bioavailability of Two Formulations in Monkeys (246-TX-013)
The relative bioavailability of tecovirimat was evaluated in fasted female cynomolgus monkeys following single oral doses of drug (50 mg capsules; 4 females/group). Two formulations, [redacted] (b) (4) capsule and [redacted] (b) (4) capsule, were used. All pharmacokinetic parameters were equivalent with both formulations (AUC_{last} = 24.5-25.5 µg•hr/mL, C_{max} = 2.5-3.0 µg/mL, T_{max} = 3.8-4.3 hrs).

Study Title: ST-246: A Single Dose Oral Pharmacokinetic Study in Monkeys [redacted] (b) (4) 1151-045)
The pharmacokinetics of tecovirimat were evaluated in cynomolgus monkeys following single oral gavage doses of drug (3, 10, 30 or 100 mg/kg/dose; 2/sex/group). Pharmacokinetic parameters are presented in **Table 10**.

Table 10 - PK Parameters From the Single-Dose Monkey Study

Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{24hr} (ng•hr/mL)	AUC_{inf} (ng•hr/mL)	$t_{1/2}$ (hr)
3	1.75	322.5	2416	2524	5.53
10	2.25	1475	9516	9823	5.17
30	3.50	4652.5	38905	44002.5	8.30
100	3.25	9902.5	105426.5	130057	9.51

Study Title: Binding of Metabolites to Human, Mouse, and Monkey Plasma Proteins (15SIGAP2)
Protein binding of three tecovirimat metabolites (SG-1 hydrazide (M4), SG-1 isoindole (M5) and trifluoromethylbenzoic acid (TFMBA)) were evaluated in plasma from humans, CD-1 mice and cynomolgus monkeys. Protein binding of M4 and M5 was low in all three species (M4: 20.7%, 4.6% and 15.8% in human, mouse and monkey plasma, respectively; M5: 33.0%, 16.6% and 17.6% in human, mouse and monkey plasma, respectively). TFMBA was highly protein-bound in human and monkey plasma (98.6% and 97.1%, respectively), but was moderately bound in mouse plasma (50.0%).

Study Title: Binding to Rabbit Plasma Proteins (17SIGAP1)
Protein binding of tecovirimat was evaluated in plasma from New Zealand White rabbits. Tecovirimat was 88.7% protein-bound at a concentration of 5 µM.

Study Title: Plasma Protein Binding in Mouse (BALB/c), Rat (Sprague-Dawley), Dog (Beagle), Monkey (Cynomolgus) and Human Plasma for [^{14}C]-ST-246 (246-AD-001)
Protein binding of tecovirimat was evaluated in plasma from mice, rats, dogs, monkeys and humans. Tecovirimat was highly protein-bound in all five species (>87% in mice, >95% in rats, >89% in dogs, >87% in monkeys, and >77% in humans) at the concentrations tested (0.03 to 50 µM).

Study Title: Pilot Mass Balance and Tissue Distribution of [^{14}C]ST246 After Oral Administration to Mice (246-AD-005)
Tissue distribution and excretion of tecovirimat were evaluated in male and female BALB/c mice following single oral gavage doses of radiolabeled drug ([^{14}C]-tecovirimat;

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100 mg/kg/dose). Tecovirimat was widely distributed to all tissues by 24 hours post-dose, with the highest concentrations in the gall bladder, liver and nasal turbinates, and the lowest concentrations in the CNS, bone, skeletal muscle and uvea. By 96 hours post-dose, tecovirimat was still detectable in the liver, gall bladder and nasal turbinates. 58-77% of the administered dose was excreted within the first 24 hours, and about 95% was excreted by 96 hours post-dose. 71.6-75.1% of the initial dose was recovered in the feces, 18.2-24.2% was recovered in the urine, and less than 0.05% was excreted with the expired CO_2. The parent compound was excreted largely intact in the feces, but only trace amounts of the parent compound were detected in the urine. Seven metabolites were detected in the urine, with the N-glucuronide and diglucuronide forms of tecovirimat being the predominant forms. Overall, however, the metabolite data from both urine and feces in this study were considered preliminary and tentative.

Study Title: Tissue Distribution of [^{14}C]-Tecovirimat Following Oral Administration to Mice (246-AD-006)

Tissue distribution of tecovirimat was evaluated in select tissues (blood, bone, bone marrow, brain, eye, kidney, liver, lungs, pigmented skin, testes and thyroid) from male pigmented C57BL/6 mice following single oral gavage doses of radiolabeled drug ([^{14}C]-tecovirimat; 100 mg/kg/dose). Peak tecovirimat concentrations were observed in the bone marrow within 1 hour post-dose, and in all other tissues around 4 hours post-dose. tecovirimat was not detectable in most tissues beyond 24 hours post-dose. By 168 hours, tecovirimat was only detectable in the bone marrow and liver. The highest concentrations were observed in the blood, bone marrow and liver, and the lowest were observed in the brain and eyes. Tecovirimat may therefore cross the blood-brain barrier in this species, though at low levels. Tecovirimat was detectable in the blood for up to 96 hours post-dose, and the blood:plasma ratio ranged from 0.504 to 0.585 throughout. Tissue:plasma concentrations were generally <0.6 throughout the study with the notable exception of the bone marrow.

Study Title: ST-246®: Placental and Milk Transfer Study in Mice (b) (4) -2083-003-001-SN9)

Distribution of tecovirimat to the placenta and milk were evaluated in pregnant or lactating CD-1 mice, respectively, following single oral gavage doses of radiolabeled drug ([^{14}C]-tecovirimat; 100, 300 and 1000 mg/kg/dose). Pregnant mice were treated on Gestation Day 15 (12 females/group), and lactating mice were treated on Postnatal Day 10 or 11 (5 females/group). In the pregnant animals, the mean percentage of maternally administered radioactivity detectable in the fetuses was generally inversely proportional to dose between 1 and 8 hours post-dose (~3-fold higher at the low dose than at the mid- and high doses), with peak concentrations around 4 hours post-dose in the low-dose group. By 24 hours post-dose, tecovirimat levels in the fetuses were highest in the high-dose group (~6-fold higher than the low dose). Similarly, in the lactating animals, tecovirimat levels in milk were inversely dose-proportional 6 hours post-dose (~3-fold higher at the low dose relative to the mid- and high doses). At 24 hours post-dose, however, tecovirimat levels in milk were highest at the high dose (~4-fold higher than the low dose). The milk:plasma ratios at the low, mid- and high doses were 0.43, 0.40 and 0.83 at 6 hours post-dose, and 0.67, 0.83 and 0.72 at 24 hours

Reference ID: 4258909

post-dose, respectively. Tecovirimat can therefore be transferred across the placenta during pregnancy, and into milk during lactation, in this species.

Study Title: ADME-Tox Study of ST-246 (100010334)
The potential of tecovirimat to be a substrate for human UGT enzymes (1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15) was evaluated *in vitro*. Of these, tecovirimat was identified as a substrate for UGT1A1, 1A3 and 1A4.

Study Title: Metabolic Stability and Metabolite Characterization in Mouse (CD-1), Rat (Sprague-Dawley), Dog (Beagle), Monkey (Cynomolgus), and Human Microsomes for [^{14}C]-ST-246 (246-AD-002)
The metabolic stability and characterization of tecovirimat was evaluated in mouse, rat, dog, monkey and human liver microsomes treated with radiolabeled drug ([^{14}C]-tecovirimat). [^{14}C]- tecovirimat was considered stable in dog, monkey and human microsomes, but was metabolized in rat and mouse microsomes with turnover rates of 18% and 6%, respectively. A single metabolite (M1) was detected in rat, mouse and dog microsomes at very low levels. The metabolism of tecovirimat in these 3 species was determined to be NADPH-independent.

Study Title: Multiple Dose Pharmacokinetic Study to Detect Parent Tecovirimat and Three Metabolites, M4, M5, and TFMBA in Mice Following Oral Administration of Tecovirimat (8320258)
The pharmacokinetics of tecovirimat and three metabolites previously identified in humans (M4, M5 and TFMBA) were evaluated in BALB/c mice following daily oral gavage doses of drug for 14 days (1000 mg/kg/day; 57/sex/group). Pharmacokinetic parameters for all 4 compounds are presented in **Table 11**.

Table 11 - PK Parameters From the Single-Dose Mouse Study

		C_{max} (ng/mL)		T_{max} (hr)		AUC_{0-24} (ng·hr/mL)		$t_{1/2}$ (hr)	
		Day		Day		Day		Day	
Analyte	Sex	1	14	1	14	1	14	1	14
ST-246	Male	52200	48700	12.0	12.0	983000	845000	NC	6.39
	Female	69200	45500	12.0	8.00	1200000	809000	NC	8.76
4-TFMBA	Male	424	466	2.00	4.00	6640	7400	NC	6.09
	Female	1430	2090	1.00	1.00	23400	22000	NC	4.61
M4	Male	2240	2740	12.0	2.00	40800	44500	NC	7.99
	Female	4190	2580	4.00	4.00	67500	37300	NC	5.31
M5	Male	927	1850	12.0	12.0	17900	30900	NC	NC
	Female	1930	2500	12.0	8.00	35700	32000	NC	NC

NC Not calculated.
Note: Values represent 3 animals/sex/group/time point.

Reference ID: 4258909

Study Title: Multiple Dose Pharmacokinetic Study to Detect Parent Tecovirimat and Three Metabolites, M4, M5, and TFMBA in Monkeys Following Oral Administration of Tecovirimat (8320257)

The pharmacokinetics of tecovirimat and three metabolites previously identified in humans (M4, M5 and TFMBA) were evaluated in cynomolgus monkeys following daily oral gavage doses of drug for 14 days (300 mg/kg/day; 3/sex/group). Pharmacokinetic parameters for all 4 compounds are presented in **Table 12**.

Table 12 - PK Parameters From the Single-Dose Monkey Study

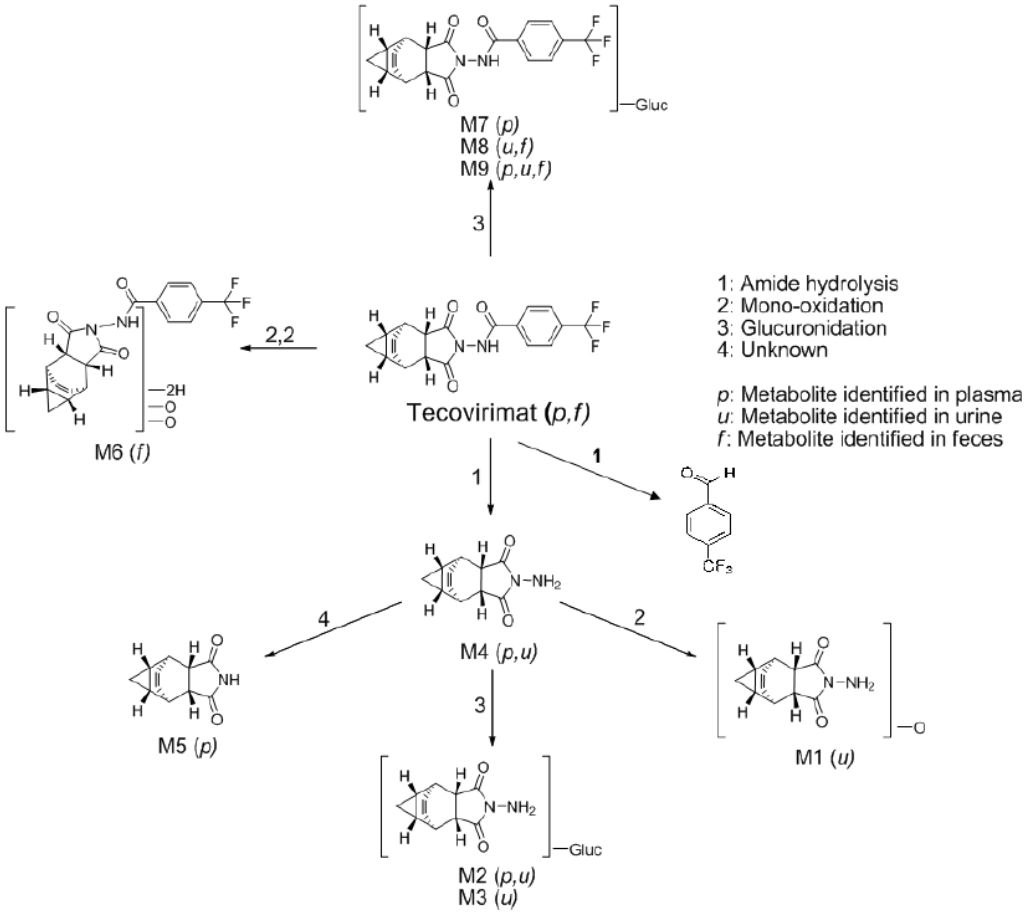
			C_{max} (ng/mL)		T_{max} (hr)		AUC_{0-24} (ng·hr/mL)		$t_{1/2}$ (hr)	
			Day		Day		Day		Day	
Analyte	Sex		1	14	1	14	1	14	1	14
ST-246	Male	Mean	1830	2210	2.67	1.67	20000	23100	NA	19.0
		SD	645	570	1.15	0.577	2460	4360	NA	3.22
	Female	Mean	1900	2480	2.00	2.00	21900	27600	NA	15.2
		SD	255	480	0.00	0.00	4260	4790	NA	5.00
TFMBA	Male	Mean	1780	2360	6.00	2.00	27400	31600	NA	17.8
		SD	629	756	3.46	0.00	7350	7980	NA	3.46
	Female	Mean	1500	1850	5.00	1.67	21400	27000	NA	14.8
		SD	382	193	6.08	0.577	2470	6130	NA	4.16
M4	Male	Mean	310	483	3.33	2.67	4810	5590	NA	12.2
		SD	39.3	119	1.15	1.15	617	1300	NA	NA
	Female	Mean	343	345	8.00	3.33	4680	5050	NA	15.5
		SD	118	57.2	0.00	1.15	752	196	NA	3.92
M5	Male	Mean	439	1300	24.0	5.67	6480	28400	NA	35.8
		SD	41.3	287	0.00	5.69	307	5460	NA	7.78
	Female	Mean	302	1000	24.0	13.3	4670	22100	NA	44.6
		SD	14.0	274	0.00	9.24	560	5400	NA	10.5

Notes: Values represent N=3 animals/sex.
Elimination half –life ($t_{1/2}$) was not able to be calculated on Day 1.

