BRIEFING BOOK

Background Package for FDA Advisory Committee Meeting On December 14-15, 2011

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TABLE OF CONTENTS

Page

LIST OF ABBREVIATIONS 7

EXECUTIVE SUMMARY 10

1. INTRODUCTION 19

1.1 Smallpox as a Human Disease 19

1.2 Smallpox Response Plan 20

1.3 Clinical Features of Human Smallpox Disease 21

1.4 Challenges to Developing Drugs for Smallpox Under the Animal Rule 23
1.4.1 Evaluation of Smallpox Antivirals in Humans Infected With Closely Related Orthopoxviruses 24

2. ANIMAL MODELS FOR SMALLPOX 27

2.1 Small Animal Model Summary 28

2.2 NHP Models of Human Smallpox Disease 28
2.2.1 General Overview of NHP Models 28
2.2.2 IV VARV Model 30
2.2.3 IV MPXV Model 32
2.2.4 Aerosol MPXV Model 33
2.2.5 Intranasal MPXV Model 33
2.2.6 Intratracheal MPXV Model 34
2.2.7 Subcutaneous MPXV Model 35
2.2.8 Marmoset Model Development 35
2.2.9 Conclusions on NHP Models 36
2.2.10 Expert Advisory Panel Report – Summary Evaluation of Animal Models of Variola Virus Disease 39

2.3 Proposed Pivotal Efficacy Study in NHPs 40

2.4 PK/PD Modeling 44
2.4.1 Use of PK/PD Modeling to Determine ST-246 Efficacious Dose 44
2.4.2 PK Modeling and Bridging the Effect of Infection to Humans 44
2.4.3 Survival Analysis: Dose-Survival and PK/Survival Modeling 48
2.4.4 Receiver Operator Characteristic Analysis and Optimal Cut-Offs for Exposures 51
2.4.5 Overall PK/PD Conclusions 51

2.5 Resistance Monitoring Plan 52

3. DEVELOPMENT OF ST-246 UNDER THE ANIMAL RULE 54

3.1 Overview of ST-246 54
3.1.1 ST-246 Mechanism of Action 54
3.1.2 Metabolism and Pharmacokinetics 56
3.1.2.1 *In Vitro* ADME 56
3.1.2.2 Metabolism 57
3.1.2.3 Pharmacokinetics 57

Table of Contents - Continued

Page
3.1.3 Effect of MPXV Infection on ST-246 PK in NHPs ... 58
3.1.4 Toxicological Safety Summary ... 59
3.1.4.1 *In Vitro* Toxicity Studies ... 59
3.1.4.2 Genotoxicity ... 59
3.1.4.3 Safety Pharmacology ... 59
3.1.4.4 General Toxicology ... 59
3.1.4.5 Reproductive Toxicology ... 60
3.1.5 Completed ST-246 Clinical Study Overview ... 60
3.1.5.1 Key Safety Results From Completed Clinical Studies ... 60
3.1.6 Planned Clinical Studies ... 63
3.1.7 Emergency Use in Humans ... 63
3.1.7.1 E-IND No. 112,324 ... 63
3.1.7.2 E-IND No. 106,338 ... 63
3.1.7.3 E-IND No. 104,793 ... 63
3.1.7.4 E-IND No. 74,773 ... 64
3.1.7.5 Compassionate Use in Europe ... 65

3.2 ST-246 Efficacy *In Vivo* ... 65
3.2.1 Summary of IV MPXV Nonhuman Primate Studies ... 66
3.2.1.1 FY-06-035 Efficacy in Lethal Challenge ... 66
3.2.1.2 AP-06-21 Experiment 1 and 2 Efficacy At Different Dose Levels ... 68
3.2.1.3 AP-06-021G Efficacy (Therapeutic Window and Dose) ... 70
3.2.1.4 AP-06-021E6 Efficacy ... 72
3.2.1.5 AP-09-026G Efficacy and Minimum Effective Dose ... 75
3.2.1.6 FY10-087 Pharmacokinetics in Infected NHPs ... 79
3.2.1.7 SR10-037F Efficacy in Delayed Dosing ... 85
3.2.1.8 SR10-038F Efficacy and Duration of Dose ... 88
3.2.2 Summary of Variola Nonhuman Primate Studies ... 91
3.2.2.1 1470HUGMONC Efficacy ... 91
3.2.2.2 ST246-1745 Efficacy ... 92
3.2.3 Summary of Respiratory MPXV Nonhuman Primate Studies ... 98
3.2.3.1 FY11-096 Evaluation of Aerosol Model ... 98

4. ST-246 AND THE ANIMAL RULE ... 103
4.1 Summary ... 106

REFERENCES ... 108

APPENDICES ... 114

Appendix A: Small Animal Models of Poxvirus Infection ... 114

Appendix B: Expert Advisory Panel Report - Summary Evaluation of Animal Models of Variola Virus Disease and Bios of Panel Members ... 114

Appendix C: Report on Emergency Use of ST-246, May 2011 ... 114

Appendix D: MMWR Report on Emergency Use of ST-246, November 2009 ... 114

Appendix E: MMWR Report on Emergency Use of ST-246, May 2009 ... 114

Table of Contents - Continued

Page

Appendix F: MMWR Report on Emergency Use of ST-246, May 2007 114
Appendix G: University of Chicago Account of Emergency Use of ST-246..... 114
Appendix H: Small Animal Studies With ST-246..... 114

TABLE OF TABLES

Page

Table 1: Nonhuman Primate Studies 15
Table 1.2-1: Comparison of Best and Worst Case Scenarios in a Smallpox Outbreak 21
Table 2-1: Summary of ST-246 in Small Animal Models 27
Table 2-2: Insufficiency of Small Animal Models 28
Table 2.2.1-1: Non-Human Primate Models for Lethal Poxvirus Infection 30
Table 2.2.9-1: Comparative Pathologies of Human Smallpox and NHP Models 37
Table 2.2.9-2: Comparison of NHP Models and Surrogate Endpoints for Human Smallpox 38
Table 2.3-1: Study Group Assignment 41
Table 2.4.3-1: Dose Group Number Breakdown for MPXV Efficacy Studies Used in Survival PD Analysis 49
Table 3.1-1: ST-246 Antiviral Specificity 54
Table 3.1.5.1-1: TEAEs Reported In Clinical Studies 61
Table 3.2-1: Summary of ST-246 Activity in Animal Models 65
Table 3.2.1.1-1: FY06-035 Total Lesion Count 68
Table 3.2.1.3-1: AP-06-021G Treatment Groups 71
Table 3.2.1.3-2: AP-06-021G AUCs for Viral Load and Total Lesion Count 72
Table 3.2.1.3-3: AP-06-021G Time to Maximum Viral Load and Total Lesion Count 72
Table 3.2.1.4-1: AP-06-021E6 Onset of Lesion Formation 73
Table 3.2.1.5-1: AP-09-026G Survival up to Day 42 76
Table 3.2.1.6-1: FY10-087 Study Group Layout 80
Table 3.2.1.6-2: FY10-087 Daily Schedule for Observations and Sample Collection 80
Table 3.2.1.6-3: FY10-087 Plasma Sampling Schedule for PK Analysis 80
Table 3.2.1.6-4: FY10-087 Average C_{max} (ng/mL) Pharmacokinetic Results 82
Table 3.2.1.6-5: FY10-087 Average T_{max} (hr) Pharmacokinetic Results 83
Table 3.2.1.6-6: FY10-087 Average AUC_{last} (hr*ng/mL) Pharmacokinetic Results 83
Table 3.2.1.6-7: FY10-087 Average AUC_{0-24} (hr*ng/mL) Pharmacokinetic Results 84
Table 3.2.1.6-8: FY10-087 Average $t_{1/2}$ (hr) Pharmacokinetic Results 84
Table 3.2.1.7-1: SR10-037F Study Group Assignment 85
Table 3.2.1.7-2: SR10-037F Number of Animals Surviving to Scheduled Termination 86
Table 3.2.1.8-1: SR10-038F Study Group Assignment 89
Table 3.2.3.1-1: FY10-096 Study Design 99

TABLE OF FIGURES

Page

Figure 1: IV NHP Models Compared to Human Smallpox Disease 12
Figure 1.3-1: Human Smallpox Disease Progression 22
Figure 1.4.1-1: Monkeypox Virus Photos 25
Figure 2.4.2-1: Overlay of ST-246 Exposures – Uninfected Monkeys and Humans 45
Figure 2.4.2-2: Effect of Infection on ST-246 Clearance in NHP 46
Figure 2.4.2-3: Human Dose of 400 mg ST-246 With Overlaid Equivalent Monkey Dose at Steady State 47
Figure 2.4.2-4: Human Dose of 600 mg ST-246 With Overlaid Equivalent Monkey Dose at Steady State 48
Figure 2.4.3-1: Kaplan-Meier Survival Curve Analysis Showing Placebo (N = 3), 0.3 (N = 5), 1 (N = 5), 3 (N = 14), 10 (N = 24), and 20 (N = 12) mg/kg Doses of ST-246 50
Figure 2.4.4-1: ROC Analysis 51
Figure 3.1.1-1: Orthopoxvirus Life Cycle 55
Figure 3.1.1-2: ST-246 Molecular Mechanism of Action 56
Figure 3.2.1.4-1: AP-06-021E6 Total Body Poxvirus Lesion Count 74
Figure 3.2.1.4-2: AP-06-021E6 Blood Viral DNA (Genome/mL) 75
Figure 3.2.1.5-1: AP-09-026G Total Lesion Count Through Day 42 78
Figure 3.2.1.5-2: AP-09-026G Viral Load (Genomes/mL) Through Day 42 79
Figure 3.2.1.6-1: FY10-087 Quantified MPXV HA Gene in Whole Blood 81
Figure 3.2.1.7-1: SR10-037F Viral Load (Genomes/mL) Through Day 56 87
Figure 3.2.1.7-2: SR10-037F Total Lesion Count Through Day 56 88
Figure 3.2.1.8-1: SR10-038F Viral Load (Genomes/mL) Through Day 28 90
Figure 3.2.1.8-2: SR10-038F Total Lesion Count Through Day 28 91
Figure 3.2.2.2-1: ST246-1745 Total Pox Lesion Count by Treatment Day 94
Figure 3.2.2.2-2: ST246-1745 Least Square Mean of Total Lesion Count 94
Figure 3.2.2.2-3: ST246-1745 Least Square Mean of Total Lesion Count by Treatment Day 95
Figure 3.2.2.2-4: ST246-1745 Viral Load (Genomes/mL) by Treatment Day 96
Figure 3.2.2.2-5: ST246-1745 Least Square Mean Viral Load (Genomes/mL) 96
Figure 3.2.2.2-6: ST246-1745 Least Square Mean Viral Load (Genomes/mL) by Treatment Day 97
Figure 3.2.3.1-1: FY-096 Kaplan-Meier Survival Plot by Treatment Group 100
Figure 3.2.3.1-2: FY-096 Average Lesions per Animal 101

LIST OF ABBREVIATIONS

ABSL	Animal Biosafety Level
ADME	Absorption, Distribution, Metabolism and Elimination
AML M0	Acute Mylegenous Leukemia M0
ATCC	American Type Culture Collection
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-}24}$	Area under the plasma concentration-time curve from 0-24 hours
$AUC_{0\text{-}INF}$	Area under the plasma concentration-time curve from 0 hours to infinity
AUC_{last}	Area under the plasma concentration-time curve from time zero to the time of the last measurable concentration
AUC/WT	Area under the plasma concentration-time curve normalized for body weight
BCS Class II	Biopharmaceutics Classification System Class II
b.i.d.	Two doses daily
BLQ	Below the Limit of Quantification
BSL	Biosafety Level
CBER	Center for Biologics Evaluation and Research
CC_{50}	50% Cytotoxic concentration
CEV	Cell associated enveloped virus
CDC	Centers for Disease Control and Prevention
CDV	Cidofovir
CF	Complement-fixing
CL/F	Apparent total clearance of the drug from plasma during terminal phase
C_{max}	Peak plasma drug concentration
C_{min}	Minimum plasma drug concentration
C_{min1}	Minimum first dose plasma drug concentration
C_{minss}	Minimum steady state plasma drug concentration
CNS	Central nervous system
CPXV	Cowpox virus
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
dpi	Days post-infection
DRC	Democratic Republic of the Congo
EC_{50}	50% Effective concentration
ECG	Electrocardiogram
ECTV	Ectromelia virus
EEG	Electroencephalogram
EEV	Extracellular enveloped virus
E-IND	Emergency Investigational New Drug Application
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FOB	Functional Observational Battery
GLP	Good laboratory practice
GMP	Good manufacturing practice

List of Abbreviations - Continued

HA gene	Hemagglutinin gene
HED	Human equivalent dose
hERG	Human ether-a-go-go-related gene
HHS	Health and Human Services
HI	Hemagglutinin-inhibiting
h.p.i.	Hours post inoculation
HPLC	High-performance liquid chromatography
hr	Hours
ID	Intradermal
IEV	Intracellular enveloped virus
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMV	Intracellular mature virus
IN	Intranasal
IND	Investigational new drug
IOM	Institute of Medicine
IP	Intraperitoneal
IT	Intratracheal
IV	Intravenous
Ka	Absorption rate constant
kg	Kilogram
L	Liter
LD_{50}	Lethal dose
LE	Late endosomal
LLOQ	Lower limit of quantification
LS	Least square
mg	Milligram
mg.hr/L.kg	Exposure
MDD	Mean days to death
MID	Minimal infectious dose
MMWR	Morbidity and Mortality Weekly Review
MPXV	Monkeypox virus
MTD	Mean time to death
MVA	Modified vaccinia Ankara
n	Number of independent determinations
N	Number of subjects
N/A	Not Applicable
NDA	Non-disclosure Agreement
NHP	Nonhuman primates
NIAID	National Institute of Allergy and Infectious Diseases
NOAEL	No observable adverse effect level
NOEL	No observable effect level

List of Abbreviations - Continued

NS	Not significant
NZW	New Zealand White
OPV	Oral Poliovirus Vaccine
OPXV	Orthopoxviruses
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD	Pharmacodynamics
pfu	Plaque-forming units
P-gp	P-glycoprotein
p.i.	Post-infection
P.I.	Post-inoculation
PK	Pharmacokinetics
POP	Population
PV	Progressive vaccinia
P-value	Probability, with a value ranging from zero to one
QC	Quality control
Q/F	Inter-compartmental clearance
qPCR	Quantitative polymerase chain reaction
QTc	Corrected QT interval
ROC	Receiver operator characteristic
RPXV	Rabbitpox virus
SAE	Severe adverse events
SD	Standard deviation
s.i.d.	One dose daily
$t_{½}$	Elimination half-life
TBD	To be determined
TEAE	Treatment Emergent Adverse Event
T_{max}	Time to reach the maximum plasma concentration
Tx	Treatment
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
μL	Microliters
μM	Micromoles
VACV or VV	Vaccinia virus
VARV	Variola virus
Vc/F	Central volume of distribution
Vector	Russian State Research Centre of Virology and Biotechnology
VIGIV	Vaccinia Immune Globulin Intravenous (Human)
Vp/F	Peripheral volume of distribution
WHO	World Health Organization
WT	Weight

EXECUTIVE SUMMARY

Smallpox, caused by either of two variola virus (VARV) variants, variola major or variola minor, is a highly contagious and lethal disease. Smallpox was declared eradicated from the natural environment worldwide in 1980 after an aggressive global surveillance, vaccination and containment campaign by the WHO. Despite its eradication, the accidental or deliberate release of smallpox virus via an act of bioterrorism into today's largely unvaccinated and highly mobile population could be catastrophic.

In 1999, an Institute of Medicine panel recommended the development of new antiviral drugs against smallpox, especially medications that could be taken orally. The panel noted the need for a number of drugs with different mechanisms of action that could protect against the emergence of a drug-resistant virus. Ideally, these new antiviral drugs would also be used in the treatment of other Orthopoxvirus (OPXV) disease and zoonotic OPXV disease, such as those caused by vaccinia virus (VACV), monkeypox virus (MPXV) and cowpox virus (CPXV). This recommendation is supported by the WHO Advisory Committee on Variola Virus Research.

Though the best way to prevent smallpox is vaccination, the current FDA-approved vaccine is contraindicated in the general public due to its association with severe adverse effects. Therefore, the development of a new antiviral drug for the treatment of smallpox, preferably a drug that is highly stable and could be administered orally, is a priority. However, a development program for such a drug candidate is complex, especially in light of the fact that VARV is strictly a human pathogen and does not cause smallpox in any other host. Ideally, the supportive model should provide a sufficiently accurate recapitulation of the human disease course, provide a dosing trigger such as lesion onset, provide sufficient pharmacokinetic information to support dosing and duration of treatment and produce quantifiable and reproducible results and endpoints.

<u>Small Animal Models - Differences in Small Animals</u>
While small animal models are appropriate for early stage efficacy evaluation due to their availability and cost, they are insufficient for pivotal studies. Most small animal models with high mortality rates do not exhibit smallpox-like pathologies and lesions are not apparent at the time of death (lesions occasionally appear on survivors). In respiratory challenge models, death is most often due to inflammatory respiratory disease (necrotizing bronchopneumonia, bronchitis, bronchiolitis, etc). In addition, selection of human dose based on the extrapolation of small animal pharmacokinetics to humans may prove difficult. Given the physiological, immunological, and other similarities between humans and nonhuman primates (NHPs), a NHP model of OPXV infection that closely resembles human smallpox disease would be preferable over that of small animal models.

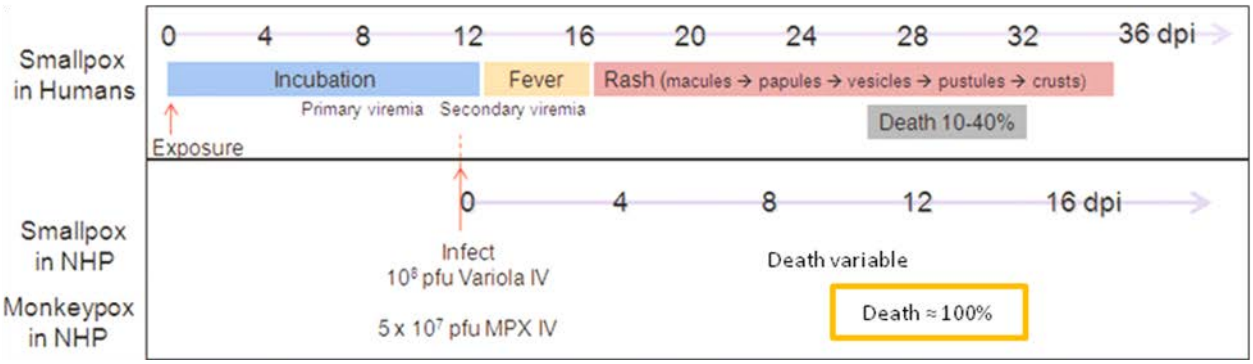
<u>NHP Animal Models - The Complex Issue Regarding NHPs</u>
Attempts have been made to administer natural strains of variola virus to produce a surrogate disease in NHPs, but in the three development and efficacy studies completed to date, only a high IV dose (~ 10^8 pfu) of VARV produces disease that resembles human smallpox. This model bypasses the prodomal phase of human disease but produces skin lesions that can be used as an appropriate trigger for treatment. However, it does not generate reproducible significant mortality to empower a reasonable statistical analysis. At an even higher viral inoculum

(10^9 pfu), while producing near uniform lethality, the disease does not represent typical smallpox, but rather resembles late-stage hemorrhagic smallpox that has been observed in very few humans. In addition, death can occur prior to lesion onset, complicating interpretation of a treatment outcome and complicating definitive dosing criteria. While the current variola model provides useful and supportive data, it has deficiencies and requires significant improvements if it were to be considered for use in support of statistically significant, quantifiable efficacy studies.

For the past 40 years, scientists have attempted to inoculate variola virus into a large number of laboratory animals, including every species of captive primate, and have uniformly failed to develop a useful animal model. Only recently, by mirroring late stage viremia through the inoculation of high doses of variola virus directly into the bloodstream, has any morbidity and mortality been induced in a primate. Variola is a strictly human pathogen, that replicates poorly, if at all in the primate and as a result the primate is a poor surrogate host. This is the reason for the model development conundrum. It is not due to a lack of effort that a better variola model has not been developed, rather it likely reflects the biology of the virus. The general consensus in the poxvirus research community is that NHPs infected with MPXV is the best available model. In addition, it is unlikely that significant improvements to the VARV NHP model will be forthcoming since experts have not yet been able to identify a definitive path forward for this model. Furthermore, all research with VARV is governed by the Smallpox Research Advisory Committee of the WHO. This committee has continued to limit the nature and scope of allowed experimentation, and steadfastly refused to permit any genetic alterations of the VARV genome that might improve its replication in animal hosts. Approved research is to be "outcome oriented" and supportive of antiviral or vaccine licensure. Lastly, the World Health Assembly continues to deliberate on the eradication of all existing variola stocks. If this resolution were passed, no additional VARV research would be possible.

In contrast, the monkeypox model, specifically the Zaire 79 strain IV challenge, provides the best surrogate model for studying a drug for treatment of smallpox. SIGA is developing a small molecule OPXV inhibitor, ST-246, and this model has been used for nine development and efficacy studies. The model has proven to be robust and reproducible, with consistent and quantifiable endpoints. Infection of NHPs with 5×10^7 pfu monkeypox virus administered via the IV route reproducibly results in a systemic febrile rash followed by an OPXV disease that mimics the exanthemous phase of both monkeypox and "ordinary" variola infection in humans. Additionally, rapid disease onset following intravenous challenge, and the resultant high mortality rate set a very high standard for drug evaluation. Lesions follow the typical smallpox rash progression (macules/papules/vesicles/pustules/umbilication/crust/scab) and provide an appropriate trigger for initiation of treatment. Viral DNA and lesion counts correlate well and are excellent indicators of disease severity and mortality. Mortality in this model is consistently very high. While the monkeypox model also bypasses the prodomal phase of human smallpox, the tight correlation between lesion counts, viral DNA and mortality in this model creates definable, consistent, reproducible results/endpoints for the measurement of efficacy and determination of optimal dosing. A comparison of the MPXV and VARV models to the course of human smallpox disease is illustrated in Figure 1.

Figure 1: IV NHP Models Compared to Human Smallpox Disease



To support registration, SIGA plans to conduct a pivotal GLP efficacy study in NHPs in the lethal IV monkeypox model with ST-246. This will be a randomized, placebo-controlled, double-blind study conducted at more than one investigational site. The primary endpoint of this study will be survival through Day 56. Secondary endpoints will be 1) the impact of drug on viremia; 2) impact of drug on lesion counts; 3) impact of drug on immune responses to monkeypoxvirus; and 4) evaluation of occurrence and frequency of drug resistance. The number of NHPs to be studied to provide statistical significance will be determined and reviewed with FDA.

Application of the Animal Rule – A Path to Approval

Over the course of development, SIGA has worked collaboratively with the FDA regarding the path to approval for a therapeutic countermeasure such as ST-246. Although the path for FDA approval under the Animal Rule has evolved, the guidance currently states that when it is neither ethical nor feasible to conduct human efficacy studies, the FDA can invoke the Animal Rule to evaluate evidence of countermeasure effectiveness derived from animal studies. The following is the mechanism to date by which SIGA has applied its development efforts to satisfy each of the 4 tenets of the Animal Rule. Development efforts will culminate in the conduct of a definitive animal efficacy study to be conducted in the appropriate animal model as well as a definitive human safety clinical trial and the completion of a thorough human QTc study.

Tenet 1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product.

The replication cycle of OPXVs is well-characterized and the understanding of the mechanism by which OPXVs cause systemic infection is documented in the literature.[1, 2] The virus initially infects respiratory tract mucosa and spreads to local lymph nodes and subsequently to the lymphoid organs via a transient viremia. It then replicates in reticuloendothelial cells through an asymptomatic incubation period of 10-12 days. The high fever prodrome period begins after the incubation period, and lasts for 2-3 days. After this, a second wave of viremia results in dissemination of the virus to the dermal epithelium, oropharyngeal mucous membranes and other organs of the body. This dissemination leads to the eruption of lesions over the tongue and oropharynx and rashes that start at the face and extremities and may eventually envelope the whole body. The disease pathology is based on the systemic spread of virus and is the result of the viral cytopathic effect.

OPXVs are present within infected cells in two distinct infectious forms: intracellular mature virus and intracellular enveloped virus. It is the latter type that buds through the cell membrane and causes systemic disease. OPXV particles that are unable to progress to the extracellular enveloped stage demonstrate markedly reduced pathogenesis, both *in vitro* and *in vivo*, and are considered avirulent in animal hosts with intact immune systems. In order to produce extracellular virus particles and induce systemic spread, a small percentage of intracellular mature OPXVs induce a wrapping complex derived from intracellular host membranes. The wrapped virus is then transported to the cell surface and released. SIGA has determined that ST-246 inhibits the envelopment and egress stage of the viral replication cycle. ST-246 prevents the formation of the wrapping complex by inhibiting the function of a conserved protein specific to OPXVs that is required for this process.[(3)] In the absence of extracellular enveloped forms of OPXVs, systemic disease is prevented. Further, studies in mice have demonstrated that protective immunity is achieved even in the absence of systemic virus, a circumstance that does not occur with countermeasures that inhibit virus replication.

OPXVs do not recrudesce once the initial infection has been cleared. Additionally, OPXVs do not set up persistent infection in immune-competent hosts and fluctuation analyses have shown that OPXVs are genetically stable and are not prone to frequent mutation even in the presence of selective pressure. Virus populations grown in the presence of ST-246 did not demonstrate a propensity for resistance and study results indicated that mutations that confer compound resistance occurred at a frequency of about 1.3 in every 10^6 pfus and were likely to have pre-existed in the virus population. SIGA has not experienced any treatment failures to date that are attributable to the emergence of a resistant virus population. Efficacy studies utilizing the MPXV NHP model of human smallpox disease have allowed SIGA to implement a resistance monitoring program in which virus is extracted from blood and tissue samples of infected NHPs and evaluated for resistance to ST-246, a scenario that is not possible for studies conducted with variola in a BSL4 setting. The one instance where resistant variants were identified, occurred in the case of a severely immune-compromised patient suffering from a progressive vaccinia infection and receiving ST-246 both orally (75 days) and topically (68 days). Resistant variants were recovered only from the patient's satellite lesions. The patient recovered and continues to do well.

Tenet 2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans.

OPXVs infect a multitude of animal species. While disease manifestation and progression vary widely between species, death, as an endpoint for pre-clinical studies is possible, using appropriate combinations of specific challenge viruses and challenge dose. SIGA has conducted, or participated in, 28 small animal studies utilizing four small animal species (various strains of mice, rabbits, prairie dogs and ground squirrels) to demonstrate ST-246 efficacy against five types of OPXVs (ectromelia virus, rabbitpox virus, cowpox virus, vaccinia virus and MPXV). In each study where the primary endpoint was the prevention of death from a lethal OPXV challenge, treatment with ST-246 increased survival with statistical significance over vehicle controls. In cases where drug was administered prophylactically (i.e. prior to, or concurrent

with, challenge), disease was virtually non-existent. These results suggest ST-246 is efficacious against the OPXV family as a whole.

However, when examining OPXV disease progression, there is much dissimilarity between human OPXV disease and OPXV disease in small animal species. One glaring example is a lack of raised pustular skin lesions over the entire body, a signature characteristic of human smallpox disease. Where lesions do play a role in disease manifestation in small animal models, they require immune-compromised hosts or emerge late in infection and then only in animals that are destined to survive challenge. OPXV disease in NHPs addresses this shortcoming. OPXV disease progression in NHPs closely follows that of human smallpox beginning at the secondary viremic stage of disease. NHPs infected via the IV route with MPXV or VARV develop the characteristic skin rash within 3-4 days post-challenge and, in the case of the IV MPXV NHP model, become progressively more ill, ultimately succumbing to infection within 7-17 days post-challenge. It is the emergence of lesions, reminiscent of human smallpox disease, which serves as the trigger for therapeutic intervention. SIGA has participated in eleven ST-246 efficacy studies in NHPs, 9 in the IV MPXV model and 2 in IV VARV model.

In nine studies utilizing the IV MPXV NHP model, 93 of the 109 animals (85.3%) treated with ST-246 at 3 or more days post-challenge demonstrated lesions prior to treatment initiation and of those, 80.1% (75 of 93) survived challenge. In some of these studies, treatment was delayed up to two days after lesions were first noted and in others treatment was administered for a sub-optimal period of time. In the two IV variola studies 7 of 13 animals (53.8%) were treated at lesion onset and of those the survival rate was 85.7% (6 of 7).

Tenet 3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.

IV inoculation of NHPs with either VARV or MPXV produces a systemic, lesional disease that closely resembles human smallpox. Infection of NHPs with monkeypox virus appears to produce a more severe infection with higher mortality than VARV and thus, represents a more rigorous model for establishing efficacy of an antiviral therapeutic. A description of the NHP efficacy studies SIGA has conducted to date is located in Section 3.2, and summarized in Table 1.

Table 1: Nonhuman Primate Studies

Study Identifier	Challenge Agent/ Study Location	ST-246 Dosage (mg/kg/day)	ST-246 R_x (h.p.i.)	Died or Euthanized as Moribund at Endpoint		
				Placebo	ST-246	
FY06-035 (Non-GLP)	MPXV/LRRI	30, 300 for 14 days	1	50% (2 of 4)	0% (0 of 8)	
AP-06-21 Experiment 1 (Non-GLP)	MPXV/USAMRIID	300 for 14 days	24 and 72	100% (2 of 2)	0% (0 of 6)	
AP-06-21 Experiment 2 (Non-GLP)	MPXV/USAMRIID	3, 10, 30, 100 for 14 days	72	100% (3 of 3)	0% (0 of 12)	
AP-06-021G (GLP)	MPXV/USAMRIID	10, 20 for 14 days	72 and 96	100% (4 of 4)	0% (0 of 12)	
AP-06-21E6 (Non-GLP)	MPXV/USAMRIID	10 for 14 days	120	100% (1 of 1)	0% (0 of 5)	
AP-09-026G (GLP)	MPXV/USAMRIID	0.3, 1, 3, 10 for 14 days	Lesion onset	100% (7 of 7)	0.3	80% (4 of 5)
					1	100% (5 of 5)
					3	20% (1 of 5)
					10	20% (1 of 5)
FY10-087 (GLP)	MPXV/LRRI	10, 20 for 14 days	96 h.p.i.	100% (6 of 6)	0% (0 of 18)	
SR10-037F (Non-GLP)	MPXV/Southern Research Institute	10 for 14 days	96, 120 or 144	100% (3 of 3)	96 h.p.i.	16.7% (1 of 6)
					120 h.p.i.	16.7% (1 of 6)
					144 h.p.i.	50% (3 of 6)
SR10-038F (Non-GLP)	MPXV/Southern Research Institute	10 for 3, 5, 7, or 10 days	96	75% (3 of 4)	3 doses	50% (2 of 4)
					5 doses	0% (0 of 6)
					7 doses	0% (0 of 6)
					10 doses	20% (1 of 5)
1470HUGMONC (Non-GLP)	VARV/CDC	300 for 14 days	0 and 24	50% (1 of 2)	0% (0 of 6)	
ST246-1745 (Non-GLP)	VARV/CDC	10 for 14 days	Lesion onset (72, 96)	0% (0 of 7)	14.2% (1 of 7)	

KEY: GLP = Good Laboratory Practice; MPXV = monkeypox virus; LRRI = Lovelace Respiratory Research Institute; USAMRIID = United States Army Medical Research Institute of Infectious Disease; CDC = Centers for Disease Control and Prevention; h.p.i. = hours post infection; VARV = variola virus

SIGA has participated in nine MPXV NHP studies that evaluated the efficacy of various doses of ST-246 (placebo, 0.3, 1, 3, 10, 20, 30, 100, or 300 mg/kg/day) given once daily to a total of 154 cynomolgus macaques challenged with MPXV (Zaire 79 strain) via the IV route (Table 1). In all cases, the primary endpoint was protection from death. ST-246 was administered to a total

of 120 NHPs for 3, 5, 7, 10 or 14 consecutive days by oral or nasogastric gavage. Delays in initiation of ST-246 treatment ranged from 1 hour to 6 days post-challenge with the treatment of 109 of 120 (90.8%) NHPs being delayed to at least 3 days post-challenge. Total study durations ranged from 28-56 days. Nineteen deaths occurred among NHPs treated with ST-246 during all 9 MPXV studies (19 of 120; 15.8%). The majority of these deaths occurred in animals treated sub-optimally: 9 of 19 (47.4%) were treated with 1 mg/kg or less of ST-246 beginning 4 days post-challenge, 2 of 19 (10.5%) received only 3 days of treatment beginning 4 days post-challenge and 3 of 19 (15.8%) did not receive treatment until 6 days post-challenge. Of the remaining 5 deaths, 2 were reported as not due to typical MPXV disease (see Sections 3.2.1 and 3.2.3). There was no evidence of disease recrudescence during any of the studies. All of the studies indicated statistically significant decreases in major morbidity in the form of mean total body lesion counts for a majority of ST-246 treatment groups versus placebo. Viral DNA levels in the blood were also reduced with statistical significance for ST-246 treatment groups versus placebo.

SIGA has participated in two variola NHP studies that evaluated the efficacy of 300 mg/kg or 10 mg/kg doses of ST-246 given once daily to 22 cynomolgus macaques challenged with VARV (Harper strain) via the IV route (Table 1). In all cases, the primary endpoint was survival. Treatment with ST-246 was administered to 13 NHPs over a course of 14 days, via oral gavage. Delays in initiation of ST-246 treatment ranged from concurrent with challenge to 4 days post-challenge. Total study durations ranged from 23-28 days. No deaths attributable to smallpox disease occurred among NHPs treated with ST-246, although one animal did succumb on treatment Day 6 (Study Day 9 post-challenge) following an anesthetic procedure (1 of 13; 7.7% - see Section 3.2.2). Similar to the treatment group, only 2 deaths occurred among the placebo-treated animals (2 of 9; 22%), although only one was attributed to smallpox infection (see Section 3.2.2). A statistically significant decrease in mean total body lesion counts and viral DNA levels in the blood for ST-246 treatment groups versus placebo was observed for specific time points during the studies. A number of experts in the field agree that since humans are the only natural host of the VARV and that VARV does not replicate efficiently in NHPs, the variola NHP model of disease may not be the most appropriate model for assessing efficacy based upon an endpoint of survival. Likewise, reductions in major morbidity in the form of mean total body lesion counts and viral load are also affected by the inability of the virus to establish a productive infection in NHP hosts.

Tenet 4. The data or information on the (pharmaco) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

The primary efficacy animal model must provide a quantitative assessment of the therapeutic potential of a drug candidate that is predictive for outcome in humans. Models developed in NHPs infected with MPXV via IV injection produce a lethal infection with high viremia and exanthematous disease that closely resembles human smallpox. Given the similarity in drug pharmacokinetics between NHPs and humans, the MPXV NHP model offers an ideal tool for the assessment of antiviral efficacy. Further, the MPXV NHP model allows for an evaluation of the effect that OPXV infection has on pharmacokinetics (PK), a scenario that is not feasible in the variola NHP model due to restrictions for conducting studies in a Biosafety Level-4 (BSL4) environment. Performance of assays is restricted to the availability of equipment and trained

personnel within the ABSL-4 facilities as biological samples potentially containing live variola may not leave the BSL-4 space. As such, pharmacokinetic evaluation of ST-246 in a variola-infected NHP is not possible since those capabilities presently do not exist at the CDC.

ST-246 safety and PK have been evaluated in numerous nonclinical studies, as well as three Phase I clinical studies and one Phase II clinical study. The PK of ST-246, and its underlying metabolism, was very similar in NHPs and humans. In both species, the drug has approximately 50% oral bioavailability, when administered in the fed state. Peak plasma concentrations occur 3 and 4 hours after administration for NHPs and humans, respectively. The terminal elimination is slightly shorter in NHP than humans, 8 versus 20 hours. Single daily oral administration results in steady state after 5-6 days with minimal (approximately 20%) higher exposure for both species.

Data obtained from studies conducted in both uninfected, as well as in MPXV-infected NHPs, has been used to develop a population (POP) PK model of ST-246 in NHPs. MPXV infection resulted in a minimal (< 10%) increase in ST-246 clearance, although inter-individual clearance variability was 32%. The effect of MPXV infection on clearance was translated to a similarly developed POP PK model of ST-246 in healthy humans. Pharmacokinetic exposure parameters (AUC, C_{max}, C_{min}) were then predicted for OPXV-infected humans on Day 1 of treatment and at steady state. Finally, simulated exposure parameters (AUC, C_{max}, C_{min}) in infected NHPs over the range of doses used in efficacy evaluations (3-168 mg/m^2, 0.25-14 mg/kg) were overlaid with the same parameters predicted for OPXV-infected humans after doses of either 400 mg or 600 mg daily. Doses of 96-120 mg/m^2 (8-10 mg/kg) and 144-168 mg/m^2 (12-14 mg/kg) administered to infected NHPs gave ST-246 exposures (AUC) equivalent to 400 and 600 mg doses, respectively. A simple comparison of the *in vitro* IC_{50} values for ST-246 against MPXV and Variola compared to the plasma concentrations of the compound in NHPs and humans demonstrates the more than sufficient concentration of this compound. ST-246 has an average IC_{50} against tested strains of MPXV *in vitro* of 0.029 μM (10.8 ng/mL) and 0.033 μM (12.3 ng/mL) against different Variola strains (see Table 3.1-1). The mean C_{max} and C_{min} concentrations for ST-246 after oral administration of the minimum therapeutic dose of 3 mg/kg in NHP were 395 and 32 ng/mL, respectively. The C_{ave} concentration was 128 ng/mL, more than 10-fold higher than the IC_{50} for both MPXV and Variola strains (LRRI FY10-087). Humans administered a 400 mg daily dose, which is equivalent to 8-10 mg/kg in NHP had plasma ST-246 concentrations at steady state that were much higher than the *in vitro* IC_{50} values for Variola virus, with mean C_{max} and C_{min} values of 1300 and 174 ng/mL, equivalent to 100- and 10-fold higher than the average *in vitro* IC_{50} values for Variola strains. POP PK model development is presented in detail in Section 2.4.2.

In addition to the POP PK modeling, which identified equivalency of doses between infected NHPs and humans, pharmacodynamic (PD) analysis has been used to evaluate the correlation between ST-246 dose and exposure in NHPs and survival. The analysis used survival data from all MPXV infection studies that had survival data available at the time that the analysis was started. The total number of NHPs for the combined dose versus survival analysis was 96, of which 23 animals were placebos, with the remainder having received doses of ST-246 that ranged from 0.3 mg/kg-300 mg/kg. Among the 96 NHPs evaluated for the initial PD survival analysis, 34 NHPs died before the end of the study. Most of these animals were administered placebo, 0.3 mg/kg, and 1 mg/kg ST-246.

The Dose-Survival relationship was explored using the Kaplan Meier approach. In this analysis a nonparametric survival curve estimates the probability of survival over time. For dose levels that were associated with death, 35 NHPs started their treatment on Day 4, 9 started their treatment on Day 3, and 5 NHPs started treatment on Day 5. Probabilities of survival were greater than 90% versus placebo at doses of 10 mg/kg and 3 mg/kg. Survival associated with doses of 0.3 mg/kg and 1 mg/kg versus placebo did not demonstrate statistical significance.

Recursive receiver operator characteristic (ROC) analysis was conducted in order to determine the optimal cut-off points for dose or exposures levels that best correlate with the survival outcome. The advantage of this analysis is that an optimal cut-off is determined based on statistical criteria that optimize sensitivity and specificity. All results converged on the same overall conclusion, showing that doses of 3 mg/kg and higher were associated with maximum survival whereas doses of less than 3 mg/kg had very low survival rate. The actual cut-off values corresponded to a dose level of 100 mg for humans of a median weight of 78.35 kg (obtained from SIGA's Phase II clinical study). This analysis strongly indicates that a dose of 400 mg of ST-246 will provide more than sufficient exposure to protect humans against OPXV disease. Addition information is presented in Section 2.4.4.

Summary

To summarize, SIGA has utilized a number of animal species, types and variants, inoculation routes and challenge inoculum titers to evaluate the efficacy of ST-246. The fact of the matter remains that there is no ideal animal model that recapitulates human smallpox disease to the letter. ST-246 is extremely safe, is taken orally once daily for two weeks and could be distributed widely, without fear of complication, in a situation that warrants availability of a countermeasure to the masses. In the absence of an NDA approval, this solution does not exist.

That said, the data presented in this document support the IV MPXV model as closely representing the course of a VARV infection in humans. The fact that treatment is withheld until a productive systemic infection is established only strengthens the model's rigor and raises the efficacy bar. Animals present with lesions and the model is almost uniformly lethal. Finally, the IV MPXV model is reproducible among multiple investigators, can be conducted under GLP and contains clearly defined endpoints making it an ideal model in which to conduct definitive, NDA-supporting efficacy studies. With the acceptance of this model under the animal rule pathway to U.S. NDA approval, ST-246 could be made available for treatment use by any and all infected individuals across the United States who contract an orthopoxvirus disease via incidental contact, or in the event of a declared emergency due to an accidental or deliberate release of an orthopoxvirus.

1. INTRODUCTION

1.1 Smallpox as a Human Disease

Smallpox is a highly contagious and lethal disease caused by either of the two VARV variants, variola major or variola minor, which belong to the family Poxviridae, subfamily Chordopoxvirinae, and the genus Orthopoxvirus. Smallpox was declared eradicated from the natural environment worldwide in 1980 after an aggressive global surveillance, vaccination, and containment campaign conducted by the WHO from 1966-1977. Smallpox vaccination of the general public in the United States was discontinued in 1971. Currently, known VARV stocks are held only in two high-security, maximum containment WHO reference laboratories, one at the Centers for Disease Control and Prevention (CDC) in the U.S.A. and one at the Russian State Research Centre of Virology and Biotechnology (Vector). Despite its extinction in nature, smallpox as a catastrophic epidemic disease remains a concern owing to the potential for a deliberate release of VARV as an act of war or bioterrorism.[4,5] A single case of smallpox anywhere in the world would be considered a global health emergency. The deliberate or accidental release of the smallpox virus into today's largely unvaccinated and highly mobile population would wreak far-reaching medical, social, and economic havoc. For this reason, variola is now classified as a “Category A Bioterror Threat.”

The best way to prevent smallpox is through vaccination. The vaccine used to eradicate smallpox is a live attenuated vaccinia virus, a close relative of variola. Properly administered as a *pre-exposure prophylactic*, the vaccine is approximately 95-98% effective; however, it has the highest adverse event rate of any FDA-approved vaccine and is associated with a number of severe adverse side effects such as progressive vaccinia, eczema vaccinatum, post-vaccinial encephalitis, fetal vaccinia, peri/myocarditis, generalized vaccinia, auto-inoculation, and erythema multiforme, and death among other more common but less severe reactions.[6,7] Vaccination within 3 days post-exposure will prevent, or greatly reduce, the severity of smallpox in most people. Vaccination will not protect against smallpox disease in patients who already have a rash. Currently, due to concern over adverse events, the vaccine is contraindicated for the general public. There are no FDA-approved therapeutics for smallpox.

The principal use for a self-administered antiviral drug against smallpox would be to provide immediate protection to unvaccinated persons in the event of a biological weapon attack. The 10-14 day incubation period of smallpox favors the success of such a strategy. In contrast to vaccination, which acts indirectly by eliciting the development of an adaptive immune response and must occur soon after exposure to be effective, antiviral therapy could arrest viral spread immediately, and could potentially be effective when begun at any time during the incubation period. Drug treatment would therefore be especially important for individuals who are too late in the incubation period to benefit from vaccination. The current Smallpox Response Plan to begin mass vaccination only after VARV has been released aims to “rescue” persons already infected and immunize the rest of the population, but this assumes that the vaccine can be distributed, health care workers will be available to provide it, and the population will be able to reach it within 3 days after a large-scale biological attack. This approach clearly leaves many areas of vulnerability that could be reduced if an effective antiviral drug were available for immediate use. As with other types of antimicrobial therapy, drug treatment would be most effective if begun before the development of full-blown disease, when the clinical picture is dominated by host inflammatory responses.

Ideally, a self-administered antiviral drug would supplement, rather than replace vaccination. As the newly produced live-virus vaccine will presumably remain available for use for many years, the addition of an anti-smallpox drug to the public health armamentarium becomes even more attractive if the same drug could be used to prevent or treat complications arising during the course of a mass vaccination campaign. In addition, a self-administered drug with broad anti-OPXV activity could also be used to increase the safety of vaccination for the 9-30% of the population with atopic dermatitis/eczema who may be at risk for disseminated VACV infection (eczema vaccinatum) following administration of the current live-virus vaccine.

1.2 Smallpox Response Plan

A Material Threat Assessment by the Department of Homeland Security states that smallpox may be used as a biological weapon and poses a serious risk to the security of the U.S. There are numerous contributing factors that make smallpox an ideal biological weapon: First, smallpox is highly contagious. Second, the U.S. population is highly susceptible to the virus and will remain so for the foreseeable future. Third, publicly accessible scientific literature yields relatively simple and inexpensive methods for the growth, purification and genetic engineering of smallpox,[16, 17] including proven methods for increasing the virulence of poxviruses.[18] Fourth, the widespread dispersal of the virus may be enhanced by "weaponization"[19] or aerosolization.[20] Fifth, decontamination may be difficult. Sixth, there is no FDA-approved effective therapy for smallpox. Seventh, a smallpox outbreak would result in severe socio-economic disruption. Eighth, stability of shed virus will pose a long term health hazard.[86]

A single case of smallpox anywhere in the world would be considered a global health emergency. The deliberate or accidental release of the smallpox virus into today's largely unvaccinated and highly mobile population would wreak far-reaching havoc (medical, social, and economic). A comparison of best and worst case scenarios in a potential smallpox outbreak is shown in Table 1.2-1. In the event of a smallpox outbreak in the U.S. the CDC would immediately implement the Smallpox Response Plan.[21-25] As there is currently no FDA-approved effective treatment for smallpox, the Plan largely seeks to contain the outbreak. In a large urban setting, the policy is to isolate suspected cases, trace and vaccinate their contacts ("race to trace"), quarantine any of those contacts who are feverish, but then vaccinate more widely if the outbreak is not contained by these measures. The vaccine used to eradicate smallpox is a live attenuated vaccinia virus, a close relative of variola. While the vaccine was proven to be extremely effective, it has the highest adverse event rate of any FDA-approved vaccine and was associated with a number of severe adverse side effects such as progressive vaccinia, eczema vaccinatum, post-vaccinial encephalitis, fetal vaccinia, peri/myocarditis, generalized vaccinia, auto-inoculation, and erythema multiforme among other more common but less severe reactions.[6, 7] The overall adverse event rate is approximately 1,000 per million vaccinations and between 14 and 52 per million vaccinations will be potentially life threatening. In a 1968 survey, vaccination was found to result in 1-2 deaths per million vaccinations[26] although a more recent analysis suggests that the adverse event rate following vaccination was strain-dependent and that there may have been as many as 8 deaths per million vaccinations with the commonly used Lister strain.[27] The risk-benefit ratio of the smallpox vaccine prevents its use in the general population in the absence of risk from smallpox but in the event of a smallpox outbreak, the vaccine would be administered to everyone at risk of exposure regardless of

previously existing contraindication. Without the availability of an antiviral, the risk of adverse events due to vaccination would be acceptable given the lethality of smallpox.

Table 1.2-1: Comparison of Best and Worst Case Scenarios in a Smallpox Outbreak

Parameter	Best Case[a]	Worst Case[b]
Number of Index Cases	10-100	1,000's (sporting venues, multiple simultaneous attacks, etc.)
Spread From Index Cases	Up to 3 individuals (CDC model)	10-20 individuals[(4)]
Transmissibility	26%	> 96%[(20)]
Potential Number Infected Before Final Containment	4,200	Millions[(24)]
Time to Containment	1 year	Smallpox becomes endemic; years[(24)]

[a]Based on Centers for Disease Control and Prevention estimates.
[b]Based on scenarios presented in selected references

1.3 Clinical Features of Human Smallpox Disease

Smallpox disease is marked by the dissemination of VARV within the infected host and the appearance of skin lesions or pocks.[(8, 9)] The virus is mainly transmitted between humans by aerosol droplets; however, contact with VARV-contaminated clothing or bedding may also contribute to transmission. Once inhaled, VARV appears to first infect the upper or lower respiratory tract mucosa and spread to and replicate within the local lymph nodes. The virus then appears to disseminate most likely to the lymphoid organs (spleen, bone marrow, and lymph nodes) via a transient viremia and replicate in reticuloendothelial cells through an asymptomatic incubation period of 7-17 days (average of 10-12 days). The prodrome period that begins after the incubation period is characterized by symptoms of high fever (> 104°F), severe headache, lasts for 2-3 days. After that, a second wave of viremia occurs and results in the dissemination of the virus to the dermal epithelium of the skin and mucous membranes of the mouth and pharynx. This dissemination leads to the eruption of lesions over the tongue, mouth, and oropharynx and of rashes that start at the face and extremities and may eventually envelope the whole body. The rashes begin as small, reddish macules but progress to papules (2-3 mm), vesicles (2-5 mm), and pustules (4-6 mm in diameter) within seven days and remain for an additional 5-8 days before becoming umbilicated, crusted, drying into scabs, and finally falling off after 3-4 weeks, if the patient survives. Lesions appear denser on the face and extremities than on the trunk and lesions at a specific part of the body tend to be at the same stage of progression. In addition, a second spike in fever (> 101°F) tends to occur 5-8 days after the onset of the rash. Human smallpox disease progression is outlined in Figure 1.3-1.

Epidemiological studies suggest that transmission from smallpox patients to their contacts occurs only from the time of the earliest appearance of rash and that excretions from the mouth and nose are the most important source of infectious virus for transmission.[(4)] The transmission rate of smallpox to unvaccinated contacts has been estimated to be in the range of 37-88 percent.[(9)]

Figure 1.3-1: Human Smallpox Disease Progression

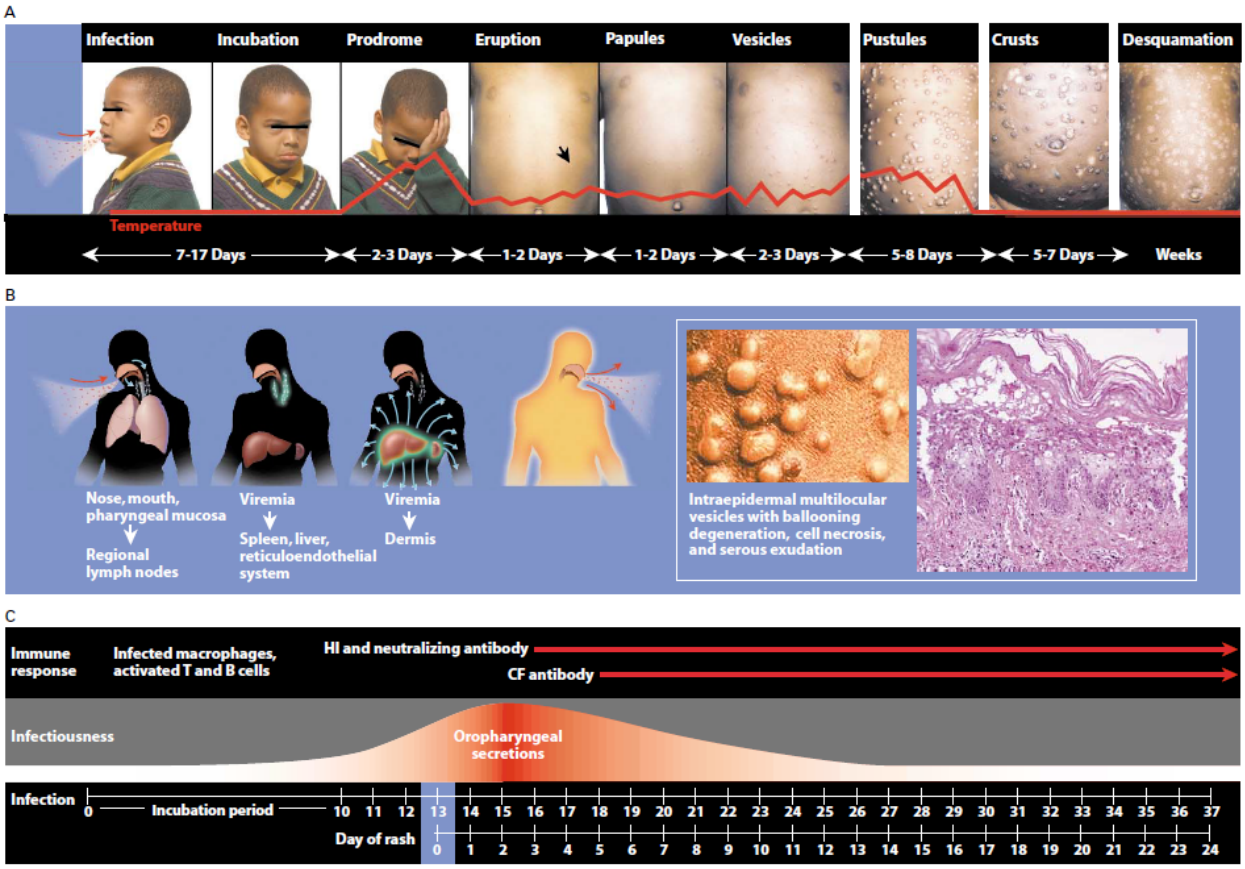


Figure 1 (facing page). Clinical Manifestations and Pathogenesis of Smallpox and the Immune Response.
Panel A shows the initial phases of infection and the clinical manifestations, which include temperature spikes and progressive skin lesions (photographs of lesions courtesy of Dr. David Heymann, World Health Organization). Panel B shows the pathogenesis of the infection. The photographs at the right-hand side of the panel show the characteristic features of the vesicles caused by smallpox (hematoxylin and eosin, ×90; reprinted from Strano[14]). Panel C shows the immune response to smallpox and the period of infectiousness. HI denotes hemagglutination inhibition, and CF complement fixation.
Figure and legend obtained from Breman and Henderson.[9]

There are four major types of clinical presentations of variola major infection as defined by lesion morphology and severity of disease symptoms.[10] Ordinary smallpox (~ 90% of cases and a fatality rate of 30% or more) is characterized by raised pustular skin lesions that can be discrete (~ 10% mortality) or confluent (~ 60% mortality). Flat or malignant smallpox (~ 7% of cases) characterized by soft, flat, confluent or semi-confluent lesions and hemorrhagic smallpox (~ 2% of cases) characterized by widespread hemorrhages in the skin and mucous membranes lack the typical pustular rash and are uniformly lethal (~ 93-100%). Modified smallpox characterized by superficial and pleiomorphic skin lesions that evolve more quickly occurs in ~ 25% of previously vaccinated individuals and is rarely, if ever, fatal. In addition, smallpox characterized by fever without rash (variola sine eruptione) occurred in previously vaccinated contacts but can only be confirmed by virus culture or serological testing.

Death from smallpox usually occurred during the second week of illness but may occur as early as the fifth day of illness in hemorrhagic smallpox.[4] Even survivors may be left with long-term side-effects, such as disfiguring skin scars from pockmarks, blindness from corneal infections, arthritis from infection of the metaphysis of growing bones, and infertility in males.[8, 9, 11] It is

believed that smallpox survivors completely eliminated the virus (i.e. persistent infection or reactivation by immunosuppression did not occur) and developed lifelong immunity against re-infection.[8] Neutralizing and hemagglutinin-inhibiting (HI) antibodies were detectable by the sixth day of rash onset and were followed about two days later by complement-fixing (CF) antibodies.[12] Neutralizing antibody levels persisted at high titers for several years, whereas HI and CF levels fell to low levels within 5 and 1 years post-infection, respectively.[8] Although the type and extent of cellular immunity in smallpox patients is unknown, peripheral blood T-cell counts were found to be consistently lower in smallpox patients than healthy controls.[13]

Due to the "toxic" appearance of variola major patients and the readily detectable levels of viral antigens in their plasma, it was generally believed that the cause of death in smallpox patients was toxemia (what is now considered to be "cytokine storm" and the resulting septic shock) associated with the formation of immune complexes.[14] However, examination of surviving case series of smallpox pathology and review articles from the last 200 years by D.B. Martin provides strong support to the assertion that death from smallpox resulted from the cytopathic effect of the virus rather than toxemia.[15] It appears that death was most likely due to "renal failure, shock secondary to volume depletion, and difficulty with oxygenation and ventilation as a result of viral pneumonia and airway compromise, respectively."[15] Observations that supported this view included:[15]

a) Viral damage and inflammatory responses caused lesions of the skin and mucosal membranes. During vesicular and pustular lesion formation, intravascular volume could be reduced due to the accumulation of large amounts of subcutaneous fluid. In addition, airway difficulties and difficulty with eating could be caused by the pseudomembranes covering mucosal lesions.
b) Bronchitis, hyperemia, degeneration of alveolar lining cells, and alveolar congestion were evident in the lungs. These pathologies are consistent with a potentially lethal interstitial pneumonitis that could compromise oxygenation.
c) The kidneys exhibited degeneration of the endothelium, interstitial edema, and lymphocyte infiltration consistent with virus-induced acute tubulointerstitial nephritis. Combined with reduced intravascular volume, tubulointerstitial nephritis could significantly impair renal function.

Although areas of liver degeneration caused by virus infected hepatocytes were evident and may have resulted in some level of functional impairment, liver failure did not appear to have contributed to the cause of death. Otherwise, in the majority of cases, the spleen and lymph nodes did not exhibit major pathological abnormalities and appeared to have responded appropriately to the viral infection.

1.4 Challenges to Developing Drugs for Smallpox Under the Animal Rule

Considering that naturally occurring smallpox no longer occurs, humans are the only natural host, and it is unethical to test vaccines or antiviral drugs in experimentally infected humans, surrogate models are the only option to demonstrate efficacy. Two possibilities exist:
1) evaluate drug efficacy in humans infected with closely related orthopoxviruses, with additional evaluations for safety and pharmacokinetics also conducted in infected and healthy humans, or 2) evaluate safety, pharmacokinetics, and drug efficacy in animal models that closely recapitulate human smallpox and conduct additional evaluations for safety and pharmacokinetics

in healthy humans, which would serve to "bridge" the animal studies to humans. These options are discussed below.

1.4.1 EVALUATION OF SMALLPOX ANTIVIRALS IN HUMANS INFECTED WITH CLOSELY RELATED ORTHOPOXVIRUSES

In addition to variola, other OPXVs including monkeypox, cowpox, buffalopox and vaccinia can cause disease in man; however, humans are not the natural host for any of these other viruses. Except for monkeypox virus, human infections with cowpox, buffalopox and vaccinia viruses in very few ways recapitulate human smallpox disease. Although the viruses are genetically very similar to variola virus, the route of infection, clinical disease manifestations, lack of transmission and a very low fatality rate all distinguish them as very different. Clinical trials with these viruses would have very limited benefit in determining whether a drug would be efficacious as a smallpox therapeutic.

Monkeypox, however, is unique among the naturally occurring OPXVs in that it causes disease in humans that is nearly indistinguishable from smallpox. Human monkeypox infection can be acquired naturally by means of varied routes (e.g., the respiratory tract, skin, and mucous membrane) and from varied sources (e.g., rodents, humans, and nonhuman primates).[28]

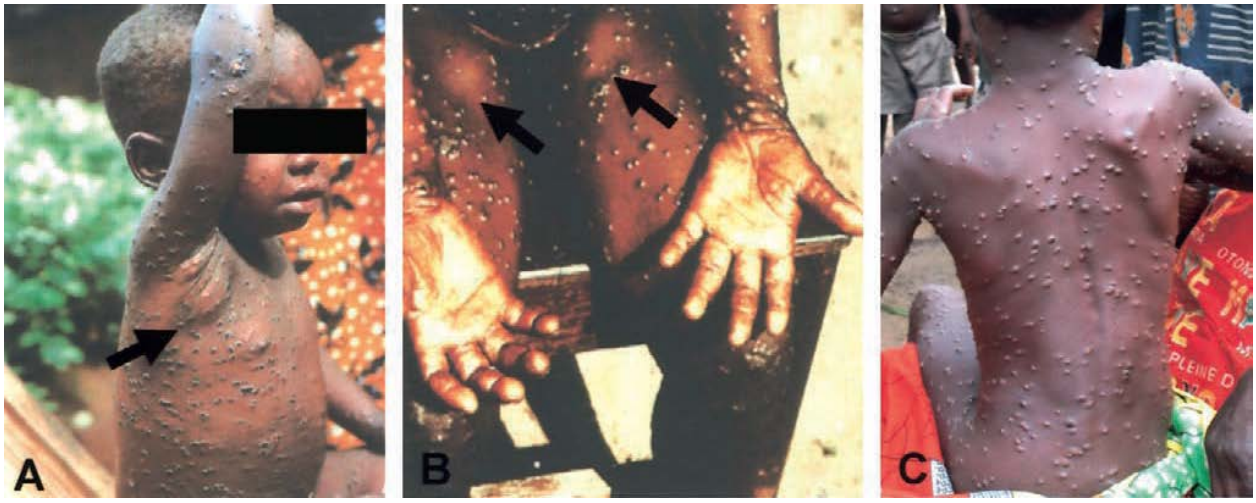
The clinical features of human monkeypox closely resemble those of ordinary smallpox.[29] After a 10–14-day incubation period, prodromal illness with fever, malaise, and swollen lymph nodes is observed in most of the patients before the development of a rash.[30] Other signs and symptoms of monkeypox include chills and/or sweats, headache, backache, sore throat, cough, and shortness of breath. Lymphadenopathy, which has been observed in 90% of unvaccinated patients, is not a common feature of smallpox and is therefore considered to be a key distinguishing feature of monkeypox (Figures 1.4.1-1A and 1.4.1-1B). Lymph node enlargement can occur in the submandibular and the cervical or inguinal regions.[28] The prodromal period generally lasts 1–3 days before the occurrence of the typical maculopapular rash. During the first week of the rash, the patient is considered to be infectious and should be isolated until all scabs separate and results of throat swab PCR are negative. The mean diameter of the skin lesions is 0.5-1 cm, and the clinical progress is very similar to that of ordinary smallpox lesions. During a 2–4-week period, lesions progress from macules to papules, vesicles, and pustules, followed by umbilication, scabbing, and desquamation (Figure 1.4.1-1).[28] Although the rash starts mainly on the trunk, it can spread in a peripheral distribution to the palms and soles of the feet. Lesions can be observed on mucous membranes, in the mouth and tongue, and on the genitalia. In addition to skin lesions, extracutaneous manifestations, such as secondary skin and/or soft-tissue infection (19% of cases), pneumonitis (12%), ocular complications (4%–5%), and encephalitis (< 1%) can be observed in patients infected with MPXV.[30] The fatality rate is 10%, and death generally occurs during the second week of the disease.[28, 31]

In Figure 1.4.1-1A, a 3-year-old African boy with monkeypox and axillary lymph node enlargement (arrow). In Figure 1.4.1-1B, a 7-year-old African girl with monkeypox and bilateral inguinal lymphadenopathy (arrows). For both patients, lymphadenopathy was the main differential diagnostic criterion that distinguished monkeypox from smallpox. Photographs were kindly provided by Mark Szczeniowski, World Health Organization. In Figure 1.4.1-1C, a 7-year-old girl from Tokondo village, Kasai-Oriental province, Democratic Republic of Congo,

with reported exposure to a dead monkey. Note the characteristic pustules on her back. Photograph was taken on 04 October 2004 and kindly provided by Dr. Robert Shongo.

The quoted text above and "Figure 1.4.1-1" are directly lifted from Nalca *et al*.[32]

Figure 1.4.1-1: Monkeypox Virus Photos



Would it be feasible and ethical to conduct a clinical trial to evaluate the efficacy of an antiviral countermeasure in humans infected with monkeypox? It would certainly be unethical to experimentally infect humans with monkeypox, so a trial would have to be conducted within the context of naturally occurring disease. With the exception of an outbreak in the U.S. in 2003 due to the importation of exotic pets,[33] monkeypox infections are generally restricted to West Africa and the Congo Basin. While the seroprevalence of anti-monkeypox antibodies is similar for both regions, more than 90% of reported cases are from the Congo Basin. Case fatality rates in humans and virulence testing in NHPs demonstrate that the Congo Basin clade is more virulent than the West African clade.[34] As such, it is likely that most infections in West Africa are very mild or subclinical. If a clinical trial were to be conducted, it would be most appropriate to evaluate antiviral therapeutics in the Congo Basin considering that most cases occur there, facilitating accrual, and the likelihood of demonstrating a statistically significant impact on disease is increased, since the Congo Basin clade of viruses cause more severe disease.

SIGA has discussed with the FDA the possibility of obtaining human data in a setting of naturally occurring orthopoxvirus disease in the Democratic Republic of the Congo (DRC). Although currently infeasible, because the health care infrastructure is unsuitable, political instability in the region, sporadic disease occurrence, inadequately trained local personnel, and regulatory and ethical concerns, SIGA will consider conducting a clinical trial in the future as it is possible. SIGA sent a representative to Kole, DRC in June 2008 to evaluate the clinical setting where the U.S. Army (USAMRIID) is collecting epidemiology data on monkeypox cases in humans. His observations showed that the site is poorly accessible and the current infrastructure is insufficient for a clinical study. The disease incidence is low and sporadic (5.3/10,000),[35] mortality is extremely low (0% in adults and approximately 1% in pediatrics), thereby not reflecting the typical smallpox disease course. Most of the patients admitted to the study are pediatrics and adolescents (< 15 yrs of age)[35] for which SIGA does not yet have an appropriate GMP formulation or the pharmacokinetic data on which to justify an appropriate

dose. In discussions with Col. Mark Withers, an MD with the US Army and principal investigator for the monkeypox study at the Kole site, and SIGA's observations, it is clear that patients typically arrive with advanced lesional disease (late in the disease course), their viral load is modest (10^4-10^5 genomes/mL of blood) and immunological clearance of the virus has likely already been initiated. There is a high incidence of underlying disease in these patients from other agents and many of the patients are on other therapeutic modalities. Drug/drug interactions may complicate the issue as the local standard of care for patients that come to the clinic includes anti-malarial drugs and multi-vitamins. Also, shipping samples out of the DRC to the United States has been very problematic. Shipment of samples is an absolute requirement as the Kole site is not equipped with advanced clinical, diagnostic or analytical capabilities. The cold-chain necessary for the preservation of samples is intermittently intact as it relies on unreliable power sources such as gasoline generators and solar power cells.

SIGA believes that the human monkeypox studies in the DRC in the future could be very valuable; however, the present infrastructure is not adequate for running clinical studies and with the ongoing political instability it is not safe for patients or staffing personnel. SIGA plans to continue to monitor the situation and stay in contact with each of the organizations researching monkeypox in the DRC to determine if and when clinical studies with ST-246 will be feasible. SIGA is therefore restricted to seeking approval based on efficacy data in animal models and safety and pharmocokinetic data in humans.

2. ANIMAL MODELS FOR SMALLPOX

In the absence of an appropriate clinical setting in which ST-246 could be evaluated, animal models remain the only option for drug efficacy studies. As requested by FDA, and due to cost effectiveness and availability, small animal models were employed initially. A multitude of animal were developed and characterized using a wide range of OPXVs by a large number of investigators. SIGA has utilized many of these models (data presented in Appendix A) both independently and in collaboration with others and, based upon this wealth of experience, SIGA contends that the IV MPXV NHP model of human smallpox disease is the best representation of human smallpox available in animals. This model, used for nine ST-246 efficacy studies, has been shown to be robust and reproducible, with consistent and quantifiable endpoints. In addition, the IV MPXV NHP model contains a predictable therapeutic trigger in the form of lesions. Efficacy data obtained from this model (supported by data obtained from many other models, including studies in NHPs using VARV), combined with safety data obtained from toxicology studies in animals and human clinical studies, should provide the required information to support licensure of a smallpox therapeutic. The following table summarizes our experience in small animal models. In each lethal challenge model, ST-246 was found to be efficacious even when initiated following the onset of clinical symptoms. Furthermore, body surface area scaling, typically used in predicting human exposure, shows that the efficacious dose in small animals is supportive of the proposed human dose of 400 mg, once daily for 14 consecutive days.

Table 2-1: Summary of ST-246 in Small Animal Models

Virus	Animal	Route of Infection	Oral Treatment Regimen (days)	Challenge Dose (PFU)	Protective Efficacy
VAC	Balb/C	Intranasal	14	1×10^6 ($10 \times LD_{50}$)	Yes
ECTV	A/NCR	Intranasal, Footpad	14	3×10^4 ($40{,}000 \times LD_{50}$)	Yes
CPX	Balb/C	Intranasal	7	1×10^6 ($10 \times LD_{50}$)	Yes
VAC	*Nu/Nu*	Lumbosacral scarification	10	5×10^5	Yes
VAC	NMRI	Tail Vein	5	4×10^3	Yes
VAC	*Nu/Nu*	Tail Vein	10	4×10^3	Yes
MPX	*Ground squirrel*	SubQ	7	1×10^3	Yes
RPV	*Rabbit*	Aerosol	7	1×10^3	Yes
MPX	*Praire dog*	Aerosol	14	1×10^5	Yes

28 studies; 5 poxviruses

Further details on each of these animal models can be found in Appendix A.

While each small animal model has provided valuable data demonstrating ST-246 efficacy against orthopoxviruses, they are not suitable for pivotal efficacy studies. It is most notable that small animal physiology is dissimilar in many aspects from human physiology and the disease progression and response to drugs may differ significantly. Additionally, small animal models require the use of surrogate viruses that, while causing lethal disease, do not mimic the progression of pathogenic events typically seen in human smallpox cases. Table 2-2 summarizes the insufficiencies in each small animal model.

Table 2-2: Insufficiency of Small Animal Models

Virus	Host	Similar or Dissimilar to Human Smallpox (Yes/No)				
		Challenge Route	Challenge Dose	Disease Kinetics	Lesional Disease	Cause of Death
Vaccinia	Mouse	Yes - intranasal	No	No	No	No
Cowpox	Mouse	Yes - intranasal	No	No	No	No
Ectromelia	Mouse	No - Footpad	Yes	Yes	No	No
		Yes - intranasal	Yes	Yes	No	No
Monkeypox	Squirrel	Yes - intranasal	No	No	No	Yes/No
	Prairie Dog	Yes - intranasal	No	No	No – high dose Yes – low dose	No – high dose None – low dose
Rabbitpox	Rabbit	Yes - aerosol	Yes	No	Yes	No
		No - intradermal	Yes	No	Yes	No

2.1 Small Animal Model Summary

In summary, small animal models are appropriate for early stage efficacy evaluation due to their availability and cost but are insufficient for pivotal studies. The majority of small animal models with high mortality rates do not exhibit smallpox-like pathologies and lesions are not apparent at the time of death (lesions occasionally appear on survivors). In respiratory challenge models, death is most often due to inflammatory respiratory disease (necrotizing bronchopneumonia, bronchitis, bronchiolitis, etc). In addition, selection of a human dose based on the extrapolation of small animal pharmacokinetics to humans may prove difficult.

2.2 NHP Models of Human Smallpox Disease

2.2.1 General Overview of NHP Models

Given the physiological, immunological, and other similarities between humans and NHPs, a NHP model of OPXV infection that closely resembles human smallpox disease would be preferable over that of small animal models. In evaluating which NHP model for human smallpox is most appropriate for demonstrating the efficacy of antiviral post-exposure therapeutics, a number of parameters have to be considered. It is important to determine the time in the course of human smallpox at which treatment is no longer considered prophylactic and is then considered therapeutic. The distinction between the two phases of treatment is not an easy decision: Human smallpox is characterized by a relatively long incubation period, a prodromal pre-eruptive phase and an eruptive phase. Historically, any treatment that was initiated prior to the formation of pocks would be considered post-exposure prophylaxis. With the development of modern technologies, a definitive diagnosis of smallpox would likely be made prior to pock formation and probably as early as the prodromal fever (when secondary viremia would be easily detected by qPCR) if exposure was suspected. In the event of a suspected outbreak of smallpox, this is almost certainly true for the contacts of index cases but it is likely that index cases would

not be diagnosed or treated until after pock formation. Nevertheless, this suggests that by current standards, treatment started any time post-diagnosis should then be considered "therapy." An acceptable animal model must be characterized by post-exposure fever and viremia followed by the development of a significant number of skin lesions. Human smallpox skin lesions progress through characteristic developmental and resolutional phases and are distributed over the dermis in specific centrifugal patterns. Although human smallpox lesions may present as hemorrhagic, atypical, flat or non-specific rashes, these are not the norm. Animal models should be chosen such that following challenge, numerous lesions form that develop in phases similar to human smallpox lesions with a distribution similar to human smallpox lesions. In addition to typical lesion formation, it is also important that other human clinical features of smallpox disease, including death, are also presented in the selected model.

To identify the most appropriate NHP model, both MPXV and VARV infections have been evaluated by several investigators. The various administration parameters and survival outcomes from those investigations are summarized in Table 2.2.1-1. The detailed description for each NHP model is provided below.

Table 2.2.1-1: Non-Human Primate Models for Lethal Poxvirus Infection

Challenge Virus	Challenge Strain	Route	Dose	Survival Outcome (Untreated Animals)	Ref.
MPXV	Zaire 79	IV	$5x10^6$	100% - 0 of 2 dead	(62)
			$2x10^7$	0% - 3 of 3 dead, Days 7-14	
			$3.8x10^7$	0% - 8 of 8 dead by Day 9	(70)
			$5x10^7$	67% - 2 of 6 dead by Day 18	(63)
				67% - 1 of 3 dead, Day 12	(67)
				< 5% survival	(71)
				0% - 10 of 10 dead, Days 11-21[a]	(64)
				0% - 3 of 3 dead, Day 9	(65)
				0% - 1 of 1 dead, Days 1-14	(66)
				100% - 0 of 2 dead	(68)
				0% - 2 of 2 dead, Day 13	(69)
			$5x10^8$	0% - 2 of 2 dead, Day 6	(62)
		aerosol	$1x10^4 – 1.41x10^5$[bc]	0% - 15 of 15 dead, Days 9-17	(56)
		intratracheal	$3.42x10^6$	67% - 1 of 3 dead, Day 12	(57)
			$8.37x10^6$	33% - 2 of 3 dead, Day 12, 16	
			$3.53x10^7$	33% - 2 of 3 dead, Day 8	
	MSF#6	intratracheal	$1x10^6$	100% - 0 of 3 dead	(58)
			$1x10^7$	0% - 3 of 3 dead, Days 15-19	
				0% - 14 of 14 dead, Days 9-19	(59)
	Liberia	intranasal	$1x10^6$	100% - 0 of 2 dead	(60)
	Zr-599	subcutaneous	$1x10^6$	0% - 2 of 2 dead, Days 14, 18	
	Ultrecht	subcutaneous	$3.2x10^5 + 2.5x10^7$	100% - 0 of 1 dead[d]	(61)
Variola	Harper	IV	$1x10^8$	67% - 2 of 6 dead	(71)
				67% - 1 of 3 dead, Day 11	(55)
			$1x10^9$	0% - 7 of 7 dead, Days 4-10	
		IV/aerosol	$1x10^9$	25% - 3 of 4 dead, Days 4-6	
	India 7124	IV	$1x10^7$	100% - 0 of 3 dead	
			$1x10^8$	100% - 0 of 3 dead	
			$1x10^9$	17% - 5 of 6 dead, Days 3-10	
		IV/aerosol	$1x10^9$	0% - 4 of 4 dead, Days 3-13	

[a]Some of these animals had been vaccinated with MVA or Dryvax but were immunodeficient (CD4 counts less than 300 mL) due to SIV infection.
[b]The Zaire strain used in this study is officially known as Monkeypox virus strain V79-I-005.
[c]Aerosol dosages varied from animal to animal depending on the efficiency of delivery.
[d]Animals were challenged first with a low dose then rechallenged 3 days later with the high dose.

2.2.2 IV VARV MODEL

"The use of i.v. inoculation effectively eclipses the incubation and prodromal phases of naturally acquired smallpox infection in humans by creating instantaneous viremia and systemic spread of virus to target tissues."(55) Infection with doses of 10^9 pfu was required in NHPs for near uniform mortality while lower doses (10^8 pfu and lower) were non-lethal in the majority of animals challenged. Both the high and low dose challenges resulted in viremia by Day 2, which represented cell-associated virus as no virus was found to be free-floating in the serum. Infectious virus was isolated from throat swabs by Day 3 post-challenge. Cutaneous erythema

was evident by Day 2, and by Day 3, early development of vesicles and pustules was evident. All animals developed fever by Day 3. Skin lesions, which can serve as a therapeutic trigger and become evident on Day 3 or Day 4 post-challenge, were characterized by centrifugal distribution with concentration on the extremities and varied with dose. "At necropsy, in addition to cutaneous hemorrhage, features of coagulopathy were observed, which included hemorrhagic effusions in the thoracic and abdominal cavities and the ocular anterior chamber, in addition to visceral and mucosal hemorrhage" suggesting that the disease caused by variola in monkeys closely recapitulates *hemorrhagic* smallpox in humans. "Typical" smallpox-like disease (i.e., non-hemorrhagic) did not occur with the high-dose challenge. In addition, data from QPCR showed a complex pattern of viral DNA accumulation in most tissues with little evidence of significant increases in virus replication over time. While DNA levels were significantly different from baseline (i.e., prior to infection), accumulation of viral DNA in most tissues over time was not observed suggesting VARV does not replicate efficiently in a primate host. Investigators often refer to the NHP VARV model as having 33% mortality among placebo-treated control animals. The figure of 33% mortality was derived from early studies in which NHPs were challenged with $1x10^8$ pfu of the Harper strain of variola by the IV route. Although this figure was published very early in model development and after only 6 animals had been evaluated, this has established this model as a "standard" of sorts for the evaluation of smallpox therapeutics. However, recent studies conducted by USAMRIID in the ABSL-4 facilities at the CDC (1470HUGMONC and ST246-1745) designed to evaluate the efficacy of ST-246 in this model did not confirm this mortality rate (presented in greater detail in Section 3.2.2). In the first study 2 of 2 vehicle treated controls were euthanized due to severe advanced disease, while in the second study, none (0 of 7) of the vehicle treated controls died although lesional disease was evident in all monkeys. This underscores the irreproducibility of mortality in this model. Further, while lesions reminiscent of human smallpox disease are a clinical manifestation of this model, the fact remains that viral titers do not appear to increase following challenge and animals are able to recover from illness, albeit in a slightly delayed timeframe, in the absence of therapeutic intervention.

The high-dose and low-dose variola challenge models are each insufficient for unique reasons. The high-dose challenge results in uniform mortality but the kinetics of disease progression are extremely accelerated relative to human disease and death is due to a hemorrhagic form of disease which is rare in humans. There is no therapeutic trigger for antiviral intervention in this model. The low-dose variola challenge results in a lesional disease similar to human smallpox although viremia is not pronounced suggesting inefficient replication of variola in NHPs. Additionally, and most importantly, the fatality rate in this model is not predictable as it has been irreproducible in a number of studies. High mortality, or at least predictable mortality, would be necessary for antiviral evaluation. Without that, in order to provide adequate statistical power for comparison of treated animals to untreated controls, large numbers of monkeys would be required – a condition that could not be met considering the limitations in conducting research in the ABSL-4 at the CDC.

Research with live variola virus in the US is limited to the BSL-4 facilities at the CDC. Smallpox research activities must be approved by the WHO and are conducted according to priorities set by WHO and CDC. Research at the CDC is not GLP-compliant and will likely not be for the foreseeable future. Performance of assays is restricted to the availability of equipment and trained personnel within the ABSL-4 facilities as biological samples potentially containing

live variola may not leave the BSL-4 space. As such, pharmacokinetic evaluation of ST-246 in a variola-infected NHP is not possible since those capabilities presently do not exist at the CDC. All procedures must be conducted by personnel wearing a one-piece positive pressure suit ventilated with a life support system which limit mobility and agility in handling dangerous infectious samples and sharp objects.