The proposed metabolic pathway of tecovirimat in humans is presented in **Figure 30**.

Reference ID: 4258909

Figure 30 - Proposed Metabolic Pathway of Tecovirimat in Humans



6 General Toxicology

6.1 Single-Dose Toxicity

Study Title: ST246: Acute Oral Toxicity Study in Mice (246-TX-002; GLP)
Mortality, clinical exams, body weights, food consumption, hematology, clinical chemistry, gross pathology, organ weights and histopathology were performed or evaluated in BALB/c mice following single oral gavage doses of either vehicle (1% HPMC/0.5% Tween® 80) or tecovirimat (2000 mg/kg/dose; 10/sex/group). Animals were treated once on Day 1 and were euthanized on Day 15. One male and 3 females in the treatment groups were either found dead or euthanized *in extremis* on Day 1. Cause of death was the result of dosing error (foreign material present in the lungs and thoracic cavity), and was not attributed to tecovirimat. Food consumption was decreased 46% on Day 1, and body weight was decreased 4% on Day 2, in the tecovirimat -treated females. A large subcutaneous abscess with moderate erosion/ulcer and exudate on the epidermal surface of the skin, and associated mild subacute inflammation in the liver and moderate to severe granulocytic hyperplasia in the bone marrow and spleen, were all observed in 1 treated male. The skin lesion and related findings are likely incidental and unrelated to treatment. NOAEL = 2000 mg/kg.

Reference ID: 4258909

Study Title: ST246: Acute Oral Toxicity Study in Monkeys (246-TX-005; GLP)
Mortality, clinical exams, body weights, electrocardiography, hematology, clinical chemistry, gross pathology, organ weights, histopathology and toxicokinetics were performed or evaluated in cynomolgus monkeys following single oral gavage doses of either vehicle (1% HPMC/0.5% Tween® 80) or tecovirimat (1000 or 2000 mg/kg; 2/sex/group). Animals were treated once on Day 1 and were euthanized on Day 15. There were no unscheduled deaths. Decreased activity and ataxia were observed in all treated animals within 4 hours post-dose. Sinus bradycardia (heart rate <160 bpm) occurred in 1 high-dose male 4 hours post-dose. Increased QT (4-8%) and QTcB (4-10%) intervals were also increased at both dose levels 4 hours post-dose. A NOAEL was not defined in this study.

6.2 Repeat-Dose Toxicity

Study title: ST246: Bioavailability and a 7-Day Oral Repeat Dose Toxicity Study in Mice

Study no.:	**246-TX-001**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 21st, 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot #PCCS 1391, purity not provided)

Summary

Mortality, clinical signs, body weights, food consumption, hematology and clinical chemistry, gross pathology, organ weights and pharmacokinetics were evaluated in BALB/c mice. Phase A animals were received a single IV or oral gavage dose of tecovirimat (1 or 3 mg/kg IV and 30, 100, 300 or 1000 mg/kg oral; 18-30/sex/group). Phase B animals were treated by oral gavage once daily for 7 days (30, 100, 300 or 1000 mg/kg; 6-18/sex/group). A total of 9 animals died throughout the study likely due to the blood collection procedure (no relation to treatment was observed). Non-adverse increases in sorbitol dehydrogenase (2-fold) and thymus weight (87%; males only) were observed at 1000 mg/kg/dose. Bioavailability of up to 44.5% was observed at 30 mg/kg/dose, but was decreased to 3.5-4.0% at 1000 mg/kg/dose. Plasma concentrations of tecovirimat increased with dose, however, indicating that maximum absorption was not reached.

NOAEL = 1000 mg/kg/dose (AUC_{0-t} = 470.3 µg•hr/mL, C_{max} = 57.8 µg/mL at Day 8)

Reference ID: 4258909

Study title: ST246: A 28-Day Oral Toxicity Study in Mice

Study no.: **246-TX-006**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: June 14th, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #5C020, purity = 99.8%)

Summary

Mortality, clinical exams, body weights, ophthalmology, hematology, clinical chemistry, gross pathology, organ weights, histopathology and toxicokinetics were performed or evaluated in BALB/c mice following oral gavage doses of either vehicle (1% HPMC/0.5% Tween® 80) or tecovirimat (500, 1000 or 2000 mg/kg/dose; 10/sex/primary groups, 5/sex/recovery groups, and 18/sex/satellite groups). Animals were treated once daily for 28 days, and were euthanized on either Day 28 or 42. A total of 4 main study animals (1 control, 1 low-dose and 2 mid-dose males) and 20 satellite animals (3 low-dose, 4 mid-dose and 4 high-dose males, and 1 low-dose, 5 mid-dose and 3 high-dose females) died prematurely. 3 of the main study deaths were attributed to gavage-related trauma, while a cause of death for the fourth animal was not determined. The satellite animal deaths were likely the result of the orbital bleeding procedure. All deaths were considered unrelated to treatment. Liver weight was increased 15-16% in high-dose males and in all treated females, and spleen weight was increased 15-23% in high-dose males and females. These changes were considered non-adverse due to absence of pathological correlates. A non-adverse decrease in albumin (9%) was seen in mid- and high-dose males. No adverse findings were observed in the recovery groups.

NOAEL = 2000 mg/kg/dose (AUC_{0-t} = 858.6 µg•hr/mL, C_{max} = 53.0 µg/mL at Day 28)

Study title: 3-Month Oral (Gavage) Toxicity Study in Mice

Study no.: **2083-003-001-SN3**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: September 18th, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #6A003, purity = 97.7%)

Reference ID: 4258909

Key Study Findings

NOAEL = 1000 mg/kg/day (AUC_{0-24hr} = 700.6 µg•hr/mL, C_{max} = 64.9 µg/mL at Week 13). A lymphosarcoma in the thymus with metastatic lesions in the spleen, lung, and bronchial lymph node were observed in a single high-dose male at Day 120. Red fluid in the abdominal and thoracic cavities, pale discoloration of the pancreas, and ulceration of the penis were also observed in this animal. It is unclear if these findings are related to treatment. Increased reticulocytes (percentage and total number) and cholesterol were also observed at the high dose. These changes may be drug-related, but were considered non-adverse.

Methods

Doses:	0, 300, 600 & 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	BALB/c mice
Number/Sex/Group:	20/sex/group for main study groups, 10/sex/group for recovery groups (control and high dose only)
Age:	8 weeks
Weight:	Males – 16.7 to 24.0 g; Females – 15.2 to 21.3 g
Satellite groups:	39/sex/group (treated animals only; no controls)
Unique study design:	Main study and satellite animals were dosed once daily for 91 and 84 consecutive days, respectively. Recovery animals were euthanized on Day 120.
Deviation from study protocol:	9 extra satellite mice were assigned to the treatment groups to replace animals that died from gavage-related trauma within the first 4 days of dosing. These animals received 89-90 daily doses, rather than the protocol-specified minimum of 91. This was not considered to have adversely affected the outcome of the study.

Observations and Results

Mortality

Morbidity and mortality were assessed at least once daily. Aside from the 9 animals which died from gavage-related trauma and were replaced within the first 4 days of dosing (2 low-dose, 3 mid-dose, and 4 high-dose), an additional 9 animals died over the course of the study. Of these, 7 deaths (3 control, 2 mid-dose and 2 high-dose animals) were attributed to gavage-related trauma between Days 5 and 21. All gavage-related deaths were confirmed by necropsy. One high-dose female was accidentally crushed by the food hopper on Day 63. One control male was found dead on Day 35 (cause of death was not determined). All control and high-dose main study animals that died prematurely were replaced with animals from the corresponding group of recovery animals at the terminal necropsy.

Reference ID: 4258909

Clinical Signs

Clinical exams were performed at least once daily. There were no drug-related findings.

Body Weights

Body weights were recorded once weekly. There were no drug-related changes.

Food Consumption

Food consumption was recorded once weekly. There were no drug-related changes.

Hematology

Blood samples were collected prior to necropsy on Day 92/93 and 120. Increased total reticulocytes (10-32%) were observed in all treated females at Day 92/93 (significant only at the high dose). Increased percent reticulocytes (27%) were also observed in high-dose females at Day 92/93. This may be drug-related, but was considered non-adverse due to the lack of a pathological correlate in the bone marrow.

Clinical Chemistry

Blood samples were collected prior to necropsy on Day 92/93 and Day 120. Creatinine levels were increased 22-39% in all treated males, and cholesterol levels were increased 17-22% in mid- and high-dose females, on Day 92/93. The change in creatinine may be attributed to abnormally low levels in the control animals at this time point. The increase in cholesterol may be drug-related, but was considered non-adverse.

Gross Pathology

Gross pathology was evaluated at the time of necropsy on Day 92/93 and Day 120. The following were observed at Day 92/93:

- Foci in the heart were observed in 3 low-dose and 1 mid-dose males, and 2 high-dose females (also 1 control female).
- Dilatation of the uterus in 2 low-dose and 3 high-dose females (also 1 control female).

The following were observed at Day 120:

- Mass in the thymus, enlarged spleen and bronchial lymph node, pale pancreas, red fluid in the abdominal and thoracic cavities, and crust on the penis in a single high-dose male.

The recovery male determined to have a lymphosarcoma in the thymus with metastatic lesions in the spleen, bronchial lymph node and lung. It is unclear if the findings in this animal were related to treatment. The heart findings correlate to mineralization of the epicardium (see Histopathology section), and were considered incidental and unrelated to treatment.

Organ Weights

Organ weights were recorded at the time of necropsy on Day 92/93 and Day 120. Increased liver (10-16%) and spleen weights (9-27%) were observed in mid- and high-dose females on Day 92/93. These changes were not considered adverse due to a lack of pathological correlates.

Reference ID: 4258909

Histopathology

Adequate Battery Yes

Peer Review No

Histological Findings Histopathology was evaluated at the time of necropsy on Day 92/93 and Day 120. For the low- and mid-dose groups, only tissues with gross lesions were evaluated. For the control and high-dose groups, all main study tissues were evaluated, and only those with gross lesions were evaluated from the recovery groups. However, lung tissues were evaluated from all groups at both time points. The following were observed at Day 92/93:

- Adenoma in lung in 1 high-dose male and 1 high-dose female. Minimal osseous metaplasia in lung in 1 high-dose male. Minimal chronic inflammation in lung in 1 low-dose, 1 mid-dose and 4 high-dose males, and in 6 low-dose and 7 high-dose females (also 3 male and 1 female controls). Minimal to mild acute inflammation in lung in almost all animals, including almost all controls.
- Minimal to mild mineralization of the epicardium in 3 low-dose, 1 mid-dose and 2 high-dose males, and 2 high-dose females (also 1 male and 2 female controls).

The following were observed at Day 120:

- Lymphosarcoma in thymus with metastases in the lung, spleen and bronchial lymph node in 1 high-dose male. Moderate ulceration and mild acute inflammation of the penis were also noted in this animal.
- Adenoma in lung in 1 high-dose male.
- Minimal to mild acute inflammation in lung in almost all animals, including almost all controls.

It is unclear if the lymphosarcoma was related to treatment. Adenomas of the lung are a common finding in this species and were considered incidental. The mineralization in the epicardium corresponds to the gross findings in the heart, and was considered incidental and unrelated to treatment.

Toxicokinetics

Blood samples were collected 0.5, 1, 2, 4, 8 and 24 hours post-dose on Day 1 and Week 13, and pretreatment at Week 13 only. Week 13 toxicokinetic parameters are presented in **Table 13**.