2.2.3 IV MPXV MODEL

There are numerous published reports in which experimental MPXV infection of *M. fascicularis* and *M. mulatta* by IV injection using 1 – $5x10^7$ pfu ($5x10^7$ most common) are utilized as a model for human smallpox disease (Table 2.2.1-1). The model is most commonly used because "a consistent upper respiratory tract monkeypox model is not available… (W)e used intravenous injection, which effectively bypasses the incubation and prodromal phases of naturally acquired orthopoxviral infections in humans, by creating instantaneous viremia and systemic spread of virus to target organs. Intravenous inoculation is probably the most rigorous challenge route."[(63)] According to Hooper *et al*[(62)] "The most dramatic clinical manifestation of monkeypox in the challenged monkeys, other than death (which is > 95% in this model), was the generalized vesiculopustular rash. The rash was first evident 6 days post-challenge and progressed from macules to papules, to vesicles, to pustules and finally to crusts over 10 days." In recent well-controlled NHP studies using the standard challenge dose of $5x10^7$ pfu, rash is first evident in 3-4 days post challenge. "As in naturally occurring human monkeypox and smallpox, the distribution of the lesions was primarily on the face and hands but rarely on the abdomen. The sex skin, nipples, and buttocks were also commonly affected." Additionally, "The monkeys that died…presented a disseminated exanthematous rash, marked lymphadenopathy, mild splenomegaly, mild pulmonary edema, and a notable absence of remarkable pathology in other organs. Except for lymphadenopathy, which is a clinical symptom of monkeypox, the necropsy findings are similar to those of autopsies of human smallpox fatalities." In a separate study[(70)] "All (monkeypox infected animals) had pathological findings consistent with death due to monkeypox infection… Lesions considered related to monkeypox infection also include necrosis of the liver, lymph nodes, spleen, testes and ovaries, and incidence of focal to multifocal fibrinohemorrhagic bronchitis and aveolitis." In all studies examined, viremia is detectable by qPCR as early as Day 2 post-challenge and throat swabs are positive starting as early as Day 4 post-challenge. Some animals develop fever as early as 2 days post-infection while all animals have fever by at least 4 days post-infection.

Recent studies conducted by USAMRIID and by several other contract research organizations have found very similar results as those described above. Animals challenged with $5x10^7$ pfu of a standardized, commercially available strain of MPXV form lesions, which serve as the therapeutic trigger, within 3-4 days post challenge. Untreated control animals or those animals treated sub-optimally become progressively more ill and die within 7-17 days post challenge. All animals demonstrate an increase in viral load post-challenge indicating that the MPXV is replicating in tissues resulting in a productive infection. Thus, it is the IV MPXV NHP model that is characterized by post-exposure fever, a productive viremia and the eruption of a significant number of skin lesions that progress through the characteristic developmental and resolutional phases of human smallpox disease. Further, the model is consistently lethal, highly reproducible and sets a high standard for the therapeutic evaluation of a drug, all of which are lacking in the IV VARV model.

2.2.4 AEROSOL MPXV MODEL

"The principal distinguishing feature of infection by aerosolized monkeypox virus in cynomolgus monkeys was severe fibronecrotic bronchopneumonia."[56] According to Zaucha *et al.*, the clinicopathologic features of aerosolized monkeypox resembled features observed in humans infected with monkeypox or smallpox. Following infection, "monkeys began to show evidence of exanthema, enanthema, mild anorexia, fever, cough and nasal discharge on Days 6 and 7 post-exposure. Dyspnea, noted as early as Day 8 post-exposure, was evident in all animals by Day 10. By Days 9 and 10, all animals had exanthema and enanthema, were depressed and severely anorexic, and showed signs of weakness. Clinical signs progressed until all animals died naturally or were killed 9-17 days post-exposure." While the route of infection may more closely resemble human smallpox disease, the presence of the major clinical manifestations and therapeutic trigger, skin lesions, were absent, or widely variable at best. Skin lesion formation varied from barely detectable small papules to extensive involvement of the inguinal regions, chest and stomach. No other therapeutic trigger has been identified. "Systemic viral dissemination appeared to occur through a monocytic cell-associated viremia (no cell-free viremia was noted at any time), resulting in lesions affecting the lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive system, and liver." Viremia was detected in 1 of 8 animals on Day 6, 9 of 11 animals on Day 9, and 2 of 7 on Days 12 or 13. Death was attributable to bronchopneumonia, with airway epithelial cells serving as the primary target of infection. This model is insufficient for evaluation of antivirals since it does not mimic human smallpox in a number of ways: First, the cause of death is fibronecrotic bronchopneumonia. Pneumonia was often observed in human smallpox but it was most commonly characterized as interstitial pneumonia. While the fatality rate is high, it is significantly less than 100% and may be variable depending on the reproducibility in delivering the intended challenge dose. Second, aerosol challenge produces a mildly lesional disease at best. In most cases, death may occur prior to the formation of lesions or after the formation of < 10. A high number of lesions may form, but only in those animals that survive the challenge. In this model, no therapeutic trigger has been identified.

2.2.5 INTRANASAL MPXV MODEL

This model is non-lethal for the majority of animals challenged with virus doses of $1x10^7$ pfu or lower. Reproducible, high mortality rates require challenge doses of $4x10^8$ pfu or higher. Following IN challenge with 10^6 pfu of MPXV strain Liberia, body weight decreased sharply after challenge and by Day 10 post-challenge, monkeys exhibited a loss of appetite, rhinorrhea, conjunctival discharge, diarrhea, and a skin rash.[58, 59] All symptoms disappeared by Day 20 post-challenge except for the skin lesions. Time to resolution of skin lesions was not reported for this study as animals were sacrificed for virological and histological examination at 21 days post-challenge. In a separate study, utilizing the Zaire 79 strain as the challenge agent, it was determined that challenge doses of $1x10^7$ pfu or lower caused mild disease, few lesions (< 25 total) and low mortality (25%). The estimated challenge dose (based on an in vivo titration) for severe/lethal disease in 90% (SD90) of the animals following intranasal challenge was 1.7x109 pfu. A dose of 4x108 pfu was used since the stock concentration of virus would not allow for a higher challenge. Challenge with $4x10^8$ pfu resulted in high mortality (5 of 6 animals) although the surviving animal exhibited only mild disease manifestations. Death or euthanasia occurred between Days 8 and 14 post-challenge which corresponded with peak lesion counts. The initial abnormal clinical signs following challenge were present on Day 2 (although not uniform for all animals) and increased through Day 14 (including coughing, sneezing,

hunched posture, lethargy, nasal or ocular discharge), with lesion development on the arms, legs, ventral torso, and head not observed until Day 6. All animals challenged at this dose showed an average decrease in weight over time that correlated with animals exhibiting signs of monkeypox infection. Virus was detected in whole blood, peripheral blood mononuclear cell, nasal swab, throat swab, and terminal tissues using PCR and plaque assay. Essentially, no aspect of this model is suitable for drug evaluation.

2.2.6 INTRATRACHEAL MPXV MODEL

Intratracheal challenge with 10^6 pfu of MPXV strain MSF#6 also was not lethal. Animals became feverish at Day 6 post-challenge and on Day 11 post-challenge skin lesions were evident and increased in number to more than 100 per animal by Day 14. Lesions fully resolved by Day 28 post-challenge. Animals were lethargic on Days 19-21. Body temperatures were elevated from Day 6-12 post-challenge. The lungs showed mild pulmonary lesions comprising small foci of necrosis surrounded by inflammatory cells. A one-log increase in the challenge inoculum resulted in uniformly lethal infection. Animals challenged with 10^7 pfu had elevated temperatures from Day 5 until death. On Days 8-10, lesions formed that were more pronounced than the animals challenged with the lower dose and progressed to typical pustules. Clinical signs deteriorated until animals died or were euthanized between Days 15 and 19 post-challenge. A combined analysis of survival data from two reports[58, 59] results in a mean day of death of 12.6 days (range 9–19). Histological examination of the lungs revealed severe macroscopic lesions, which were characterized as fibronecrotic bronchopneumonia. Other changes noted in these animals included tracheitis, necrotizing glossitis, lymphadenitis, and splenitis with lymphoid depletion. Throat swabs were positive for virus by Day 4 post-challenge at both challenge doses. Viremia was detected by qPCR from Day 4-16 for the low dose challenge and from Day 4 until death for the high dose challenge. The cause of death was attributed to fibronecrotic bronchopneumonia, which is not the cause of death in human smallpox disease. These results are somewhat contradictory to the data reported by other investigators. An intratracheal challenge with the high dose (10^7 pfu) of MPXV Zaire 79 resulted in lethal disease with the development of only a few number of skin lesions (less than 25 per animal).[72] Challenge with lower doses was inconsistently lethal but resulted in the formation of numerous skin lesions. This study concluded that intratracheal challenge, however, likely was not the optimal route of infection since the animals succumbed to respiratory distress due to bronchopneumonia before systemic pox infection developed.

Recently a new intratracheal challenge model was introduced.[57] Prior intratracheal challenges involved delivery of a bolus dose of virus in liquid suspension while the new method delivered virus directly to the tracheal carina as a large particle aerosol via microspray device. While the particle size (~ 8 µm) is larger than that typically generated by collison nebulizers (~ 1-3 µm), this method consistently allowed for a dose-dependent incubation period before onset of clinical disease, allowed for development of a disease that resembles classic, ordinary smallpox disease with systemic dissemination, and resulted in pathology that is consistent with inhalation of monkeypox virus. In addition, the amount of virus required to produce significant disease was lowered by at least a log compared to the intravenous infection model although mortality was not uniform at any challenge dose. Major pathological findings, which included viremia, lesion counts, histology and immunohistochemistry of all major organ systems, also were not dose-dependent. The day of the first appearance of lesions was dose dependent: At high doses, lesions appeared starting on Day 4 post-challenge, while low-dose challenge resulted in lesions

appearing first on Day 8. Lesion counts were similar regardless of challenge dose although animals in the high-dose challenge group were more varied in number. Challenge doses less than 10^7 pfu resulted in maximum lesion counts of ranging from 250-600 per animal while the high dose challenge resulted in lesion counts ranging from 25-350. Additionally, no dose evaluated ($3.42x10^6$ pfu-$3.53x10^7$ pfu) produced uniform mortality. One of 3 animals died following the low dose challenge (Day 12 post-challenge) while 2 of 3 died following the high dose challenge (Day 8 post-challenge). However, the disease does not closely resemble the ordinary human smallpox disease because approximately one third of the animals (regardless of the challenge dose) had clear manifestations of hemorrhagic disease and the cause of death was likely fibronecrotic pneumonia, although there was clear evidence of viral pathology in numerous organ systems. Without further development, this model is insufficient for antiviral evaluation. The inconsistent mortality, lack of correlation between clinical observations of disease severity (such as lesion counts) and survival outcome, death due to fibronecrotic pneumonia, and atypical disease (hemorrhagic) in one third of the animals (regardless of challenge dose) undermine the validity of this model.

2.2.7 SUBCUTANEOUS MPXV MODEL

Two reports describe the use of a subcutaneous monkeypox challenge as a model for human smallpox disease. One report[61] does not contain sufficient information to allow an evaluation of the model. The second report[60] is more detailed. Subcutaneous infection of *M. fascicularis* with 10^6 pfu of MPXV Zr-599 resulted in uniform lethality (2 of 2 dead) by Days 14-18 post-challenge. Body weight decreased 15% from the time of challenge until death. Papulovesicular skin lesions appeared on Day 7 post-challenge (390-1150 lesions per animal). In addition to the skin lesions, "lesions were detected in the lymphoid systems (lymph nodes, thymus, and tonsils), respiratory tract structures (lung and trachea), digestive organs (stomach, small intestine, colon, rectum, and liver), urogenital tract (bladder, uterus, and ovary)" at the time of death. C-reactive protein (CRP) levels were measured as an indicator of inflammatory processes. CRP levels were significantly increased in challenged animals. The pro-inflammatory cytokines IFN-γ and IL-6 were elevated in challenged animals. Viremia was analyzed by qPCR and all animals were positive between Days 3 and 18 post-challenge. The cause of death is not reported. In addition to the incomplete development and characterization, this model remains insufficient due to the subcutaneous challenge route.

2.2.8 MARMOSET MODEL DEVELOPMENT

A cowpox-like virus was isolated from a lethal orthopoxvirus ("calpox") outbreak in New World monkeys.[73, 74] In attempts to establish a model using this virus, early studies demonstrated that marmosets infected with calpox virus, not only via the intravenous but also the intranasal route, reproducibly develop symptoms resembling smallpox in humans.[74] Infected animals died within 1-2 days after onset of symptoms (12-15 days post-challenge), even when very low infectious viral doses of $5x10^2$ pfu were applied intranasally, although there was 60% survival at this challenge dose. Infectious virus was demonstrated in blood, saliva and all organs analyzed. *In vivo* titration resulted in an MID_{50} (minimal monkey infectious dose 50%) of $8.3x10^2$ pfu of calpox virus. Very few lesions were noted following intravenous challenge and were observed only upon post-mortem examination. Following intranasal challenge, multiple lesions were evident on the face, abdomen, and thighs 1-2 days prior to death. With death occurring within 1-2 days after the first appearance of any clinical symptom, it would seem that there is no therapeutic trigger for intervention in this model of disease. This model has not been fully

established as valid although these initial studies seem to indicate that there are some features that might make it an alternate model for studying the pathology of smallpox. The use of a surrogate virus such as calpox (derived from cowpox), which causes mild disease in humans, as well as the short time to death (in marmosets) following the appearance of disease symptoms underscore that this model is not ideal for evaluation of antiviral therapeutics. Antiviral efficacy (or vaccine efficacy) has not been evaluated in this model nor is it known how well pharmacokinetics in the marmoset will translate into human. In addition to the significant differences in the kinetics of disease, and the use of a surrogate virus that does not cause significant disease in humans, no immunological reagents are available for this model. The role of immunity in human smallpox is well-known but this cannot be evaluated in this model while it is possible in other models.

2.2.9 CONCLUSIONS ON NHP MODELS

An appropriate animal model should recapitulate various aspects of human disease. Specifically, the virus should cause a systemic disease and not be mainly restricted to a single organ as is the case in the aerosol and intratracheal challenge models. Viral infection should target multiple organ systems with significant pathology attributable to inflammatory mediators in addition to direct viral cytotoxicity. Lesions should form and develop in phases similar to human smallpox lesions with a similar distribution, which would serve as a therapeutic trigger. A comparative summary of pathology findings in human smallpox and those in variola and monkeypox NHP models is presented in Table 2.2.9-1 and a comparison of NHP models and surrogate endpoints for human smallpox is shown in Table 2.2.9-2.

Table 2.2.9-1: Comparative Pathologies of Human Smallpox and NHP Models

Features	Smallpox[a]	IV MPX in NHP[b]	IV VARV in NHP[c]
Pox Lesions			
Skin lesion appearance	papule,vesicular,pustule following secondary viremia (~ Day 16 post-exposure)	papule,vesicular,pustule starting 3-4 days post-IV inoculation	papule,vesicular,pustule starting 3-4 days post-IV inoculation, 1 at 2 dpi
Stages of lesion development	rash/papule/vesicle/pustule/ scab	same	same
Other locations	Pharynx, uvula, larynx, tongue, bronchi, trachea, esophagus, stomach, intestine, bladder, urethra, uterus, vagina	oral mucosa, soft/hard palate, tongue, gum, lung, esophagus, stomach, ileum, colon, bladder	oral mucosa, tongue, gum and colon
Lesion Counts	Average > 250 (peak 7-10 days post-rash formation)	Peak means range from 1,147-1,492 (Days 10-12)	Peak means range from 350-> 1,000 (Days 7-9)
Principal Organs Affected[d]			
Spleen	congestion, lymphocytic expansion probably in response to infection	congestion, lymphocytolysis/lymphoid depletion followed by increased plasma cells indicating immune response to infection	congestion, lymphocytolysis/lymphoid depletion followed by increased plasma cells indicating immune response to infection
Liver	enlarged, hepatocellular degeneration, fatty metamorphosis, necrosis, lymphocytic/macrophage infiltration	hepatocellular degeneration, lipid-type vacuolation of hepatocytes, necrosis, leukocytosis	hepatocellular degeneration, lipid-type vacuolation of hepatocytes, necrosis, leukocytosis
Lung	interstitial pneumonia	pneumonia/interstitial pneumonia (edema, congestion, necrosis)	pneumonia/interstitial pneumonia
testis	necrotic/hyperemia/ lymphocytic inflitration	necrotic/hemorrhage/ inflammation	degeneration/necrosis/ hemorrhage/inflammation
Lymph Nodes	edema/necrosis/hemorrhage/ mononuclear response	necrotizing lymphadenitis/ lymphoid hyperplasia/ edema	lymphoid hyperplasia
Bone marrow	necrosis, proliferation of premyelocytes	myeloid hyperplasia	myeloid hyperplasia
Kidney	acute tubulointerstitial nephritis	non-specific chronic interstitial nephritis	non-specific lymphoplasmacytic inflammation
Central Nervous System	edema, perivascular infiltration, congestion in brain & spinal cord	lymphoplasmacytic inflammation with no viral staining	non-specific lymphoplasmacytic inflammation
Lymphadenopathy[e]	not reported	yes	yes

[a]From Martin D.B. 2002 {Ref. 15} and Fenner F. et al 1988 {Ref. 8}
[b]Untreated animal data from Studies VO4-16 and AP-06-21 (SN:0045), FY06-035 (SN:0098), and 463-G607607 and 466-G607607 (PIND 109254 cross-reference submission)
[c]Untreated animal data from Study 1400JAHMONC (SN: 0045), ST246-1745 (SN: 0146), and Wahl-Jensen V. et al 2011 {Ref. 85}
[d]List of pricipal organ affected is based on smallpox disease described in Martin D.B. 2002 {Ref. 15}
[e]Lymphadenopathy is added to the list since it has been shown to be a clear difference between MPXV/VARV in NHPs and smallpox in humans

Table 2.2.9-2: Comparison of NHP Models and Surrogate Endpoints for Human Smallpox

Model	Lethality	Cause of Death	Fever	Viremia	Lesion
Variola IV	10^9 pfu: 100%, ~ d6	Organ dysfunction, multisystem failure, coagulopathy, hemorrhage	Day 3+	Day 2+	Day 3+ (hemorrhagic)
Variola IV	10^8 pfu: 0-33%	Organ dysfunction, multisystem failure, hemorrhage in some animals	Day 2+	Day 3+	Day 3+ ("classical")
Variola IV	10^8 pfu: 0%	Not applicable	Day 2+	Day 3+	Day 3+
MPXV IV	100%: d7-14	Organ dysfunction, multisystem failure	Day 2+	Day 3+	Day 3+ ("classical")
MPXV aer.	80-100%: d9-17	Fibronecrotic bronchopneumonia	Day 6+	Day 6+	Day 6+ (modified)
MPXV IT	10^6 pfu: 0% 10^7 pfu: 100% d15-19	10^6 pfu: N/A 10^7 pfu: Fibronecrotic bronchopneumonia	Day 5+	Day 4+	Day 7+
MPXV IN	0%	N/A	Day 10+	Day 4+	Day 10+
MPXV SC	100%: d14-18	Not reported	Not reported	Day 3+	Day 7+

In addition, the challenge model system should result in uniform (near 100%) lethality. The IV MPXV NHP model satisfies all the above considerations. There has been a suggestion that the variola challenge in monkeys is an appropriate model for human smallpox specifically because it resulted in ~ 33% mortality similar to human smallpox in a single study of 6 cynomolgus monkeys. This observation presents a number of challenges.

- First, in order to demonstrate efficacy in this model, much larger group sizes would be required in order to achieve statistically significant outcomes. Smaller group sizes would be required if using an animal model in which lethality is closer to 100%.
- Second, although variola infection in monkeys in many ways recapitulates human smallpox disease, the lethal form of the disease in NHPs was characterized as more similar to hemorrhagic smallpox in humans as opposed to the much more commonly occurring typical smallpox (variola vera).
- Third, in evaluating therapeutic efficacy, it will be difficult to attribute survival to drug action since VARV is a human-specific pathogen and does not replicate efficiently in NHPs.

There are additional advantages to using the IV MPXV model, in addition to being lethal and reproducible while mimicking many aspects of human smallpox, including a therapeutic trigger. Biosafety Level 3 (BSL-3) is required for MPXV research while BSL-4 is required for variola. Therefore, MPXV studies may be conducted according to GLP standards at a number of research organizations while variola research is limited to a single facility in the U.S. (CDC, Atlanta), which is not GLP compliant. Approval to conduct variola research must be granted from the WHO. There are limited analytical capabilities within the BSL-4 suite at CDC and many types of samples may not be removed from containment, for example virus samples cannot be removed from the BSL-4 suite for resistance analysis and PK analyses cannot be carried out in VARV-infected animals since samples cannot be aerosolized to allow for quantitation by mass

spectrometer. These types of limitations do not exist with the MPXV NHP model. Based on this, we conclude that the only NHP model that is appropriate for definitive demonstration of the efficacy of anti-smallpox therapeutics is the MPXV Zaire 79 $5x10^7$ pfu IV challenge in *M. fascicularis* monkeys.

2.2.10 EXPERT ADVISORY PANEL REPORT – SUMMARY EVALUATION OF ANIMAL MODELS OF VARIOLA VIRUS DISEASE

Our conclusions are supported by leading scientists in the field. In response to a request by FDA, SIGA convened an "Advisory Panel" on March 24, 2010 in Washington, DC, to assess the strengths and weaknesses of two NHP models (variola vs. monkeypox) in terms of their likelihood of predicting efficacy and optimal dosage of ST-246 to treat cases of human smallpox disease. The Panel Members include: Inger Damon, M.D., Ph.D., Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention; Arthur J. Goff, PhD., Virology Division, US Army Medical Research Institute of Infectious Diseases; and Grant McFadden, PhD. Molecular Genetics and Microbiology, University of Florida and WHO Variola Advisory Committee (past member – currently an advisor to the committee). After review of the relevant literature and recent data from studies initiated by SIGA they conclude:

"From our review of the literature and examination of the newer data provided by SIGA, the design of an animal model to faithfully reproduce "human smallpox," or a particular illness manifestation of the previously characterized human disease, has not been fully successful. Similarly, the Institute Of Medicine report "Live Variola Virus: Considerations for Continuing Research" released in 2009 recommended that the variola nonhuman primate challenge be re-evaluated for potential refinement. If variola studies, using the 10^8 pfu challenge model, were to be supportive of efficacy claims of an antiviral treatment, additional information that would be important to contrast across the various variola and monkeypox nonhuman primate studies to understand the apparent non-reproducibility of all disease parameters in the variola studies would include, but not be limited to: the animal weights, ages, sources, and baseline laboratory studies used in the various challenge studies. Ideally, the demonstration of efficacy of an antiviral against variola in an *in vivo* challenge study would be important to support efficacy of an antiviral treatment for human smallpox. CDC is working with HHS to assemble a panel of experts to review and suggest refinements to the variola challenge/non human primate model, as suggested by the IOM (Institute of Medicine). It is not possible to predict when these findings will be available, nor if their recommendations will be predictive of a model with greater reproducibility, or greater similarity to human smallpox. Similarly, the timeline for WHO approval to use live variola in any new revised proposal is not possible to predict. In contrast, the monkeypox Zaire 79 $5x10^7$ infectious particle IV challenge, with initiation of treatment at lesion onset, has been reproducibly used to evaluate survival, and overall benefit/efficacy, of antiviral treatment for systemic, febrile rash during orthopoxvirus illness in nonhuman primates. Furthermore, the sensitivity of monkeypox and variola virus to ST-246 in cultured cells is very similar, consistent with the close sequence similarity between the orthologous viral protein (F13L) that is targeted by this drug. Although there are differences in manifestations of human monkeypox and human smallpox disease pathogenesis (most notably the presence of lymphadenopathy in human monkeypox, also demonstrated in the monkeypox nonhuman primate model), we conclude that the reproducibility of the monkeypox/nonhuman primate model, and its close similarity to the exanthematous phases of human monkeypox/variola disease, makes it the best currently available nonhuman primate model for evaluation of potential

antiviral efficacy of ST-246 for treatment of human smallpox." (The full report and a biography of each member are provided in Appendix B.)

2.3 Proposed Pivotal Efficacy Study in NHPs

Considering the endorsement by the scientific community and specifically, the stated conclusions of the independent Expert Advisory Panel, that the $5x10^7$ pfu IV challenge with the Zaire 79 strain of MPXV in NHPs is the best available model for drug evaluation, SIGA proposes to conduct a pivotal efficacy study in NHPs utilizing this lethal IV MPXV model treated therapeutically with the human equivalent dose of ST-246. The protocol design will be a randomized, placebo-controlled, double-blind, GLP study conducted at more than one site.

Title: Randomized Double-Blind Placebo-Controlled Study to Evaluate the Efficacy of ST-246 Therapy Following Lethal Intravenous Monkeypox Virus Challenge in Cynomolgus Macaques

Objective: This randomized double-blind placebo controlled study will evaluate the therapeutic efficacy of the human equivalent dose of orally administered ST-246 against a lethal intravenous challenge of monkeypox virus. ST-246 therapy will be initiated at lesion onset. Survival will be monitored for up to four times the mean time to death (56 days) in this model. Animals will be evaluated daily for clinical signs of disease (including lesion counts) and blood will be collected to assess viremia, hematology, clinical chemistry, and for bioanalysis of ST-246. Additionally, immune responses (serum humoral responses and cytokine responses) to the challenge virus will be evaluated. Throat swab samples will be collected to assess viral shedding. Euthanasia of severely ill/moribund animals will be performed according to prospectively defined objective criteria. Necropsy will be performed on all animals dying at unscheduled intervals and on those animals surviving to study termination, which will be scheduled for euthanasia. Tissue viral load will be evaluated in tissues collected at necropsy. A Resistance Monitoring Plan will be employed to monitor the occurrence and determine the frequency of drug resistant virus arising in the context of ST-246 therapy of ongoing MPXV disease. Study personnel will remain blinded to all aspects of the study to including but not limited to, challenge, treatment, and sample and tissue identification. A prospective Statistical Analysis Plan will be employed in the evaluation of data from this study.

Primary Endpoint

a) Survival, i.e., the proportion of those animals that survive to those animals that die (including those animals euthanized due to a moribund condition) through Day 56 in both the ST-246 and placebo groups.

Secondary Endpoints

a) Impact of ST-246 on viremia, i.e., viral DNA in the blood (log_{10} viral load; genomes/mL/day), where specific criteria will include: (i) the average observed viral load over the treatment period; (ii) daily change from Day 2 in viral load observed at each post-treatment day through Day 56; and (iii) the maximum viral load observed from Day 2-56 (inclusive).

b) Impact of ST-246 on lesion counts, i.e., the total lesion count will be evaluated to measure the severity of MPXV disease and its clinical symptoms. The total lesion count will be calculated over the treatment period, i.e., from post-challenge Day 2-56 (inclusive).

Additionally, time (days) to scabbing of dermal lesions from infection day (Day 0) will be assessed, where specific criteria will include: (i) the onset of lesion scabbing in any body region; and (ii) the maximum value of lesion scab counts.

c) Impact of ST-246 on immune responses to MPXV. Humoral immune responses will be evaluated in blood samples collected at various intervals following challenge to monitor the induction of protective immunity in the presence and absence of ST-246. Additionally, the presence of inflammatory cytokines will be evaluated in serum samples to monitor the inflammatory response to infection and the impact of ST-246 on inflammatory responses.

d) Evaluation of the occurrence and frequency of ST-246 resistant virus in NHP/MPXV studies arising as a result of treatment in a disease setting.

Study Overview: Healthy male and female cynomolgus macaques, pre-screened and determined to be orthopoxvirus naïve will be randomly distributed into two groups. Within each group, the male:female ratio will be 1:1. Study personnel will remain blinded to all aspects of the study to including but not limited to, challenge, treatment, and sample and tissue identification prior to assay or evaluation. Baseline measurements for all parameters evaluated in this study will be determined prior to infection. All animals will be lethally challenged with $5x10^7$ pfu MPXV Zaire 79 by the IV route on Day 0. At lesion onset, placebo treatment will be initiated in Group 1 and ST-246 treatment will be initiated in Group 2. Study personnel will be blinded to the treatment. Clinical observations, lesion counts, photographs, blood collections, and swabs will occur at regularly scheduled intervals. Animals in moribund condition will be euthanized according to prospectively defined objective criteria. The study will be terminated at 56 days post-challenge, which is 4 times the mean time to death for untreated controls in this model. All animals will be necropsied at euthaniasia (unscheduled or scheduled) and viral load in tissues determined by qPCR and the presence of viral antigen evaluated by immunohistochemistry. All tissues will be examined by a pathologist and an independent Pathology Report will be generated. Survival, lesion counts and viremia (qPCR) data will be analyzed according to the Statistical Analysis Plan. Swab samples will be evaluated by qPCR to evaluate virus shedding from the oral mucosa. Anti-orthopoxvirus ELISA and plaque-reduction neutralizing titers as well as inflammatory cytokine responses will be evaluated in serum samples collected throughout the study. DNA isolated from the blood for the purpose of evaluating viremia will also be utilized to monitor drug resistance according to the Resistance Monitoring Plan in Table 2.3-1.

Table 2.3-1: Study Group Assignment

Group	MPXV Challenge Dose (Day 0)	ST-246 Dose Level	Dosing Schedule
1 – Placebo	$5x10^7$ pfu IV	0 mg/kg	14 daily doses starting at lesion onset
2 – ST-246 @ lesion onset	$5x10^7$ pfu IV	10 mg/kg	14 daily doses starting at lesion onset

KEY: pfu = plaque-forming units; IV = intravenous

This animal study is designed to specifically address the central tenets of the Animal Rule, and takes into account Guidances and Concept Papers published by the FDA on the Animal Rule and

developing drugs for treatment or prevention of smallpox infection. SIGA has conducted or sponsored 28 small animal efficacy studies and 11 NHP efficacy studies (presented in Appendix A, and Section 3.2). In these studies, we have evaluated ST-246 antiviral efficacy against 6 different orthopoxvirus species in 5 animal species. Based on these experiences, we conclude that the IV MPXV challenge in NHPs represents the best available model for drug evaluation, although it has some limitations. These are addressed below:

- ***Authentic Etiologic Agent***

MPXV is being used as a surrogate for the authentic etiological agent of smallpox, i.e. VARV, in this study. MPXV is an appropriate surrogate for a number of reasons. The progression of lethal pathogenic events following MPXV infection of NHPs is more similar to human smallpox than challenge with high dose VARV, since atypical hemorrhagic death occurs before the onset of lesions at high dose VARV challenge. In addition, although low dose VARV challenge produces lesional disease like smallpox, the death rate is highly variable and irreproducible. Most importantly, there is significant genetic conservation between VARV and MPXV and the genomic sequence of the protein targeted by ST-246 is 98% conserved between the two viruses with a 100% conservation in the region of the protein targeted by the drug.

- ***Clinical Manifestations of Disease and Outcome***

As discussed in detail above, smallpox was characterized by a relatively long (7-17 days) asymptomatic incubation phase during which a low-level primary viremia was detectable, followed by a febrile prodromal stage associated with high-level secondary viremia, then the appearance of numerous lesions progressing through characteristic stages of severity, and a case fatality rate of 30%. The asymptomatic incubation phase is essentially absent in MPXV infected NHPs (as it is in VARV-infected NHPs). Following IV inoculation of MPXV in NHPs, virus is immediately detectable in the blood stream more closely mimicking the secondary viremia of human disease. Within 2 days post-infection, the animals become febrile and by Days 3-4 post-infection lesions become evident. The mean time to death is 14 days. The absence of an incubation phase and immediate high-level viremia followed by rapid onset of clinical symptoms establishes this animal model as more demanding than the clinical scenario, setting a high bar for antiviral efficacy. Regarding the mortality rate, although the case fatality rate for human smallpox was ~ 30%, this would be undesirable in an animal model as it would require very large numbers of animals in order to provide statistical power. The IV MPXV in NHPs is nearly uniformly lethal allowing for the use of the smallest number of animals in order to establish a statistically significant impact of ST-246 on disease parameters such as survival, lesions counts, and viremia.

- ***Challenge Route***

Smallpox was most often transmitted via inhalation of large particle aerosols. As such, the aerosol route of exposure is of particular relevance in establishing smallpox disease models in animals. As discussed in Section 2.2.4, aerosol exposure of NHPs with MPXV results in lethal disease in the majority of animals challenged with an appropriate dose ($1x10^5$ pfu) but clinical manifestations of disease and the cause of death are different from the cause of death for human smallpox. As discussed in Section 1.3, the cause of death in smallpox was attributed to toxemia (likely what is now referred to as cytokine storm) and (based on the pathology record reviewed by Martin) direct cytopathic effect on multiple organs. *Interstitial* pneumonia was certainly a feature of human smallpox. The cause of death following aerosol challenge with MPXV is

fibronecrotic pneumonia. Although virus disseminates to additional organs based on the detection of viral DNA, there is little evidence of direct cytopathic effect in those organs. Additionally, aerosol challenge does not produce a significant number of lesions characteristic of classical smallpox. In some instances, death occurs prior to the appearance of any lesions and, if lesions appear prior to death, they may be as few as 10 or less over the entire body and form approximately 3 days prior to death. In contrast, the IV challenge route reproducibly results in the appearance of hundreds, even thousands, of lesions distributed in a centrifugal pattern characteristic of smallpox. Lesions appear 3–4 days post challenge, approximately 10 days prior to death as was typical for human smallpox. Clinical manifestations of human smallpox were not the result of the initial aerosol exposure but were the result of the secondary viremia – which is accurately modeled by the IV MPXV challenge.

- ***Challenge Dose***

The infectious dose of smallpox in a susceptible individual was thought to be between 10 and 100 infectious particles and may even have been as low as 1 infectious particle. Animal models in which infection is established following exposure to a very low dose of virus are therefore desirable. However, an infection established by the intravenous administration of a high dose of virus sets a high bar for the evaluation of antiviral efficacy since it results in the seeding of all major organs from the onset. The uniform clinical disease and mortality observed in NHPs challenged with $5x10^7$ pfu MPXV Zaire 79 by the IV route, thus, represents a highly vigorous model for evaluation of ST-246 efficacy.

- ***Challenge Strain***

The Zaire 79 strain of MPXV is the most appropriate strain for use in animal efficacy studies, conducted in NHPs. This is based on 1) review of the relevant scientific literature and records of MPXV outbreaks involving West and Central African strains, 2) comparison of genomic sequences from West African, Central African, and US strains of MPXV, variola virus (VARV), and other orthopoxviruses, and 3) comparison of MPXV virulence in animals. While the Zaire 79 virus isolate is not derived from a fatal human case, it was isolated during a geographically limited outbreak in which the case fatality rate was as high or higher than other recorded outbreaks by other Central African strains. Virus isolates of other strains from fatal cases cannot be reliably regarded as more virulent as, in each case, co-morbidities were present, which may have contributed significantly in each death. Collectively, these observations strongly support the view of the scientific community that Zaire 79 is a highly pathogenic virus and is not attenuated compared to ZAI-1996-016 or other Central African Strains of MPXV. The overwhelmingly extensive uses of Zaire 79 in intravenous NHP challenge studies in the literature, including for FDA CBER's approval of the smallpox vaccine ACAM2000™ as a replacement for Dryvax,®[70] serve as testimonies for the scientific community's acceptance of the pathogenesis of this MPXV strain as a rigorous disease model for the evaluation of prophylactic or therapeutic interventions against OPXV infections. NIAID has commissioned Biodefense and Emerging Infection Research Resources Repository (managed by the ATCC) to produce and supply to researchers in the field the Zaire 79 strain as the standard stock strain for challenge studies.

2.4 PK/PD Modeling

2.4.1 USE OF PK/PD MODELING TO DETERMINE ST-246 EFFICACIOUS DOSE

Given the necessity of using animal efficacy data to provide support for the licensure of a smallpox therapeutic, two assumptions must be made. First, the effect of orthopoxvirus infection on the pharmacokinetics of a therapeutic compound will be similar in different hosts infected with different species of orthopoxviruses. Second, the primary efficacy animal model must provide a quantitative assessment of the therapeutic potential of a drug candidate that is predictive for outcome in humans. Since variola virus has a limited host range, few animal models have been developed and existing models have highly variable endpoints making these models inadequate for definitive assessment of an antiviral product. Thus, models with well defined endpoints that use surrogate orthopoxviruses will provide critical data for antiviral evaluation. Models developed in non-human primates (NHPs) infected with monkeypox virus via intravenous injection produce a uniformly lethal infection with high viremia and exanthematous disease resembling human smallpox. Given the similarity in ST-246 drug pharmacokinetics between NHPs and humans, this animal model offers an ideal tool for assessment of antiviral efficacy.

The IV infection monkeypox virus model of orthopox disease in NHP is ideal for evaluating efficacy of a smallpox therapeutic. The disease caused by monkeypox virus infection is almost uniformly lethal with a mean time to death of 14 days after IV infection, lesion onset between Day 3 and 5 post-infections serves as a useful therapeutic trigger, and disease progression (viremia and lesion formation) resembles that of human smallpox. Given the uniform lethality, survival serves as the primary and preferred endpoint in this model system.

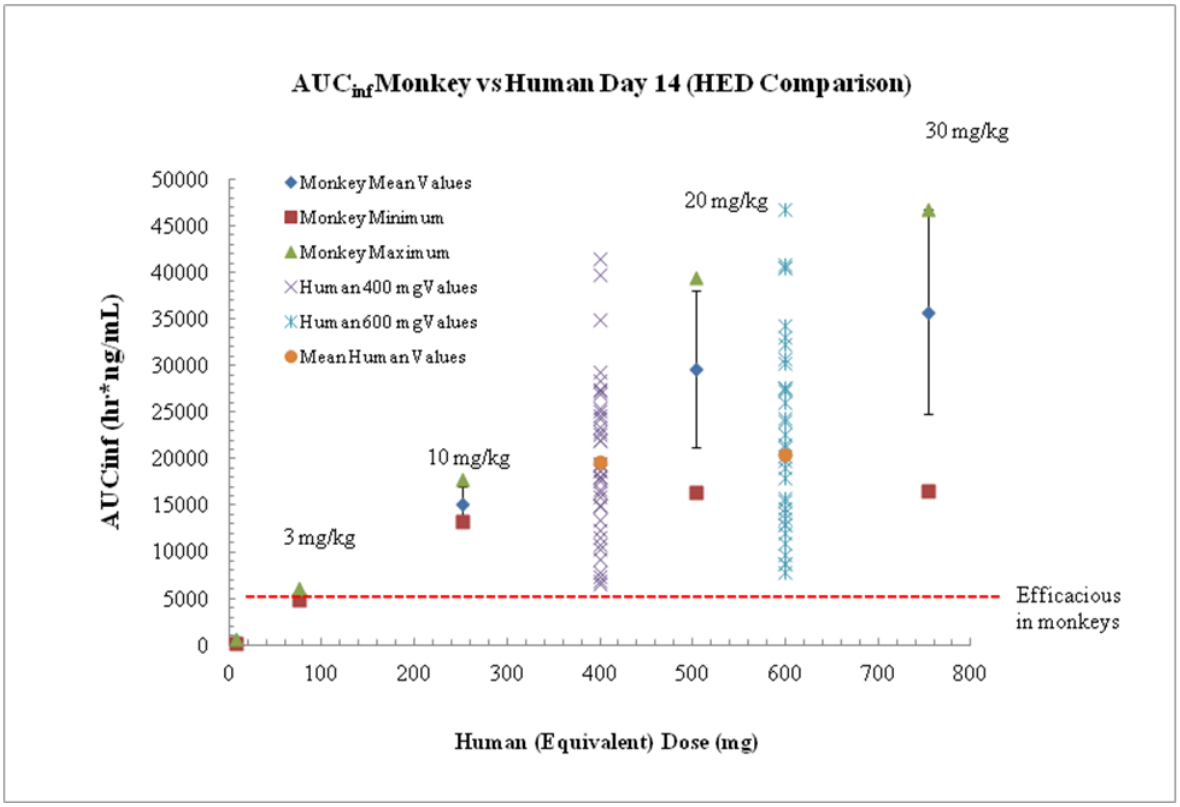
2.4.2 PK MODELING AND BRIDGING THE EFFECT OF INFECTION TO HUMANS

ST-246 safety and pharmacokinetics has been evaluated in numerous nonclinical safety and pharmacokinetic studies as well as several clinical studies. The pharmacokinetics of this compound, as well as the underlying metabolism, were very similar in NHP and humans. This can be clearly illustrated by the linearity obtained when steady state exposures ($AUC_{0\text{-}INF}$) for monkeys (MPI-1151-065) are overlaid with that observed in the most recent clinical study of healthy human volunteers (SIGA-246-004). Individual values for humans are shown, while the mean and standard deviations for the monkeys are plotted. The doses are compared after conversion to the human equivalent dose, based on standard allometric conversions using mg/m^2 and the mean body weight for humans in this clinical study of approximately 78.5 kg.