Table 13 - TK Parameters From the 3-Month Mouse Study (Week 13)

Group	Dose (mg/kg)	Sex	λ_z (1/hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}24\,hr}$ (hr*ng/mL)	$AUC_{0\text{-}\infty}$ (hr*ng/mL)	V_z/F (mL/kg)	CL/F (mL/hr/kg)
2	300	Male	0.207	3.3	2	60500	504990	509286	2843	589
3	600		0.142	4.9	2	58900	551137	569610	7427	1053
4	1000		0.151	4.6	4	58700	663767	684899	9672	1460
2	300	Female	0.216	3.2	2	70300	523563	526511	2637	570
3	600		0.253	2.7	2	69300	660848	662986	3583	905
4	1000		0.224	3.1	1	71100	737469	741652	6014	1348

Reference ID: 4258909

Dosing Solution Analysis

The test article formulations were within acceptable limits of concentration (98-107%) and homogeneity (100-104%), and were considered stable for up to 19 days (94-104%). No test article was detected in the control formulation.

Study title: ST246: Bioavailability and A 12-Day Oral Repeat Dose Study in Rats

Study no.:	**246-TX-003**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 18th, 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot #PCCS 1391, purity = 97.7%)

Summary

Mortality, clinical exams, body weights, food consumption, hematology, clinical chemistry, gross pathology and organ weights were performed or evaluated in Crl:CD®(SD) rats following oral gavage doses of either vehicle (1% HPMC/0.5% Tween® 80) or tecovirimat (30, 100, 300 or 1000 mg/kg/dose; 5/sex/primary groups). Animals were treated once daily for 12 days, and were euthanized on Day 12. In addition, toxicokinetics were evaluated in satellite rats (10/sex/group) following either a single intravenous dose (3 mg/kg) or a single oral gavage dose (30, 100, 300 or 1000 mg/kg) of tecovirimat. One 1000 mg/kg female was found dead on Day 5 due to a dosing error, which was considered unrelated to treatment. Body weights were decreased up to 19% from Days 4 to 12 in both males (300 mg/kg/day and above) and females (100 mg/kg/day and above). Corresponding decreases in food consumption were observed in the same groups from Days 1 to 4. Total lymphocytes were decreased in males and females at 300 and 1000 mg/kg/day (14-45%), and total basophils were decreased in males at 100 and 1000 mg/kg/day (49-63%). In females at 1000 mg/kg/day, total neutrophils were increased 50% and total reticulocytes were increased 41%. Increases in total bilirubin (36-73%), creatinine (5-19%) and cholesterol (20-54%; females only) were observed in males and females at 300 and 1000 mg/kg/day, and females at 100, 300 and 1000 mg/kg/day. Mild liver discoloration was noted in 1 male at 1000 mg/kg/day. Liver weights were increased 5-30% in all treated animals, and thyroid/parathyroid weights were increased 25-32% in males at 300 and 1000 mg/kg/day. Exposure, half-life and oral bioavailability of tecovirimat were about 5 times greater in males than in females, likely due to increased first pass metabolism in females. Bioavailability was inversely proportionally to dose (90% in males and 33.2% in females at 30 mg/kg/day vs. 5.9% in males and 2.7% in females at 1000 mg/kg/day).

<u>NOAEL = 30 mg/kg/day</u> ($AUC_{0\text{-}inf}$ = 7.6 µg•hr/mL, C_{max} = 1.4 µg/mL at Day 9) due to changes in body weight, food consumption, organ weights and clinical pathology parameters.

Reference ID: 4258909

Study title: ST246: A 7-Day Toxicokinetic Study in Dogs (Non-GLP)

Study no.:	**246-TX-014**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 20th, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot number and purity not provided)

Summary

Mortality, clinical exams, body weight, food consumption, hematology, clinical chemistry, gross pathology and toxicokinetics were evaluated in Beagle dogs following oral gavage doses of tecovirimat (30 or 100 mg/kg/dose; 3 males/group). Animals were to be treated once daily for 7 days, and euthanized on Day 7. However, about 5-8 hours after the first dose, all high-dose animals displayed adverse clinical signs including emesis, excessive salivation, decreased activity, pale gums, rapid/labored breathing and red nasal discharge, and 2 of these 3 had tremors and convulsions, ~5-8 hours post-dose on Day 1. All three high-dose males were euthanized ~12 hours post-dose on Day 1, and blood samples for clinical pathology and toxicokinetic analyses could not be reliably obtained. Gross findings in these animals included discolored lungs, thymus and duodenum, and abnormal contents in the trachea. At the low dose, only one animal displayed transient emesis and excessive salivation. The low dose animals were euthanized as scheduled without gross examination. All six animals had previously received another antiviral compound 5 weeks before being placed on this study, and it is unknown if the prior treatments had somehow sensitized them to tecovirimat.

MTD = 30 mg/kg/dose (TK data not calculated) due to deaths at the high dose. A NOAEL could not be defined due to the limited number of animals per group, and the absence of histopathology data. However, 30 mg/kg/dose was considered the NOAEL for convulsions in this study.

Reference ID: 4258909

Study title: ST246: A Maximum Tolerated Dose Oral Toxicity Study in Dogs

Study no.:	**246-TX-015**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 4th, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot #06-051213-04/04-28-01, purity = 99.3%)

Summary

Mortality, clinical exams, body weight, electrocardiography, echocardiography, blood pressures, electroencephalography, hematology, clinical chemistry, gross pathology, organ weights and toxicokinetics were recorded or evaluated in Beagle dogs following oral gavage doses of tecovirimat (30, 100 or 300 mg/kg/dose; 3/sex at the low dose, 1/sex at the mid- and high doses). Low-dose animals were treated once daily for 8 days, and euthanized 2 hours post-dose on Day 8. Mid-dose animals were treated once daily for 7 days, and euthanized 2 hours post-dose on Day 7. High-dose animals received a single dose on Day 1 and were to be euthanized on Day 10. Clinical signs at the high dose included ataxia, blinking, emesis, jerky head movements, salivation, tremors, panting, stereotypic walk and vocalization, and the high-dose male was euthanized *in extremis* on Day 1 due to convulsions. An increase in QTc interval of 15-30 milliseconds was noted at the high dose 6-8 hours post-dose (increased up to 336 milliseconds in the high-dose male). EEG analysis indicated that doses of 300 mg/kg can result in lowered seizure thresholds and/or frank seizures on the first day of dosing, though no differences were observed at 100 mg/kg/dose. Mid-dose clinical signs included tremors, face twitching, vocalization, and excessive salivation and licking. Low-dose clinical signs were limited to soft feces, red discolored ears and vulva discharge. Drug was also detected in the brain and cerebrospinal fluid at all dose levels, indicating that tecovirimat crosses the blood-brain barrier in this species. At 30 and 100 mg/kg/dose, brain/plasma ratio was ~0.35 and CSF/plasma ratio was ~0.06. Brain and CSF concentrations were not measured in the high-dose group.

<u>MTD = 100 mg/kg/dose</u> (AUC_{0-t} = 10.8 µg•hr/mL, C_{max} = 2.0 µg/mL at Day 5) due to adverse clinical signs including tremors and convulsions at the mid- and high doses, and euthanasia *in extremis* at the high dose. A NOAEL could not be defined due to the limited number of animals per group, the lack of concurrent control groups, and the absence of histopathology data. However, <u>100 mg/kg/dose was considered the NOAEL for convulsions</u> in this study (highest C_{max} reported at this dose = 5575 ng/mL in the male at Day 1).

Reference ID: 4258909

Study title: ST246: A 12-Day Oral Toxicity Study in Monkeys

Study no.: **246-TX-016**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: October 26th, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #06-051213-04/04-28-01, purity = 99.3%)

Summary

Mortality, clinical exams, body weights, electrocardiography, electroencephalography and pharmacokinetics were performed or evaluated in cynomolgus monkeys following oral gavage doses of tecovirimat (300 mg/kg/dose; 6/sex). Animals were treated once daily for 12 days, and were euthanized on Day 12. There were no unscheduled deaths. A decrease in heart rate (8-13%), and increases in RR (8-17%), QRS (12-20%), QT (8-22%) and QTc (5-14%) intervals were observed in both males and females within 2 hours post-dose on Day 1. No EEG findings were observed except for one period of isolated sharp waves in one female 6 hours post-dose on Day 12. Tecovirimat was detected in the brain and cerebrospinal fluid 8 hours post-dose on Day 12 (brain/plasma and CSF/plasma ratios were roughly 0.6 and 0.1, respectively).

A NOAEL was not defined due to the limited number of dose levels and absence of control groups.

Study title: A 14-Day Toxicity, Toxicokinetics, Local Tolerability and Safety Pharmacology of ST-246 by Intravenous Infusion in Cynomolgus Monkeys with a 2-Week Recovery Period

Study no.: **246-TX-021**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: November 5th, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #SG-10A11-Q, purity = 95.4%)

Summary

Mortality, clinical exams, body weights, food consumption, ophthalmology, neurologic exams, electrocardiography, hemodynamics, respiratory impedance, body temperature, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology and toxicokinetics were performed or evaluated in cynomolgus monkeys

Reference ID: 4258909

following intravenous doses of either vehicle (13.3% hydroxypropyl β-cyclodextrin) or tecovirimat (3, 10 or 30 mg/kg/dose; 3/sex/primary groups and 2/sex/recovery groups). Animals received a 6-hour infusion of drug once daily for 14 days via a battery-powered infusion pump, and were euthanized on either Day 15 or 29. A total of 9 animals died between Days 6 and 16 due to infection following surgical implantation of the venous access port used to administer the daily infusions. Necropsy in these animals identified purulent fluid accumulation in the skin surrounding the implant, and minimal to mild granuloma/thrombi formation at the catheter entrance/tip. These deaths were not related to either tecovirimat or vehicle. Of these 9 animals, only 3 could be replaced with alternates. Six animals (2 control females, 1 low-dose male, 1 low-dose female, and 2 mid-dose males) were therefore omitted from the study. Kidney weight was increased 18% in high-dose males at Day 15, but no clear histopathology correlate was identified.

NOAEL = 30 mg/kg/dose (AUC_{0-t} = 76.3 µg•hr/mL, C_{max} = 12.3 µg/mL at Day 14)

Study title: ST246: A 28-Day Oral Toxicity Study in Monkeys

Study no.:	**246-TX-007**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 10th, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #5C020, purity = 99.8%)

Summary

Mortality, clinical exams, body weights, ophthalmology, electrocardiography, hematology, clinical chemistry, urinalysis, immune cell phenotyping, gross pathology, organ weights, histopathology and toxicokinetics were performed or evaluated in cynomolgus monkeys following oral gavage doses of either vehicle (1% HPMC/0.5% Tween® 80) or tecovirimat (30, 100 or 300 mg/kg/dose; 3/sex/primary groups and 1/sex/recovery groups). Animals were treated once daily for 28 days, and were euthanized on either Day 28 or 42. There were no unscheduled deaths. Clinical signs consisted of emesis after dosing, soft feces and inappetence in some animals at all dose levels, including controls, but these findings were not dose-related and were considered non-adverse. Prostate weight was increased 4-fold in one high-dose male at Day 28, but there was no pathological correlate. Similarly, lung weight was increased 2.8-fold in one high-dose female at Day 28, but no pathological correlate was seen aside from minimal pigmentation. These findings were considered non-adverse.

NOAEL = 300 mg/kg/dose (AUC_{0-24hr} = 43.0 µg•hr/mL, C_{max} = 3.3 µg/mL at Day 27)

Reference ID: 4258909

Study title: 3-Month Oral (Gavage) Toxicity Study in Monkeys

Study no.:	**2083-003-001-SN6**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 13th, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Key Study Findings

NOAEL = 300 mg/kg/dose (AUC_{0-24hr} = 77.7 µg•hr/mL, C_{max} = 5.4 µg/mL at Week 13).

Methods

Doses:	0, 30, 100 & 300 mg/kg/dose
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/group for main study groups, 1/sex/group for recovery groups
Age:	2-2.5 years
Weight:	2-3 kg
Satellite groups:	None
Unique study design:	Main study animals were euthanized on Day 92. Recovery animals were euthanized on Day 120/121.
Deviation from study protocol:	None which affected interpretation of the study

Observations and Results

Mortality

Morbidity and mortality were assessed at least once daily. One high-dose male died on Day 43 due to gavage-related trauma, which was confirmed following a full necropsy.

Clinical Signs

Clinical exams were performed at least once daily. The following were observed:

- Labored/rapid breathing, ataxia and hypoactivity in one high-dose male on Day 43. This animal went comatose and died prematurely on the same day due to gavage-related trauma.
- Tremors, labored breathing, thin appearance, ataxia, hypoactivity, hunched posture, red nasal discharge, and coughing in a second high-dose male between Days 11 and 43. This animal also exhibited decreased food consumption on Days 23-44. The applicant attributed this to aspiration of the drug formulation.

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The clinical signs and death of the first animal were the result of gavage-related trauma. The findings in the second animal were attributed to aspiration of the drug formulation, and were likely unrelated to treatment.

Body Weights

Body weights were recorded once weekly. There were no drug-related changes.

Food Consumption

Food consumption was evaluated qualitatively once daily. Loss of or suppression of appetite were observed in a single high-dose male on Days 23-44 (except Day 36). Clinical signs including tremors, labored breathing and ataxia were also observed in these animals within this time frame. This was attributed to aspiration of the test article formulation, and is likely unrelated to treatment.

Ophthalmoscopy

Ophthalmic exams were performed pretreatment and on Weeks 13 and 17. There were no drug-related changes.

Electrocardiography

ECGs were performed pretreatment, on Day 1, and on Weeks 6, 13 and 17. There were no drug-related changes.

Electroencephalography

EEGs were performed pretreatment, on Day 1, and on Weeks 6, 13 and 17. Evidence of elevated slow wave rhythmic activity consistent with mild sedation was observed in one high-dose male on Week 6. This animal also exhibited labored/rapid breathing, ataxia and hypoactivity, and died following a coma on Day 43. These findings were attributed to gavage-related trauma and were unrelated to treatment.

Hematology

Fasted blood samples were collected prior to necropsy on Weeks 13 and 17. There were no drug-related changes.

Clinical Chemistry

Fasted blood samples were collected prior to necropsy on Weeks 13 and 17. There were no drug-related changes.

Urinalysis

Urine samples were collected prior to necropsy on Weeks 13 and 17. There were no drug-related changes.

Gross Pathology

Gross pathology was evaluated at the time of necropsy on Day 92 or 120/121. Adhesions of pulmonary parenchyma to the thoracic wall were observed in 1 male and 1 female at the mid-dose at Day 92 (also 1 male and 1 female controls), and in the mid-dose male and both high-dose animals at Day 120/121. Fibrosis of the pleura was also

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observed in these animals following histological evaluation. The significance of this finding is unclear, but is likely incidental.

Organ Weights

Organ weights were recorded at the time of necropsy on Day 92 or 120/121. There were no drug-related findings.

Histopathology

Adequate Battery Yes

Peer Review No

Histological Findings Histopathology was evaluated at the time of necropsy on Day 92 and Day 120/121. The following were observed at Day 92:

- Minimal fibrosis of the pleura in the lung in 1 mid-dose male and 1 mid-dose female (also 1 male and 1 female controls).
- Minimal acute inflammation and mild cilia loss in the trachea in 1 high-dose male. Minimal chronic inflammation in the trachea in 1 low-dose, 3 mid-dose and 2 high-dose males, and in 2 low-dose, 2 mid-dose and 1 high-dose females (also 2 female controls).
- Minimal chronic inflammation in the esophagus in 1 low-dose female, 2 mid-dose males, and all high-dose animals (also 1 female control).

In addition, minimal chronic inflammation was observed in multiple organs in almost all animals, including controls. These findings were considered incidental and unrelated to treatment. The findings in the trachea and esophagus may be treatment-related, but were considered non-adverse. The fibrosis of the pleura corresponds to the gross findings in the lung in the same animals. The significance of these findings is unclear, but they are likely incidental. There were no findings in the recovery animals.

Immunophenotyping

Blood samples were collected on Day 90/91 and Weeks 13 and 17, and immune cell phenotyping was conducted via FACS analysis. There were no drug-related changes.

Toxicokinetics

Blood samples were collected on Days 1 and 62 and Week 13 pretreatment and 1, 2, 4, 6, 8, 12 and 24 hours post-dose. In addition, blood samples from recovery animals were collected on Days 92, 98 and 120/121. Day 89 toxicokinetic parameters are presented in **Table 14**.

Table 14 - TK Parameters From the 3-Month Monkey Study (Day 89)

Dose (mg/kg)	Sex	$AUC_{0\text{-}24hr}$ (ng•hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
30	Male	30079	2805	3.5
	Female	21268	1515	1.5
100	Male	44552	3920	3.5
	Female	41511	3380	2.5
300	Male	79541	5993	3.3
	Female	75837	4785	3.5

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Dosing Solution Analysis

The test article formulations were within acceptable limits of concentration (96-103%) and homogeneity (100-101%), and were considered stable for up to 15 days (94-104%). No test article was detected in the control formulation.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay

Study no.: **246-TX-008**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: June 24th, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #5C020, purity = 97.9%)

Key Study Findings

Tecovirimat is not mutagenic as determined by the bacterial reverse mutation assay.

Methods

Strains: TA98, TA100, TA1535, TA1537 and WP2 *uvrA*
Concentrations in definitive study: 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate
Basis of concentration selection: At least 3 doses up to 5000 µg/plate which did not result in cytotoxicity or precipitation.
Negative control: DMSO
Positive control:

Control (µg/plate)	S9	Strain
2-Nitrofluorene (1)	-	TA98
Sodium azide (1)	-	TA100 & TA1535
9-Aminoacridine (1)	-	TA1537
Methyl methanesulfonate (1000)	-	WP2 *uvrA*
2-Aminoanthracene (10)	+	WP2 *uvrA*

Formulation/Vehicle: DMSO
Incubation & sampling time: Plates were incubated at 37°C for 48-72 hours with and without S9-induced metabolic activation.

Study Validity/Results

No tecovirimat-related increases in the number of colonies were observed in any strain in either the presence or absence of S9 at any concentration tested. Precipitation was observed at concentrations ≥500 µg/plate. No appreciable cytotoxicity was observed. Positive and negative controls were appropriate and produced expected responses. Background levels were within historical ranges. All criteria for a valid study were met.

Reference ID: 4258909

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Study no.: **246-TX-009**
Study report location: EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: June 24th, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #5C020, purity = 97.9%)

Key Study Findings

Tecovirimat was negative in the L5178Y TK$^{+/-}$ mouse lymphoma assay.

Methods

Cell line: L5178Y TK$^{+/-}$-3.7.2C mouse lymphoma cells

Concentrations in definitive study:

Time (hr)	S9	Concentration (µg/mL)
4	-	10, 15, 25, 50 & 75
4	+	5, 15, 25, 50 & 75
24	-	5, 10, 25, 50 & 100

Basis of concentration selection: At least 3 doses up to 75 µg/mL which did not result in cytotoxicity or precipitation.
Negative control: DMSO
Positive control: Methyl methanesulfonate (5-20 µg/mL) in absence of S9. 7,12-Dimethylbenz(a)anthracene (2.5 & 4 µg/mL) in presence of S9.
Formulation/Vehicle: DMSO
Incubation & sampling time: Cells were incubated at 37°C for 4 and 24 hours without S9-induced metabolic activation, and for 4 hours with S9-induced metabolic activation.

Study Validity/Results

No increases in mutant frequency were observed in either the presence or absence of S9 at either time point and any concentration tested. Precipitation was observed at 75 µg/mL in the 4-hour groups with and without metabolic activation, and at 100 µg/mL in the 24-hour group without metabolic activation. Positive and negative controls were appropriate and produced expected responses. Background levels were within historical ranges. All criteria for a valid study were met.

Reference ID: 4258909

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

Study title: Mammalian Erythrocyte Micronucleus Test

Study no:	**246-TX-010**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 21st, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #5C020, purity = 97.9%)

Key Study Findings

Tecovirimat was negative in the *in vivo* bone marrow micronucleus assay.

Methods

Doses in definitive study:	0, 500, 1000 & 2000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	ICR mice
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	Doses were based on toxicity data from the Applicant demonstrating that a dose of 2000 mg/kg is well-tolerated in this species.
Negative control:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Positive control:	Cyclophosphamide (50 mg/kg)

Study Validity/Results

No increases in the percentage of micronucleated cells or in the polychromatic erythrocyte:total erythrocyte ratio were noted up to 48 hours post-dose and up to the highest concentration tested (2000 mg/kg). Positive and negative controls were appropriate and produced expected responses. Background levels were within historical ranges. All criteria for a valid study were met.

8 Carcinogenicity

Carcinogenicity studies were not performed. The drug is not expected to be used chronically and thus carcinogenicity studies are not necessary for an acute/treatment indication.

Reference ID: 4258909

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: ST-246®: Study of Fertility and Early Embryonic Development to Implantation in Mice (Segment I)

Study no.:	(b) (4) **-2083-003-001-SN7**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 2nd, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Key Study Findings

NOAEL = 1000 mg/kg/dose in females (TK parameters not calculated). The NOAEL in males was not defined due to increased percent abnormal sperm and decreased sperm motility at the high dose (spermatological parameters were not evaluated at the low and mid-doses). Decreases in fertility and fecundity indices were also observed at all dose levels. Since no female fertility findings were observed in this study, these decreases were likely attributed male reproductive toxicity.

Methods

Doses:	0, 100, 300 & 1000 mg/kg/dose
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	Crl:CD1®(ICR) mice
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Males were treated for 4 weeks prior to mating, and throughout mating up to euthanasia on Week 9. Females were treated for 2 weeks prior to mating and up to Gestational Day (GD) 7, and were euthanized on GD 13.
Deviation from study protocol:	None which affected interpretation of the study

Observations and Results

Mortality

Mortality was assessed at least once daily. Two control males were found dead on Days 16 and 36, and one low-dose female died on GD 3. Clinical signs in the control animal on Day 16 included rough coat, emaciation, ataxia and coldness to touch, and necropsy revealed red pigmentation in the lungs. No clinical signs were observed in the

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control animal that died on Day 36, though gross findings included clotted blood in the thoracic cavity and a thymus cyst. Clinical signs in the low-dose female included hypoactivity and coldness to touch, and necropsy of this animal revealed a ruptured esophagus and fluid in the thoracic cavity. Death of the low-dose female was attributed to gavage error. All three deaths were considered incidental and unrelated to treatment.

Clinical Signs

Clinical exams were performed at least once daily. Hypoactivity, wet inguinal fur and alopecia were each observed in a single high-dose male at least once throughout dosing. Rough coat was observed in all males throughout the study, though the number of animals affected increased 22-44% in mid- and high-dose males relative to controls. These findings may be drug-related, but were considered non-adverse.

Body Weight

Body weights were measured twice weekly in all animals, and once every 1-3 days in pregnant females. Body weight was decreased 4% in low- and high-dose females on Day 7 prior to mating. Body weight gain was increased in high-dose males on Days 17 and 24, and was decreased in mid- and high-dose males on Day 21.

Food Consumption

Food consumption was measured weekly twice weekly in all animals, and once every 1-3 days in pregnant females. Food consumption was decreased 10% in low- and mid-dose males on Day 28. It is unclear if this is drug-related.

Gross Pathology

Gross pathology was evaluated in all animals at the time of necropsy. Bilateral small testis and epididymis were observed in 1 mid-dose male. Dilatation of the lumen of the urinary bladder was seen in 2 high-dose males. These findings were considered incidental and unrelated to treatment.

Organ Weights

Testis, epididymis, seminal vesicle and prostate weights were measured at the time of necropsy. No drug-related changes were observed.

Spermatological Parameters

Sperm count, motility and morphology were assessed in 10 control and 10 high-dose males only at the time of necropsy. Increased percent abnormal sperm (1.7% at the high dose vs. 0.9% in controls), and decreased sperm motility (68% at the high dose vs. 83% in controls) were observed at the high dose.

Estrus Determination

Vaginal smears were collected daily up to the end of cohabitation. The number of females exhibiting all stages of estrus was decreased 24% in the mid-dose group. It is unclear if this is drug-related.

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Fertility Parameters

Mating, fertility and fecundity indices, and time to insemination were evaluated. Fertility and fecundity indices were decreased 8% at the low dose, 17% at the mid-dose, and 26% at the high dose.

Caesarean Section

Cesarean sections were performed at the time of necropsy. Endpoints included pregnancy status, number of corpora lutea, implantation sites and live/dead fetuses, pre-/post-implantation loss and early/late resorptions. The number of pregnant dams and dams with viable fetuses decreased in a dose-dependent fashion (21 low-dose, 19 mid-dose and 17 high-dose females vs. 23 female controls).

Dosing Solution Analysis

The high-dose formulation on Week 5 was 117% of the target concentration. All other test article formulations were within acceptable limits of concentration and homogeneity (97-106% of the target concentration), and were stable for up to 7 days. No test article was detected in the control formulation.

9.2 Embryonic Fetal Development

Study title: ST-246: A Pilot Embryo-Fetal Developmental Toxicity Study in Mice

Study no.:	(b) (4) **2083-003-001-SN1**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 9th, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Summary

Mortality, clinical signs, body weights, food consumption, gross pathology, cesarean sections and fetal variations/malformations (external only) were performed or evaluated in pregnant female Crl:CD-1(CR)BR mice following daily oral gavage dosing with either vehicle (1% Hydroxypropyl methylcellulose/0.5% Tween® 80) or drug (30, 100, 300, 500 or 1000 mg/kg/day; 10 females/group). Mated females were treated from GD 6 to 15 (during organogenesis), and were euthanized on GD 18. There were no unscheduled deaths. Two animals at 500 mg/kg/day had no viable fetuses, though this was not observed at 1000 mg/kg/day. Slight increases in early resorptions and post-implantation loss were observed at 500 and 1000 mg/kg/day, though these changes were not significant and not entirely dose-dependent, so it is unclear if they are incidental or treatment-related.

NOAEL = 1000 mg/kg/day for both maternal and fetal toxicity (TK data unavailable).

Reference ID: 4258909

Study title: ST-246®: An Embryo-Fetal Developmental Toxicity Study in Mice

Study no.: (b) (4) **-2083-003-001-SN4**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: November 21st, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ST-246 (lot #6A003, purity = 97.7%)

Key Study Findings

NOAEL = 1000 mg/kg/dose for both maternal and fetal toxicity (AUC_{0-24hr} = 661.3 µg•hr/mL, C_{max} = 60.8 µg/mL at GD 19). Slight increases in the number of external, visceral and skeletal malformations were observed at the mid- and high doses, though due to the overall low incidence, it is unclear if these changes are incidental or related to treatment.