Exposure (AUCinf) from NHP pharmacokinetic study (MPI 1151-065) is compared to the human pharmacokinetic data using simple allometric scaling in which the NHP doses were converted to human equivalent doses by first multiplying the NHP doses by 0.32 as per FDA guidance table (Table 1, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, July, 2005), and then multiplying that value by the mean weight of the volunteers in the clinical study, which was 78.4 kilograms. Individual human exposure at the 400 and 600 mg doses are the purple and blue “x”symbols in the graphs, respectively. The solid orange circles are the mean human values for the two doses. The blue diamonds are the mean NHP (monkey) values, while the green triangles are the maximum values and the red square shows the minimum NHP value at each dose.

This graph (Figure 2.4.2-1) demonstrates the similarity of exposure between humans and NHPs over a similar dose range and also graphically shows that the healthy volunteers in the clinical study (SIGA-246-004) all have exposure levels that exceed that of NHP that had received the minimum efficacious dose of 3 mg/kg.

Figure 2.4.2-1: Overlay of ST-246 Exposures – Uninfected Monkeys and Humans

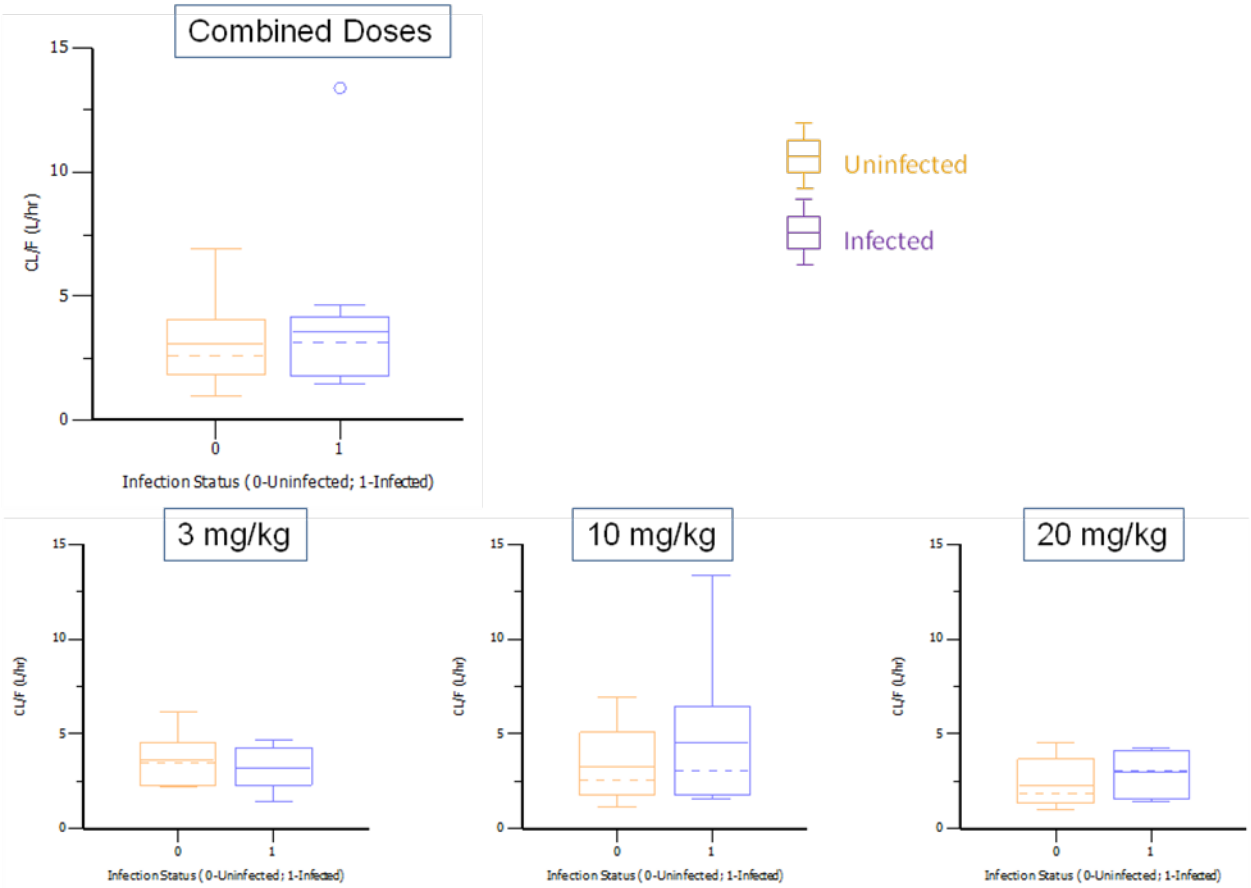


In order to select an efficacious dose, however, it was necessary to evaluate the potential impact of infection on the pharmacokinetics of ST-246. A recent study determined the pharmacokinetics during a two-week course of treatment in both uninfected as well as in monkeypox virus-infected NHP (LRRI FY10-87). This data, along with data from an uninfected monkey pharmacokinetic study (MPI 1151-065) and a third in which sparse pharmacokinetic sample data was available (AP-06-021G) were used to develop a population pharmacokinetic (POP PK) model of ST-246 in NHP. The final model fit a two-compartment model best, with a lag-time on absorption, an effect of weight (WT) on the pharmacokinetic parameters of Clearance (CL/F) and inter-compartmental clearance (Q/F) with the standard power function of 0.75, and an effect of WT on the both the central and peripheral volumes of distribution (Vc/F and Vp/F) with a power function of 1. Infection status was the most significant covariate, resulting in a small increase in CL/F as well as the absorption rate constant (Ka) for ST-246. Monkeypox virus infection resulted in a modest (< 10%) increase in ST-246 clearance. Inter-individual variability was 32% for clearance.

The effect of MPXV infection in clearance was evaluated during the generation of the monkey (NHP) POP PK model. Box plots of clearance values derived from the model for infected (blue)

or uninfected (orange) animals for individual or combined doses, are shown in the Figure 2.4.2-2. Overall there was only a modest increase in the clearance.

Figure 2.4.2-2: Effect of Infection on ST-246 Clearance in NHP



This determined effect of MPXV infection on clearance was translated to a similarly developed POP PK model of ST-246 in healthy humans, which was independently developed using data from the clinical study SIGA-246-004, and resulted in a two-compartment model as the best fit for the pharmacokinetic data. This step required the assumption that the effect of MPXV infection in humans would be the same as that observed in NHP. The human POP PK model was also a two-compartment model in which both weight and dose were covariates. Overall the final human POP PK model was similar to the monkey.

PK profiles of ST-246 were simulated using the POP PK model in humans taking into account the infection effect. Pharmacokinetic exposure parameters (AUC, C_{max}, C_{min}) were then predicted for orthopoxvirus-infected humans on Day 1 of treatment and at steady state. Finally, simulated exposure parameters (AUC, C_{max}, C_{min}), in infected NHP over the range of doses used in efficacy evaluations (3-168 mg/m^2, 0.25-14 mg/kg) were overlaid with the same parameters predicted for orthopoxvirus infected humans after doses of either 400 or 600 mg daily.

Doses of 96-120 mg/m^2 (8-10 mg/kg) and 144-168 mg/m^2 (12-14 mg/kg) administered to infected NHP gave exposures (AUC) of ST-246 equivalent to 400 and 600 mg doses, respectively. These doses were equivalent on both Day 1 and at steady state. The median

predicted C_{max} values for infected humans would be slightly higher than that observed in NHP by those doses on both Day 1 and at steady state. The median predicted C_{min} values, however, were slightly lower in humans than NHP at these exposure-equivalent doses on Day 1, but equivalent at steady state. Overall, doses of 8-10 mg/kg and 12-14 mg/kg in NHP were equivalent to 400 and 600 mg in humans, respectively. Figure 2.4.2-3 shows an overlay of simulated parameters for infected monkeys at discrete doses of 3, 6, 8, 10, 12, and 14 mg/kg on simulated parameters predicted for infected humans for a 400 mg human dose at steady state.

Figure 2.4.2-3: Human Dose of 400 mg ST-246 With Overlaid Equivalent Monkey Dose at Steady State

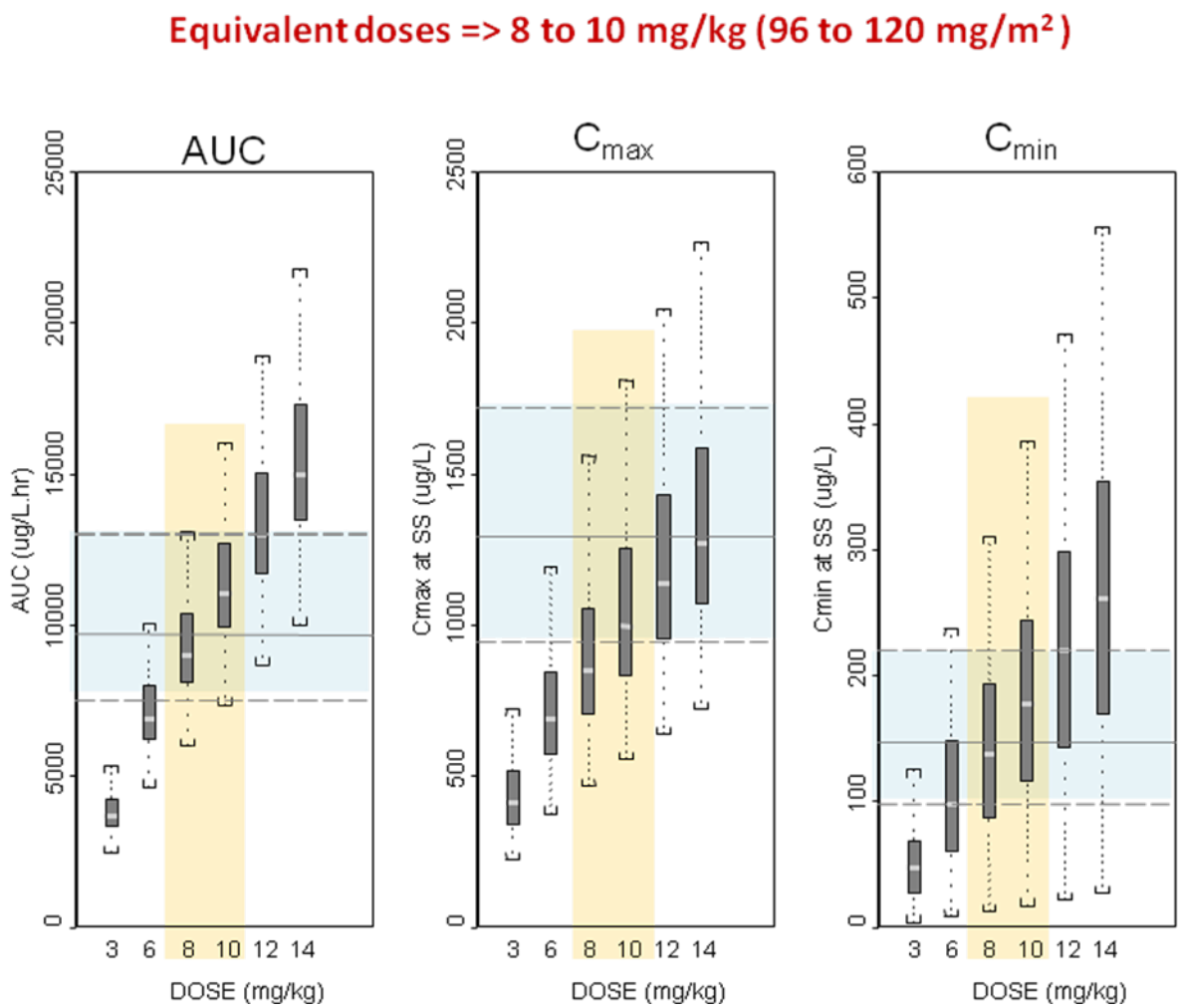
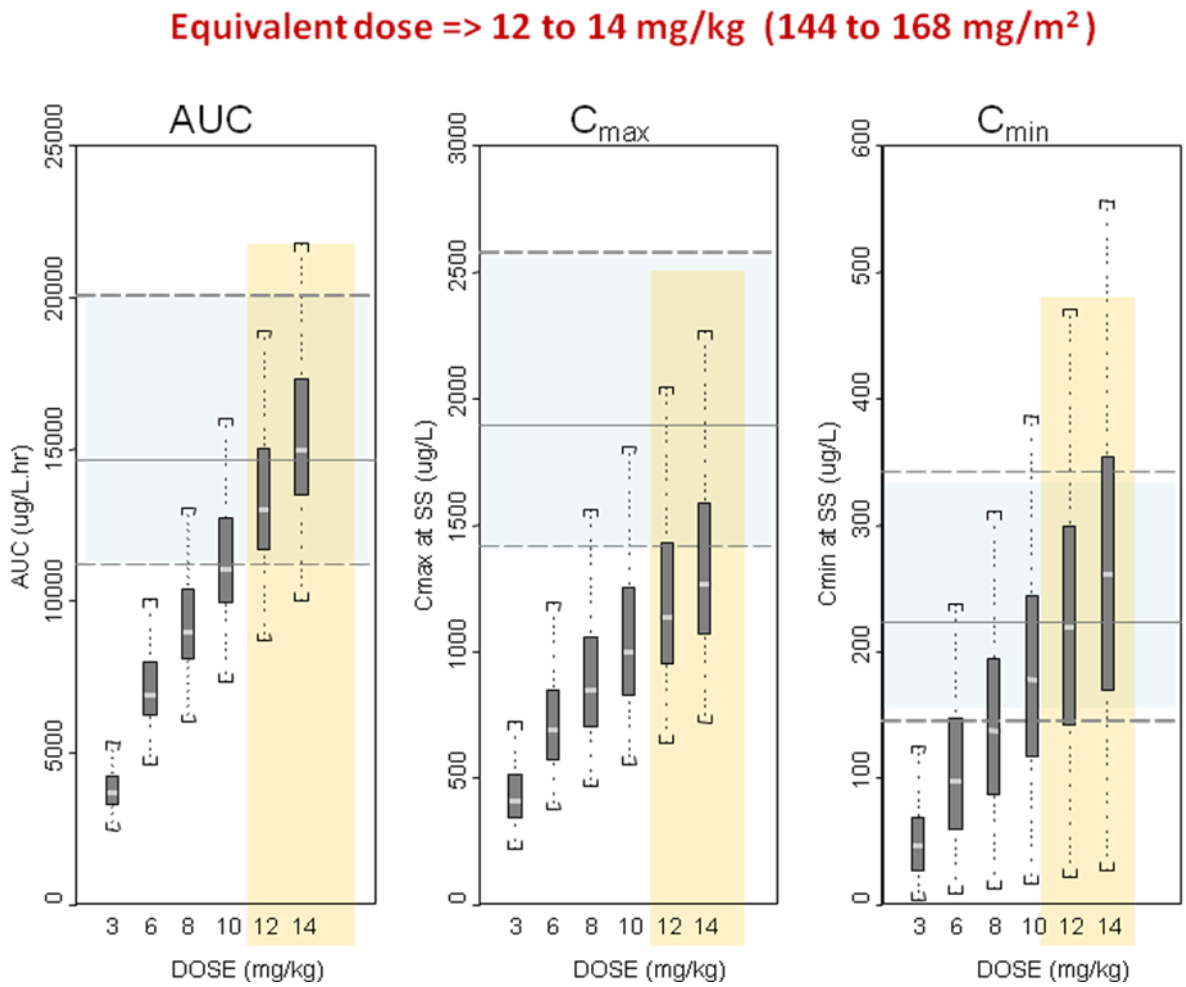


Figure 2.4.2-4 shows an overlay of simulated parameters for infected monkeys at discrete doses of 3, 6, 8, 10, 12, and 14 mg/kg on simulated parameters predicted for infected humans for a 600 mg human dose at steady state.

The effect of infection on the POP PK model in monkeys was applied to the human POP PK model. A simumlation was used to estimate the exposure (AUC), C_{max}, and trough concentration (C_{min}) at steady state after administration of the 400 mg dose in humans. The blue squares show the predicted levels for AUC, C_{max} and C_{min} from the 5% to the 95% of the population, all based

on the median weight of the human population of 78.4 kg. The solid line is the predicted median value for each of the parameters. The small box plots overlaid on each graph are the simulated predicted values for infected monkeys at each of the doses shown on the bottom of the graphs. The orange boxes show the doses in monkeys which are the best overall fit to the human dose.

Figure 2.4.2-4: Human Dose of 600 mg ST-246 With Overlaid Equivalent Monkey Dose at Steady State



The effect of infection on the POP PK model in monkeys was applied to the human POP PK model. A simulation was used to estimate the exposure (AUC), C_{max}, and trough concentration (C_{min}) at steady state after administration of the 600 mg dose in humans. The blue squares show the predicted levels for AUC, C_{max} and C_{min} from the 5% to the 95% of the population, all based on the median weight of the human population of 78.4 kg. The solid line is the predicted median value for each of the parameters. The small box plots overlaid on each graph are the simulated predicted values for infected monkeys at each of the doses shown on the bottom of the graphs. The orange boxes show the doses in monkeys which are the best overall fit to the human dose**.**

2.4.3 Survival Analysis: Dose-Survival and PK/Survival Modeling

In addition to the population pharmacokinetic modeling, which predicted equivalency of doses between infected NHP and humans, pharmacodynamic analysis was used to evaluate the

correlation between dose and exposure in NHP and survival. The analysis used survival data from all MPXV infection studies that had survival data available at the time that the analysis was started. While some of the studies were double-blinded placebo controlled, other studies were not blinded. The total number of NHP for the combined dose versus survival analysis was 96, of which 23 animals were placebos, with the remainder having received doses of ST-246 that ranged from 0.3 mg/kg to 300 mg/kg (see Table 2.4.3-1). A subset of the studies included some pharmacokinetic sampling and these studies were subsequently further analyzed to evaluate pharmacokinetic parameter correlation versus time of survival using quantitative modeling.

Table 2.4.3-1: Dose Group Number Breakdown for MPXV Efficacy Studies Used in Survival PD Analysis

	Study						
	AP-06-021-E1	AP-06-021-E3	AP-06-021-E6	AP-06-021-G	AP-09-026G	FY10-087	
Treatment	Number of Monkeys (n =)						**Total**
0.3 mg/kg	0	0	0	0	5	0	5
1 mg/kg	0	0	0	0	5	0	5
3 mg/kg	0	3	0	0	5	6	14
10 mg/kg	0	3	5	6	5	6	25
20 mg/kg	0	0	0	6	0	6	12
30 mg/kg	0	3	0	0	0	0	3
100 mg/kg	0	3	0	0	0	0	3
300 mg/kg	6	0	0	0	0	0	6
Placebo	2	3	1	4	7	6	23
Total/Study	8	15	6	16	27	24	96
PK Data	No	No	No	Yes (sparse)	Yes (sparse)	Yes (rich)	
Male/Female	8/0	15/0	4/2	16/0	27/0	12/12	82/14

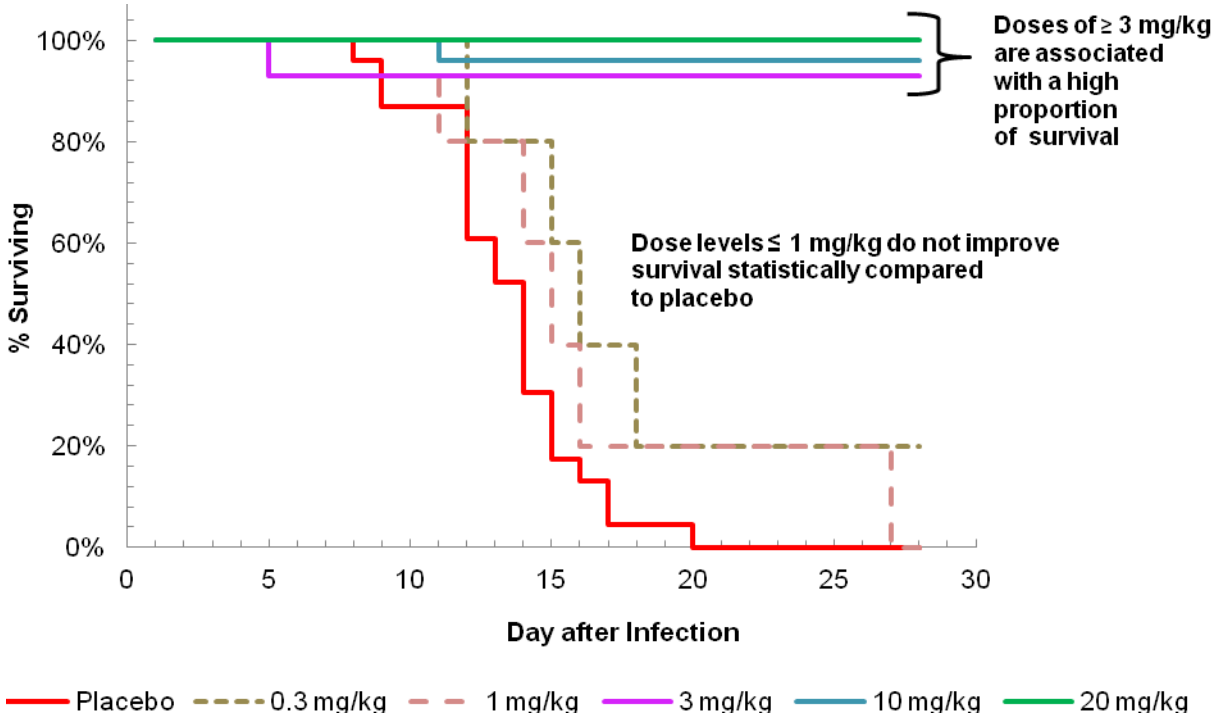
Among the 96 NHP evaluated for the initial PD survival analysis, 34 NHP died before the end of the study. Most of these animals were administered placebo, 0.3 mg/kg, and 1 mg/kg ST-246. In one study, AP-09-026G, one animal died at a dose level of 3 mg/kg, 5 days post-infection, after having received one dose of ST-246. This death was 3 days earlier than the first death observed for the Placebo group in that study. At the 10 mg/kg dose, one animal in the same study died 11 days after infection. These two cases were further investigated and histopathological analysis from that particular study (Study AP-09-026G) indicated specifically that the two animals from that study that had received doses of 3 mg/kg and 10 mg/kg did not have monkeypox virus infection severe enough to be consistent with a cause of death from the Pathologist's report for AP-09-026G ("One ST-246-treated animal in the 10 mg/kg group and one in the 3 mg/kg group died after undergoing anesthesia on Days 11 and 5 post-infection, respectively. Anesthesia and/or an underlying co-morbidity likely played a role in the death of these animals, as the monkeypox-induced lesions were very mild and not severe enough to have caused or been associated with death. The character of the histologic lesions in the 10 mg/kg animal related to monkeypox virus infection was unusual at this time point post-infection. It is possible that the ST-246 treatment altered the disease course in this animal, allowing for the development of lesions atypical of those ordinarily seen in an animal at Day 11 post-infection.

The character of the lesions present in the animal in the 3 mg/kg group was expected for this early time point post-infection.”). Overall, the median day of death was 14 days after infections, from doses ranging from 0-10 mg/kg.

The Dose-Survival relationship was explored using the Kaplan Meier approach. In this analysis a nonparametric survival curve estimates the probability of survival over time. For dose levels that were associated with the death, 35 NHP started their treatment on Day 4, 9 started their treatment on Day 3, and 5 NHP started treatment on Day 5. Due to small numbers of Days 3 and 5 no analysis could be done to explore the effect of “day of treatment start.”

At the 10 mg/kg dose a single animal from Study AP-09-026G died at Day 11. At Day 11 and after, the probability of survival was 96.0%. One NHP from Study AP-09-026G at the 3 mg/kg dose died at Day 5. At and after Day 5 the probability of survival was 92.9%. At the 0.3 and 1 mg/kg doses survival was not statistically significantly higher than Placebo treatment. In summary, the survival probability versus days after infection demonstrated a large difference in the survival probability for doses of 1 mg/kg and lower compared to that of 3 mg/kg doses and above. The 3 mg/kg dose of ST-246 appeared to be the threshold dose for increased survival, and in fact provided a large increase in survival compared to the next lower dose evaluated (Figure 2.4.3-1).

Figure 2.4.3-1: Kaplan-Meier Survival Curve Analysis Showing Placebo (N = 3), 0.3 (N = 5), 1 (N = 5), 3 (N = 14), 10 (N = 24), and 20 (N = 12) mg/kg Doses of ST-246



The exposure-survival relationship analysis included only 67 NHP, as these studies including plasma sampling for ST-246 concentrations during the course of the studies. These animals received ST-246 doses that ranged from 0.3 mg/kg to 20 mg/kg. Individual post-hoc parameters were estimated from the final population pharmacokinetic model developed for NHP. The

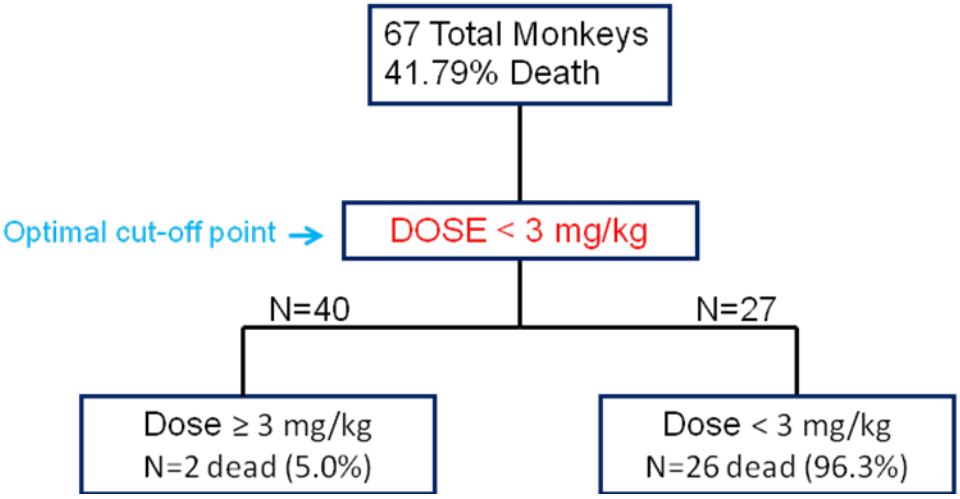
individual total exposure of ST-246 was estimated using the individual values for clearance and administered dose, in mg/kg. C_{min} and C_{max} were estimated by simulating individual profiles with available post-hoc parameters.

Consistent with dose, survival probability of infected monkeys increased with increased ST-246 exposure. Based on visual explorations, the following empirical cut-offs for AUC/WT (0.5 mg.hr/(L.kg) and C_{min} (200 µg/L) were determined for maximal survival rate. These cutoffs were further refined using the receiver operator characteristic analysis in the next section.

2.4.4 Receiver Operator Characteristic Analysis and Optimal Cut-Offs for Exposures

Recursive receiver operator characteristic (ROC) analysis was conducted in order to determine the optimal cut-off points for dose or exposure levels that best correlate with the survival outcome (Figure 2.4.4-1). For this analysis the covariates analyzed were dose, AUC/WT, C_{min} after first dose, and C_{min} at steady state. The advantage of this analysis is that an optimal cut-off is determined based on statistical criteria that optimize sensitivity and specificity. All results converged to the same overall conclusion showing that doses above 3 mg/kg and corresponding AUC/WT, C_{min1}, and C_{minss}, were associated with maximum survival whereas doses of less than 3 mg/kg and corresponding AUC/WT, C_{min1}, and C_{minss}, have very low survival rate. The two animals that died under anesthesia in the AP-09-026G study were identified as false negatives by the ROC analysis. The actual cut-off values of dose (3 mg/kg), exposure (0.5 mg.h/(L.kg)), C_{min} after the first dose and at steady state (21.22 µ/L) all corresponded to a dose level of 100 mg for humans of median weight from the SIGA-246-004 study (78.35 kg).

Figure 2.4.4-1: ROC Analysis



Receiver operator characteristic analysis determined doses of less than 3 mg/kg did not improve survival, while those of 3 mg/kg and higher were associated with higher incidence of survival.

2.4.5 Overall PK/PD Conclusions

Combined pharmacodynamic analysis of the MPXV efficacy studies was done using several independent methods of analysis and all demonstrated that the threshold for efficacy was 3 mg/kg. This dose was predicted to be equivalent to 100 mg in infected humans of the average weight of 78.35 kg from the recent clinical study (SIGA-246-004). Comparison of the predicted

exposure (AUC), maximum plasma concentration (C_{max}) and trough plasma concentration (C_{min}) for infected monkeys and humans demonstrated equivalence of the 400 mg human dose to 8-10 mg/kg ST-246 in monkeys, and the 600 mg human dose to 12-14 mg/kg. This analysis strongly indicates that a dose of 400 mg ST-246 will provide more than sufficient exposure to protect humans against disease due to orthopox-induced illness.

2.5 Resistance Monitoring Plan

Our plan is to evaluate the emergence of ST-246 resistant viruses in NHP/MPXV studies.

- **Animal Studies to be Evaluated**
  - AP-026G (completed)
    - ST-246 treatment initiated at lesion onset
    - Sub-therapeutic dosing: 0.3 mg/kg and 1 mg/kg
      - These animals failed treatment and succumbed to disease
    - Therapeutic dosing: 3 mg/kg and 10 mg/kg
      - These animals were successfully treated and survived
  - Pivotal animal efficacy study (future)
    - ST-246 treatment will be initiated at lesion onset
    - ST-246 dosing will be at the proposed Human Equivalent dose
  - We do not plan to evaluate ST-246 resistance in any of our other animal studies
- **Samples to be Evaluated**
  - Stock challenge virus – to determine the frequency of resistance in the challenge virus pool
  - Blood from select placebo treated animals – to determine the frequency of resistance generated *in vivo* in the absence of selective pressure
  - Blood from ST-246 treated animals in which treatment failed
    - Animals treated with *sub-therapeutic doses* of ST-246 that subsequently died of monkeypox and (if available) animals treated with ST-246 at *the human equivalent dose* which failed treatment
      - At least 2 blood samples will be evaluated for each animal tested: one sample from within 72 hours of treatment initiation and the second sample from the terminal bleed at euthanasia
  - Blood from animals in which treatment was successful
    - Animals treated with *therapeutic doses* of ST-246 that survived infection to study termination
      - At least 2 blood samples will be evaluated for each animal tested: one sample from within 72 hours of treatment initiation and the second sample from within 72 hours post treatment cessation
- **Method of Evaluation**
  - MPXV genomic DNA or PCR amplicon corresponding to the MPXV C19L gene (homolog of the F13L gene in vaccinia and the C17L gene of variola) which encodes the p37 protein target of ST-246, will be sent to Floragenex (our contractor for these analyses) for deep sequence analysis
  - Deep sequence analysis will be used to determine the frequency of nucleotide mutations corresponding to amino acid substitutions known to mediate resistance to ST-246

  - The assay is expected to identify nucleotide mutations occurring at a frequency of 1% or greater of the total population
- **Additional Evaluations**
  - ST-246 resistant virus samples were obtained from an immunosuppressed smallpox vaccine recipient that developed progressive vaccinia and was treated with ST-246
  - Mutations occurring in the F13L gene (of the ACAM2000 live vaccinia virus vaccine) leading to amino acid substitutions mediating ST-246 resistance were identified by deep sequence analysis similar to our proposed methods above
  - ST-246 resistance was confirmed by changes in the EC_{50} of plaque-purified virus

These same mutations will be engineered into the Western Reserve and ACAM2000 strain of vaccinia so that changes in virulence (if any) and susceptibility to ST-246 may be evaluated *in vivo* in murine models. If it is deemed necessary, mutations leading to ST-246 resistance detected by deep sequencing in MPXV samples collected in the course of our pivotal study, will be engineered into vaccinia virus for analysis of virulence and ST-246 susceptibility in murine models.

3. DEVELOPMENT OF ST-246 UNDER THE ANIMAL RULE

3.1 Overview of ST-246

ST-246 was identified in a high-throughput screen of 356,240 compounds in a chemically diverse library.[54] ST-246 was tested against numerous viruses *in vitro* and found to be potently active and specific for orthopoxviruses (Table 3.1-1). It is most notable that ST-246 was highly active against pathogenic variola and monkeypox. ST-246 has been evaluated for poxvirus inhibition in primary cell lines including human embryonic lung fibroblasts, primary human keratinocytes, and organotypic endothelial raft cultures[75] as well as standard tissue culture cells such as Vero, BSC-40 , and SIRC[54] and was found to display similar activity regardless of cell type. Cytotoxicity was evaluated in L929, SIRC, BSC-40, Vero, TREx, MRC5 cells. The CC_{50} was > 50 μM in all cell lines tested.

Table 3.1-1: ST-246 Antiviral Specificity

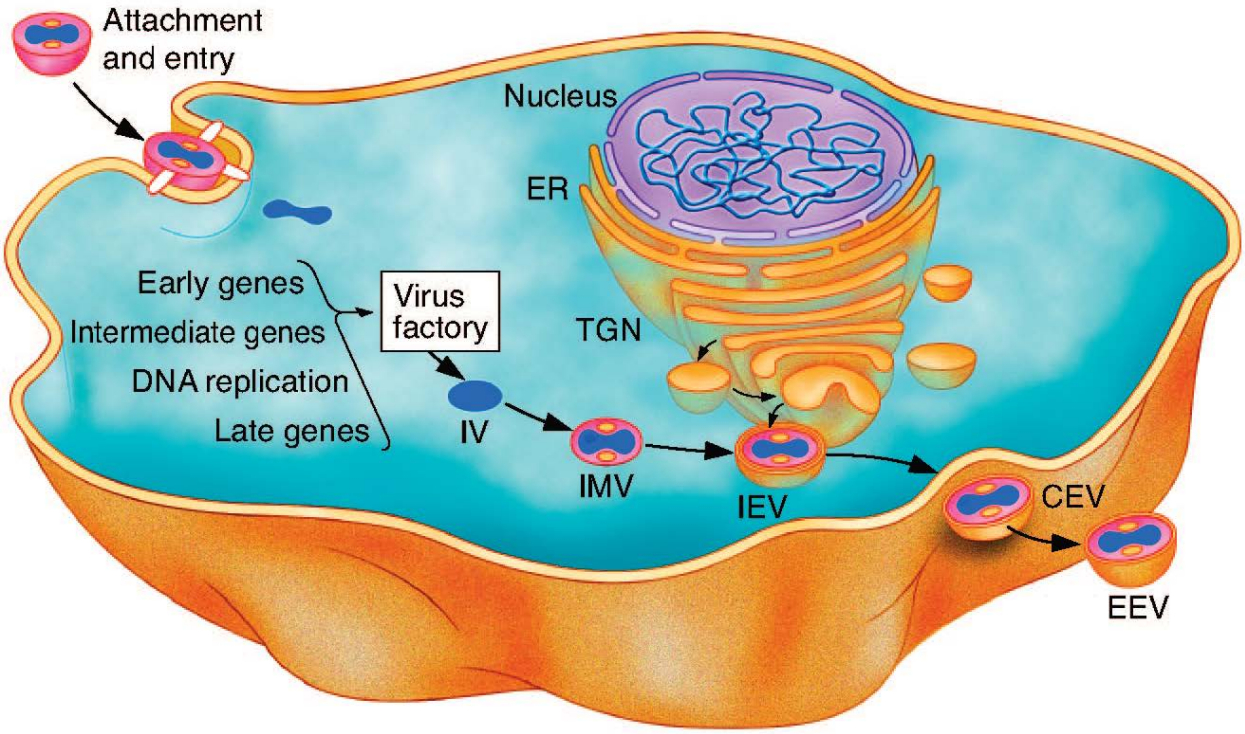
Virus	Strain	EC_{50} (μM)
Study #: 246-PC-007		
Vaccinia	Copenhagen	0.010
Cowpox	Brighton Red	0.050
Cowpox CDVr	Brighton Red	0.030
Ectromelia virus	Moscow	0.068
Camelpox		0.012
Monkeypox	Zaire 69	0.014
Variola	Butler	0.016
	Bangladesh	0.046
Smith et al., 2009[76]		
Variola	BRZ66-39	0.067
	SLN68-258	0.037
	SUD47-juba	0.019
	BSH74-sol	0.028
	NEP73-175	0.021
	SOM77-ali	0.028
Monkeypox	V78-I-3945	0.023
	V81-I-179	0.032
	2003-USA-039	0.036
	V77-I-823	0.030
	V1979-I-005	0.039

3.1.1 ST-246 MECHANISM OF ACTION

Like many other viruses, the replication cycle of OPXVs is stringently regulated by a sequence of events beginning with the attachment and fusion of viral particles to the host cell plasma membrane followed by penetration of the particle core.[77, 78] The commencement of DNA synthesis occurs two to five hours post infection and results in the production of approximately 10,000 nascent genomes per infectious particle.[79] Productive OPXV replication leads to the formation of a number of phenotypically distinct, infectious virion forms 1) intracellular mature virus (IMV), 2) intracellular enveloped virus (IEV), 3) cell associated enveloped virus (CEV)

and 4) extracellular enveloped virus (EEV).[80] IMV particles make up the majority of infectious virus and remain within the intracellular environment until cell lysis. IEV and CEV virions are IMV that have become enwrapped in two additional membranes. Viral components located within the IEV membranes function to transport the nascent virion along microtubules to the cell periphery and, once released, to shield the particles from antibody and complement.[2] A portion of the CEV particles are liberated from the cell as EEV. EEV forms of OPXVs are responsible for the long range dissemination of virus and systemic disease.[2] Mutant viruses defective for enveloped virus production show reduced virulence and poor dissemination *in vivo*. A schematic representation of each stage of the OPXV replication cycle is shown in Figure 3.1.1-1.[81]

Figure 3.1.1-1: Orthopoxvirus Life Cycle

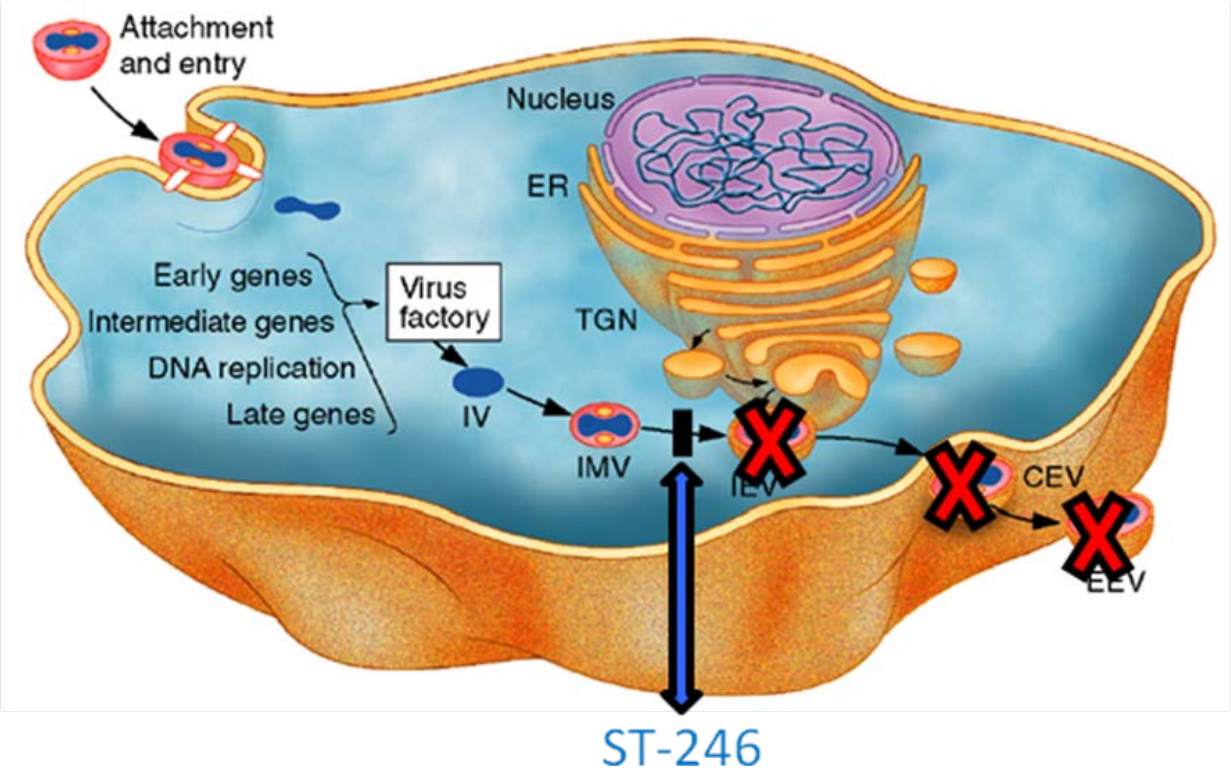


KEY: ER = endoplasmic reticulum; TGN = transgolgi network; IV = intracellular virus; IMV = intracellular mature virus; IEV = intracellular enveloped virus; CEV = cell-associated enveloped virus; EEV = extracellular enveloped virus

In a study using vaccinia virus, ST-246 was shown to target the p37 protein (encoded by the F13L gene in vaccinia, homologous to the C17L gene in VARV and the C19L gene in MPXV).[54] P37 is a 37 kDa peripheral membrane protein required for the production of extracellular forms of virus. P37 sequence identity is greater than 98% conserved amongst OPXVs and it has no mammalian homologues. P37 functions by nucleating the formation of a wrapping complex derived from late endosomal (LE) membranes. This wrapping complex catalyzes the envelopment of IMV particles to produce egress-competent, enveloped forms of virus. The wrapping complex is formed through interactions of p37 with components of LE-derived transport vesicles. Late-endosomal-derived transport vesicles shuttle cellular "cargo" between the LE and Golgi compartment. These transport vesicles assemble around cargo proteins through specific interactions with the Rab9 GTPase and TIP47, a Rab9-specific effector protein. The p37 protein acts like cellular cargo and interacts with Rab9 and TIP47 to nucleate a virus-

specific wrapping complex required for assembly of extracellular forms of virus. ST-246 blocks interaction of p37 with Rab9 and TIP47, but not p230, a Golgi-specific marker protein.[3] Thus, ST-246 prevents wrapping complex formation by inhibiting interaction of p37 with components of LE-transport vesicle biogenesis arresting the virus replication cycle at the IMV stage and preventing the formation and dissemination of EEV particles (Figure 3.1.1-2). In the absence of systemically disseminated virus, disease is prevented.