Methods

Doses: 0, 100, 300 & 1000 mg/kg/dose
Frequency of dosing: Once daily
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain: Crl:CD-1(CR)BR mice
Number/Sex/Group: 45 females/group
Satellite groups: 57-59 females/group
Study design: Pregnant females were treated daily from GD 6 to 15 (during organogenesis) and were euthanized on GD 18. Only 25 females/group were to be used initially (Cohort 1), though an additional 20 females/group (Cohort 2) were acquired due to the low rate of pregnancy in all groups, including controls. Of the 45 females in each group, only 25-30/group were confirmed to be pregnant.
Deviation from study protocol: None which affected interpretation of the study

Observations and Results

Mortality

Morbidity and mortality were assessed twice daily. One high-dose female died on GD 10 due to gavage error. Necropsy revealed a white substance in the thoracic cavity.

Clinical Signs

Clinical exams were performed at least once daily. No drug-related findings were observed.

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Body Weight

Maternal body weights were measured on GD 4, 6, 9, 12, 15 and 18. Body weight was decreased 3-8% relative to controls from GD 9 to 18 at the high dose in Cohort 1 (not statistically significant), though no differences were observed in Cohort 2. As this was only seen in Cohort 1, it is unclear if this is drug-related.

Food Consumption

Food consumption was measured on GD 4, 6, 9, 12, 15 and 18. No drug-related changes were observed.

Toxicokinetics

Blood samples were collected pretreatment on GD 15, and 0.5, 1, 2, 4, 8 and 24 hours post-dose on GD 6 and 15. GD 15 Toxicokinetic parameters are presented in **Table 15**.

Table 15 - TK Parameters From the Mouse Embryofetal Development Study (GD 15)

Dose (mg/kg)	λ_z (1/hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24hr} (ng•hr/mL)	AUC_{0-inf} (ng•hr/mL)	V_z/F (mL/kg)	CL/F (mL/hr/kg)
100	0.178	3.9	2.0	36400	403641	410445	1370	244
300	0.287	2.4	2.0	48200	476171	476907	2196	629
1000	0.132	5.2	2.0	60800	661256	694872	10896	1439

Dosing Solution Analysis

All test article formulations were within acceptable limits of concentration and homogeneity (94-108% of the target concentration), and were stable for up to 13 days. No test article was detected in the control formulation.

Necropsy

Gross pathology was evaluated in all animals at the time of necropsy. Pale liver was seen in 1 low-dose female. Uterus dilatation was seen in 1 low-dose and 2 mid-dose females (also 1 control animal). Ovarian cyst was seen in 1 low-dose female. These findings were considered incidental and unrelated to treatment.

Cesarean Sections

Cesarean sections were performed at the time of necropsy. Endpoints included pregnancy status, number of corpora lutea and implantation sites, pre-/post-implantation loss, early/late resorptions, and gravid uterine weight. No drug-related findings were observed.

Offspring

Fetal endpoints included viability, sex ratio, body weight and external/visceral/skeletal variations and malformations. Fetal variations and malformations are presented in **Tables 16 & 17**, respectively. Slight increases in the number of malformations were observed at the mid- and high doses, though due to the low incidence and lack of statistical significance, it is unclear if these changes are related to treatment.

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Table 16 - Fetal Variations From the Mouse Embryofetal Development Study

Fetal Variations		Control	Low Dose	Mid-Dose	High Dose
External	Club Foot; Hindlimb	1 (1)	2 (2)	0 (0)	1 (1)
	Number with External Variations	1 (1)	2 (2)	0 (0)	1 (1)
Visceral	Displaced Ophthalmia	0 (0)	0 (0)	0 (0)	1 (1)
	Kidney(s) – Variation	0 (0)	0 (0)	1 (1)	1 (1)
	Testis/Testes Small	0 (0)	0 (0)	1 (1)	0 (0)
	Number with Visceral Variations	0 (0)	0 (0)	2 (2)	2 (2)
Skeletal	Hyoid Body/Arch(es) Unossified	0 (0)	0 (0)	0 (0)	1 (1)
	25 Presacral Vertebrae	0 (0)	2 (2)	2 (2)	1 (1)
	27 Presacral Vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
	Sternebra(e) Malaligned – Slight to Moderate	2 (2)	1 (1)	0 (0)	0 (0)
	7th Sternebra(e)	3 (2)	0 (0)	1 (1)	1 (1)
	Sternebra(e) 5 and/or 6 Unossified	3 (2)	0 (0)	0 (0)	0 (0)
	Additional Sternebra(e) Adjacent to the First	0 (0)	0 (0)	0 (0)	1 (1)
	Cervical Rib(s) – Present	0 (0)	2 (2)	0 (0)	1 (1)
	Rib(s) 13th Rudimentary	0 (0)	0 (0)	0 (0)	1 (1)
	Rib(s) 14th Full	35 (18)	43 (16)	13 (6)	30 (11)
	Rib(s) 14th Rudimentary	28 (18)	26 (14)	11 (7)	21 (12)
	Rib(s) Incomplete Ossification	0 (0)	1 (1)	0 (0)	0 (0)
	Carpal/Metacarpal Unossified	7 (3)	6 (3)	0 (0)	4 (2)
	Tarsal/Metatarsal Unossified	6 (3)	5 (2)	0 (0)	4 (2)
	Number with Skeletal Variations	71 (22)	74 (22)	26 (12)	59 (19)
Total Number of Variations		72 (22)	76 (22)	28 (14)	61 (20)
Total Number of Fetuses/Litters		315 (27)	341 (27)	282 (22)	256 (24)

Results are presented as "# fetuses (# litters)"

Table 17 - Fetal Malformations From the Mouse Embryofetal Development Study

Fetal Malformations		Control	Low Dose	Mid-Dose	High Dose
External	Open Eyelid(s)	0 (0)	0 (0)	2 (2)	1 (1)
	Exencephaly	0 (0)	0 (0)	1 (1)	1 (1)
	Domed Head	1 (1)	0 (0)	0 (0)	0 (0)
	Tail Bent	0 (0)	0 (0)	0 (0)	1 (1)
	Number with External Malformations	1 (1)	0 (0)	2 (2)	2 (2)
Visceral	Cleft Palate	0 (0)	0 (0)	2 (1)	1 (1)
	Testes Undescended	0 (0)	0 (0)	1 (1)	0 (0)
	Number with Visceral Malformations	0 (0)	0 (0)	3 (2)	1 (1)
Skeletal	Sternebra(e) Malaligned – Severe	1 (1)	0 (0)	0 (0)	1 (1)
	Sternebra(e) Fused	0 (0)	1 (1)	0 (0)	3 (2)
	Number with Skeletal Malformations	1 (1)	1 (1)	0 (0)	4 (3)
Total Number of Malformations		2 (2)	1 (1)	5 (4)	7 (6)
Total Number of Fetuses/Litters		315 (27)	341 (27)	282 (22)	256 (24)

Results are presented as "# fetuses (# litters)"

Reference ID: 4258909

Study title: ST-246: A Pilot Embryo-Fetal Development Study in Rabbits via Oral Administration

Study no.:	(b) (4) **-1151-024**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 11th, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #PCCS1391, purity not stated)

Summary

Mortality, clinical signs, body weights, food consumption, gross pathology, cesarean sections, fetal variations/malformations (external only) and toxicokinetics were performed or evaluated in pregnant female New Zealand White Hra:(NZW)SPF rabbits following daily oral gavage dosing with either vehicle (1% Hydroxypropyl methylcellulose/0.5% Tween® 80) or drug (300, 600 or 1000 mg/kg/day; 4-5 females/group). Mated females were treated from GD 6 to 18 (during organogenesis), and were euthanized on GD 29. Three low-dose, 1 mid-dose, and all five high-dose animals were found dead between GD 8 and GD 26. Cause of death was not definitively determined, but was considered treatment-related. One low-dose animal experienced tonic convulsions prior to death on GD 15. Additional clinical signs included decreased activity (1 mid-dose and 3 high-dose animals), up-and-down head movements (1 high-dose animal), rapid breathing (1 low-dose, 1 mid-dose and 3 high-dose animals), little/no feces (3 low-dose, 4 mid-dose and 4 high-dose, and 2 control animals), thin appearance (2 low-dose, 3 mid-dose and 1 high-dose animals), red/brown material in cage (2 mid-dose animals), red material around nose and mouth (1 low-dose and 2 high-dose animals), and discolored/sparse hair in multiple treated animals. Body weight was decreased 11-14% from GD 13 to 25 at the low dose, 9-25% from GD 10 to 29 at the mid-dose, and 11-17% from GD 10 to 13 at the high dose (all high-dose animals died by GD 15). Food consumption was also decreased 58-99% at all dose levels from GD 6 to 25 (GD 6 to 13 at the high dose). Minimal to moderate hemorrhage in the ventral cervical region of the skin was observed in 4 low-dose, 1 mid-dose and 4 high-dose animals (attributed by the Applicant to the bleeding procedure used to collect TK samples). As all high-dose animals died by GD 15, no cesarean or fetal data are available from this group. Of the surviving mid-dose animals, one animal delivered prematurely on GD 28, and another experienced early resorption of all fetuses. Decreased gravid uterine weight (46%) and fetal weight (41%) and late resorption of 1 fetus were observed in the surviving mid-dose animal. No fetal variations or malformations were observed, but it is not possible to evaluate fetal toxicity in this study due to the low number of animals and the extent of maternal toxicity.

<u>Maternal and fetal NOAELs were not determined</u> due to severe maternal toxicity at all dose levels. As a result, lower doses were selected for subsequent embryofetal development studies in this species.

Reference ID: 4258909

Study title: ST-246: A Pilot Embryo-Fetal Developmental Toxicity Study in Rabbits

Study no.:	(b) (4) **-2083-003-001-SN2**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 16th, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Summary

Mortality, clinical signs, body weights, food consumption, hematology, coagulation, clinical chemistry, gross pathology, cesarean sections and fetal variations/malformations (external only) were performed or evaluated in pregnant female New Zealand White rabbits following daily oral gavage dosing with either vehicle (1% Hydroxypropyl methylcellulose/0.5% Tween® 80) or drug (30, 100 or 300 mg/kg/day; 5/main study group and 5/satellite group). Mated females were treated from GD 6 to 19 (during organogenesis), and were euthanized on GD 29. One low-dose female was found dead on GD 29, though cause of death was attributed to excessive blood loss and was unrelated to treatment. Two high-dose animals were found dead on GD 8 and 9, and the remaining three were euthanized *in extremis* on GD 10 due to maternal toxicity. The animal found dead on GD 9 also exhibited convulsions and cyanosis on GD 9, and one of the euthanized females had tremors on GD 9. Additional clinical signs included diarrhea and/or scant/no feces in all mid-dose and high-dose animals, emaciated appearance in 1 mid-dose animal. Two of the high-dose animals were also not pregnant at the time of death. Decreased body weight (12%) and food consumption (97%) were observed at the high dose prior to death on GD 9. Decreased body weight (7-11%) and food consumption (38-89%) were also seen at the mid-dose from GD 9 to GD 18. Clinical pathology findings included decreased RBCs (12%), hemoglobin (13%), hematocrit (11%) and percent lymphocytes (16%), and increased reticulocytes (46%), ALP (61%) and total bilirubin (49%), at the mid-dose (high-dose data unavailable due to maternal toxicity). Globulin levels were also increased 100-133% at the low and mid-doses. Gravid uterine weight was decreased 18% at the mid-dose (not statistically significant). As all high-dose animals died prior to delivery, no cesarean or fetal data are available at this dose level.

<u>NOAEL = 30 mg/kg/dose for maternal toxicity, and ≥100 mg/kg/dose for fetal toxicity</u> (TK data unavailable).

Reference ID: 4258909

Study title: ST-246®: An Embryo-Fetal Developmental Toxicity Study in Rabbits

Study no.:	(b) (4) **-2083-003-001-SN5**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6th, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Key Study Findings

NOAEL = 10 mg/kg/dose for maternal toxicity (AUC_{0-24hr} = 4.2 µg•hr/mL, C_{max} = 0.4 µg/mL at GD 19), and 100 mg/kg/dose for fetal toxicity (AUC_{0-24hr} = 10.8 µg•hr/mL, C_{max} = 1.1 µg/mL at GD 19). Maternal toxicities included mortality, adverse clinical signs (red vaginal and nasal discharge, redness/discoloration around nose and mouth), decreased body weight and food consumption, hematology findings (increased WBC subsets, decreased RBCs), premature delivery, total implant loss, decreased number of live fetuses, increased post-implantation loss and early resorptions, and decreased gravid uterine weight. A slight increase in the number of female fetuses was also observed, but it is unclear if this is incidental or related to treatment.

Methods

Doses:	0, 10, 30 & 100 mg/kg/dose
Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	New Zealand White Hra:(NZW)SPF rabbits
Number/Sex/Group:	22 females/group
Satellite groups:	4 females/group. Due to a shipment error, all TK samples from the original satellite animals (TK Cohort 1) were lost. The TK analysis was repeated with an additional 4 females/group (TK Cohort 2).
Study design:	Pregnant females were treated daily from GD 6 to 19 (during organogenesis) and were euthanized on either GD 29 (main study groups and TK Cohort 1) or GD 22 (TK Cohort 2).
Deviation from study protocol:	None which affected interpretation of the study

Observations and Results

Mortality

Morbidity and mortality were assessed twice daily. Nine high-dose animals in the main study group were found dead between GD 8 and 24, and these deaths were attributed to test article. Gross pathology findings in these animals included discolored liver, red

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areas in lungs, and red material/fluid in throat and thoracic cavity. The animal that died on GD 24 also delivered prematurely. At the mid-dose, one main study animal died on GD 20 due to gavage error (subcutaneous pocket of apparent test article found in thoracic cavity), one TK Cohort 2 animal was found dead on GD 20, and a second TK Cohort 2 animal was euthanized *in extremis* on GD 19 due to labored breathing, cyanosis and seizures. Two of the mid-dose deaths (the main study animal and the satellite animal euthanized *in extremis*) were attributed to gavage error, and the third was considered by the study director to be spontaneous due to the lack of clinical signs. However, due to a lack of histopathology data, it is unclear to this reviewer whether any of the mid-dose deaths were treatment-related.

Clinical Signs

Clinical exams were performed at least once daily. The following were observed:

- Premature delivery in 1 mid-dose and 1 high-dose animals.
- Red vaginal discharge in 1 mid-dose and 2 high-dose animals.
- Discoloration around mouth in 2 animals, red nasal discharge in 1 animal, and redness around nose fur in 3 animals, at the high dose.
- Rapid respiration in 1 high-dose animal.
- Emaciated appearance in 1 mid-dose and 2 high-dose animals.
- Hypoactivity in 1 mid-dose animal.
- No feces in 1 low-dose, 5 mid-dose and 5 high-dose animals. Scant feces in 5 low-dose, 12 mid-dose and 18 high-dose animals (also 2 controls).

Emaciated appearance and hypoactivity were observed in the same mid-dose animal, and this animal died prematurely due to gavage error. No/scant feces may be drug-related (likely related to the decreases in body weight and food consumption), but were considered non-adverse. Premature delivery and all other findings were considered drug-related and adverse.

Body Weight

Maternal body weights were measured on GD 4, 6, 9, 12, 15, 18, 21, 24, 27 and 29. Body weight in the main study animals was decreased 9-17% at the high dose from GD 9 to 29, and 5-7% at the mid-dose from GD 9 to 24. Body weight gain was decreased at the mid-dose on GD 9, and at the high dose on GD 9 and 12. Total body weight gain was also decreased 72% at the high dose relative to controls.

Food Consumption

Food consumption was measured on GD 4, 6, 9, 12, 15, 18, 21, 24, 27 and 29. At the mid-dose, food consumption was decreased 24-50% from GD 9 to GD 18, and was increased 44% on GD 27. At the high dose, food consumption was decreased 49-83% from GD 9 to GD 18, and was increased 51-63% on GD 27 and GD 29.

Hematology & Coagulation

Blood samples were collected from satellite animals approximately 24 hours after the last dose (GD 20), and from 5 main study animals at the time of necropsy on GD 29. The GD 20 data are of limited use due to the lack of concurrent controls. The following were observed at GD 29:

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- Increased WBCs (94%), neutrophils (2-fold), lymphocytes (86%), monocytes (3.5-fold), eosinophils (2-fold) and basophils (83%) at the high dose.
- Decreased RBCs (13%), hemoglobin (8%) and hematocrit (5%), and increased mean corpuscular volume (9%), at the high dose.
- Increased platelets (15%) at the high dose.

Only the increase in mean corpuscular volume was statistically significant. These findings were considered adverse.

Clinical Chemistry

Blood samples were collected from satellite animals approximately 24 hours after the last dose (GD 20), and from main study animals at the time of necropsy on GD 29. No drug-related changes were observed.

Toxicokinetics

Blood samples were collected from satellite animals pretreatment and 0.5, 1, 2, 4, 8 and 24 hours post-dose on GD 6 and 19, and also 48 and 72 hours post-dose on GD 19. Due to a shipping error, all TK samples from the original satellite groups (designated TK Cohort 1) were lost. TK analysis was therefore repeated with an additional 4 females/group (designated TK Cohort 2). GD 19 toxicokinetic parameters for TK Cohort 2, from 0 to 24 hours post-dose, are presented in **Table 18**.

Table 18 - TK Parameters From the Rabbit Embryofetal Development Study (GD 19)

Dose (mg/kg)	λ_z (1/hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (ng•hr/mL)	$AUC_{0\text{-}inf}$ (ng•hr/mL)	V_z/F (mL/kg)	CL/F (mL/hr/kg)
10	0.1569	4.5	3.0	423	4226	4356	14912	2305
30	0.0682	10.2	2.2	479	5757	7607	179701	13382
100	0.0988	9.6	1.8	1087	10817	16405	143539	15513

Dosing Solution Analysis

Dose formulations for the mid- and high doses collected on May 23rd, 2007, were 111% and 116% of the target concentrations. These formulations were administered only to the TK Cohort 2 animals. All other test article formulations were within acceptable limits of concentration and homogeneity (100-107% of the target concentration), and were stable for up to 16 days. No test article was detected in the control formulation.

Necropsy

Gross pathology was evaluated in all main study animals at the time of necropsy. The following were observed in the animals that survived:

- Abscess in neck in 1 mid-dose animal. Subcutaneous abscesses in neck and thoracic cavity in in 3 high-dose animals.
- Dark parenchyma and multiple red foci in lungs in 1 mid-dose animal. This animal also delivered prematurely.
- Pale adrenal glands in 1 low-dose, 2 mid-dose and 1 high-dose animals (also 1 control).

It is unclear if these findings were incidental or treatment-related. The abscesses may be related to the increases in WBC levels at the high dose.

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Cesarean Sections

Cesarean sections were performed at the time of necropsy. Endpoints included pregnancy status, number of corpora lutea and implantation sites, pre-/post-implantation loss, early/late resorptions, and gravid uterine weight. The following were observed in the animals that survived to the scheduled necropsy:

- Total implant loss in 1 low-dose and 3 high-dose animals.
- Decreased number of live fetuses per animal at the high dose (5.3 at the high dose vs. 7.9 in the control group).
- Increased post-implantation loss at the high dose (35.1% of implants per animal at the high dose vs. 3.2% in the control group).
- Increased early resorptions at the high dose (31.6% of implants per animal at the high dose vs. 1.4% in the control group).
- Decreased gravid uterine weight at high dose (32%; not statistically significant).

All high-dose findings were considered drug-related and adverse. The single incidence of total litter loss at the low dose may be incidental.

Offspring

Fetal endpoints included viability, sex ratio, body weight and external/visceral/skeletal variations and malformations. A slight increase in the percentage of female fetuses was observed at the high dose (59.6% at the high dose vs. 48.3% in the control group; not statistically significant), but it is unclear if this was incidental or related to treatment. All fetal variations and malformations are presented in **Tables 19 & 20**, respectively. No drug-related variations or malformations were observed.

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Table 19 - Fetal Variations From the Rabbit Embryofetal Development Study

Fetal Variations		Control	Low Dose	Mid-Dose	High Dose
External	Hyperflexive – Forelimbs	0 (0)	1 (1)	0 (0)	0 (0)
	Number with External Variations	0 (0)	1 (1)	0 (0)	0 (0)
Visceral	Iris – Hemorrhagic Ring	3 (2)	1 (1)	0 (0)	0 (0)
	Major Blood Vessel – Variation	8 (4)	6 (5)	2 (1)	0 (0)
	Retroesophageal Subclavian Artery	1 (1)	0 (0)	0 (0)	1 (1)
	Gall Bladder Variation	3 (3)	11 (7)	7 (6)	3 (2)
	Retrocaval Ureter(s)	0 (0)	3 (3)	4 (4)	1 (1)
	Small Spleen	0 (0)	1 (1)	0 (0)	0 (0)
	Ovary – Cyst	0 (0)	0 (0)	1 (1)	0 (0)
	Number with Visceral Variations	15 (9)	20 (13)	14 (10)	5 (4)
Skeletal	Hyoid Arch(es) Bent	12 (8)	18 (6)	14 (8)	2 (2)
	Hyoid Body/Arch(es) Unossified	1 (1)	1 (1)	11 (4)	1 (1)
	Skull Bones – Reduced Ossification	2 (2)	5 (5)	1 (1)	1 (1)
	27 Presacral Vertebrae	19 (8)	28 (11)	31 (10)	40 (8)
	29 Presacral Vertebrae	0 (0)	0 (0)	0 (0)	1 (1)
	Extra Site of Ossification	0 (0)	1 (1)	0 (0)	0 (0)
	Sternebra(e) 5 and/or 6 Unossified	35 (13)	22 (10)	35 (11)	9 (3)
	Additional Sternebra(e) Adjacent to the First	1 (1)	0 (0)	0 (0)	0 (0)
	Clavicle, Bent	0 (0)	1 (1)	2 (2)	1 (1)
	Additional Sternebra(e)	0 (0)	1 (1)	0 (0)	1 (1)
	Sternebra(e) Malaligned – Slight to Moderate	1 (1)	0 (0)	0 (0)	2 (2)
	Rib(s) 13th Full	62 (17)	87 (18)	94 (16)	56 (8)
	Rib(s) 13th Rudimentary	27 (16)	27 (16)	17 (11)	3 (3)
	Rib(s) 14th Full	0 (0)	0 (0)	0 (0)	1 (1)
	Carpal/Metacarpal Unossified	0 (0)	1 (1)	1 (1)	0 (0)
	Phalanges Unossified	0 (0)	1 (1)	0 (0)	0 (0)
	Number with Skeletal Variations	114 (20)	127 (20)	126 (18)	60 (9)
Total Number of Variations		116 (20)	133 (20)	127 (18)	61 (9)
Total Number of Fetuses/Litters		159 (20)	160 (20)	150 (18)	64 (9)

Results are presented as "# fetuses (# litters)"

Table 20 - Fetal Malformations From the Rabbit Embryofetal Development Study

Fetal Malformations		Control	Low Dose	Mid-Dose	High Dose
External	Umbilical Hernia/Omphalocele	0 (0)	1 (1)	0 (0)	1 (1)
	Number with External Malformations	0 (0)	1 (1)	0 (0)	1 (1)
Visceral	Hydrocephaly	0 (0)	1 (1)	0 (0)	0 (0)
	Kidney Absent	0 (0)	0 (0)	0 (0)	1 (1)
	Ureter Absent	0 (0)	0 (0)	0 (0)	1 (1)
	Asplenia	0 (0)	1 (1)	0 (0)	0 (0)
	Number with Visceral Malformations	0 (0)	2 (2)	0 (0)	1 (1)
Skeletal	Vertebral/Rib Anomaly	1 (1)	0 (0)	0 (0)	1 (1)
	Malaligned Vertebra(e)	0 (0)	0 (0)	0 (0)	1 (1)
	Sternebra(e) Fused	3 (3)	2 (2)	0 (0)	0 (0)
	Rib(s) – Forked	0 (0)	0 (0)	0 (0)	1 (1)
	Number with Skeletal Malformations	4 (3)	2 (2)	0 (0)	3 (3)
Total Number of Malformations		4 (3)	5 (5)	0 (0)	4 (3)
Total Number of Fetuses/Litters		159 (20)	160 (20)	150 (18)	64 (9)

Results are presented as "# fetuses (# litters)"

Reference ID: 4258909

9.3 Prenatal and Postnatal Development

Study title: ST-246®: Study for Toxic Effects on Pre- and Postnatal Development, Including Maternal Function, in Mice (Segment III)

Study no.:	(b) (4) **-2083-003-001-SN8**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 26th, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ST-246 (lot #6A003, purity = 97.7%)

Key Study Findings

NOAEL = 1000 mg/kg/dose for both maternal and fetal/neonatal toxicity (TK data unavailable).

Methods

Doses:	0, 100, 300 & 1000 mg/kg/dose
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	1% Hydroxypropyl methylcellulose/0.5% Tween® 80
Species/Strain:	Crl:CD-1(CR)BR mice
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	Pregnant F_0 females were treated daily from GD 6 to Postnatal Day (PND) 20 (from implantation through weaning). F_1 and F_2 animals were untreated. F_0 animals were euthanized on PND 21. F_1 animals were euthanized on either PND 4 or PND 21, or were selected for mating and paired at 11-12 weeks old. Remaining F_1 males were euthanized once mating was complete. F_1 females were euthanized either on PND 21 (if pregnant) or 24 days post-cohabitation (if not pregnant). F_2 pups were euthanized on either PND 4 or 21.
Deviation from study protocol:	None which affected interpretation of the study

Reference ID: 4258909

Observations and Results

F_0 Generation

Mortality

Morbidity and mortality were assessed twice daily. One mid-dose animal cold to touch on PND 15 and was found dead on PND 16. Cause of death was not determined. It is unclear if this was incidental or related to treatment.

Clinical Signs

Clinical exams were performed once daily. No drug-related effects were observed.