Figure 3.1.1-2: ST-246 Molecular Mechanism of Action



KEY: ER = endoplasmic reticulum; TGN = transgolgi network; IV = intracellular virus; IMV = intracellular mature virus; IEV = intracellular enveloped virus; CEV = cell-associated enveloped virus; EEV = extracellular enveloped virus

3.1.2 METABOLISM AND PHARMACOKINETICS

3.1.2.1 *IN VITRO* ADME

The evaluation of ST-246 ADME properties showed it has moderate to high plasma protein binding (77.3-96.3% in all species) which was independent of concentration over the range evaluated, from 0.3-50 µM. ST-246 is very stable and not metabolized to any significant degree by P450 isozymes. There was no significant CYP induction at 10 µM ST-246 (3,700 ng/mL) but at a 100 µM concentration induction was observed for the following CYP enzymes: 2B6, 2C9, 3A4, and 2C19. Inhibition of nine CYP enzymes that were tested (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) was less than 50% at a 300 µM concentration. Thus, the potential for drug-drug interactions via induction or inhibition of human cytochrome p450 enzymes is low. The *in vitro* patch clamp assay for potential hERG channel inhibition showed interaction with the potassium ion channel was quite low. The highest ST-246 concentration, 30 µM, showed only 7% inhibition. ST-246 was judged to be

highly permeable in the *in vitro* Caco-2 assay, and did not interact with P-gp glycoprotein *in vitro*, indicating that drug transporter interactions are unlikely for this compound.

3.1.2.2 METABOLISM

While the evaluation of metabolic stability in isolated microsomal fractions showed small amounts of ST-246 were cleaved, liberating 4-trifluoromethylbenzoic acid from the parent compound in rats, mice and dogs, these metabolites were not seen in either the monkey or human microsomes. No other metabolites were identified from the *in vitro* studies. An *in vivo* mass balance study in mice showed that radioactivity associated with ^{14}C-ST-246 was nearly completely eliminated within 96 hours after oral administration. At 24 hours post dose the radioactivity was broadly distributed to all organs, including the brain, with the highest concentration outside of the intestinal tract observed in the gallbladder. By 96 hours after oral administration approximately 72% of the radioactivity had been eliminated through the feces and 24% was eliminated in the urine. Whereas all of the radiolabel in the feces co-eluted on HPLC with intact ST-246 none of the radiolabel in the urine did. The urine contained multiple metabolites; however, the only ones that could be identified, in addition to trifluorobenzoic acid, were glucuronidated ST-246 metabolites.

3.1.2.3 PHARMACOKINETICS

Although the solubility of ST-246 is low (approximately 20 µM in the absence of protein) it is highly permeable (BCS Class II) and has high levels of oral bioavailability, which increases when the compound is co administered with food. In rats the bioavailability was 90% and 33%, respectively, for males and females after oral administration of 30 mg/kg ST-246. The lower concentrations of ST-246 exposure observed in female rats were consistent with first pass metabolism while multiple dose administration resulted in much lower exposure in both male and female rats, suggesting induction of metabolism. Rats, as well as rabbits were the only two species evaluated that had significant and reproducible gender differences in exposure. ST-246 did not demonstrate any consistent gender differences between mice, monkeys or human. Some differences in studies in other species were noted, but they were generally not consistent across doses and varied from study to study. In addition, rats were the only species for which multiple dose administration resulted in significantly lower exposure, indicative of induction of metabolism in this species. Over the course of extensive repeat dose studies in mice there was no consistent evidence of induced metabolism, suggesting that this phenomenon was rat specific. The predominant cause of nonlinearity in the pharmacokinetics of ST-246 observed in mice appeared to be decreased absorption with increasing dose, with no notable differences in the pharmacokinetics between genders. The decreased absorption was observed in both the observed maximum plasma concentrations as well as the exposure (as determined by AUC values). Thus, as the doses were increased, exposures also increased but not dose proportionally.

Due to this apparent saturation of absorbance of ST-246 in mice the apparent clearance and volume of distribution increased with increasing doses after oral administration. The compound was dosed once a day with the terminal elimination half-life in mice of approximately 4 hours. In the 28 day repeat dose study in mice ST-246 showed a modest amount of accumulation over the course of the study for the higher two of the three doses, approximately 25% increase in AUC values at the 1,000 mg/kg dose and approximately 50% at the 2,000 mg/kg dose. However, in the 90 day study the AUC values were lower on Day 90 than Day 1 by 25%-50%.

As was observed in mice, the bioavailability in monkeys increased when the animals were fed, doubling from approximately 25% when animals had been fasted to roughly 50% when the animals were administered ST-246 in the fed state. Dose proportional exposure was observed at lower doses but, as mentioned above, it appeared that absorption may have limited exposure increases at higher doses. The pharmacokinetics are dose-dependent, with increasing doses having lower bioavailability. Monkeys administered ST-246 at 3, 10, 20, and 30 mg/kg had bioavailability values of 68, 49, 23, and 21%, respectively, compared to identical doses administered by IV infusion. Higher doses had lower apparent bioavailability, possibly due to decreased absorption as the dose was increased. The exposure (AUC_{0-24}) increased approximately 60-fold as the dose was increased from 0.3-30 mg/kg, a 100-fold increase in dose. The exposure levels at steady state in that study were approximately 20% higher than on Day 14 than on Day 1 of the study, indicating a limited potential for accumulation. In the 28 day study the exposure, as assessed by AUC, at the end of the study tended to be lower than on Day 1 of the study, with values that ranged from 25% of the Day 1 exposure to equivalent exposure. In contrast, in the 3 month monkey study the exposure at the last time point was nearly double that observed on the first day of the study. Thus, in multiple dose studies in both mice and monkeys there was no strong evidence of significant accumulation of ST-246 nor induction of metabolism. It may be that the differences observed in the different studies were more related to the different absorption properties of dose formulation suspensions used on a given day than of any metabolic differences between the animals throughout the course of the safety studies.

The steady state volume of distribution in monkeys was calculated after a 1 mg/kg IV dose administered during the initial bioavailability study. The values for both male and female monkeys were greater than the total body water volume in monkeys (693 mL/kg-reference Davies and Morris added below), indicating a broad distribution of the compound, consistent with the radiolabel distribution and mass balance study in mice. The clearance for both male and female monkeys was close to 1,100 mL/hr/kg. Although in some studies there were statistical trends indicating gender differences in the pharmacokinetics, as with the level of accumulation during multi-dose studies, these differences were not consistently observed.

3.1.3 EFFECT OF MPXV INFECTION ON ST-246 PK IN NHPS

A study was designed utilizing the IV MPXV model of human smallpox disease specifically to assess the impact of orthopoxvirus infection on the pharmacokinetics of ST-246. Because the selection of the efficacious dose for humans will require extrapolation from animal studies, it was important to understand to the degree possible, the effect of orthopoxvirus infection on ST-246 pharmacokinetics. The pharmacokinetics were initially assessed pre-infection (Day -10), in order to be able to compare intra-animal variability due to infection, and again on Days 4, 10, and 17 after infection, which corresponded to treatment Days 1, 7, and 14. Doses of ST-246 assessed in this study were 3, 10, and 20 mg/kg, which had previously been shown to be highly efficacious. The results of the study indicated a limited impact of orthopoxvirus infection on ST-246 pharmacokinetics, with clearance increasing less than 10%, resulting in a slight decrease in exposure. The inter-individual variability for ST-246 was over 30%, much greater than the predicted impact of infection on clearance.

3.1.4 TOXICOLOGICAL SAFETY SUMMARY

3.1.4.1 *IN VITRO* TOXICITY STUDIES

ST-246 showed no inhibition of hERG-mediated potassium currents at concentrations of 0.03, 0.1, 1, and 10 µM concentrations. A maximum inhibition of 7.1% was observed at 30 µM. The highest concentration of ST-246 observed in clinical studies was approximately 10 µM (3,700 ng/mL).

3.1.4.2 GENOTOXICITY

ST-246 was not genotoxic in either bacterial or mammalian cell genotoxicity assays. In addition ST-246 did not cause chromosomal damage or bone marrow toxicity in the mouse micronucleus test. Because of the completely negative results in the evaluation of genotoxicity tests and the limited duration of dosing planned for ST-246, carcinogenicity tests are not planned.

3.1.4.3 SAFETY PHARMACOLOGY

Safety pharmacology studies for ST-246 were conducted. Plethysmography was used to measure tidal volume in mice after single oral dose administration of ST-246 of up to 2,000 mg/kg. There were no abnormal values associated with the administration of ST-246 and the NOEL was determined to be the highest administered dose, or 2,000 mg/kg. An initial study in mice used the Functional Observational Battery (FOB) to evaluate the potential for CNS toxicity. As with the pulmonary safety pharmacology the top dose of 2,000 mg/kg in mice did not elicit any behavioral or other functional changes such as core body temperature. However, studies in dogs found an association between ST-246 administration and behavioral and electroencephalogram (EEG) evidence of seizures. The maximum tolerated dose in dogs, based on this observed toxicity, was 30 mg/kg. A follow up study in primates found no evidence of altered electroencephalograms or lowered seizure thresholds in NHP after administration of 12 daily doses of 300 mg/kg of ST-246, indicating that dogs are uniquely sensitive to ST-246. In addition, analysis of brain and CSF levels of ST-246 were found to be much higher in dogs than NHP, suggesting a reason for the specific toxicity in dogs is much higher CNS penetration of the compound. Cardiovascular safety evaluation in NHP showed no prolongation of QTc at the highest dose administered in repeat dose studies, 300 mg/kg.

3.1.4.4 GENERAL TOXICOLOGY

SIGA has conducted safety evaluations in multiple species: mice, rats, rabbits, dogs, and NHP. The two species used for repeat dose toxicity studies were mice and NHP, as these species are also used in efficacy evaluation so that a thorough knowledge of safety in these species helped with the design of efficacy pharmacology studies. The maximum tolerated dose in mice was 2,000 mg/kg for both single dose as well as the 28 day study. In the single dose study there was a slight loss of body weight but recovery by the second day. Although both liver and spleen weights were slightly increased at the end of the 28 day study in mice for the 2,000 mg/kg dose of ST-246 there were no corresponding histological correlates and no alterations in clinical chemistry or liver enzymes. The NOAEL was 2,000 mg/kg. The three month study evaluated a top dose of 1,000 mg/kg of ST-246 in mice and the same slight increase in liver weight with no accompanying histopathology was observed, meaning the NOAEL was 1,000 mg/kg for the three month study. A phototoxicity study in mice evaluated potential phototoxicity in mice after a single administration of ST-246 at doses of 500, 1,000, and 2,000 mg/kg. No indication of phototoxicity was observed.

The highest single dose administered orally to NHP was 2,000 mg/kg, which resulted in decreased activity and ataxia in both male and female NHP. This was also observed after a single dose administration of 1,000 mg/kg. At 300 mg/kg ataxia was observed 4 hours post dose in one female NHP. Because of these observations the highest dose used in multiple dose safety studies in NHP was 300 mg/kg. This was used for both the 28 day and 3 month safety studies and the NOEL was the top dose of 300 mg/kg in both of these studies. ST-246 is a well-tolerated compound and seems to elicit very little toxicity at doses that are much higher than those required for the antiviral activity.

3.1.4.5 Reproductive Toxicology

Definitive Segment I, II, and III reproductive studies were conducted in both mice and rabbits. There was no evidence in either mice or rabbits of decreased fertility or fetal resorptions, fetal abnormalities or toxicity. The highest dose used in the rabbit Segment II study, 100 mg/kg, resulted in some maternal toxicity. Administration of ^{14}C-ST-246 to pregnant mice resulted in only very low levels of radiolabel in the placenta and to nursing dams very low levels (< 1%) in the milk.

3.1.5 Completed ST-246 Clinical Study Overview

Four clinical studies with ST-246 have been completed to date.

SIGA-246-001 was a single-center, Phase I, randomized, placebo-controlled, double-blind, ascending, single-dose study to assess the safety, tolerability, and pharmacokinetics (PK) of ST-246.[26,27] In this study, ST-246 was administered as a single oral dose to a total of 38 healthy adult volunteers (31 active, 7 placebo), male and female, who were in the fasted state (Group 1 [500 mg], Group 2 [1,000 mg], Group 3 [2,000 mg]), or fed (Group 4 [1,000 mg]).

SIGA-246-002 was a Phase I double-blind, randomized, placebo-controlled, escalating multiple dose trial to assess the safety, tolerability and PK of 3 different doses of ST-246 (250 mg, 400 mg, and 800 mg). In this study, ST-246 was administered as a single daily oral dose for 21 days in 30 healthy fed volunteers. Twenty-four subjects were randomized to active drug (8 per dose group), while the 6 remaining volunteers (2 per dose group) received placebo.

SIGA-246-005 was a Phase I double-blind, randomized, crossover, study of the PK, safety, and tolerability of a single dose of 400 mg of Form I (hemihydrate form) versus 400 mg of Form V (monohydrate form) of ST-246 in healthy fed volunteers. In this study, each formulation of ST-246 was administered as a single oral dose, 10 days apart, in 12 healthy non-fasting volunteers (n = 6 received Form I followed by Form V and n = 6 received Form V followed by Form I).

SIGA-246-004 was a Phase II, double-blind, randomized, placebo-controlled, multi-center (3 sites) trial to assess the safety, tolerability, and pharmacokinetics of 400 mg and 600 mg of Form I ST-246 when administered as a single daily oral dose for 14 days to 107 healthy, fed volunteers between 18 and 75 years of age.

3.1.5.1 Key Safety Results From Completed Clinical Studies

Overall, ST-246 has an excellent safety profile and no deaths or serious adverse events have been reported. In total, 187 healthy volunteers have participated in these studies. Single doses of

ST-246 ranging from 250-2,000 mg and 400-1,000 mg have been administered to subjects in fasted and fed states, respectively. Repeated doses of ST-246 ranging from 250-800 mg have been administered to subjects for up to 21 days. A total of 158 subjects have been exposed to at least 1 dose of ST-246: 23 subjects have received a single dose in the fasted state; 9 subjects have received a single dose in the fed state; and 126 subjects have received multiple doses (at least 2) in the fed state.

Of the 187 subjects who have participated in the clinical studies of ST-246, 62 of the 158 subjects (39.2%) in the active treatment groups and 12 of the 29 subjects (41.4%) in the placebo groups reported at least 1 TEAE (Treatment Emergent Adverse Event). Overall, 30 subjects (19.0%) in the active treatment groups reported 82 drug-related TEAEs and 5 subjects (17.2%) in the placebo groups reported 12 drug-related TEAEs. Headache was the most commonly reported TEAE and the most commonly reported drug-related TEAE. Most TEAEs were mild or moderate; only 3 TEAEs were severe in intensity and only 1 (headache) was considered to be related to study drug. Table 3.1.5.1-1 lists TEAEs reported in clinical studies conducted to date that were of moderate intensity occurring in ≥ 2% of the subjects treated with ST-246 as compared to placebo.

Table 3.1.5.1-1: TEAEs Reported In Clinical Studies

TEAE	% Occurring in ST-246 Treated Subjects	% Occurring in Placebo Treated Subjects
Headache	16.5	10.3
Nausea	5.1	3.4
Back Pain	3.2	3.4
Dizziness	2.5	0
Fatigue	2.5	0
Flatulence	2.5	0

Overall, there have been no clinically significant abnormalities or changes from Baseline in laboratory test results or vital sign measurements. Analysis of ECGs performed during the 4 completed clinical studies has revealed no clinically significant abnormalities. No clear signal of any effect on AV conduction or cardiac depolarization as measured by the PR and QRS interval durations has been detected. In addition, no significant effect on cardiac repolarization, as measured by the lack of a significant change in QTc, has been noted. No clinically relevant new morphologic changes have been seen.

PK data is provided in Section 2.4 on PK/PD Modeling and in the short summaries of each of these studies as presented below.

INDIVIDUAL CLINICAL STUDY DATA

SIGA-246-001

In the first human study, ST-246 was safe and well-tolerated when administered orally as a single dose to healthy normal volunteers in a fasted (500-2,000 mg) or fed (1,000 mg) state. No deaths, SAEs, or discontinuations due to treatment-emergent AEs occurred. There appeared to be no dose relationship in the incidence of AEs. There were no clinically meaningful changes in vital signs. ECG analysis did not reveal any clinically significant QTcB changes (no QTcB

changes from screening/baseline were greater than ≥ 30 msec) or changes in interpretation in individual subjects.

The plasma pharmacokinetics of ST-246 were well characterized for all dose levels, 500, 1,000, and 2,000 mg in fasted subjects and 1,000 mg in fed subjects. The single-dose plasma PK of SIGA-246 in fasted subjects showed dose proportionality over the dose levels of 500 and 1,000 mg, but not so over the 1,000 and 2,000 mg dose levels. At the 1,000 mg dose level of ST-246, fed subjects had greater apparent C_{max}, T_{max}, and AUC_{inf} than fasted subjects.

There were minimal gender differences in ST-246 plasma concentrations based on visual inspection of the linear concentration-time profiles.

SIGA-246-002
In the second human study, an ascending multiple dose study in fed volunteers, ST-246 was safe, and well-tolerated. No deaths or SAEs occurred; 2 subjects withdrew due to an AE (or treatment of an AE). There appeared to be no dose relationship in the incidence of AEs. Headache, the most commonly reported AE, was experienced with all treatments (placebo and 250, 400, and 800 mg/day ST-246). Most headaches were mild or moderate in intensity; only 1 was rated as severe in a subject who had received ST-246 800 mg/day for 16 days, and for whom the headache resolved. This subject discontinued treatment on his own and discontinued from the study after completing end-of-study procedures.

The multiple-dose plasma and urine pharmacokinetics of ST-246 at dose levels of 250, 400, and 800 mg given once per day for 21 days were well-characterized in this study. ST-246 was measurable in all 24 active-treated, healthy volunteers. Since blood samples were collected on Days 1, 6, and 21, and urine samples on Days 1 and 21, pharmacokinetic parameters after the first dose through 24 hours, at an intervening time point (Day 6), and for 72 hours after the last dose on Day 21 were determined. In particular, the Day 21 elimination half-life was, calculated. Steady state was reached by Day 6 (within 3-5 half-lives), saturable absorption was observed at the 800 mg dose level, and very little drug was excreted in urine. Regarding dose proportionality, as the dose increased from 250-400 mg (1.6-fold), the mean Day 21 exposure also increased 1.6-fold; when the dose was further increased to 800 mg (3.2-fold, from 250-800 mg), the mean exposure increased only 2.25-fold. Thus, a conclusion of dose proportionality could not be confirmed across the full dose range (from 250-800 mg).

SIGA-246-PO-005
In the third human study, a single dose study of Form I versus Form V ST-246 in fed healthy volunteers, ST-246 was safe and very well tolerated. Adverse events were low in frequency and mild in intensity and no events were considered to be related to treatment with the study medication in either form. Both forms exhibited comparable plasma concentration versus time profiles. The extent of absorption (as defined by $AUC_{0-\infty}$) of Form I was approximately 12% lower than Form V.

SIGA-246-004
In the fourth human study (Phase II study), ST-246 was safe and well-tolerated. The dose proportionality analysis showed that the ratios of dose-normalized C_{max} and AUC_{τ} values ranged from 80%-85%, and the 90% confidence intervals did not include 1.0. Therefore, dose

proportionality could not be concluded. Analysis of data from the dose-normalized C_{max} and AUC_{τ} values indicate that absorption of ST-246 was not dose proportional within the 400-600 mg dose range. The most commonly reported TEAEs for subjects in the ST-246 400 mg and ST-246 600 mg groups were headache and nausea. There were no deaths or SAEs reported during this study. There were no remarkable or clinically significant results from laboratory assessments, vital sign measurements, physical examinations, or ECGs.

3.1.6 PLANNED CLINICAL STUDIES

SIGA is planning two additional clinical studies to support its New Drug Application as follows: an expanded safety study to further assess the safety of a single daily dose of ST-246 administered for 14 days and a Phase I thorough QT/QTc study to assess the potential of ST-246 to delay cardiac repolarization.

3.1.7 EMERGENCY USE IN HUMANS

3.1.7.1 E-IND NO. 112,324

On May 13, 2011, a possible contact vaccinia infection involving a 25 year old healthy, immunocompetent woman with a history of acne was reported to the CDC by the (b) (6) Department of Health. A week to 10 days previously, the woman was believed to have contracted a live VACV infection on her chin while changing a bandage covering the smallpox vaccination site of her boyfriend, a military contractor. The first lesion was noticed on May 9, with 3 more lesions developing shortly thereafter. The patient was treated on May 14 with VIGIV and was placed under house quarantine. A daily oral dose of ST-246 (400 mg) was administered for 14 days, under E-IND # 112,324, starting on May 19, 2011. The patient responded well to treatment, with no apparent side-effects. As of August, 2011, the patient was doing well and lesions had completely healed with minimal scarring. A detailed report can be found in Appendix C.

3.1.7.2 E-IND NO. 106,338

On August 15, 2009, SIGA was notified by the CDC that ST-246 would be needed to treat a 37-year old female patient who developed vesiculopustular skin lesions on her arm and hand due to accidental exposure to recombinant-vaccinia-based rabies vaccine in a bait-sponge found by the patient's dog. The patient had a history of Crohn's disease and was undergoing treatment with daily Imuran, and Remicade every 6 weeks. On August 22, 2009, after vaccinia-positive PCR results, the patient was given VIGIV and started on a daily oral dose of ST-246 (400 mg) for 14 days under E-IND # 106,338. The lesions healed and the patient was discharged on (b) (6). A detailed report can be found in Appendix D.

3.1.7.3 E-IND NO. 104,793

On March 2, 2009, a possible progressive vaccinia (PV) case involving a 20-year old male military smallpox vaccinee at a (b) (6) was reported to the CDC. Progressive vaccinia (PV) is a rare and often fatal adverse event following smallpox vaccination with live vaccinia virus. The patient's history included post-vaccination neutropenic fever which was diagnosed on January 28, 2009 as acute mylegenous leukemia M0 (AML M0). Approximately two weeks after a second round of induction chemotherapy, the patient's vaccination site deteriorated to a deep bulla, 4 cm in diameter, with a raised edge and central bleeding crust. Viral culture of a lesion swab and PCR viral analysis confirmed the presence of orthopoxvirus. Serum showed equivocal to absent anti-orthopoxvirus IgG or IgM by an ELISA

test. Based on the patient's history and test results, a diagnosis of PV was made. After receiving an initial dose of VIGIV, SIGA's oral and topical ST-246 was administered under E-IND # 104,793. The initial oral dose of 400 mg was increased to 1,200 mg during the treatment period. Analysis of ST-246 plasma concentrations indicated lower exposure than that previously observed in healthy volunteers thus the dose was increased until the samples taken indicated ST-246 concentrations had reached a range consistent with likely efficacy. There is no compelling evidence to explain the anomalously low concentrations. One possibility is that absorption of ST-246 may have been lower due to 1) lack of food intake and 2) recent chemotherapy which might have altered the intestinal muscosa. In addition, measured ST-246 concentrations were much lower compared to healthy volunteers during the time that the patient was on hemodialysis.

The patient also received repeated doses of VIGIV, topical Imiquimod, and six oral doses of CMX001 (lipidated cidofovir). During the course of treatments, the patient developed methicillin-resistant *Staphylococcus aureus* infection in vaccination satellite lesions, and *Pseudomonas aeruginosa* sepsis and multiorgan failure, requiring excessive vasopressor support resulting in bilateral trans-tibial amputation. Despite the patient's protracted clinical course with superinfections and sepsis, probably due to cellular immunodeficiencies, the patient was discharged after testing negative for VACV. A detailed report can be found in Appendix E.

3.1.7.4 E-IND NO. 74,773

In March 2007, SIGA provided ST-246 to support an Emergency Investigational New Drug Application (E-IND # 74,773) sponsored by the CDC. The E-IND was filed for the one-case use of ST-246 for treatment of a child with eczema vaccinatum. The patient, a 28-month old male child with a history of eczema and failure to thrive, was exposed to virus through direct contact with a vaccinee on February 16-20, 2007. He presented to the emergency room on (b) (6) with high fever and severe eczema. Vesicular skin scrapings and viral culture supernatant from vesicles on the child's skin were obtained. The (b) (6) Department of Public Health found the samples to be positive for non-variola orthopoxvirus by polymerase chain reaction (PCR). The child's condition continued to worsen despite initial treatment with VIGIV on hospital Day 6, and he exhibited progressive metabolic then respiratory acidosis, hypoalbuminemia, hypothermia, and hypotension through (b) (6) Following issuance of the E-IND, ST-246 was orally administered once daily for 14 days via a nasogastric tube. The dosing regimen of ST-246 was initially 50 mg/day, increased after two days to 75 mg, and increased a third time to 100 mg. Plasma samples were analyzed for ST-246 levels and when weight normalized were within the range of the concentrations observed at similar times in healthy adult volunteers (SIGA-246-004).

The child also received Cidofovir once and repeated doses of VIGIV, with the last dose of VIGIV administered on (b) (6). Clinical signs of the child's improvement were observed within 1 week of the antiviral intervention (ST-246, Cidofovir, and VIGIV). The child was extubated on (b) (6) moved out of intensive care on (b) (6), and discharged to home on (b) (6) (hospital Day 48). There were no adverse events that could be attributed to ST-246. As of February 2008 (most recent data available), the child is thriving and continues to do well. A detailed report and further information can be found in Appendices F and G.

3.1.7.5 COMPASSIONATE USE IN EUROPE

In December 2009, SIGA was contacted by the Division of Infectious Diseases at (b) (6) to request provision of ST-246 for compassionate use in a 32-year old female patient who was suffering from severe keratoconjunctivitis. The patient had been on various treatments since September 2009 and the Chief Physician reported the possibility of orthopoxvirus infection, as she tested PCR-positive for ocular CPXV (cowpox virus). The patient was given 400 mg for 14 days. Plasma, serum and tear ST-246 concentrations were monitored during treatment, and appeared adequate. There were no serious drug-related adverse events reported. As of April 2010, the patient's corneal inflammation had improved. Orthopoxviral culture remained negative, but the PCR assay still tested positive.

3.2 ST-246 Efficacy In Vivo

In vivo studies in small animal models for multiple species of orthopoxvirus, including multiple VACV strains, ECTV strain Moscow, CPXV strain Brighton, RPXV strain Utrecht and MPXV strain Zaire 79, have been necessary to evaluate the antiviral activity and dosing regimens of ST-246, providing the framework for design and testing of more definitive animal efficacy studies, particularly in NHP models more reminiscent of human disease. ST-246 has been shown to provide protective efficacy in many different animal species models of orthopoxvirus disease, by different challenge routes, and in studies conducted by multiple investigators in different laboratories. Each model was designed to mimic different aspects of orthopoxvirus disease, including local replication, systemic lesional disease, progressive cutaneous infection, and infection of an immunocompromised host. In all cases, ST-246 protected animals from lethal infection and reduced or eliminated orthopoxvirus-induced disease.

Initially, ST-246 was evaluated in several murine models of orthopoxvirus disease.[37, 42, 54, 82-84] In mice, the optimal dosing regimen for both VACV and ectromelia virus infections was found to be 100 mg/kg/day for ≥ 5 days. Treatment could be initiated as late as 72 h post-infection to provide 100% protection. However, for CPXV infection, at least 7 days of 100 mg/kg/day dosing was required for 93% efficacy, and this treatment needed to be initiated by 48 h post-infection. ST-246 also protected ground squirrels and prairie dogs from MPXV infection, and rabbits from RPXV infection. A summary of small animal studies is presented in Table 3.2-1 and the studies, including objectives, study design and results, are located in Appendix H.

Table 3.2-1: Summary of ST-246 Activity in Animal Models

Virus	Host	Optimal Oral Dose mg/kg, q.d.	Dose Duration (% Efficacy)	Time of Treatment (% Survival)
Vaccinia	Mouse	100	≥ 5 days (100%)	72 h.p.i (100%)
Cowpox	Mouse	100	≥ 7 days (93%)	48 h.p.i. (93%)
Ectromelia	Mouse	100	≥ 5 days (100%)	72 h.p.i. (100%)
Monkeypox	Squirrel	100	Not done	72 h.p.i. (100%)
	Prairie Dog	30	Not done	240 h.p.i. (100%)
Rabbitpox	Rabbit	40	Not done	72 h.p.i. (83%)

KEY: h.p.i. = hours post-infection; q.d. = one dose daily for 14 days

A number of *in vivo* studies have also been conducted in NHPs using IV inoculation of MPXV and VARV strain Harper. Clinical signs and pathology of these diseases in NHPs are similar to that of monkeypox and smallpox infections in humans, although the IV MPXV NHP model demonstrates a higher mortality rate in NHPs compared to VARV, which does not replicate as efficiently in NHPs. However, in all challenge experiments to-date, ST-246 administered as an oral suspension at doses ≥ 3 mg/kg at the time of infection or < 96 hours post-infection, not only protected monkeys from death, but significantly decreased the amount of viral DNA present in the blood, as compared to outcomes after administration of a vehicle.

ST-246 delivered at 3, 10, 30, 100 and 300 mg/kg/day at 3 days after infection for a period of 14 days suppressed lesion formation and protected NHPs infected with a lethal intravenous dose of MPXV (SIGA AP-06-21). ST-246, delivered at 10 or 20 mg/kg/day starting at 3 or 4 days post -infection, also suppressed lesion formation and protected monkeys infected with a lethal dose of MPXV administered intravenously (AP-06-21G). In a separate study, treatment with ST-246 at 10 mg/kg/day could be initiated as late as 120 h post-infection and still provide 100% protection (AP-06-21E6). In a recent study (FY10-087), we have evaluated the impact of ongoing MPXV infection on the pharmacokinetic parameters of ST-246. Infection had no significant impact on the PK of ST-246 when treatment was initiated at 4 days post-infection at doses of 3, 10, and 20 mg/kg for 14 doses. All three dose levels (3, 10 and 20 mg/kg) resulted in 100% survival. Additional studies evaluated the impact of ST-246 treatment at 10 mg/kg for 14 days following dose delays of 4, 5, or 6 days post-infection (SR10-037F) and ST-246 treatment at 10 mg/kg for dose durations of 3, 5, 7, and 10 doses (SR10-038F) when treatment was initiated at 4 days-post-infection. Dose delays of 4 or 5 days post-infection resulted in 83% survival, while a delay of 6 days resulted in 50% survival. Treatment durations of 3, 5, 7, or 10 doses resulted in 50%, 100%, 100% and 80% survival respectively. ST-246 delivered at 10 mg/kg/day protected NHPs from VARV-induced disease when treatment was initiated even as late as lesion onset at 4 days post-infection in this non-lethal model. A summary of the NHP studies conducted to date is shown in Table 1 and complete study descriptions, including objectives, study design and results, is presented in Sections 3.2.1 (Summary of IV MPXV Nonhuman Primate Studies) and 3.2.2 (Summary of Variola Nonhuman Primate Studies)

Recently, SIGA has participated in a blinded, MPXV respiratory challenge study carried out at Lovelace Respiratory Research Institute (LRRI). Animals were given placebo or 10 mg/kg ST-246 by oral gavage once daily for 14 days beginning 1, 2, 3 or 4 days post-challenge. Preliminary results show that 100% of ST-246-treated animals survived compared to only 25% of controls. A description of the study, including objectives, study design and preliminary results, is presented in Section 3.2.3

3.2.1 SUMMARY OF IV MPXV NONHUMAN PRIMATE STUDIES

3.2.1.1 FY-06-035 EFFICACY IN LETHAL CHALLENGE

Title: ST-246: Efficacy Evaluation of an Antiviral Agent in an Intravenous Monkeypox Virus Challenge Model Using Cynomolgus Macaques (*Macaca fascicularis*)

Objective: To determine the potential protective efficacy of the antiviral drug candidate ST-246 in a lethal MPXV challenge (6.57×10^6 pfu) model in 4 male and 8 female, non-fasted cynomolgus macaques (*Macaca fascicularis*).

Study Design: This was a randomized, placebo-controlled, repeat-dose study in cynomolgus macaques. Twelve infected monkeys were randomized into 3 treatment arms: 2 males and 2 females in the high group (ST-246 300 mg/kg/day), 4 females in the low group (ST-246 30 mg/kg/day), and 2 males and 2 females in the control group (placebo). Monkeys were challenged intravenously with a target dose of $1x10^7$ pfu of MPXV on Day 0. One hour after the virus challenge, non-fasted monkeys were dosed with either placebo or 1 of the 2 dose levels of ST-246, according to each animal's study group assignment. Food was not administered via nasogastric tube immediately following administration of ST-246 or placebo. Dose administration continued once daily from Day 0-13. Evaluation of the animals continued through Day 28 after MPXV challenge and included twice daily (b.i.d.) cage-side clinical observation and qualitative assessment of food consumption. Body weights were obtained at predetermined times during the experiment (Days -12, 0, 6, 9, 11, and 20). A physical examination of each monkey was conducted by a licensed veterinary technician under the supervision of a veterinarian before challenge and at scheduled necropsy. Subcutaneous body temperatures were obtained before challenge and b.i.d. thereafter. The number, location, and characteristics of dermal lesions were assessed and documented periodically (Days 2, 4, 6, 8, 10, 12, 14, 16, 20, and 28). Clinical pathology (hematology and serum chemistry) was assessed periodically before and after challenge (Days -12, 0, 6, 12, 20, and 28). Viral load was determined in whole blood and buccal swabs at defined intervals (Days 0, 2, 4, 6, 8, 10, 12, 14, 16, 20, and 28) and in selected tissues (adrenal gland, brain, inguinal lymph node, kidney, liver, lung, and spleen) from all monkeys that died or were euthanized. At necropsy, selected tissues were obtained, weighed, fixed in formalin, and processed for histopathologic evaluation. All surviving study monkeys were euthanized after Day 28.

Efficacy Results:

Mortality: Analysis of the challenge material indicated that animals were infected with $6.57x10^6$ pfu of MPXV. Two untreated monkeys were euthanized as moribund on Days 13 and 17 after infection. No deaths or euthanizations due to moribund states occurred among monkeys treated with ST-246 during the study.

Total Body Lesion Counts: Mean total body lesion counts were summarized by treatment group and study day in Table 3.2.1.1-1. Pox lesions were not observed on any study animals treated with ST-246.

Table 3.2.1.1-1: FY06-035 Total Lesion Count

Group	Study Day											
	2	**4**	**6**	**8**	**10**	**12**	**13[a]**	**14**	**16**	**17[a]**	**20**	**28**
Control												
Mean	0	68	517	866	1147	1053	645	578	419	420	214	40
SD	0	25.7	61.2	53.4	84.9	31.6	–	52.2	65.7	–	–	–
N	4	4	4	4	4	4	1	3	3	1	2	2
Low ST-246												
Mean	0	0	0	0	0	0	–	0	0	–	0	0
SD	0.0	0.0	0.0	0.0	0.0	0.0	–	0.0	0.0	–	0.0	0.0
N	4	4	4	4	4	4	–	4	4	–	4	4
High ST-246												
Mean	0	0	0	0	0	0	–	0	0	–	0	0
SD	0.0	0.0	0.0	0.0	0.0	0.0	–	0.0	0.0	–	0.0	0.0
N	4	4	4	4	4	4	–	4	4	–	4	4

[a]Additional unscheduled lesion counts were performed on animals upon euthanasia.
KEY: N = number of subjects; pfu = plaque-forming unit; SD = standard deviation

Viral Load: MPXV was not recovered at any time from the blood of monkeys treated with ST-246; therefore, blood MPXV titers for treated animals are absent from the figure. MPXV was recovered from the blood of all 4 control animals, and viral titers increased initially, dropped, increased, and dropped again. Viral titers were highest in the 2 animals that became moribund and were euthanized. Surviving control animals had no MPXV in the blood from Day 20 to the end of the study. No MPXV was identified in the tissues of animals treated with ST-246; however, MPXV was identified in the inguinal lymph nodes of 2 control animals and in the lung tissue of another animal.

Conclusion: Clinical observations for all animals treated with ST-246 were essentially normal throughout the study with occasional body temperature fluctuations. All control animals had significant clinical observations that included reduced grooming, dull/rough coat, body weight loss of up to 16%, elevated body temperature, decreased body temperature, decreased food intake, no food intake, subdued behavior (hypoactive), enlarged inguinal lymph nodes, dehydration, unresponsiveness, weakness, and pox lesions.

All 4 untreated monkeys developed typical MPXV dermal lesions by Days 4 to 6 after infection, which peaked on Days 12 to 16, as well as lymphadenopathy beginning on Day 8. Two untreated monkeys were euthanized as moribund (Days 13 and 17), and MPXV was detected in the tissues of both animals.

All 8 of the animals treated with ST-246 remained healthy until euthanasia. No dermal lesions were observed in either ST-246 treatment group, but lymphadenopathy was present, more so in the 30 mg/kg group than the 300 mg/kg group. MPXV was not found in the blood or tissues of MPXV-infected animals treated with ST-246.

3.2.1.2 AP-06-21 Experiment 1 and 2 Efficacy At Different Dose Levels

Title: Evaluation of Oral ST-246 in the Lesional Monkeypox Cynomolgus Monkey Model

Objective: To evaluate and compare the ability of ST-246 at 5 different dose levels administered orally in 14 daily doses to treat the classic signs of MPXV and the severe rash illness and to observe a reduction in viral load and decrease in mortality with reference to placebo.

Study Design: Experiments 1 and 2 were randomized, double-blind, placebo-controlled, repeat-dose studies of ST-246 monotherapy, administered orally in parallel groups. Male monkeys in both experiments were infected with $5x10^7$ pfu of Zaire 79 strain of MPXV and received ST-246 or placebo for 14 days after infection. Supplemental food was not administered immediately after dosing of ST-246 or placebo. The 2 experiments employed different daily doses and delayed treatment initiation times of ST-246.

Experiment 1: Eight infected monkeys were grouped in 2 cohorts of 4 monkeys each. Within each cohort, 3 monkeys were randomized to receive ST-246 and 1 monkey to receive placebo. Monkeys in Cohort 1 started treatment on Day 1 after infection with either 300 mg/kg/day of ST-246 or placebo for 14 days, and monkeys in Cohort 2 started treatment on Day 3 after infection with either 300 mg/kg per day of ST-246 or placebo for 14 days.

Experiment 2: Fifteen infected monkeys were randomized into 5 treatment arms of 3 monkeys each. These arms comprised placebo and 3, 10, 30, and 100 mg/kg/day of ST-246. Treatment started on Day 3 after infection and continued for 14 days.

As the numbers evaluated in each treatment group were small and the 2 experiments were otherwise similar in study conduct and data collection, data from the 2 experiments were pooled together to assess the efficacy of ST-246 and dose-response against MPXV infection. There were a total of 23 monkeys infected with MPXV in the combined database. The infected monkeys were randomized to receive 1 of 5 different oral daily doses of ST-246 (3, 10, 30, 100, and 300 mg/kg/day) or placebo for 14 days. The placebo group had 5 monkeys; the 3, 10, 30, and 100 mg/kg/day groups had 3 monkeys each; and the 300 mg/kg/day had 6 monkeys. Treatment began on Day 3 (72 hours) after infection for all but 1 treatment arm (including placebo). Three of the 6 monkeys in the 300 mg/kg/day ST-246 dose group received treatment on Day 1 (24 hours) after infection.

Efficacy Results:
Mortality: No deaths or euthanizations due to moribund states occurred among animals treated with ST-246 during the study. All animals treated with placebo either died (3 out of 5) or required euthanization (2 out of 5) due to moribund conditions.

Total Body Lesion Counts: Poxvirus lesion count analyses were based on the average count derived over a 4-day interval after infection (i.e., Days 1-4, 5-8, 9-12, 13-16, 17-21, and 31-33) for the infected population. The poxvirus lesion count was the sum score across lesion types. Statistically significant differences in the numbers of poxvirus lesions among the treatment groups were noted beginning with the Day 5-8 assessment ($P < 0.0001$) and continued through the endpoint assessment ($P = 0.0209$). Animals treated with ST-246 had significantly fewer poxvirus lesions than those treated with placebo at the Day 5-8 and Day 9-12 assessments. No lesions were noted among animals that received 300 mg/kg/day of ST-246 on Day 1. Animals that received 300 mg/kg/day of ST-246 on Day 3 developed a small number of lesions, which

were observed beginning at the Day 5-8 assessment; however, no lesions remained among these animals by the Day 13-16 assessment.

Viral Load: Viral load analyses were based on the average genomes/mL derived over a 4-day interval after infection (i.e., Days 1-4, 5-8, 9-12, 13-16, 17-21, and 31-33) for the infected population. Pairwise comparisons of each active dose versus placebo over the treatment time interval indicated that viral load decreased generally in a dose-dependent manner beginning with the Day 1-4 assessment. At that time point, the 300 mg/kg/day doses (Day 1 and Day 3) significantly decreased the amount of virus present when compared with placebo. All doses of ST-246 significantly decreased the amount of virus present when compared with placebo beginning with the Day 5-8 assessment, with the exception of the 3 mg/kg/day treatment versus placebo at the Day 13-16 assessment. At study endpoint, viral DNA levels in blood in the groups treated with ST-246 showed a linear dose-response, with the 3 mg/kg/day dose group yielding the lowest reduction (> 1,000-fold) in viral replication compared with the placebo group. The differences among each ST-246 treatment group compared with placebo were highly statistically significant (analysis of variance: $P < 0.0001$).

Conclusion: At this viral dose, this is a uniformly lethal model and the NHPs develop approximately 1,500 lesions before death. In Experiment 1, NHPs were dosed orally with placebo or 300 mg/kg/day of ST-246 starting at 24 or 72 hours after infection and continuing for 14 days (Days 1-14 or Days 3-16). Both ST-246 treatment groups were completely protected from disease, and viral DNA levels in blood were quickly reduced to below detectable limits.

In Experiment 2, ST-246 was tested in a dose-optimization experiment where treatment was initiated 72 hours after infection with MPXV. The NHPs were treated with 3, 10, 30, or 100 mg/kg/day of ST-246 for 14 days by oral gavage and subjected to physical examination, viral load assessment, and hematological and chemical chemistry analyses. The criteria for establishing efficacy were: the lack of mortality; suppression of viral lesions to < 20% of control lesion counts; an increase in viral load of < 100-fold as compared with the level of those just before initiation of treatment; and duration of viremia of < 50% versus control animals. Since all ST-246 treatment groups met these criteria, preliminary data suggests ST-246 can prevent death when administered 3 days after infection at a dose level of ≥ 3 mg/kg.

3.2.1.3 AP-06-021G EFFICACY (THERAPEUTIC WINDOW AND DOSE)

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

Objective: To determine the duration of the therapeutic window (i.e., delaying treatment until 72 or 96 hours after infection) and dose level (10 or 20 mg/kg/day) of oral ST-246 for therapeutic treatment of MPXV infection by using the lesional cynomolgus monkey model of smallpox.

Study Design: This was a randomized, blinded, placebo-controlled, parallel-group, longitudinal study of oral ST-246 in cynomolgus monkeys infected intravenously with MPXV. All animals participating in the study were male and were anesthetized and infected intravenously on Study

Day 0 with $5x10^7$ pfu of MPXV challenge stock (strain Zaire – CDC V79-I-005). One animal in Treatment Group 5 (ST-246 20 mg/kg Study Day 4) was incorrectly dosed and was included in Treatment Group 3 (ST-246 20 mg/kg Study Day 3) for all statistical analyses. The final treatment groups are defined in Table 3.2.1.3-1. Pox lesions appeared on 100% of animals by 4 days post-infection, although the appearance of lesions did not dictate treatment initiation.

Table 3.2.1.3-1: AP-06-021G Treatment Groups

Treatment Group	N	Study Day; Treatment Initiation	Frequency of Test/Control Dosing	Test/Control Substance	Test Substance (mg/kg/dose)
1	4	3; 72 h.p.i. ± 3 h.p.i.	Once daily	Placebo	0
2	3	3; 72 h.p.i. ± 3 h.p.i.	Once daily	ST-246	10
3	4	3; 72 h.p.i. ± 3 h.p.i.	Once daily	ST-246	20
4	3	4; 96 h.p.i. ± 3 h.p.i.	Once daily	ST-246	10
5	2	4; 96 h.p.i. ± 3 h.p.i.	Once daily	ST-246	20

KEY: h.p.i. = hours postinfection; N = number of subjects

The animals received 15 daily oral doses of blinded study medication (10 or 20 mg/kg of ST-246 or placebo), such that the placebo control article was administered to Treatment Group 1 on Study Day 3 and continued every 24 ± 3 hours for 15 consecutive days. In Treatment Groups 2 and 3, the test article was administered on Study Day 3 and continued every 24 ± 3 hours for 14 consecutive days followed by 1 day of placebo control article to maintain blinding (Study Day 17). In Treatment Groups 4 and 5, the test article was administered beginning on Study Day 4 after infection and continued every 24 ± 3 hours for 14 consecutive days. The placebo control article was administered on Study Day 3 to maintain blinding.

Efficacy Results:
Mortality: The 12 animals treated with ST-246 survived until scheduled termination at Study Day 42. All 4 animals in the placebo group were euthanized due to moribund states from typical disease between Study Days 13 and 15, inclusive.

Viral Load and Total Body Lesion Count: The area under the curve (AUC) for viral load and total lesion count are summarized in Table 3.2.1.3-2.

There was a statistically significant difference between the AUC for viral load when the combined ST-246 treatment group was compared with placebo ($P = 0.0005$), and for all other treatment groups ($P \leq 0.0286$), except for Treatment Group 5 ($P = 0.0667$). The lack of statistical significance in Treatment Group 5 was likely because of the dosing error that reduced the sample size from 3 animals to 2.

There was also a statistically significant difference between the AUC for total lesion count when the combined ST-246 treatment group was compared with placebo ($P = 0.0005$), and for all other treatment groups ($P \leq 0.0286$), except for Treatment Group 5 ($P = 0.0667$). The time to maximum viral load and total lesion count are summarized in Table 3.2.1.3-3. The results indicate there was a marked difference between the median time to maximum viral load for the placebo group (12 days) and Treatment Groups 2 and 3 (4 days).

Table 3.2.1.3-2: AP-06-021G AUCs for Viral Load and Total Lesion Count

Parameter	Placebo N = 4	Group 2 10 mg/kg 72 h.p.i. N = 3	Group 3 20 mg/kg 72 h.p.i. N = 4	Group 4 10 mg/kg 96 h.p.i. N = 3	Group 5 20 mg/kg 96 h.p.i. N = 2	Combined ST-246 Groups N = 12
Viral Load (genomes x 1,000/mL)						
Mean	66834.2	871.6	864.2	864.0	871.3	867.2
SD	23626.8	280.4	448.8	246.4	301.6	297.6
P value		0.0286	0.0143	0.0286	0.0667	0.0005
Total Lesion Count						
Mean	12744.3	2067.0	1019.5	2995.0	2162.0	1965.7
SD	2852.4	1464.5	1307.7	2347.0	1562.7	1644.4
P value		0.0286	0.0143	0.0286	0.0667	0.0005

KEY: h.p.i. = hours postinfection; N = number of subjects; SD = standard deviation.

Table 3.2.1.3-3: AP-06-021G Time to Maximum Viral Load and Total Lesion Count

Time	Placebo N = 4	Group 2 10 mg/kg 72 h.p.i. N = 3	Group 3 20 mg/kg 72 h.p.i. N = 4	Group 4 10 mg/kg 96 h.p.i. N = 3	Group 5 20 mg/kg 96 h.p.i. N = 2	Combined ST-246 Group N = 12
Time to Maximum Viral Load (Days)						
Mean	11.5	9.0	3.8	12.7	13.0	8.8
SD	1.00	9.54	0.50	7.51	7.07	6.93
Median	12.0	4.0	4.0	13.0	13.0	4.5
Min, Max	10, 12	3, 20	3, 4	5, 20	8,18	3, 20
Time to Maximum Total Lesion Count (Days)						
Mean	11.0	13.0	9.0	6.7	11.5	9.8
SD	1.15	0.00	2.71	1.15	2.12	2.98
Median	11.0	13.0	10.0	6.0	11.5	10.0
Min, Max	10, 12	13, 13	5, 11	6, 8	10, 13	5, 13

KEY: h.p.i. = hours postinfection; Max = maximum value; Min = minimum value; N = number of subjects; SD = standard deviation.

Conclusion: Oral administration of ST-246 for 14 consecutive days at doses of 10 and 20 mg/kg/day starting at 3 or 4 days after MPXV challenge prevents mortality and reduces disease symptoms (viral load and lesion number) in cynomolgus monkeys intravenously infected with a lethal dose of MPXV.

3.2.1.4 AP-06-021E6 EFFICACY

Title: Evaluation of Oral ST-246 in the Lesional Monkeypox Cynomolgus Monkey Model

Objective: To evaluate and compare the ability of ST-246 polymorph I administered orally at 10 mg/kg in 14 daily doses starting 5 days after infection to treat the classic signs of MPXV

infection and the severe rash illness and to observe a reduction in viral load and decrease in mortality with reference to placebo.

Study Design: This was an observational, parallel-group, repeat-dose, placebo-controlled study. Six infected monkeys were grouped in 2 cohorts with 5 monkeys (3 males and 2 females) in Cohort 1 receiving ST-246 treatment and 1 male monkey in Cohort 2 receiving placebo. Monkeys in Cohort 1 started treatment on Day 5 after infection with ST-246 polymorph I at 10 mg/kg per day for 14 days and the monkey in Cohort 2 started treatment on Day 5 after infection with placebo for 14 days. ST-246 and placebo were administered at a dose of 2.0 ± 0.1 mL/kg of liquid suspension followed by 5 ± 0.5 mL/kg of a suspension of hydrated homogenized monkey biscuits by oral gavage. Following MPXV exposure, the infected animals were observed by the investigator at least twice each day for up to 28 days to examine signs of illness.

Efficacy Results:
Mortality: No deaths due to moribund states occurred among animals treated with ST-246 during this study. The placebo-treated monkey was recovering from infection but was euthanized on Day 20. The monkey had an abscess on the elbow, and the study director concluded that the animal had a bacterial infection at the time of euthanasia.

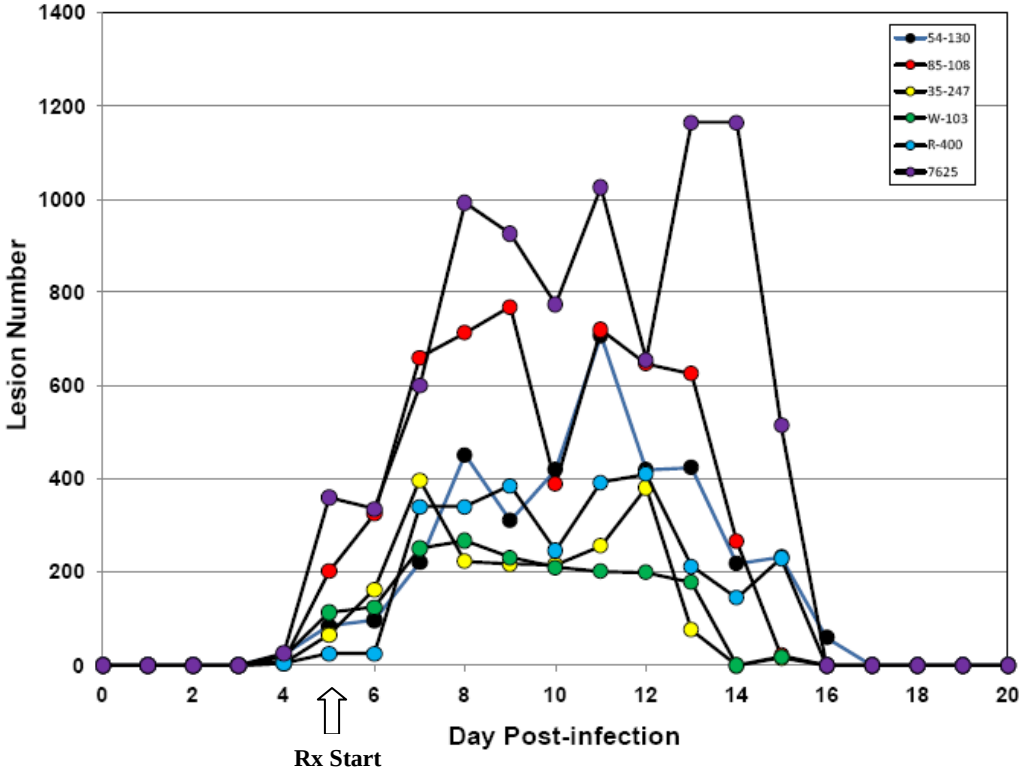
Total Body Lesion Counts: Study days on which pox lesions were first observed for each animal and the number of lesions observed that day are presented in Table 3.2.1.4-1. Total body lesion counts are depicted by treatment group and study day in Figure 3.2.1.4-1.

Table 3.2.1.4-1: AP-06-021E6 Onset of Lesion Formation

Animal ID	Treatment (mg/kg/day)	Study Day First Lesions Appeared (Lesion Number)	Number of Lesions at Treatment (120 h.p.i.)
54-130	Placebo	4 (23)	84
85-108	10	4 (8)	202
35-247	10	4 (4)	65
W-103	10	4 (19)	113
R-400	10	4 (4)	25
7625	10	4 (26)	360

KEY: h.p.i. = hours postinfection; ID = identification.

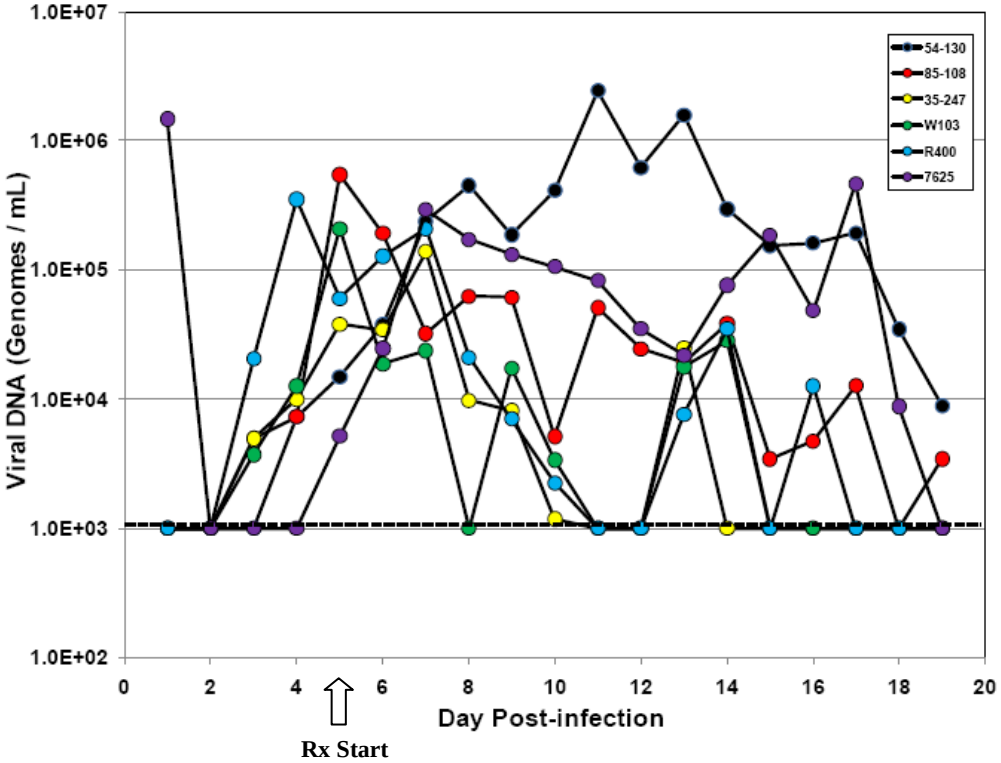
Figure 3.2.1.4-1: AP-06-021E6 Total Body Poxvirus Lesion Count



Lesions were first observed by Day 4 after infection and accumulated in number until approximately Day 8 after infection and were fully resolved by Day 17 after infection. Both cohorts began treatment on Day 5 after infection.

Viral Load: The effect of MPXV infection and ST-246 treatment on MPXV viral load in blood is shown in Figure 3.2.1.4-2. Lower levels of viral DNA were found in blood for all ST-246-treated animals between Day 8 and Day 14. Both cohorts began treatment on Day 5 after infection.

Figure 3.2.1.4-2: AP-06-021E6 Blood Viral DNA (Genome/mL)



Conclusion: All ST-246-treated animals survived the infection; however, the placebo-treated animal did not die from monkeypox infection. It died from an apparent bacterial infection at Day 20. Results suggest that oral administration of ST-246 at 10 mg/kg/day starting at Day 5 post-monkeypox virus infection could potentially protect cynomolgus monkeys from lethal infection but no clear conclusion can be drawn from the study since the placebo-treated animal did not die from monkeypox infection.

3.2.1.5 AP-09-026G Efficacy and Minimum Effective Dose

Title: Double Blind, Randomized, Placebo-Controlled, Repeat-Dose Efficacy Study of the Minimum Effective Therapeutic Dose of Oral ST-246 Polymorph I in Cynomolgus Monkeys Infected with Monkeypox Virus

Objective: To determine the minimum effective dose of oral ST-246 for the treatment of MPXV in the lesional cynomolgus monkey model of smallpox beginning individually on the day of onset of lesions in each animal.

Study Design: This was a randomized, double-blinded, placebo-controlled study of oral ST-246 in cynomolgus monkeys infected intravenously with MPXV (strain Zaire – CDC V79-I-005). All animals participating in the study were male and were anesthetized and infected intravenously on Day 0 with $5x10^7$ pfu of MPXV. Of the 27 available monkeys, 25 were randomly assigned into 2 iterations (5 treatment groups per iteration with 3 monkeys per group in iteration 1 and 2 monkeys per group in iteration 2). The 2 remaining monkeys (1 per iteration) were assigned to the placebo group. Monkeys received placebo (vehicle) or ST-246 at 0.3, 1, 3, or 10 mg/kg by oral gavage once daily beginning on the day lesions first appeared on an animal

and continuing for 14 consecutive days. ST-246 and placebo were administered at a dose of 2.0 ± 0.1 mL/kg of liquid suspension followed by 5 ± 0.5 mL/kg of a suspension of hydrated homogenized monkey biscuits by oral gavage. Following MPXV exposure, the infected animals were observed by the investigator at least twice each day for up to 42 days to examine signs of illness.

Efficacy Results:
Mortality: Table 3.2.1.5-1 summarizes the numbers of animals that died or were euthanized due to disease. In the placebo and ST-246 1 mg/kg groups, no animals (0%; 0 of 7 and 0 of 5 animals, respectively) survived to study termination, although 1 monkey in the ST-246 1 mg/kg group died on Day 27, had resolving lesions, and was reported as not typical disease. In the ST-246 0.3 mg/kg Group 1 of 5 animals (20%) in survived to study termination. In both the ST-246 3 and 10 mg/kg groups, 4 of the 5 monkeys (80%) survived to study termination. The deaths of the 2 animals in these 2 higher dose groups were reported as not due to typical disease.

Table 3.2.1.5-1: AP-09-026G Survival up to Day 42

Dose Group (mg/kg)	Number per Group	Number Completing Study	Day After Infection to Death[a]
0	7	0	9, 14 (2), 15 (2), 17 (2)
0.3	5	1	12, 15, 16, 18, 42
1	5	0	11, 14, 15, 16, 27[b]
3	5	4	5[b], 43[c] (4)
10	5	4	11[b], 42 (4)

[a]Number in parentheses indicates the number of animals necropsied on that day.
[b]Deaths of these animals was reported as not due to typical disease.
[c]The study concluded on Day 42 but some surviving animals were not actually euthanized until Day 43.

In general, the gross findings in animals that succumbed or were euthanized moribund were consistent with typical monkeypox virus infection in this species. Most of these animals (16/18) died or were euthanized between Day 9-17 post-infection. One animal in the 1 mg/kg treatment group was found dead on Day 27 post-infection. It is unusual for cynomolgus macaques to succumb this late in the disease course, and this animal had underlying co-morbidities as revealed by histopathology. The ST-246-treated animals that survived to the end of the in-life phase (n = 9) had chronic lesions frequently observed in this species after surviving monkeypox virus infection. These included desquamated skin lesions in 9/9 (100%), lymphadenopathy in one or more lymph nodes in 6/9 (67%), pleural adhesions in one 10 mg/kg animal, and nodular pleural lesions in one 3 mg/kg animal. One 10 mg/kg animal had pinpoint white foci (lymphoid hyperplasia) in the spleen. Fibrous adhesions were present in the abdominal cavity (liver and/or spleen) in 3/9 animals. These animals likely had inflammation associated with the peritoneal cavity which resolved but resulted in adhesions; however, the relationship of these lesions to infection with monkeypox virus is unknown.

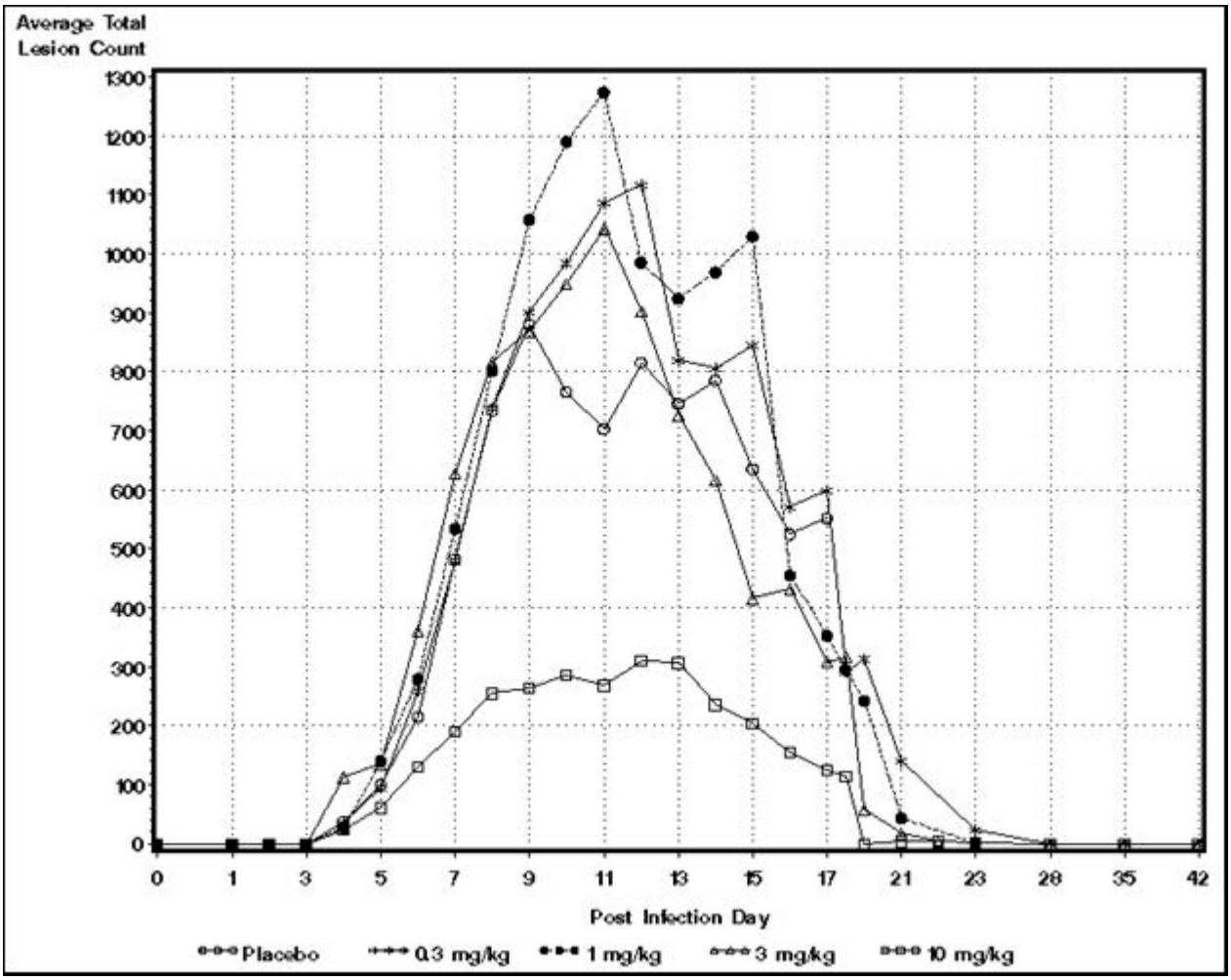
A subdural hematoms was noted grossly and confirmed histologically in the animal in the 3 mg/kg treatment group that died while under anesthesia on Day 5 post-infection. This death was not considered to be related to monkeypox virus infection. Although this may have been

associated with the cause of death there was no evidence of an underlying cause; the reason for this hemorrhage could not be determined histologically. Additionally, this animal died after undergoing anesthesia and, although it underwent anesthesia prior to this episode, it is possible that death was associated to this particular anesthetic event. In cases of an anesthesia-related death, there is often a lack of histopathologic evidence because acute hypoxia and/or metabolic abnormalities cause the animal to die before there is enough time for any lesions to develop that can be detected by routine light microscopy. Histologic lesions that were related to early monkeypox virus infection included epidermal hyperplasia in the skin, lymphocytolysis in the mandibular lymph node and tonsil, proliferative and necrotizing tonsillitis, subacute orchitis and hepatitis, and acute to subacute perivascular inflammation in the mucocutaneous junction of the lip and nose. There was also hyperplasia of the myeloid series in the bone marrow which indicates increased tissue demand for white blood cells (inflammation).

Ultimately, the cause of death of the one animal that dies in the 10 mg/kg treatment group is unknown as the clinicopathologic findings attributed to monkeypox virus were relatively mild in comparison to other animals that succumbed at this time point post-infection (Day 11). As with the animal in the 3 mg/kg group, this animal died after undergoing anesthesia and, although it underwent anesthesia prior to this episode, it is possible that death was related to this particular anesthetic event. The histologic lesions interpreted to be related to monkeypox virus infection in this animal were unusual in character. On Day 11 post infection, lesions attributed to monkeypox virus infection are usually necrotizing; however, the lesions in this animal were subacute to chronic and very mild in severity. These included lymphoplasmacytic and histiocytic orchitis, hepatitis, interstitial pneumonia, and perivascular and periadnexal dermatitis. It is possible that the test article altered the disease course in this animal, allowing for the development of atypical lesions (less severe and non-necrotizing) at this time point post-infection.

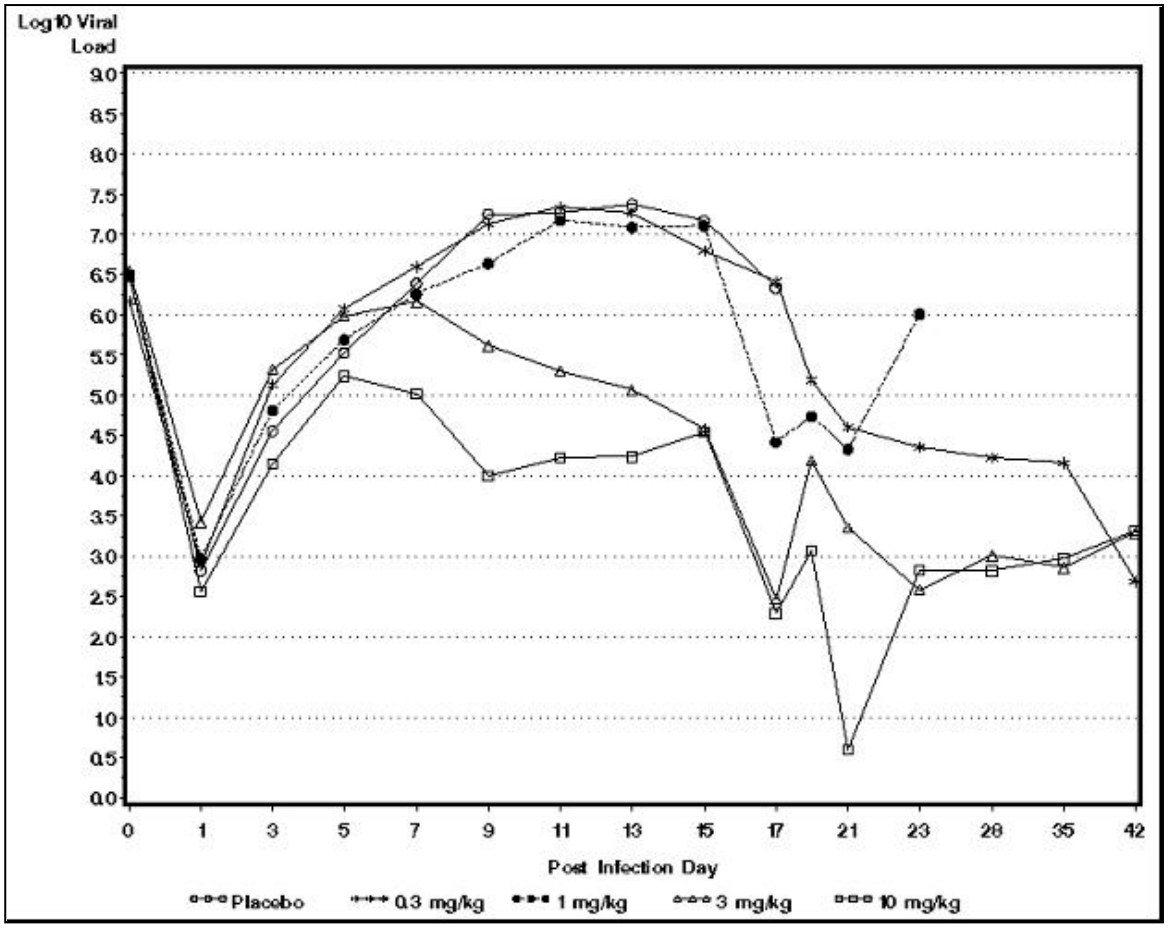
Total Body Lesion Counts: Total body lesion counts are depicted by treatment group and study day in Figure 3.2.1.5-1. The mean maximum total lesion count that occurred after Day 1 of treatment was significantly decreased (> 69%) in the ST-246 10 mg/kg group compared with that of the placebo group. Mean maximum levels were 1080.43, 1196.60, 1343.60, 865.80, and 337.40 lesions for placebo and ST-246 0.3, 1, 3, and 10 mg/kg groups, respectively.

Figure 3.2.1.5-1: AP-09-026G Total Lesion Count Through Day 42



Viral Load: The effect of MPXV infection and ST-246 treatment on MPXV viral load in blood is shown in Figure 3.2.1.5-2. The mean maximum $\log_{10}$ viral DNA level that occurred after Day 1 of treatment was significantly decreased (> 20%) in the ST-246 3 and 10 mg/kg groups compared with that of the placebo group (P = 0.0331 and P = 0.0185, respectively). Mean maximum levels were 7.61, 7.43, 7.36, 5.97, and 5.39 genomes/mL for placebo and ST-246 0.3, 1, 3, and 10 mg/kg groups, respectively.

Figure 3.2.1.5-2: AP-09-026G Viral Load (Genomes/mL) Through Day 42



Conclusion: Based on the results of this study, the minimum effective dose of oral ST-246 for the treatment of MPXV in the lesional cynomolgus monkey model of smallpox beginning individually on the day of onset of lesions in each animal was 3 mg/kg (36 mg/m^2) with corresponding plasma levels of approximately 250 ng/mL at 4 hours after dosing. Findings at 3 mg/kg included increased survival (80%), significantly decreased viremia, less severe clinical chemistry changes, and less severe and less frequent gross necropsy findings.

3.2.1.6 FY10-087 PHARMACOKINETICS IN INFECTED NHPS

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

Objective: To evaluate the pharmacokinetics of ST-246 in Cynomolgus macaques infected via intravenous injection with MPXV beginning 4 days following.

Study Design: This was a randomized, placebo-controlled study of oral ST-246 in cynomolgus monkeys infected intravenously with MPXV (strain Zaire – CDC V79-I-005). The dose groups and experimental plan are shown in Tables 3.2.1.6-1 and 3.2.1.6-2. In brief, at 10 days prior to infection the animals were orally administered vehicle or ST-246 (3, 10, or 20 mg/kg) with blood collected for pharmacokinetic (PK) analyses. ST-246 was then allowed to "wash-out" for 9 days. Following challenge on Day 0, treatment (vehicle or ST-246) began 4 days post-challenge with

PK blood sampling on Days 4, 10, and 17 days post challenge. The PK blood sampling scheme is shown in Table 3.2.1.6-3. Animal weights, observations, viral load and lesion progression were monitored throughout the study.

Table 3.2.1.6-1: FY10-087 Study Group Layout

Group	No. NHP/Group	MPXV
1 – Vehicle	6 (3 male/3 female)	$5x10^7$ pfu IV
2 – ST-246 3 mg/kg		
3 – ST-246 10 mg/kg		
4 – ST-246 20 mg/kg		

Table 3.2.1.6-2: FY10-087 Daily Schedule for Observations and Sample Collection

	Day															
Sample for Assay	**-10**	**0**	**3**	**4**	**6**	**9**	**10**	**12**	**15**	**17**	**18**	**19**	**20**	**21**	**24**	**28**
Weight	X	X	X		X	X		X	X		X			X	X	X
Lesion counts			X		X	X		X	X		X			X	X	X
Clinical Obs./Temp	X|----------Twice Daily----------|															
Whole blood (Virus) (viremia)	X	X	X		X	X		X	X		X			X	X	X
Plasma (PK)	X			X			X			X	X	X	X			

Table 3.2.1.6-3: FY10-087 Plasma Sampling Schedule for PK Analysis

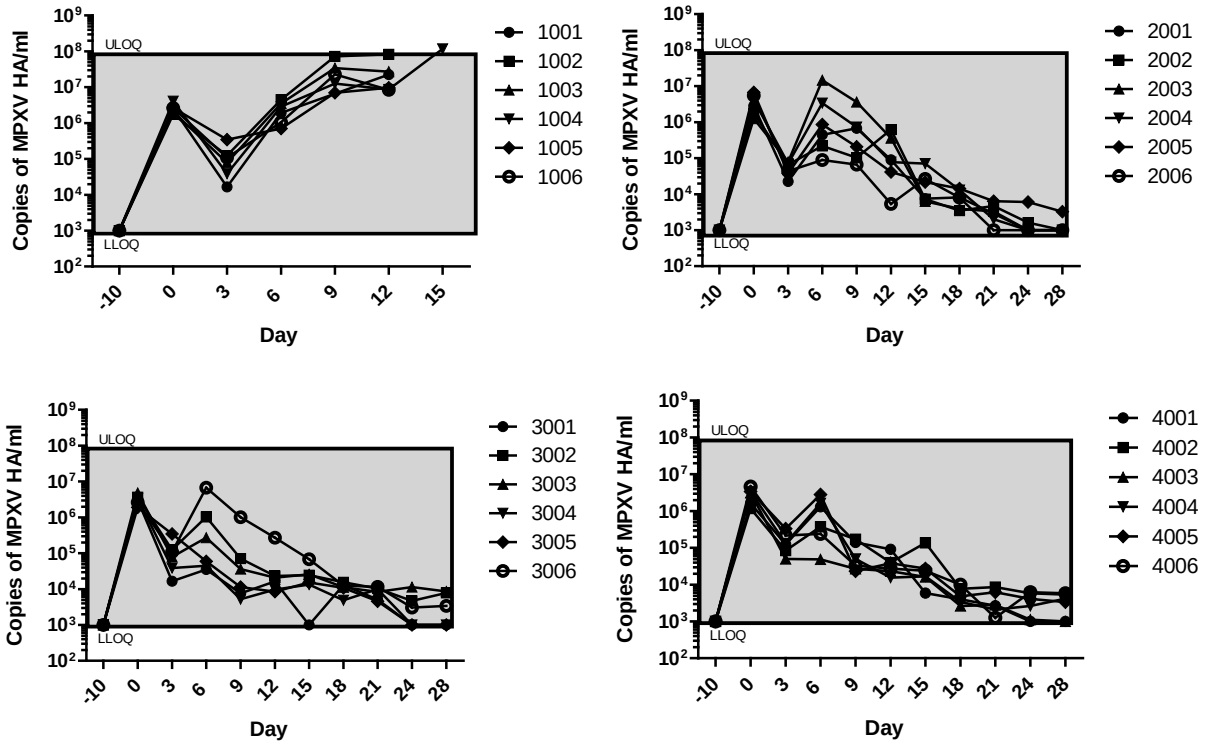
Day -10								**Day -9**	**Day -8**	**Day -7**
Time Points	**Predose**	**1 Hr**	**3 Hrs**	**4 Hrs**	**8 Hrs**	**12 Hrs**	**18 Hrs**	**24 Hrs**	**48 Hrs**	**72 hrs**
3 Males	X	X	X	X	X	X	X	X	X	X
3 Females	X	X	X	X	X	X	X	X	X	X
Day 4 (After First Dose)										
Time Points	**Predose**	**1 Hr**	**3 Hrs**	**4 Hrs**	**8 Hrs**	**12 Hrs**	**18 Hrs**	**24 Hrs**		
3 Males		X	X	X	X	X	X	X		
3 Females		X	X	X	X	X	X	X		
Day 10 (After Seventh Dose)										
Time Points	**Predose**	**1 Hr**	**3 Hrs**	**4 Hrs**	**8 Hrs**	**12 Hrs**	**18 Hrs**	**24 Hrs**		
3 Males		X	X	X	X	X	X	X		
3 Females		X	X	X	X	X	X	X		
Day 17 (After Fourteenth Dose)								**Day 18**	**Day 19**	**Day 20**
Time Points	**Predose**	**1 Hr**	**3 Hrs**	**4 Hrs**	**8 Hrs**	**12 Hrs**	**18 Hrs**	**24 Hrs**	**48 Hrs**	**72 Hrs**
3 Males	X	X	X	X	X	X	X	X	X	X
3 Females	X	X	X	X	X	X	X	X	X	X

Efficacy Results

Mortality: Animals in the control group were euthanized due to morbidity scores between Study Days 12 and 16. All other animals were euthanized per the Study Protocol on Day 28.