Body Weight

Maternal body weights were recorded on GD 4, 6, 9, 12, 15 and 17, and on PND 0, 4, 7, 10, 14, 17 and 21. No drug-related changes were observed.

Food Consumption

Food consumption was measured on GD 4, 6, 9, 12, 15 and 17, and on PND 0, 4, 7, 10, 14, 17 and 21. Food consumption was decreased 31% in low-dose animals on GD 9, 14-17% in all treated animals on PND 7, and 18% in low-dose animals on PND 10. It is unclear if this is drug-related.

Pregnancy Status

Fertility, fecundity, and gestation indices, and mean gestation length, were evaluated. No drug-related findings were observed.

Necropsy Observations

Gross pathology was evaluated, and uterine exams were performed, at the time of necropsy. No drug-related findings were observed.

Dosing Solution Analysis

The low-dose formulations on Week 1 and Week 4 were 86% and 88% of the target concentration, respectively. All other test article formulations were within acceptable limits of concentration and homogeneity (90-101% of the target concentration), and were stable for up to 14 days. No test article was detected in the control formulation.

F_1 Generation

Mortality

Survival, sex ratio and external abnormalities were examined once delivery was complete. Morbidity and mortality were assessed at least once daily. Two mid-dose females were found dead on PND 56 and 70, and 1 control male was found dead on PND 84. Cause of death was not determined, but this was considered incidental and unrelated to treatment given the lack of dose response and presence in controls.

Clinical Signs

Clinical exams were performed on PND 1, 4, 7, 14 and 21, and once weekly on select animals up to the date of euthanasia. Dehydration and emaciation were observed in 9

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mid-dose animals (1 litter) and 10 high-dose animals (1 litter) on PND 14. As these findings were limited to 2 litters at a single time point, it is unclear if this is drug-related.

Body Weight

Pup body weights were recorded on PND 1, 4, 7, 14 and 21, once weekly until mating (females) or euthanasia (males). Maternal F_1 weights were recorded on GD 0, 4, 7, 10, 14 and 17, and on PND 0, 4, 7, 14 and 21. Body weight was decreased 11-13% in high-dose males and females on PND 14 and 21 (not significant). As this was not statistically significant, and as no differences were seen throughout the remainder of the study, it is unclear if this is incidental or drug-related.

Developmental Parameters

Endpoints included pinna unfolding (starting PND 2), static righting reflex (PND 4), cliff aversion (PND 9), eye opening (starting PND 9), air drop righting reflex (PND 16), vaginal patency (starting PND 23) and preputial separation (starting PND 23). No drug-related findings were observed.

Behavioral Assessment

Endpoints included auditory startle response (PND 21), automated motor activity (PND 35), and passive avoidance (PND 60-75). A functional observational battery was also conducted on PND 22. No drug-related findings were observed.

Estrus Determination

Vaginal smears were collected daily for two weeks prior to mating. No drug-related findings were observed.

Fertility Parameters

Endpoints included gestation length, and mating, fertility, fecundity and gestation indices. No drug-related findings were observed.

Necropsy Observations

Gross pathology was evaluated, and uterine exams were performed, at the time of necropsy. No drug-related findings were observed.

F_2 Generation

Observations

Mortality, sex ratio and external abnormalities were assessed once daily. No drug-related findings were observed.

Body Weight

Pup body weights were recorded on PND 1, 4, 7, 14 and 21. No drug-related findings were observed.

Necropsy Observations

Gross pathology was evaluated at the time of necropsy. No drug-related findings were observed.

Reference ID: 4258909

10 Special Toxicology Studies

Study Title: Single Dose Oral (Gavage) Tolerance and Phototoxicity Evaluation of ST-246 (Polymorph Form I) in Hairless Mice (246-TX-017)

Oral tolerance and phototoxicity were evaluated in female Crl: SKH1-*hr* mice following single oral gavage doses of tecovirimat (0, 500, 1000 and 2000 mg/kg; 3-9 females/group). There were no unscheduled deaths, adverse clinical signs or changes in body weight, and no phototoxicity was observed within 1 hour post-dose. NOAEL = 2000 mg/kg. Positive and negative controls induced appropriate responses. The study was properly validated.

11 Integrated Summary and Safety Evaluation

New drug application 208627 was submitted in support of tecovirimat, an orthopoxvirus VP37 envelope wrapping protein inhibitor and new molecular entity for the treatment of human smallpox disease. The proposed clinical regimen is 600 mg twice daily for 14 days. The nonclinical findings for tecovirimat (animal efficacy under the animal rule and toxicology) are summarized in the following sections.

Primary Pharmacology/Efficacy:

Multiple primary pharmacology studies were submitted for this NDA as either supportive or pivotal animal efficacy data to support tecovirimat for variola. Many of the studies were mainly supportive studies which were used to derive the final two animal species that were used to assess the effect of tecovirimat in an animal model of disease. Due to difficulties in the variola model of disease (variola virus in nonhuman primates), it was decided that surrogate viruses closely related to variola in a surrogate animal model would suffice to meet the animal rule *(see Advisory Committee minutes from December 2011).* The final two animal models for tecovirimat as a surrogate for variola in humans was: 1) monkeypox in monkeys and 2) rabbitpox in rabbits.

Monkeypox in monkeys was a robust model that demonstrated efficacy across multiple laboratories (USAMRIID, [redacted] (b) (4) The monkeypox model required significant virus to induce disease (~5 x 10^7 PFU) via the IV route of exposure. However, this model limitation was determined to be acceptable in order to induce a robust disease in NHPs. The natural history data supported a robust infection model with mild effects around Day 1-3 with lesions and more severe disease occurring around Day 4-5. Many of the lesions occurred on the leg but multiple body sites were affected. Maximal serum viral load occurred around Day 7-8 with deaths beginning around Day 9. Therefore, it was decided that the intervention with tecovirimat would occur at Day 4 with the beginning of lesions and the increase in viral burden.

In the pivotal studies of monkeypox in NHPs, the animals demonstrated clinical signs of disease: pox formation, moderate fever, body weight decreases, clinical chemistry changes, pathological findings (including pox and immune endpoints). Viral load (measured by PCR in serum/blood) and pox lesion counts trended lower in animals exposed to tecovirimat. However, sampling times for viral PCR (at the time of euthanasia – meaning early in the study for moribund animals and at end of study for survivors) prevented meaningful evaluation of these endpoints.

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Overall, it appeared that treatment with tecovirimat in NHPs significantly increased survival (see **Table 21**) and conferred a survival advantage compared to placebo/vehicle. Tecovirimat increased survival when dosed for 14 days at a dose as low as 3 mg/kg. However, 10 mg/kg of tecovirimat was selected as the fully effective dose for further studies to allow for species differences between humans and animals (per the Animal Rule Guidance). Furthermore, based on the delayed treatment study, the effective dose of tecovirimat (10 mg/kg) conferred survival when dosed during severe disease (Days 4-5 post-infection) and even conferred moderate survival during later stages of disease (Day 6 post infection) in NHPs. Lastly, based on the treatment duration study, it is suggested that more than 3 days of drug would be necessary to confer a maximal survival effect in NHPs.

Table 21 - Overall Survival in the Pivotal NHP/MPXV Model

Study ID	Type	Dose / Duration	Survival Rate
AP-09-026G	Dose Range	0, 0.3, 1, 3, 10 mg/kg *QD x 14 days from Day 4*	**0, 20, 0, 80%, 80%**
FY10-087	PK	0, 3, 10, 20 mg/kg *QD x 14 days from Day 4*	**0, 100%, 100%, 100%**
SR10-037F	Delayed Treatment	0, 10, 10, 10 mg/kg *QD x 14 days from Day 4, 5, or 6*	**0, 83%, 83%, 50%**
SR10-038F	Treatment Duration	0, 10, 10, 10 mg/kg *QD on Day 4 for 3, 5, 7, or 10 days*	**20%, 50%, 100%, 100%, 80%**

The second species selected was the rabbitpox model in the NZW rabbit. The NZW rabbit had strengths to compliment the NHP model. Primarily, the dose of the challenge agent was relatively low (~1000 PFU) compared to the NHP model, which was a significant strength. Surprisingly, the rabbits did not exhibit the extent of dermal pox lesions compared to the NHP model. Several pox lesions were noted in the natural history study on the ear of the rabbit as well as on the shaved portion of the back. Unfortunately, the pox lesions were not always consistent between study rabbits, and therefore could not be used as a clinical surrogate to identify advanced rabbitpox disease. Fortunately, temperature and clinical scores (of disease) were strong indicators of disease progression in the rabbitpox model. Notably, temperature would increase with viremia and disease progression followed by a peak in temperature and usually a rapid decline in body temperature (sometimes hypothermic -- below baseline). Based on the findings in the natural history study it was determined that day 4 appeared to correlate with the peak of disease and was chosen for treatment initiation in the pivotal efficacy study and the pivotal PK study in the rabbitpox/rabbit model (see table below).

In the pivotal efficacy study for rabbitpox, rabbits exhibited clinical signs of disease (most importantly an increase in temperature and clinical signs) with disease progression. Viral load and lesion count did increase in many animals, but was not consistent. Therefore, temperature and clinical signs were the primary indicators of disease progression. Treatment as low as 20 mg/kg of tecovirimat conferred a clear survival advantage in the rabbitpox/rabbit model. However, similar to the monkeypox

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model, a higher dose (40 mg/kg in the rabbitpox model) of tecovirimat was used as the fully effective dose for human dose calculations. In the final study (the rabbitpox/rabbit PK study), a placebo group was not included. However, the primary purpose of the study was PK in diseased animals and not efficacy. Based on prior data in the efficacy study and the disease process recorded in the animals, it was clear that the animals exhibited similar disease and had a survival advantage with ≥ 40 mg/kg of tecovirimat. Overall, it appeared that treatment with tecovirimat in rabbits with rabbitpox significantly increased survival (see **Table 22**) and conferred a survival advantage compared to placebo/vehicle.

Table 22 - Overall Survival in the Pivotal Rabbit/RPXV Model

Study ID	Type	Dose / Duration	Survival Rate
SR14-008F	Dose Range	0, 20, 40, 80, 120 mg/kg *QD x 14 days from Day 4*	**0, 90%, 90%, 80%, 80%**
SR13-025F	PK	40, 80, 120 mg/kg *QD x 14 days from Day 4*	**90%, 90%, 100%**

Secondary Pharmacology:

Several studies were performed in both nonhuman primates and mice to evaluate the effect of tecovirimat on smallpox vaccines in immunocompromised hosts as well as to identify if tecovirimat may interfere with the current smallpox vaccine. Overall no effect on overall survival was identified. However, further analysis of the data by Dr. Patrick Harrington (*see Clinical Virology review*) identified a possible signal indicating that tecovirimat may interfere with a replicating smallpox vaccine, albeit the overall survival for animals on drug in the study was unchanged. The data suggests that, tecovirimat may interfere with the robustness of the live smallpox vaccine that relies on vaccinia replication while still conferring a survival advantage in this model.

Pharmacokinetics:

Increasing doses of tecovirimat generally resulted in less-than dose-proportional increases in exposure (AUC and C_{max}) in both mice and monkeys. Oral bioavailability was ~4-45% in mice, ~15% in rabbits, ~28-50% in monkeys, and serum half-life ranged between 3-5 hours in mice and 7-11 hours in monkeys. Tecovirimat was largely protein-bound (>87%) in the serum in all species tested (mice, rats, rabbits, dogs and monkeys), and was >77% protein-bound in humans. In mice, tecovirimat was widely distributed within the first 4 hours of dosing, though the highest concentrations were observed in the blood, bone marrow and liver, and the lowest were observed in the brain and eyes. Tecovirimat was not detectable in most tissues by 24 hours post-dose, but was present in the blood for up to 96 hours post-dose. Drug was detected in the brain and cerebrospinal fluid in mice, dogs and monkeys, indicating that it crosses the blood:brain barrier in these species. In pregnant mice, tecovirimat was detected in the fetuses within 24 hours post-dose, indicating that it crosses the placental barrier during pregnancy in this species. The metabolites of tecovirimat formed in humans (M4, M5 and TFMBA) were also detected in mice and monkeys, the species selected for the pivotal nonclinical safety studies. TFMBA levels in humans were about 5-fold greater

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than those of the parent compound, and were about 5- to 10-fold greater than those in mice and monkeys. However, QSAR analysis on TFMBA determined that this metabolite was negative for genotoxicity. TFMBA is also >97% protein-bound in human and monkey plasma, though is only 50% protein-bound in mouse plasma. In mice, tecovirimat is primarily excreted in the feces (~71-75%), while the remainder is largely excreted in urine. In lactating mice, tecovirimat is also excreted in milk, with milk:plasma ratios reaching 0.40-0.83 by 6 hours post-dose, and 0.67-0.83 by 24 hours post-dose.

Safety Pharmacology:

Cardiovascular assessments were conducted in a hERG assay and in general toxicology studies in Beagle dogs and cynomolgus monkeys (ECGs). Decreased heart rate and increases in QT and QTc intervals were observed in the single-dose and 12-day repeat-dose studies in monkeys, but no ECG findings were observed in this species in a 14-day IV study, or in the pivotal 28-day and 3-month studies. An increase in QTc interval was also seen at the high dose in a 7-day dog study (246-TX-015), but the significance of this is unclear given the limited number of animals per group, and the lack of concurrent controls and histopathology data.