Viral Load: Viral infection was monitored by quantitative real-time polymerase chain reaction (qPCR). All animals lacked detectable circulating MPXV prior to challenge. Within two minutes of intravenous administration of virus, all groups demonstrated high concentrations (3.11×10^6 copies/mL, SD 1.47×10^6 copies/mL) of MPXV in the blood, thereby indicating successful challenge. Average levels decreased to 1.11×10^5 copies/mL, (SD 9.92×10^4 copies/mL) at three days post-challenge. By six days post MPXV administration, animals that were treated with vehicle demonstrated a consistent increase in circulating viral load until animals succumbed to infection; terminal or near-terminal samples measured with 5.57×10^6 to 8.32×10^7 copies/mL. All animals that received ST-246 (all dose levels) presented with a gradual decline in circulating viral load, however, it was not dose dependent. Concentrations fell to the lower detectable limit around Day 15 (10 mg/kg), 18 (20 mg/kg) or 21 (3 mg/kg) post challenge. Figure 3.2.1.6-1 shows the quantified copies of MPXV HA gene in whole blood for each study group.

Figure 3.2.1.6-1: FY10-087 Quantified MPXV HA Gene in Whole Blood



Total Body Lesion Counts: The qPCR results correlate with the resolution of MPXV lesions from macule to desquamation in the ST-246 treated animals. By Day 3 lesions were noted in animals from all study groups (macule, papule, pustule, or crust). By Day 18, lesions can primarily be described as umbilicated, crusted, or desquamated with further progression by Day 21 to crusted or desquamated. Lesion counts were performed on un-anesthetized animals to ensure unperturbed pharmacokinetics and therefore, were reported as ranges. Lesion count data from this study were not compared to one another and were not considered as strong indicators of dose-dependent efficacy.

Pharmacokinetic Analysis:
Pharmacokinetic parameters were evaluated in naïve animals on Day -10 (prior to MPXV infection) as a baseline comparator for possible changes induced by MPXV infection. Analyses were performed on Day 4 (the first day of ST-246 treatment), Day 10, and Day 17 (the last day of ST-246 treatment) to account for various stages or progression of the infection as well as evaluate accumulation or enhanced clearance due to induction.

ST-246 concentrations in plasma vs. time data were analyzed in all plasma samples collected for pharmacokinetic analysis throughout the study. All data from the low dose (Group 2), mid dose (Group 3), and high dose (Group 4) groups from study Days -10, 4, 10 and 17 were grouped by study day and analyzed using WinNonLin software (Version 5.0.1, Pharsight Corporation, Mountain View, CA) model 201, uniform weighting with no limits on the calculation of the terminal half-life. No modifications to the inclusion/exclusion of time points were needed based on data analysis. No analysis was performed on the vehicle group as all the bioanalytical results were below the limit of quantification (BLQ).

The pharmacokinetic parameters that were calculated consisted of maximum observed concentration (C_{max}, ng/mL), time at which C_{max} is observed (T_{max}, hr), area under the plasma vs. time curve from zero through the last measured concentration (AUC_{last}, hr*ng/mL), and the terminal half-life ($t_{1/2}$, hr) were calculated for both sets of data. Gender correlation and accumulation throughout the study were evaluated. The summarized results for each parameter from Day -10, 4, 10, and 17 are presented in Tables 3.2.1.6-4 through 3.2.1.6-8.

Table 3.2.1.6-4: FY10-087 Average C_{max} (ng/mL) Pharmacokinetic Results

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

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

C_{max} values were dose-dependent with averages for the males and females in the low, mid, and high doses groups ranging from 335-467 ng/mL, 1048-1623 ng/mL, 1405-3343 ng/mL and 332-563 ng/mL, 570-1445 ng/mL, 1098-2667 ng/mL, respectively.

Table 3.2.1.6-5: FY10-087 Average T_{max} (hr) Pharmacokinetic Results

Exposure Group	Day -10	Day 4	Day 10	Day 17
Males[a]				
Low Exposure	1.67 (1.15)	2.33 (1.15)	2.33 (1.15)	1.67 (1.15)
Mid Exposure	2.33 (2.08)	3.00 (0.00)	3.33 (0.58)	3.00 (0.00)
High Exposure	3.67 (0.58)	3.33 (0.58)	3.33 (0.58)	3.00 (0.00)
Females[a]				
Low Exposure	5.33 (5.86)	1.67 (1.15)	1.67 (1.15)	2.33 (1.15)
Mid Exposure	4.67 (2.87)	3.00 (0.00)	3.00 (0.00)	2.33 (1.15)
High Exposure	3.00 (0.00)	4.00 (0.00)	2.67 (1.15)	3.00 (0.00)

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

ST-246 typically reached T_{max} by 3 hours post dose administration on Days -10, 4, 10 and 17. T_{max} appeared to be independent of sex or dose level across doses ranging from 3 to 20 mg/kg/day.

Table 3.2.1.6-6: FY10-087 Average AUC_{last} (hr*ng/mL) Pharmacokinetic Results

Exposure Group	Day -10	Day 4	Day 10	Day 17
Males[a]				
Low Exposure	3360 (1800)	3560 (804)	3560 (253)	2320 (640)
Mid Exposure	20000 (7000)	10300 (3150)	15300 (1960)	20400 (4490)
High Exposure	44600 (22300)	19600 (11100)	39100 (17000)	40700 (19200)
Females[a]				
Low Exposure	3320 (1490)	3420 (1570)	4330 (1180)	4380 (2390)
Mid Exposure	11000 (10000)	6210 (1450)	13100 (7370)	14600 (5160)
High Exposure	25900 (9450)	14900 (5810)	26800 (12200)	24700 (10300)

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

To readily compare each Study Day collection to one another (Day -10 vs. 4 vs. 10 vs. 17), AUC_{0-24} was calculated for Day -10 and 17 (blood was only collected to 24 hours for Day 4 and 10). Average AUC_{0-24} for the males and females in the low, mid, and high dose groups ranged from 2325-3564, 10348-16089, 19582-44539 hr*ng/mL and 3095-4384, 6209-13057, 14893-26829 hr*ng/mL, respectively.

Table 3.2.1.6-7: FY10-087 Average AUC_{0-24} (hr*ng/mL) Pharmacokinetic Results

Exposure Group	Day -10	Day 4	Day 10	Day 17
Males[a]				
Low Exposure	3050 (1310)	3560 (804)	3560 (253)	2320 (641)
Mid Exposure	13700 (2380)	10300 (3150)	15300 (1960)	16100 (2880)
High Exposure	44600 (22300)	19600 (11100)	39100 (17000)	33000 (14000)
Females[a]				
Low Exposure	3090 (1100)	3420 (1570)	4330 (1180)	4120 (1980)
Mid Exposure	9020 (7530)	6210 (1450)	13100 (7370)	12600 (5640)
High Exposure	25900 (9450)	14900 (5810)	26800 (12200)	20400 (8980)

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

Average AUC_{last} and AUC_{0-24} appeared to show a dose response in both males and females however no apparent accumulation was observed when comparing Days -10 and 4 to Day 10 or 17. Average AUC_{last} for the males and females in the low, mid, and high dose groups ranged from 2324-3564, 10348-20429, 14893-26829 hr*ng/mL and 3323-4384, 6209-14560, 14893-26829 hr*ng/mL, respectively.

Table 3.2.1.6-8: FY10-087 Average $t_{1/2}$ (hr) Pharmacokinetic Results

Exposure Group	Day -10	Day 4	Day 10	Day 17
Males[a]				
Low Exposure	6.75 (2.46)	6.69 (2.67)	7.62 (1.65)	7.02 (1.99)
Mid Exposure	15.3 (8.4)	11.0[b] (0.8)	10.1 (2.6)	9.20 (2.28)
High Exposure	7.95 (3.30)	24.1 (9.8)	9.49 (3.60)	8.60 (0.43)
Females[a]				
Low Exposure	7.03 (1.06)	9.51 (6.13)	6.86 (1.44)	6.33 (1.28)
Mid Exposure	11.6 (5.9)	14.9 (11.9)	16.9 (8.7)	8.18 (3.96)
High Exposure	12.5 (3.1)	19.7 (3.5)	15.4 (9.8)	8.91 (1.46)

[a]All data was rounded to three significant figures; shown as average (standard deviation)
[b]Animal 3002 was excluded as an outlier from the group average

After achieving maximum concentration, ST-246 clearance and $t_{1/2}$ were highly variable within each dose group making it difficult to determine apparent effects associated with MPXV infection. Independent of the Study Day, average $t_{1/2}$ for the males and females in the low, mid, and high dose groups ranged from 6.69-7.62, 9.2-15.31, and 7.95-24.13 hours and 6.33-9.51, 8.18-16.91, and 8.91-19.73 hours, respectively. In general ST-246 clearance was slowest on Day 4, however, due to animal variability, the data are likely not biologically significant.

Conclusion: Following challenge, disease-associated alterations in the animals' health were noted in all groups. Average levels of viral DNA in the blood decreased to $1.11x10^5$ copies/mL, (SD $9.92x10^4$ copies/mL) at three days post-challenge. By six days post MPXV administration, animals that were treated with vehicle demonstrated a consistent increase in circulating viral DNA levels in the blood until animals succumbed to infection; terminal or near-terminal blood samples had viral DNA levels at $5.57x10^6$ to $8.32x10^7$ copies/mL. All animals that received ST-246 (all dose levels) had gradual declines in circulating viral load however these declines

were not dose dependent. Concentrations fell to below the detectable limit around Day 15 (10 mg/kg), 18 (20 mg/kg) or 21 (3 mg/kg) post challenge. The reduction in viral DNA levels correlated with the resolution of MPXV lesions from macule to desquamation in the ST-246 treated animals. By Day 3, lesions were noted in animals from all study groups (macule, papule, pustule, or crust). By Day 18, lesions could primarily be described as umbilicated, crusted, or desquamated with further progression by Day 21 to crusted or desquamated.

Animals in the control group were euthanized due to morbidity scores on Study Day 12 (1001, 1002, 1005), Study Day 13 (1003), Study Day 14 (1006), and Study Day 16 (1004). All other animals were euthanized per the Study Protocol on Day 28. No overt changes in ST-246 pharmacokinetics were observed when Days -10, 4, 10, and 17 pharmacokinetic parameters were compared to one another (Day -10 compared to each of the infection days). No gender difference was observed in this study, either before or during infection.

3.2.1.7 SR10-037F Efficacy in Delayed Dosing

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

Objective: To identify the maximum delay post-challenge (with $5x10^7$ pfu MPXV IV) at which ST-246 (10 mg/kg) is effective at preventing mortality in nonhuman primates

Study Design: This was a randomized, double-blinded, placebo controlled study of ST-246 administered orally to cynomolgus monkeys infected intravenously with MPXV (strain Zaire – CDC V79-I-005). The dose groups and experimental plan are shown in Table 3.2.1.7-1. In brief, monkeys were challenged on Day 0 and received ST-246 at 10 mg/kg by oral gavage beginning on Day 4 (Group 2), Day 5 (Group 3), and Day 6 (Group 4) post-challenge and continued to receive once-daily doses for 14 consecutive days. Observations were carried out through Day 56 post-challenge.

Table 3.2.1.7-1: SR10-037F Study Group Assignment

Group[a]	No. of Animals		MPXV Challenge Dose	ST-246 Dose Level	Dosage Schedule[b]	
	M	F			ST-246	Placebo
1 – Placebo[c]	1	2	$5x10^7$ PFU IV	0 mg/kg	N/A	Days 4-19
2 – ST-246 Day 4	3	3	$5x10^7$ PFU IV	10 mg/kg	Days 4-17	Days 18-19
3 – ST-246 Day 5	3	3	$5x10^7$ PFU IV	10 mg/kg	Days 5-18	Days 4 and 19
4 – ST-246 Day 6	3	3	$5x10^7$ PFU IV	10 mg/kg	Days 6-19	Days 4-5

[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.
KEY: PFU = plaque-forming units; N/A = not applicable; IV = intravenous

Efficacy Results

Mortality: None of the animals (0%) in the placebo group survived to post-infection Day 56 while five of six (83%) in the Day 4 and Day 5 groups and three of six (50%) in the Day 6 group survived to scheduled euthanasia. Survival was significantly increased (p = 0.0476) in both the Day 4 and Day 5 groups. When all ST-246-treated animals were considered together (Day 4, Day 5, and Day 6 groups combined), survival was 72%, which was statistically significantly better than the placebo group (p = 0.0421).

The pattern of necropsy findings was similar in placebo-treated and ST-246-treated monkeys. However, there were noticeable differences in the extent and severity of these findings between non-survivors and survivors. The most common necropsy findings across all animals were enlarged or slightly enlarged darkened lymph nodes (axillary, inguinal, and mesenteric). Enlargement or slight enlargement, thickening, firmness, and discolorations in the spleen were noted in one monkey from each group that did not survive to scheduled euthanasia, two of which also manifested a yellowish and pale pancreas with an observed lesion. Other common findings included white focal lesions (lung, stomach), dark red lesions or petechiae (lung, stomach, heart), pale appearance (lung, liver, kidney, heart), thickening (urinary bladder, lung, colon, stomach, esophagus), gelatinous consistency (lung, liver), bloody urine, skin sloughing off the surface of the tongue, blood clots in the stomach, gas filled colon, leathery texture of the kidney, and uniform consolidation and emphysema lesion in the lung. The majority of the necropsy findings were attributable to monkeys that were found dead or euthanized early. Results are presented in Table 3.2.1.7-2.

Table 3.2.1.7-2: SR10-037F Number of Animals Surviving to Scheduled Termination

Group	Number of Animals		Statistics[a]
	Study Start	Study End	
Placebo	3	0 (0%)	--
ST-246 Day 4	6	5 (83%)	p = 0.0476[c]
ST-246 Day 5	6	5 (83%)	p = 0.0476[c]
ST-246 Day 6	6	3 (50%)	p = 0.4463
All ST-246-Treated[b]	18	13 (72%)	p = 0.0421[c]

[a]P-value obtained using Fisher's Exact Test (2-sided) vs. Placebo
[b]Day 4, Day 5, and Day 6 groups combined
[c]Significant at p = 0.05

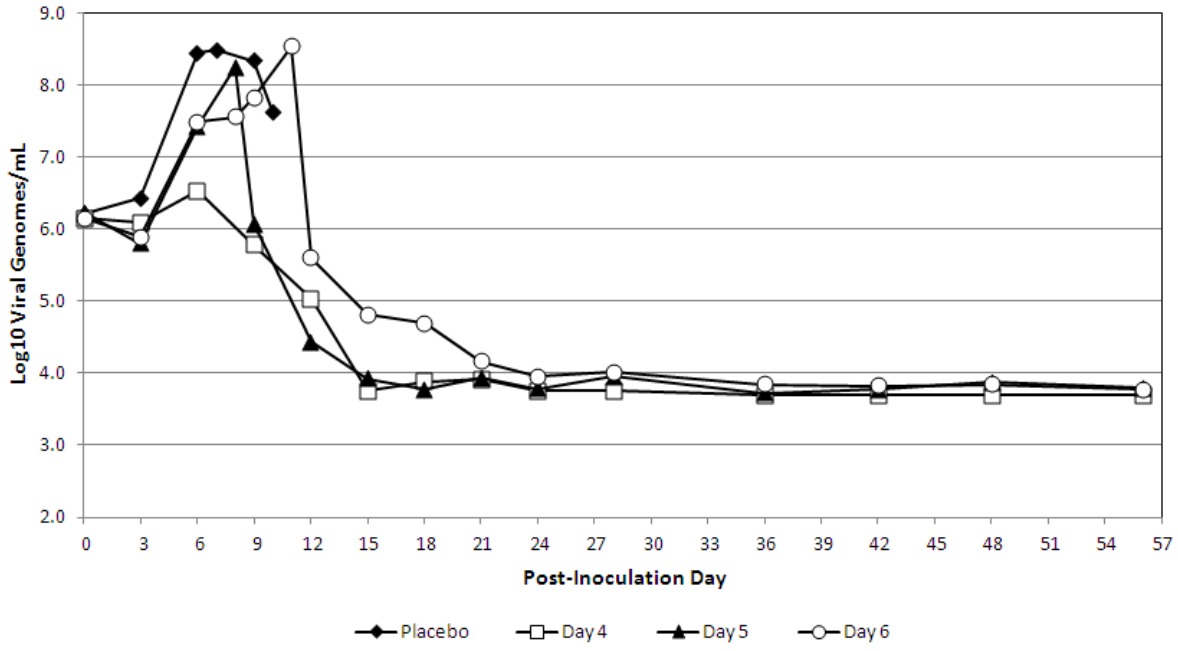
Viral Load: The mean time-weighted average of $\log_{10}$ viral DNA levels reflects the overall viral burden over the course of a specified period of time. For treatment Days 1-16 and post-infection Days 3-24, mean time-weighted average $\log_{10}$ viral DNA levels were reduced by ST-246 treatment compared to placebo; however, the decreases were not statistically significant ($p \geq 0.0888$) for any individual ST-246 treatment group relative to the placebo group. There was a significant reduction in time-weighted average viral load in all ST-246-treated animals (Days 4, 5, and 6 animals combined) compared to the placebo group (p = 0.0430) for treatment Days 1-16 and for post-infection Days 3-24. These results are presented graphically in Figure 3.2.1.7-1.

Mean (range) time-weighted average $\log_{10}$ viral DNA levels for treatment Days 1-16 were 8.35 (7.44-9.41), 5.50 (4.32-8.74), 5.71 (4.73-8.11), and 6.90 (5.13-8.11) $\log_{10}$ genomes/mL for the placebo, Day 4, Day 5, and Day 6 groups, respectively. For post-infection Days 3-24, mean

(range) values were 8.72 (7.79-9.82), 5.63 (4.42-9.26), 5.72 (4.74-8.46), and 7.02 (5.05-8.49) $\log_{10}$ genomes/mL for the placebo, Day 4, Day 5, and Day 6 groups, respectively.

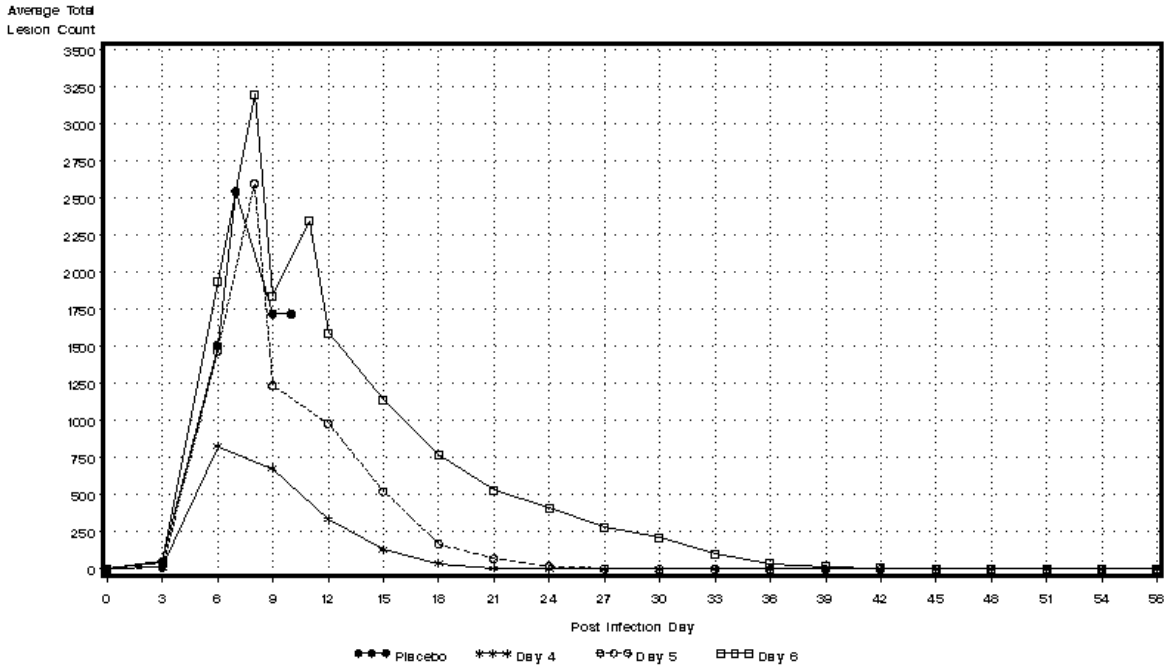
Mean (range) time-weighted average $\log_{10}$ viral DNA levels for treatment Days 1-16 for all ST-246-treated monkeys combined was 6.04 (4.32-8.74) $\log_{10}$ genomes/mL, compared to 8.35 (7.44-9.41) $\log_{10}$ genomes/mL for the placebo. For post-infection Days 3-24, mean (range) time-weighted average viral loads were 6.12 (4.42-9.26) and 8.72 (7.79-9.82) $\log_{10}$ genomes/mL for all ST-246-treated and placebo animals, respectively.

Figure 3.2.1.7-1: SR10-037F Viral Load (Genomes/mL) Through Day 56



Total Body Lesion Counts: All animals were lesional by Day 4. There were no significant differences in mean time-weighted average of total lesion counts for treatment Days 1-16 and post-infection Days 3-24 in any of the ST 246- treated groups compared to placebo. The range of total lesion counts for treatment Days 1-16 was 535.63-2240.13 counts per day across all animals in the placebo group and 86.88-2825.63 counts per day for all animals in the ST 246 treatment groups combined. For post-infection Days 3 to 24, the ranges were 554.05-2317.52 counts per day for the placebo animals and 67.29-2924.52 counts per day for the treated animals. These results are presented graphically in Figure 3.2.1.7-2.

Figure 3.2.1.7-2: SR10-037F Total Lesion Count Through Day 56



Conclusion: Based on the results of this study, oral administration of 10 mg/kg ST-246 daily for 14 days was effective in decreasing mortality from monkeypox in the lesional cynomolgus monkey model of orthopoxvirus disease when treatment was initiated on Day 4 or Day 5 after viral challenge. Commencement of ST-246 treatment on Day 4 or Day 5 was associated with increased survival (83%), significantly decreased change in viral load from baseline, milder clinical manifestations, less severe changes in clinical chemistry parameters, and fewer and less severe gross necropsy findings. None of the ST-246 treatments was 100% effective in preventing mortality; however the number of days from treatment start to day of death was significantly increased in the presence of ST-246, regardless of whether dosing commenced on Day 4, 5, or 6 after inoculation with MPXV.

3.2.1.8 SR10-038F Efficacy and Duration of Dose

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

Objective: To determine the minimal treatment duration in which ST-246 at 10 mg/kg provides full protection from lethal MPXV challenge ($5x10^7$ pfu IV) in nonhuman primates

Study Design: This was a randomized, double-blinded, placebo controlled study of ST-246 administered orally to cynomolgus monkeys infected intravenously with MPXV (strain Zaire – CDC V79-I-005). The dose groups and experimental plan are shown in Table 3.2.1.8-1. In brief, monkeys were challenged on Day 0 with $5x10^7$ pfu of MPXV IV and received ST-246 at 10 mg/kg by oral gavage beginning on Day 4 post-challenge and continued to receive once-daily doses for 3 (Group 2), 5 (Group 3), 7 (Group 4) or 10 (Group 5) consecutive days. Observations were carried out through Day 28 post-challenge.

Table 3.2.1.8-1: SR10-038F Study Group Assignment

Group	N	MPXV Challenge Dose	ST-246[a] Dose	ST-246 Dosage Schedule	Bioanalysis of ST-246 Blood Levels Post Challenge
1-Placebo[b,d]	4	$5x10^7$ pfu IV	0	Daily starting on Day 4 and up to D13 (Placebo only)	Days -7, 4, 7, 9, 11, 14
2-ST-246[c,d] 3 doses	4	$5x10^7$ pfu IV	10 mg/kg	Daily starting on Day 4 and up to Day 6, Placebo to Day 13	Days -7, 4, 7, 9, 11, 14
3-ST-246[c,d] 5 doses	6	$5x10^7$ pfu IV	10 mg/kg	Daily starting on Day 4 and up to Day 8, Placebo to Day 13	Days -7, 4, 7, 9, 11, 14
4-ST-246[c,d] 7 doses	6	$5x10^7$ pfu IV	10 mg/kg	Daily starting on Day 4 and up to Day 10, Placebo to Day 13	Days -7, 4, 7, 9, 11, 14
5-ST-246[d] 10 doses	5	$5x10^7$ pfu IV	10 mg/kg	Daily starting on Day 4 and up to Day 13	Days -7, 4, 7, 9, 11, 14

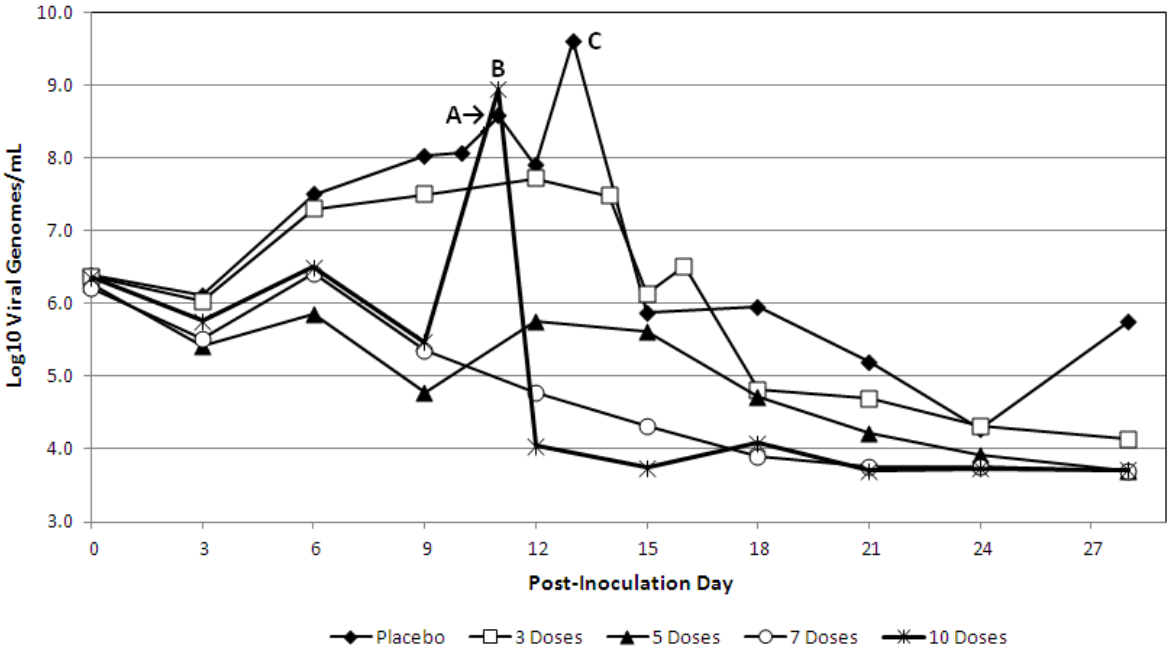
[a]Groups 2 to 5 were administered ST-246 by oral gavage on the days indicated here.
[b]Animals in Group 1 were dosed with drug vehicle plus Avicel® by oral gavage and serve as Placebo controls.
[c]In addition to being dosed with ST-246, the animals received placebo on the indicated days to accommodate blinding.
[d]The groups have been assigned Group numbers to aid in clarity in this protocol only and were not arranged by groups but rather animal number.
KEY: pfu = plaque-forming units; IV = intravenous

Efficacy Results

Mortality: The percent of animals surviving to scheduled termination was statistically significantly increased in both the 5-Dose and 7-Dose groups compared to the placebo group ($p = 0.0333$). One animal (25%) in the placebo group survived to post-challenge Day 28 while two of four (50%) in the 3-Dose group, six of six (100%) in the 5-Dose and 7-Dose groups, and four of five (80%) in the 10-Dose group survived to scheduled termination. A survival rate of 86% was observed when all ST-246-treated animals were considered together (3-Dose, 5-Dose, 7-Dose, and 10-Dose groups combined), which was statistically significantly better than the placebo group ($p = 0.0312$).

Viral Load: Viral DNA levels, measured as time-weighted average, rate of increase, maximum number, and change from pre-treatment baseline, generally decreased with treatment durations of at least 5 doses (Figure 3.2.1.8-1). A negative rate of increase, representing a decline, in viral DNA was observed in the 5-Dose, 7-Dose, and 10-Dose groups, achieving statistical significance in the 5-dose group. Maximum viral DNA levels were reduced in all ST-246-treatment groups relative to placebo, most notably in the 5-Dose, 7-Dose, and 10-Dose groups, attaining statistical significance in the 7-Dose group. A statistically significant reduction in viral load from baseline was observed for the 5-Dose treatment group compared to placebo for all treatment day windows that were evaluated except the final period (Days 10-11). Statistically significant decreases were also noted between Days 6 and 11 for the 7-Dose and 10-Dose groups.

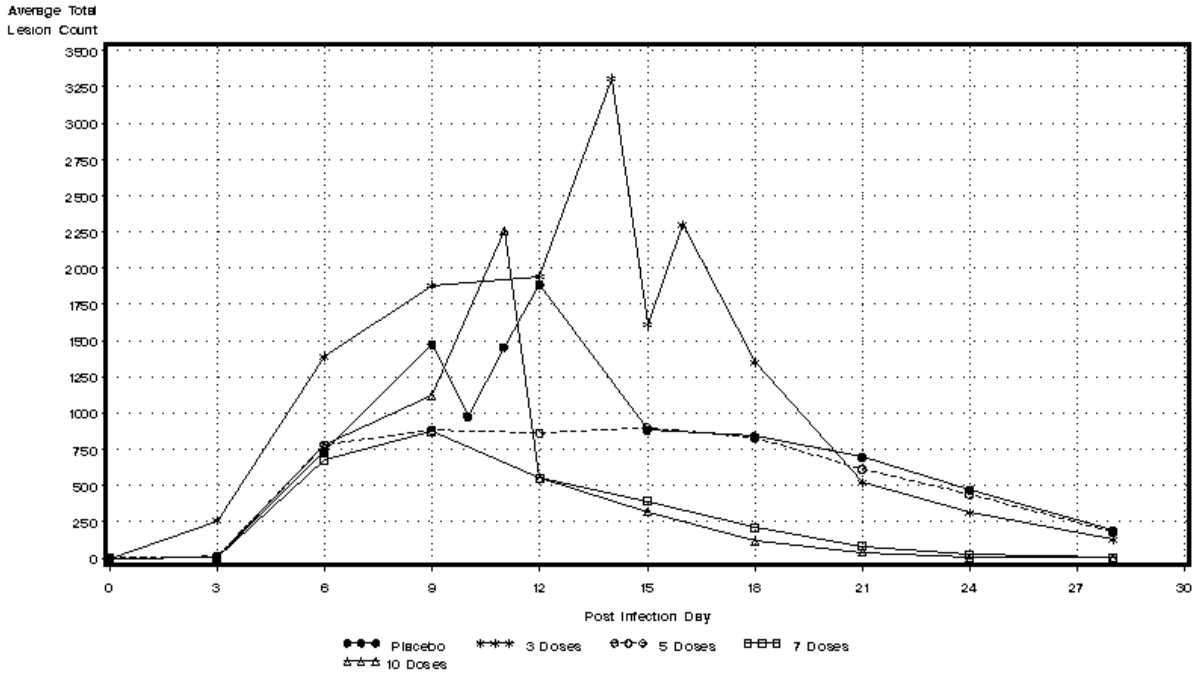
Figure 3.2.1.8-1: SR10-038F Viral Load (Genomes/mL) Through Day 28



Note: Values appear elevated for the placebo and 10-Dose groups on Days 11 and/or 13 because the plot points represent data from single animals on the day of euthanasia and not group means. **A** = Animal 4821 (placebo); **B** = 4819 (10-Dose); **C** = 4803 (placebo)

Lesion Counts: There were no statistically significant differences in lesion counts between ST-246-treatment groups and the placebo group with respect to time-weighted average, rate of increase, maximum number, and change from pre-treatment baseline. Generally these effects were greatest in the 5-Dose, 7-Dose, and 10-Dose groups. In plots of mean lesion counts (Figure 3.2.1.8-2), it is apparent that there is a trend towards a reduction in lesion number that is related to length of treatment. Within each group however, lesion counts were highly variable and individual ranges overlapped considerably between groups. Therefore, statistical significance was not achieved in comparing ST-246 treatment groups (either individually or combined) with the placebo group. Moreover, the effect on lesion count of the 10-Dose treatment appeared to be diminished compared to the 5-Dose and 7-Dose treatments, which could be ascribed in part to the very high mean value reported on Day 11; this data point represented a single moribund monkey (4819) on the day of euthanasia.

Figure 3.2.1.8-2: SR10-038F Total Lesion Count Through Day 28



Conclusion: Based on the results of this study, oral administration of five or seven 10 mg/kg ST-246 consecutive daily doses was effective in decreasing mortality from monkeypox in the lethal cynomolgus monkey model of orthopoxvirus disease, when treatment was initiated on Day 4 after viral challenge. Treatment with five or seven doses of ST-246 was associated with increased survival (100%), significantly decreased systemic viral burden according to one or more measures of viral DNA in plasma, milder clinical manifestations, and less severe changes in clinical chemistry parameters.

3.2.2 SUMMARY OF VARIOLA NONHUMAN PRIMATE STUDIES

3.2.2.1 1470HUGMONC EFFICACY

Title: Evaluation of Oral ST-246 in the Lesional Variola Cynomolgus Monkey Model (Pilot Study)

Objective: To evaluate and compare the ability of oral administration of ST-246 administered once daily for a period of 14 days to treat smallpox-virus-induced disease in NHPs, including reduction of the severe rash illness, viral load, and mortality.

Study Design: This was a randomized, placebo-controlled, parallel-group, repeat-dose study of ST-246 monotherapy administered orally in variola-infected NHPs. Monkeys in this study were first infected with $1x10^8$ pfu of Harper strain of smallpox virus and then received treatment with either placebo or a combination of placebo and ST-246 for a total of 15 days (Days 0 -14). Animals receiving ST-246 were dosed at either Day 0 or Day 1 after infection and received 14 consecutive daily doses of ST-246.

Although 9 animals were planned, 8 monkeys participated in this study. They were randomized into 3 treatment groups: 3 monkeys each for the ST-246 groups (2 males and 1 female per

group), and 2 monkeys for the placebo group (1 male and 1 female). Group A, the placebo group, was treated with a daily placebo dose starting immediately after infection on Day 0-14. Group B, the ST-246 Day 0 group, was treated with a daily dose of ST-246 at 300 mg/kg starting immediately after infection on Day 0-13, followed by 1 placebo dose on Day 14. Group C, the ST-246 Day 1 group, was treated with 1 placebo dose immediately after infection on Day 0, followed with a daily dose of ST-246 at 300 mg/kg from Day 1-14. In addition to placebo or ST-246, animals also received supplemental feeding consisting of 5 ± 0.5 mL/kg of a 30% suspension of hydrated, homogenized monkey chow via oral gavage.

Following variola exposure and over the course of the treatment, the infected animals were observed by the investigator at least twice each day for a total of up to 28 days to evaluate signs of illness. Blood samples were collected from the infected animals for virological, hematological, immunological, and chemical analyses. For those animals that died during the study, a full necropsy was performed to collect tissues for pathological examination. At the end of the study, surviving animals were anesthetized, exsanguinated, and euthanized, and a full necropsy was performed to collect tissues for pathological examination.

Conclusion: In this study, cynomolgus macaques were infected with variola virus by IV delivery to produce a lesional disease that resembles human smallpox. Treatment with ST-246 at 300 mg/kg/day prevented lesion formation, reduced viral DNA levels in blood, and reduced disease symptoms relative to placebo-treated animals. ST-246 was effective when treatment was initiated at the time of infection or 1 day after infection.

3.2.2.2 ST246-1745 EFFICACY

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

Objectives: To determine the levels of safety and efficacy of 10 mg/kg/day of oral ST-246 administration of Polymorph I (equivalent to ~ 400 mg/day in humans); and to verify Polyform I provided efficacy equivalent to Polymorph V (evaluated in previous studies).

Study Design: This was a randomized, double-blind, placebo-controlled, study in cynomolgus monkeys infected with variola virus. A total of 15 healthy male cynomolgus monkeys were selected and 14 were randomized into 2 treatment groups of 7 animals per group. The additional monkey was substituted for a monkey in the placebo group that died before the start of treatment. The cause of death was attributed to pre-existing cardiomyopathy and not associated with poxviral infection. Gross lesions in this monkey included lymphadenopathy and cutaneous pox lesions over the inner thigh. Histologically, this animal had mild, non-lethal lesions consistent with early poxviral infection, including cystitis with mucosal hyperplasia and ballooning degeneration, necrotizing orchitis, dermatitis with necrosis, epithelial ballooning degeneration and hyperplasia, cheilitis with epithelial hyperplasia and ballooning degeneration, and tonsillitis with necrosis and mucosal epithelial ballooning degeneration.

Fifteen cynomolgus monkeys (not previously exposed to any orthopoxvirus) were intravenously infected with $1x10^8$ pfu of Harper strain variola virus on Day 0. Test or control substances (10 mg/kg/day ST-246 or placebo) were administered, followed by 5 ± 0.5 mL/kg of a 30%

suspension of hydrated homogenized monkey biscuits, via an orogastric feeding tube. Treatment began on the day when lesions first appeared on an animal and continued every 24 ± 2 hours for 14 consecutive days.

Efficacy Results

Mortality: Based upon a previous study[55] investigating a range of challenge doses, a 30% mortality rate was predicted for placebo-treated animals in this study. Contrary to expectations, there were no deaths associated with the placebo animals and one animal in the ST-246 treatment group died on Study Day 9 while under anesthesia after receiving 6 doses of ST-246. While this monkey had histologic lesions consistent with variola virus infection and had four immunopositive tissues, the severity of lesions was considered insufficient to account for death. The clinical history and lack of significant histologic changes suggested physiologic impairment secondary to anesthesia. Not surprisingly, this animal presented with the greatest number of lesions directly attributed to VARV infection since it is the only animal in the study that was evaluated postmortem during the time of peak lesion formation (ordinary smallpox animal model). Grossly this animal had multiple scabbed lesions and associated histologic changes affecting haired skin of the eyebrow and nose, generalized lymphadenopathy, non-collapsing lungs with pinpoint hemorrhages, and large, friable spleen. Histologically this animal had necrotizing and inflammatory changes affecting the haired skin from the eyebrow, testicle, and popliteal lymph node. Epithelial hyperplasia and ballooning degeneration were present in sections of haired skin from the eyebrow, nose, and lip. Interestingly, many of the lesions associated with VARV infection in this animal ranged from subacute to chronic, a temporal departure from the more acute lesions typically present in affected monkeys at Day 9 post-infection. It is possible that ST-246 treatment altered the natural course of disease in this animal, allowing for the development of lesions atypical of those ordinarily seen at Day 9 post-infection.

Total Body Lesion Count: The mean and median time-weighted average of total lesion count were 48-71% smaller in the ST-246 group than in the placebo group, in both the ITT and PP populations, over the 14-day treatment period and over the entire study (post-infection Day 1-23). These differences were not statistically significant. These results are presented graphically in Figure 3.2.2.2-1. Covariate analysis revealed that the model-based least square (LS) mean of total lesion count over the period of treatment (Days 4-17) was significantly decreased ($p = 0.0280$) in the ST-246 group compared to the placebo group. These results are presented graphically in Figure 3.2.2.2-2. Covariate analysis revealed LS mean total lesion count was decreased in the ST-246 group on individual treatment Days 2-14 with statistical significance ($p < 0.05$) noted on days 3, 5, 6, 7, 8, 9, 10, and 11 compared to the placebo group. These results are presented graphically in Figure 3.2.2.2-3.