Respiratory and neurobehavioral assessments were performed in mice. No adverse effects of tecovirimat were observed in these studies. EEG analyses were also conducted in the repeat-dose studies in dogs and monkeys. In a 7-day dog study (246-TX-015), EEG analysis indicated that a single dose of 300 mg/kg can result in a lowered seizure threshold and/or frank seizures in this species. No drug-related EEG findings were observed in the pivotal repeat-dose studies in monkeys, however.

General Toxicology:

General repeat-dose toxicology evaluations consisted of GLP studies up to 3 months duration in both mice and monkeys. Additional (non-GLP) studies included one 12-day study in rats and two 7-day studies in dogs. No adverse, drug-related findings were observed in the pivotal 3-month studies in mice and monkeys, and the findings in the 12-day rat study were limited to decreased body weight and food consumption, and mild liver toxicity. Exposure multiples at the NOAELs in the pivotal, 3-month mouse and monkey studies were 24.3- and 2.7-fold, respectively, based on AUC relative to the proposed clinical regimen. The highest dose of 300 mg/kg in the monkey studies was based off results from bioavailability and dose-range finding studies in this species. Had a higher dose been selected for use in the pivotal monkey study, it is conceivable that the exposure multiple would be higher.

In the first repeat-dose dog study (246-TX-014), all 3 high-dose animals (100 mg/kg) exhibited rapid/labored breathing, decreased activity, emesis, excessive salivation, pale gums and red nasal discharge, and 2 of these 3 experienced tremors and convulsions 5-8 hours post-dose on Day 1. Findings at the low dose of 30 mg/kg were limited to transient emesis and excessive salivation. Due to the findings at 100 mg/kg, the study was stopped and the high-dose animals were euthanized on Day 1. A NOAEL could not be reliably determined given the limited number of animals lack of histopathology data, and comparisons to human exposure could not be made due to a lack of toxicokinetic data.

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In the second dog study (246-TX-015), adverse findings at the high dose of 300 mg/kg included convulsions (tonic and clonic), tremors, ataxia, stereotypic walk, excessive blinking, face-twitching, jerky head movements, panting, emesis and vocalization starting about 30 minutes post-dose on Day 1. EEG analysis also indicated that a dose of 300 mg/kg can result in a lowered seizure threshold and frank seizures on the first day of dosing. Most of these findings were isolated to the male animal in this group, which was euthanized *in extremis* on Day 1. The exposure multiple at the C_{max} in this animal (16,500 ng/mL) was 7.8-fold relative to the proposed clinical regimen. No drug-related EEG findings were observed at the mid-dose of 100 mg/kg, but tremors, face-twitching and vocalization were noted in these animals. No neurologic findings were observed at 30 mg/kg/dose. Drug was detectable in the brain and cerebrospinal fluid at all dose levels, indicating that tecovirimat crosses the blood-brain barrier in this species. At the low and mid-doses, the brain/plasma and CSF/plasma ratios were ~0.35 and ~0.06, respectively. Brain and CSF concentrations were not determined at the high dose. A NOAEL could not be reliably determined in this study due to the limited number of animals, and lack of concurrent controls and histopathology data, but 100 mg/kg/dose was considered the NOAEL for convulsions. The exposure multiple based on a C_{max} of 5,575 ng/mL, the highest C_{max} reported at this dose level, was 2.6-fold relative to the proposed clinical regimen. Based on these data, a C_{max} of 5,575 ng/mL was also selected as the highest allowable exposure level in humans.

Genotoxicology and Carcinogenicity:

Tecovirimat was negative for mutagenesis and clastogenesis as determined by an *in vitro* bacterial reverse mutation assay, an *in vitro* L5178Y/TK$^{+/-}$ mouse lymphoma assay, and an *in vivo* micronucleus assay in mice. Carcinogenicity studies with tecovirimat have not been conducted as the treatment duration is less than 6 months.

Reproductive and Developmental Toxicology:

Reproductive toxicology studies with tecovirimat consist of a fertility and early embryonic development study in mice, embryofetal development studies in mice and rabbits, and a pre/postnatal development study in mice. In the fertility and early embryonic development study, no adverse findings were observed in females up to the highest dose tested (exposure multiple = 23.8 based on AUC relative to the proposed clinical regimen). In males, increased percent abnormal sperm (1.9-fold) and decreased sperm motility (15%) were observed at the high dose. Since spermatological parameters were not evaluated in the low- and mid-dose groups, a NOAEL in males could not be defined in this study. Decreases in fertility and fecundity indices were also observed at all dose levels. Since no female fertility findings were observed in this study, these decreases were likely attributed male reproductive toxicity.

Three embryofetal developmental studies were conducted in rabbits, and extensive maternal toxicity was observed in each of them. Almost all animals across all three studies at doses ≥30 mg/kg either died prematurely or were euthanized *in extremis* due to drug-related toxicities. Importantly, one animal in study (b) (4)-1151-024 experienced tonic convulsions prior to death at 300 mg/kg/dose on GD 15, two animals in study (b) (4)-2083-003-001-SN2 had either convulsions prior to death or tremors at 300 mg/kg/dose on GD 9, and one animal in study (b) (4)-2083-003-001-SN5 had

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seizures prior to euthanasia at 30 mg/kg/dose on GD 19. The cause of these findings, and their relation to the convulsions observed in the repeat-dose dog studies, is unclear. In addition, weight loss, decreased food consumption and clinical signs (hypoactivity, up-and-down head movements, rapid breathing, thin appearance, premature delivery and red vaginal discharge) were observed at doses ≥30 mg/kg. In the pivotal rabbit embryofetal study (b) (4)-2083-003-001-SN5), cesarean findings attributed to maternal toxicity (premature delivery, total implant loss, increased post-implantation loss and early resorptions, and decreased number of live fetuses and gravid uterine weight) were observed at doses ≥30 mg/kg. However, no fetal toxicity was observed at doses that were not maternally toxic. The exposure multiples for maternal and fetal toxicity in the pivotal rabbit embryofetal study were 0.1 and 0.4, respectively, based on AUC relative to the proposed clinical dose.

No maternal or fetal toxicities were observed in the pivotal embryofetal development study in mice (exposure multiple = 23.0 based on AUC relative to the proposed clinical dose). Likewise, no adverse findings were observed in the pivotal pre/postnatal development study in mice (exposure multiple = 23.8 based on AUC for both maternal and fetal NOAELs relative to the proposed clinical dose).

Special Toxicology Studies:

Tecovirimat was not associated with any phototoxicity or intolerance when administered orally.

Exposure Margins:

Exposure multiples, based on AUC and a proposed daily dose of 600 mg BID, are presented in **Table 23**. The exposure multiples at the NOAEL for convulsions in dogs, based on C_{max}, are presented in **Table 24**.

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Table 23 - Tecovirimat Safety Factors Based on AUC

Study	NOAEL (mg/kg/dose)	Toxicities Observed	Nonclinical AUC (µg•hr/mL)	Exposure Multiple[a]
Repeat-Dose Studies				
28-day mouse oral	2000[b]	None	858.6	29.8
3-month mouse oral	1000[c]	None	700.6	24.3
28-day monkey oral	300[d]	None	43.0	1.5
3-month monkey oral	300[c]	None	77.7	2.7
Reproductive Toxicology Studies				
Fertility and Early Embryonic Development				
Mouse	1000/undefined[e]	↑ Abnormal sperm, ↓ sperm motility, ↓ fertility and fecundity indices	683.9/undefined	23.8/undefined
Embryofetal Development				
Mouse	1000/1000[f,g]	None	661.3/661.3	23.0/23.0
Rabbit	10/100[f,h]	Mortality, early delivery, total implant loss, ↑ early resorptions and post-implantation loss, ↓ gravid uterine weight	4.2/10.8	0.1/0.4
Pre/postnatal Development				
Mouse	1000/1000[f,i]	None	683.9/683.9	23.8/23.8

[a] Based on mean exposures in healthy human volunteers receiving 600 mg BID tecovirimat under fed conditions for 14 days ($AUC_{0\text{-}24hr}$ = 28.791 µg•hr/mL)
[b] Day 28 data
[c] Week 13 data
[d] Day 27 data
[e] Female and male NOAELs are presented as "female/male". Female exposure data are from the 28-day repeat-dose mouse study (Study #246-TX-006, Day 28 data).
[f] Maternal and fetal NOAELs are presented as "maternal/fetal"
[g] Gestational Day 15 data
[h] Gestational Day 19 data
[i] Female exposure data are from the 28-day repeat-dose mouse study (Study #246-TX-006, Day 28 data).

Table 24 - Tecovirimat Safety Factors Based on C_{max}

Study	NOAEL (mg/kg/dose)	Toxicities Observed	Nonclinical C_{max} (ng/mL)	Exposure Multiple[a]
Repeat-Dose Studies				
7-day dog oral	100[b]	Convulsions	5575	2.6/1.3[c]

[a] Based on exposures in healthy human volunteers receiving 600 mg BID tecovirimat under fed conditions for 14 days (average C_{max} = 2106 ng/mL; highest observed C_{max} = 4460 ng/mL)
[b] Day 1 data (highest C_{max} reported at this dose level)
[c] Exposure multiples based on average/highest C_{max}

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12 Appendix

References:

Product Development Under the Animal Rule: Guidance for Industry (Oct 2015). https://www.fda.gov/downloads/drugs/guidances/ucm399217.pdf (accessed 4/24/2018)

Antiviral Drugs Advisory Committee Meeting, December 14-15. https://wayback.archive-it.org/7993/20170404145348/https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AntiviralDrugsAdvisoryCommittee/ucm247236.htm (accessed 4/24/2018)

Guidance for Industry: Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention. (Draft Guidance, 2007). https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071179.pdf (accessed 4/24/2018)

Development of Antiviral Products for Treatment of Smallpox and Related Poxvirus Infections; Public Workshop (Sept 2009). https://www.gpo.gov/fdsys/pkg/FR-2009-08-18/pdf/E9-19781.pdf (accessed 4/24/2018)

ICH M3(R2): Guidance on Nonclinical Safety Studies for the conduct of human Clinical Trials and Marketing Authorization for Pharmaceuticals. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf (Accessed 4/24/2018)

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/s/

DAVID H MCMILLAN
05/07/2018

LAINE P MYERS
05/07/2018

HANAN N GHANTOUS
05/07/2018
I concur with Dr. Myers and Dr. McMillan on approving Tecovirimat under the animal rule.

PHARMACOLOGIST REVIEW OF GLP EIR (b) (4)

Firm Name:

City, State, Country:
EI Dates:
FDA Participants:

(b) (4)

Inspection Summary:

This was a (b) (4). The current inspection covered study (b) (4) Study No. FY10-087). No Form FDA 483 was issued at the inspection close-out. The final classification is No Action Indicated (NAI). I recommend that the audited study be accepted for review by the Agency.

Study Audited during this Inspection:

(b) (4) Study No.:	(b) (4) Study No. FY10-087)
Study Title:	Bioanalytical Sample Analysis of ST-246 in Cynomolgus Monkey Plasma to Support (b) (4) Sample Analysis Study FY10-087
Dates:	(b) (4)
Report Completion:	(b) (4)
Principal Investigator:	(b) (4)
Study Director:	
Testing Facility:	
NDA No.:	208627
Sponsor:	SIGA Technologies, Inc. (Corvallis, OR)
NDA #:	208627
Review Division:	DAVP

Background: (b) (4)

(b) (4)

Prior Inspection: The previous inspection of this facility conducted between [redacted] (b) (4) was tentatively classified as NAI pending final center [redacted] (b) (4) classification. No Form FDA 483 was issued.

Current Inspection: This was a [redacted] (b) (4) The current inspection covered study [redacted] (b) (4) (bioanalytical portion of [redacted] (b) (4) Study No. FY10-087), including documentation of study conduct, environmental conditions, personnel training, quality assurance unit, equipment, archives, and relevant standard operating procedures. A Form FDA 483 was not issued at the close out meeting and there were no discussion items.

Recommendations:

- I recommend that the data from study [redacted] (b) (4) (bioanalytical portion of [redacted] (b) (4) Study No. FY10-087) be accepted for Agency review.
- This site should be re-inspected in [redacted] (b) (4) as part of the [redacted] (b) (4) GLP surveillance program.
- Final classification: No Action Indicated (NAI).

[redacted] (b) (4)

Date Assigned: December 21, 2017
EI Dates: [redacted] (b) (4)
District Office:
FDA Investigators:

Inspection Type: _____Routine Surveillance __X__Directed
FDA-483 Issued: ___X___No _____Yes
Letter Issued: ___X___None _____Response Request Letter

Date EIR Received by OSIS: [redacted] (b) (4)
Date EIR Received by Reviewer:
1st Draft Review Completed:

Inspection Conclusion: NAI
District Decision: NAI
Final HQ Classification: NAI

cc: via DARRTS

[redacted] (b) (4)

[redacted] (b) (4)

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/s/

MARK J SEATON
03/22/2018

ZHOU CHEN
03/22/2018

CHARLES R BONAPACE
03/22/2018