Figure 3.2.2.2-1: ST246-1745 Total Pox Lesion Count by Treatment Day

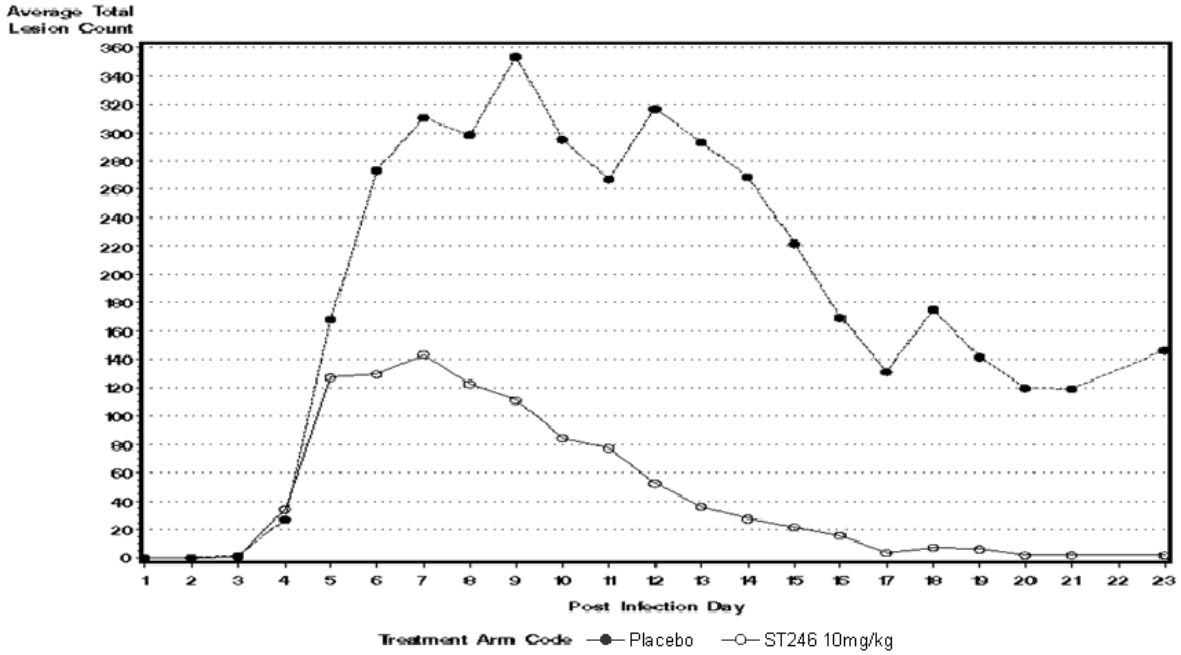


Figure 3.2.2.2-2: ST246-1745 Least Square Mean of Total Lesion Count

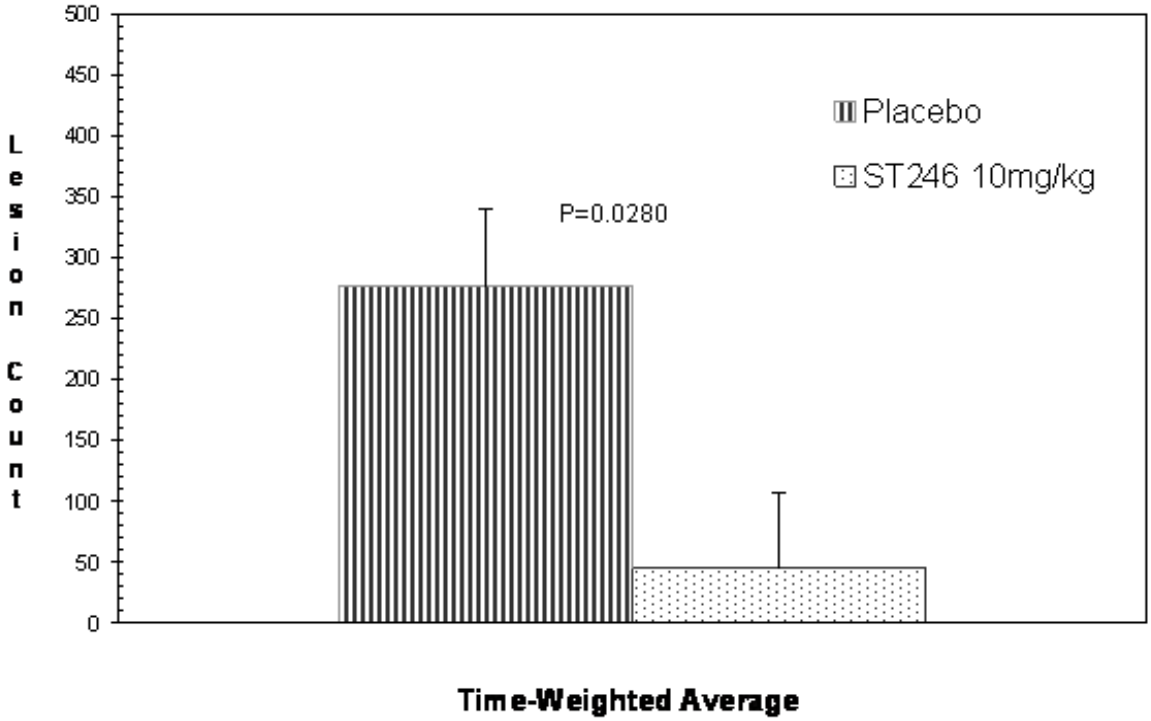
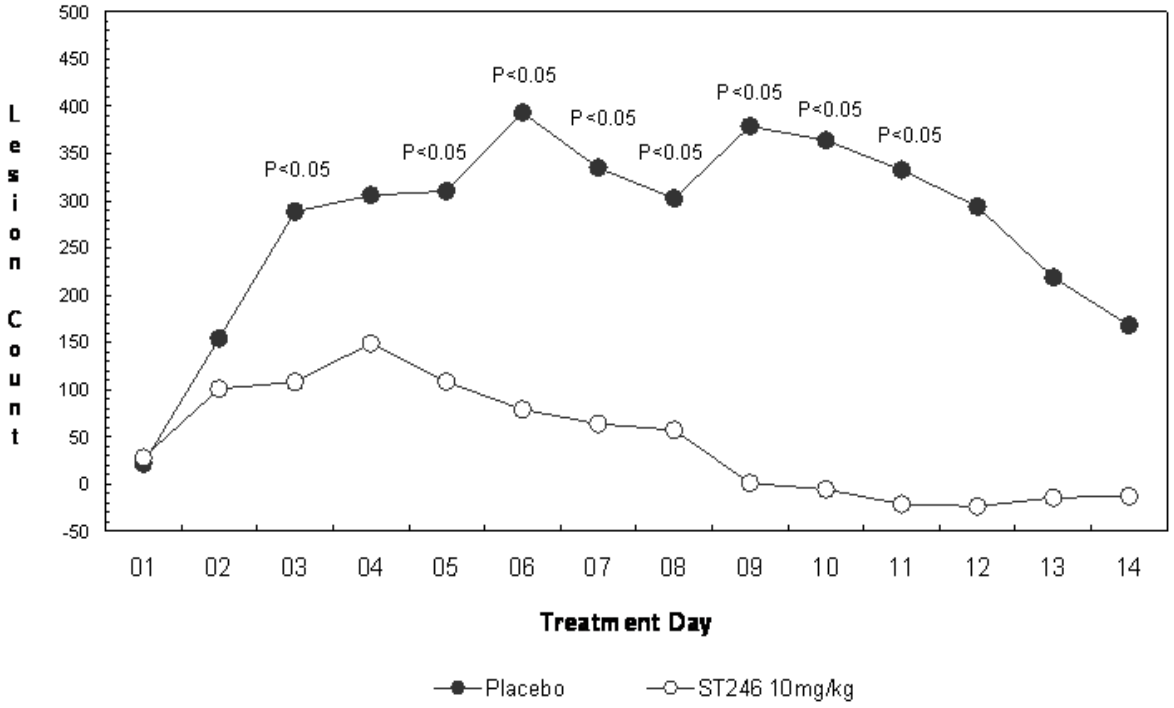


Figure 3.2.2.2-3: ST246-1745 Least Square Mean of Total Lesion Count by Treatment Day



Viral Load: The mean and median time-weighted average of $\log_{10}$ viral DNA level were 5-17% smaller in the ST-246 group than in the placebo group over the treatment period (treatment Days 1-14) and over the entire study (post-infection Day 1-23). These differences were not statistically significant. These results are presented graphically in Figure 3.2.2.2-4. Covariate analysis revealed that the model-based least-square mean of total $\log_{10}$ viral DNA level over the treatment period (Days 4-17) was significantly decreased ($p = 0.0195$) in the ST-246 group compared to placebo. These results are presented graphically in Figure 3.2.2.2-5. In addition, covariate analysis revealed total $\log_{10}$ viral DNA level by treatment day was significantly decreased in the ST-246 group on treatment Days 4-8 compared to the placebo group. These results are presented graphically in Figure 3.2.2.2-6.

Figure 3.2.2.2-4: ST246-1745 Viral Load (Genomes/mL) by Treatment Day

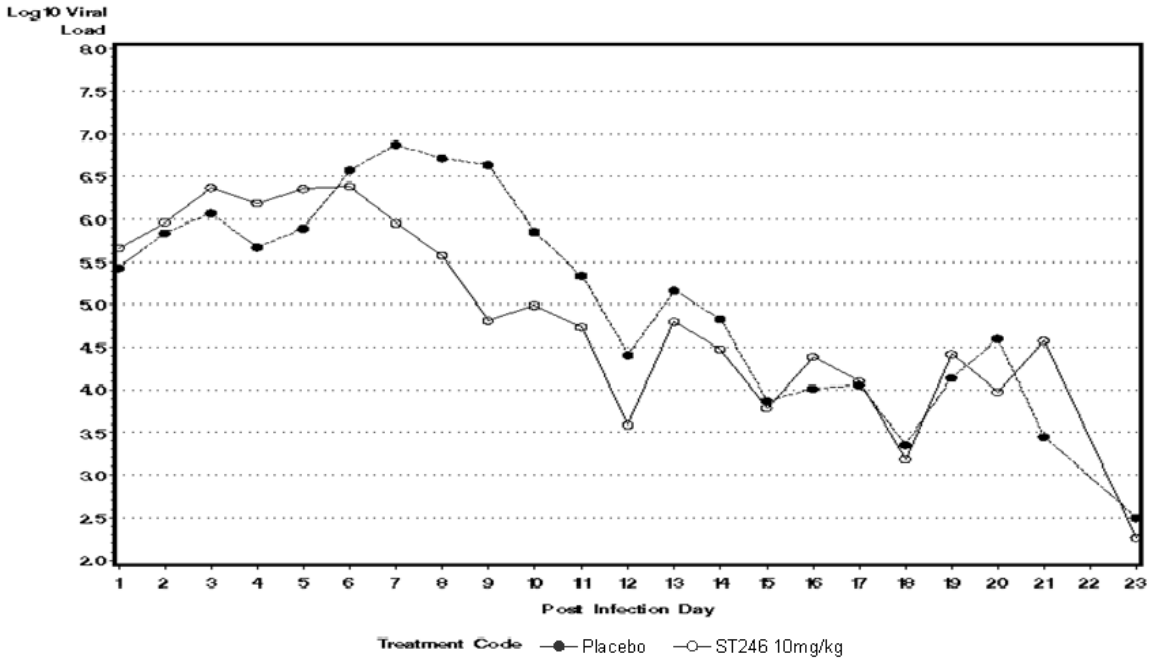


Figure 3.2.2.2-5: ST246-1745 Least Square Mean Viral Load (Genomes/mL)

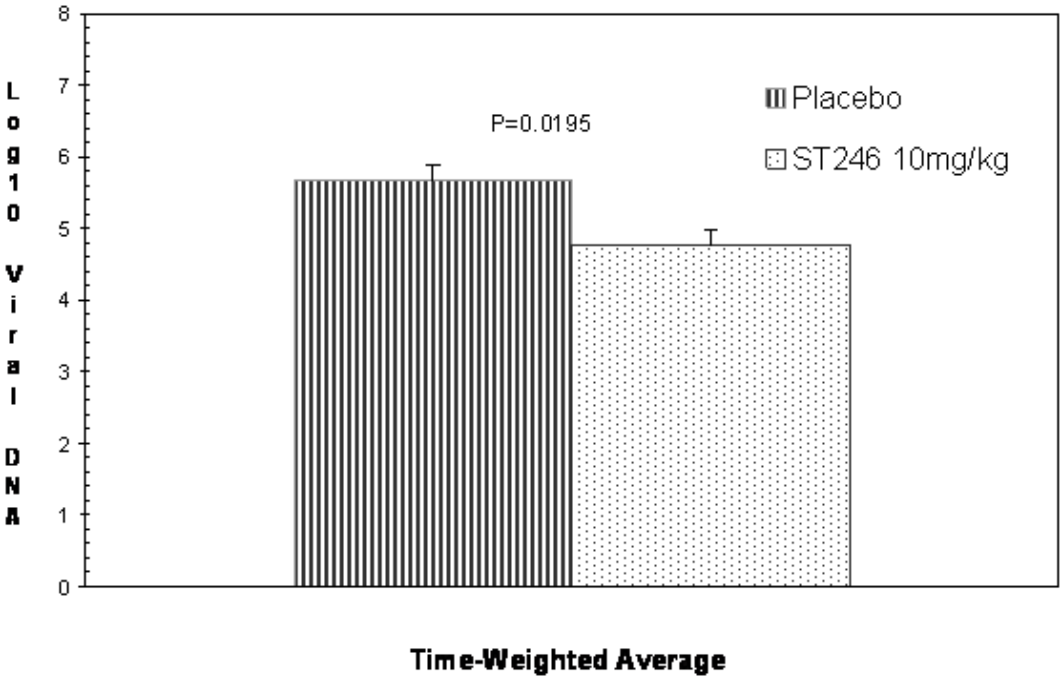
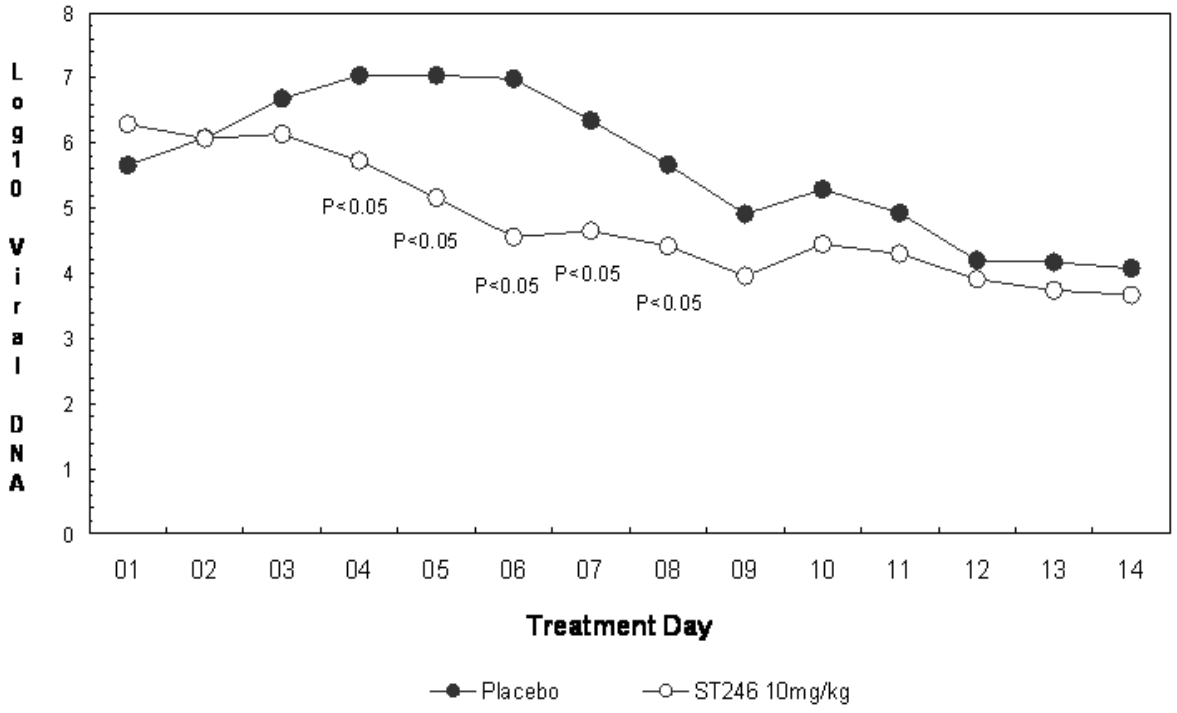


Figure 3.2.2.2-6: ST246-1745 Least Square Mean Viral Load (Genomes/mL) by Treatment Day



Conclusion: One animal in the ST-246 group died while under anesthesia; death was attributed to adverse anesthetic reaction. One animal assigned to the placebo group also died while under anesthesia prior to initiation of treatment; death was attributed to cardiac compromise associated with anesthesia and the animal was replaced. All other animals survived until the termination of the study on post-infection Day 23. The placebo group did not experience mortality as expected from previous studies (Jahrling et al., 2004; Huggins et al., 2009) so a comparison of the effect of ST-246 on survival was not possible.

Viral DNA was detected in the blood by one day after inoculation. Animals treated with ST-246 had a minimally lower time-weighted viral DNA level and maximum total viral DNA level than did placebo-treated animals. These levels were statistically significant with covariate analysis when using both the baseline disease severity and animal body weight as covariates. These results suggest that ST-246 treatment reduces viremia in monkeys infected with variola but the differences may not have reached a level of biological significance in this model.

Viral lesions were first observed by post-infection Day 3 after infection with all animals having lesions by Day 4. Animals treated with ST-246 had a lower time-weighted average of lesions, maximum total lesion count, and rate of increase in lesions than did placebo-treated animals; however, these differences did not achieve statistical significance. These levels were statistically significant with covariate analysis when using both the baseline disease severity and animal body weight as covariates. These results suggest that ST-246 treatment reduces lesion number in monkeys infected with variola.

Other than increased viral DNA level and pox lesions, VARV disease resulted in no other changes from baseline over the course of infection and the effects of ST-246 on these values were minor. In general, clinical chemistry values and physical examination findings were not informative relative to the severity of the disease. There was little gross, histologic, and immunohistochemical separation between treated and placebo groups in this study. Variola virus was not considered to be the cause of death of any animal in this study. All monkeys had mild gross and histologic findings consistent with poxviral infection, including disseminated chronic cutaneous lesions and lymphadenopathy. Excluding the animal from each group that succumbed prior to end of study, slightly more animals from the placebo group had at least one immunopositive tissue (three animals in the placebo group vs. two animals in the treated group), and slightly more tissues were immunopositive in the placebo animals than in the treated animals (four tissues in the placebo animals vs. two tissues in the treated animals). Immunopositive tissues were uncommon in the surviving treated monkeys; one monkey had a single focus of immunopositivity within the colon, and another monkey had multifocal immunopositivity within areas of bone marrow necrosis.

3.2.3 Summary of Respiratory MPXV Nonhuman Primate Studies

3.2.3.1 FY11-096 Evaluation of Aerosol Model

(**NOTE:** The provided summary is from an interim analysis of the data. Complete analysis and a Final Report are pending.)

Title: Evaluation of Aerosol Monkeypox Model for Testing Antivirals

Objectives: The objective of this study was to further develop and evaluate the aerosol monkeypox virus cynomolgus macaque model for efficacy evaluation of antiviral compounds. Evaluation of this model for antiviral testing employed a small molecular weight compound (ST-246) that inhibits poxvirus replication and has been proven effective in numerous orthopoxvirus challenge studies using other animal models and challenge routes. This study utilized ST-246 to further develop the nonhuman primate model of lethal MPXV aerosol infection.

Study Design: The experimental design for the antiviral efficacy study is shown in Table 3.2.3.1-1. Thirty Indonesian *Macaca fascicularis* (cynomolgus macaques) were placed into the five groups outlined in Table 3.2.3.1-1.

Table 3.2.3.1-1: FY10-096 Study Design

Group	Designation[a]	Test Article Treatment Dose	Animals/Group (Target)		Target Challenge Dose
			Male	Female	(pfu)
1	Vehicle Control	n/a	2	2	$1.0x10^5$
2	Day 1 Tx	10 mg/kg	2	2	$1.0x10^5$
3	Day 2 Tx	10 mg/kg	3	3	$1.0x10^5$
4	Day 3 Tx	10 mg/kg	4	4	$1.0x10^5$
5	Day 4 Tx	10 mg/kg	4	4	$1.0x10^5$

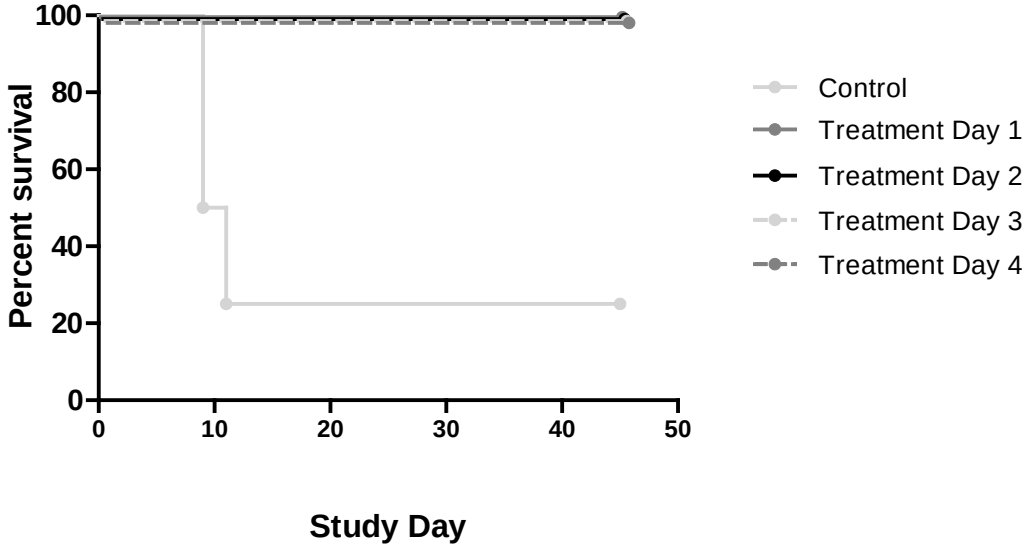
[a]The antiviral test article treatment was initiated on the specific day post-aerosol challenge via oral delivery (gavage) and continued as once-a-day dosing for 14 total doses. Group 1 animals were treated with vehicle control beginning on Study Day 1. Aerosol challenge was defined as Study Day 0.
KEY: pfu = plaque forming units

On Study Day 0, the monkeys were challenged with MPXV Zaire 79 via the aerosol route. The target presented dose was $1.0x10^5$ plaque forming units (pfu). Following challenge, drug treatment as indicated in Table 3.2.3.1-1 was initiated via oral delivery (gavage) and continued as once-a-day dosing for 14 total doses (up to and including Day 17). Animals were monitored daily for clinical signs of disease for 45 days post-challenge. The requirement for euthanasia was determined by the Study Director based on his/her understanding of disease progression with this model; examples of moribund criteria included sustained hypothermia, respiratory distress, weight loss of greater than 20%, or evidence of neurological complications (i.e. seizures). During the study period, body weights, lesion counts, and subcutaneous body temperatures were measured and viral load was assessed by quantitative PCR analysis of collected blood and pharyngeal swabs. Blood and separated serum were analyzed via select clinical pathology assays. Upon end of life (found dead, moribund, or terminal euthanasia), terminal samples were obtained and select tissues were collected for viral load processing and histopathology.

Efficacy Results

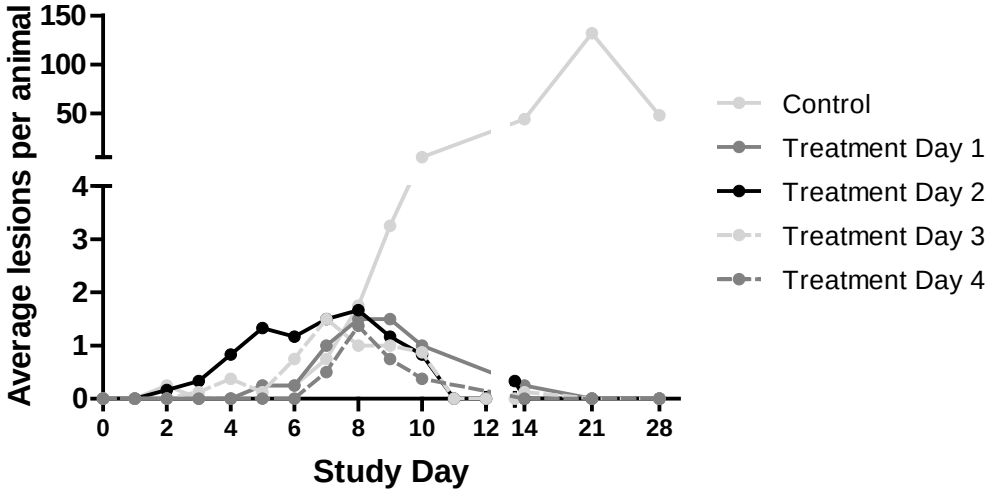
Moribundity and Mortality Observations: The antiviral efficacy of the test article (ST-246) was evaluated in four different treatment groups which varied by the day treatment was initiated post-challenge. All animals treated with ST-246 survived to the end of in-life, 45 days post-challenge, regardless of the day treatment was initiated on (Figure 3.2.3.1-1). The control group (Group 1, n = 4) was treated orally with vehicle for 14 days starting on Day 1 post-challenge. One control animal became very ill and survived to the end of in-life. Of the other three, two (one male and one female) became moribund and required euthanasia on Day 9 and the third was found dead on the morning of Day 11.

Figure 3.2.3.1-1: FY-096 Kaplan-Meier Survival Plot by Treatment Group



Lesion Counts: Figure 3.2.3.1-2 shows the average number of pox lesions at all stages of progression in all animals presented by treatment group. All groups treated with ST-246 exhibited lesions at some point after challenge with MPXV virus. Average number of lesions per animal was low, less than two per animal, regardless of how many days post-challenge treatment was initiated. We also observed that early stage lesions (i.e., macule, papule) frequently resolved spontaneously in these groups without progressing through the advanced stages of development. No lesions were observed in any treated animals past Day 14. Moreover, average lesion counts per animal in the vehicle treated control group were very similar to those observed in the treatment groups until Day 8 post-challenge. At this point, the data diverge dramatically. After Day 11, data from the vehicle treated control group represent lesion counts for the single survivor of this group. Average daily lesion counts for this group continued to rise from Day 8-21, reaching a peak of 132 lesions. Over the course of the study, all stages of lesion development were observed.

Figure 3.2.3.1-2: FY-096 Average Lesions per Animal



Clinical Observations: Listed for each group is a compilation of all clinical signs observed over the course of the study in the group, and the most severe presentation of those signs observed.

- Group 1: one animal survived in this group, subject 1001. Over the course of the study these animals presented with dry cough, reduced appetite (eating enrichment only), dehydration, ocular discharge, hunched posture, loss of appetite, weight loss, subdued activity, diaphragmatic breathing, and rapid breathing.
- Group 2: All animals survived the duration of the study. Animals presented with reduced appetite, possibly due to gavage with primate biscuit slurry as part of the study protocol. No other clinical signs were observed.
- Group 3: All animals survived the duration of the study. Animals showed slight weight change (5-20% of baseline), mild reduction in appetite, and mild increased respiration rate.
- Group 4: All animals survived the duration of the study. Animals showed slight weight change (5-10% of baseline), decreased appetite, eating enrichment only, dry cough, nasal discharge, diaphragmatic respiration, increased respiration rate (> 100 resp/min in at least one case).
- Group 5: All animals survived the duration of the study. Animals exhibited increased respiration rate, weight loss, reduced appetite, dry cough, nasal discharge, slightly elevated rectal temperature, hunched posture, subdued behavior (normal with stimulation), diaphragmatic breathing, reduced appetite (anorexic), and increased respiration rate.

As is apparent in this list, as treatment is delayed, more signs of illness are exhibited and the severity of those signs increases. Following challenge, symptoms of disease were observed in all treatment groups. Animals in Group 1 were treated with vehicle starting one day post-challenge. All animals in Group 1 became very ill and exhibited multiple severe signs of illness. Of 4 animals originally assigned to this group a single NHP survived to the end of study. All animals receiving test article treatment survived to the end of study. The severity and incidence of observed signs of disease in test article treated animals increased with increasing delay in initiation of treatment after aerosol monkeypox challenge. For example, Group 2, which initiated treatment on Day 1 post-challenge, was virtually asymptomatic. The only signs of exposure to monkeypox virus exhibited by NHPs in this group were mildly reduced appetite and

the appearance of a few lesions that typically progressed as far as the papule stage before resolving. NHPs in Group 5, which initiated treatment on Day 4 post-challenge, became very sick, nearly as sick as the vehicle treated control animals in Group 1. The animals in Group 5 exhibited changes in hematology and clinical chemistry consistent with but less severe than changes observed in these parameters in Group 1 animals and also showed moderate loss of weight and elevated respiration rates. Severity of illness in animals in Group 3 (treatment started 2 days post-challenge) and Group 4 (treatment started 3 days post-challenge) fell between the extremes of Groups 2 and 5 with the animals showing more signs of illness and increased severity of symptoms as the interval between challenge and test article treatment was increased.

Although ST-246 was clearly efficacious in this model, the model itself appears insufficient. It should be noted that this cannot be described as a "lesional model." Some animals did not form lesions before death and in the animals that did form lesions, they were very few in number (1-2 lesions per animal). The only animal with a significant number of lesions was the placebo treated control that did not die from disease. In addition, deaths occurred between 9 and 10 days post-challenge, shortly after the appearance of lesions. The lack of lesions and the shortened disease course highlight that this model is less similar to human smallpox than the IV challenge model.

4. ST-246 AND THE ANIMAL RULE

Over the course of development, SIGA has collaboratively worked with the FDA regarding the path to approval for a countermeasure such as ST-246. Although the path for FDA approval, under the Animal Rule has evolved, currently the guidance states that when it is neither ethical nor feasible to conduct human efficacy studies, the FDA can invoke the Animal Rule to evaluate evidence of countermeasure effectiveness derived from animal studies only when:

Tenet 1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product.

As discussed in previous sections, the replication cycle of OPXVs is well-characterized and the understanding of the mechanism by which OPXVs cause systemic infection is documented in the literature. OPXV particles that are unable to progress to the extracellular enveloped stage demonstrate markedly reduced pathogenesis, both *in vitro* and *in vivo*, and are considered avirulent in animal hosts with intact immune systems. In order to produce extracellular virus particles and induce systemic spread, a small percentage of intracellular mature OPXVs induce a wrapping complex derived from intracellular host membranes. The wrapped virus is then transported to the cell surface and released. SIGA has determined ST-246 inhibits the envelopment and egress stage of the viral replication cycle. ST-246 prevents the formation of the wrapping complex by inhibiting the function of a conserved protein specific to OPXVs that is required for this process.[(3)] In the absence of extracellular enveloped forms of OPXVs, systemic disease is prevented. Further, studies in mice have demonstrated that protective immunity is achieved even in the absence of systemic virus, a circumstance that does not occur with countermeasures that inhibit virus replication. OPXVs do not recrudesce once the initial infection has been cleared.

Additionally, OPXVs do not set up persistent infection in immune-competent hosts and fluctuation analyses have shown that OPXVs are genetically stable and are not prone to frequent mutation even in the presence of selective pressure. Virus populations grown in the presence of ST-246 did not demonstrate a propensity for resistance and study results indicated that mutations that confer compound resistance occurred at a frequency of about 1.5 in every 10^6 pfus and were likely to have pre-existed in the virus population. Moreover, SIGA has not experienced any treatment failures to date that are attributable to the emergence of a resistant virus population. However, efficacy studies utilizing the MPXV NHP model of human smallpox disease have allowed SIGA to implement a resistance monitoring program in which virus is extracted from blood and tissue samples of infected NHPs and evaluated for resistance to ST-246, a scenario that is not possible for studies conducted with variola in a BSL4 setting. The one instance where resistant variants were identified occurred in the case of a severely immune-compromised patient suffering from a progressive vaccinia infection and receiving ST-246 both orally (75 days) and topically (68 days). Resistant variants were recovered only from the patient's satellite lesions. The patient recovered and continues to do well.

Tenet 2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species

that represents a sufficiently well-characterized animal model for predicting the response in humans.

OPXVs infect a multitude of animal species. While disease manifestation and progression vary widely between species, many of the animal models available are lethal when the appropriate OPXV is used at the appropriate challenge dose. SIGA has conducted or participated in 28 small animal studies utilizing four small animal species, (various strains of mice, rabbits, prairie dogs and ground squirrels), to demonstrate ST-246 efficacy against five types of OPXVs, (ectromelia virus, rabbitpox virus, cowpox virus, vaccinia virus and MPXV). In each study where the primary endpoint was the prevention of death from a lethal OPXV challenge, treatment with ST-246 increased survival with statistical significance over vehicle controls. In cases where drug was administered prophylactically (i.e. prior to or concurrent with challenge), disease was virtually non-existent. These results suggest ST-246 is efficacious against the OPXV family as a whole.

However, when examining OPXV disease progression, there is much dissimilarity between human OPXV disease and OPXV disease in small animal species. One glaring example is a lack of raised pustular skin lesions over the entire body, a signature characteristic of human smallpox disease. Where lesions do play a role in disease manifestation in small animal models, they require immune-compromised hosts or emerge late in infection and then only in animals that are destined to survive challenge. OPXV disease in NHPs addresses this shortcoming. As discussed in previous sections, OPXV disease progression in NHPs closely follows that of human smallpox beginning at the secondary viremic stage of disease. NHPs infected via the IV route with MPXV or variola virus develop the characteristic skin rash within 3-4 days post-challenge and, in the case of the IV MPXV NHP model, become progressively more ill ultimately succumbing to infection within 7-17 days post-challenge. It is the emergence of lesions, reminiscent of human smallpox disease, which serves as the trigger for therapeutic intervention. SIGA has participated in eleven ST-246 efficacy studies in NHPs. In nine studies utilizing the IV MPXV NHP model, 93 of the 109 animals (85.3%) treated with ST-246 at 3 or more days post-challenge demonstrated lesions prior to treatment initiation and of those, 80.1% (75 of 93) survived challenge. In some of these studies, treatment was delayed up to two days after lesions were first noted and in others treatment was administered for a sub-optimal period of time. In the two IV variola studies 7 of 13 animals (53.8%) were treated at lesion onset and of those the survival rate was 85.7% (6 of 7).

Tenet 3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.

IV inoculation of NHPs with either variola virus or MPXV produces a systemic, lesional disease that closely resembles human smallpox. Infection of NHPs with monkeypox virus appears to produce a more severe infection with higher mortality than variola virus and thus, represents a more rigorous model for establishing efficacy of an antiviral therapeutic.

SIGA has participated in nine MPXV NHP studies that evaluated the efficacy of various doses of ST-246 (placebo, 0.3, 1, 3, 10, 20, 30, 100, or 300 mg/kg/day) given once daily to a total of 154 cynomolgus macaques challenged with MPXV (Zaire 79 strain) via the IV route (Table 1). In all cases, the primary endpoint was protection from death. ST-246 was administered to a total

of 120 NHPs for 3, 5, 7, 10 or 14 consecutive days by oral or nasogastric gavage. Delays in initiation of ST-246 treatment ranged from 1 hour to 6 days post-challenge with the treatment of 109 of 120 (90.8%) NHPs being delayed to at least 3 days post-challenge. Total study durations ranged from 28 to 56 days. Nineteen deaths occurred among NHPs treated with ST-246 during all 9 MPXV studies (19 of 120; 15.8%). The majority of these deaths occurred in animals treated sub-optimally: 9 of 19 (47.4%) were treated with 1 mg/kg or less of ST-246 beginning 4 days post-challenge, 2 of 19 (10.5%) received only 3 days of treatment beginning 4 days post-challenge and 3 of 19 (15.8%) did not receive treatment until 6 days post-challenge. Of the remaining 5 deaths, 2 were reported as not due to typical MPXV disease. There was no evidence of disease recrudescence during any of the studies. All of the studies indicated statistically significant decreases in major morbidity in the form of mean total body lesion counts for a majority of ST-246 treatment groups versus placebo. Viral DNA levels in the blood were also reduced with statistical significance for ST-246 treatment groups versus placebo.

SIGA has participated in two variola NHP studies that evaluated the efficacy of 300 mg/kg or 10 mg/kg doses of ST-246 given once daily to 22 cynomolgus macaques challenged with variola virus (Harper strain) via the IV route (Table 1). In all cases, the primary endpoint was survival. Treatment with ST-246 was administered to 13 NHPs and occurred over 14 days, with ST-246 being delivered through oral gavage. Delays in initiation of ST-246 treatment ranged from concurrent with challenge to 4 days post-challenge with the treatment of 7 of 13 (53.8%) NHPs being delayed to lesion onset. Total study durations ranged from 23 to 28 days. No deaths attributable to smallpox disease occurred among NHPs treated with ST-246, although one animal did succumb on treatment Day 6 (Study Day 10 post-challenge) following an anesthetic procedure (1 of 13; 7.7%). Similar to the treatment group, only 2 deaths occurred among the placebo-treated animals (2 of 9; 22%), although only one was attributed to smallpox infection. A statistically significant decrease in mean total body lesion counts and viral DNA levels in the blood for ST-246 treatment groups versus placebo was observed for specific time points during the studies. As discussed previously, a number of experts in the field agree that since humans are the only natural host of the variola virus and the variola virus does not replicate efficiently in NHPs, the variola NHP model of disease may not be the most appropriate for assessing efficacy based upon an endpoint of survival. Likewise, reductions in major morbidity in the form of mean total body lesion counts and viral load are also affected by the inability of the virus to establish a productive infection in NHP hosts.

Tenet 4. The data or information on the (pharmaco) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

The primary efficacy animal model must provide a quantitative assessment of the therapeutic potential of a drug candidate that is predictive for outcome in humans. Models developed in NHPs infected with MPXV via IV injection produce a near uniformly lethal infection with high viremia and exanthematous disease that closely resembles human smallpox. Given the similarity in drug pharmacokinetics between NHPs and humans, the MPXV NHP model offers an ideal tool for the assessment of antiviral efficacy. Further, the MPXV NHP model allows for an evaluation of the effect OPXV infection has on pharmacokinetics (PK), a scenario that is not feasible in the variola NHP model due to restrictions for conducting studies in a BSL4 environment.

ST-246 safety and PK have been evaluated in numerous nonclinical studies as well as three Phase I clinical studies and one Phase II clinical study. The PK of ST-246, as well as the underlying metabolism, were very similar in NHPs and humans. Data obtained from studies conducted in both uninfected as well as in MPXV-infected NHPs has been used to develop a population (POP) PK model of ST-246 in NHPs. MPXV infection resulted in a minimal (< 10%) increase in ST-246 clearance, although inter-individual clearance variability was 32%. The effect of MPXV infection on clearance was translated to a similarly developed POP PK model of ST-246 in healthy humans. Pharmacokinetic exposure parameters (AUC, C_{max}, C_{min}) were then predicted for OPXV-infected humans on Day 1 of treatment and at steady state. Finally, simulated exposure parameters (AUC, C_{max}, C_{min}) in infected NHPs over the range of doses used in efficacy evaluations (3-168 mg/m^2, 0.25-14 mg/kg) were overlaid with the same parameters predicted for OPXV-infected humans after daily doses of either 400 mg or 600 mg. Doses of 96-120 mg/m^2 (8-10 mg/kg) and 144-168 mg/m^2 (12-14 mg/kg) administered to infected NHPs gave ST-246 exposures (AUC) equivalent to 400 and 600 mg doses, respectively.

In addition to the POP PK modeling, which predicted equivalency of doses between infected NHP and humans, pharmacodynamic (PD) analysis has been used to evaluate the correlation between ST-246 dose and exposure in NHPs and survival. The analysis used survival data from all MPXV infection studies that had survival data available at the time that the analysis was started. The total number of NHPs for the combined dose versus survival analysis was 96, of which 23 animals were placebos, with the remainder having received doses of ST-246 that ranged from 0.3 mg/kg to 300 mg/kg. Among the 96 NHPs evaluated for the initial PD survival analysis, 34 NHPs died before the end of the study. Most of these animals were administered placebo, 0.3 mg/kg, and 1 mg/kg ST-246.

The Dose-Survival relationship was explored using the Kaplan Meier approach. In this analysis a nonparametric survival curve estimates the probability of survival over time. For dose levels that were associated with death, 35 NHPs started their treatment on Day 4, 9 started their treatment on Day 3, and 5 NHPs started treatment on Day 5. Probabilities of survival were greater than 90% versus placebo at doses of 10 mg/kg and 3 mg/kg. Survival associated with doses of 0.3 mg/kg and 1 mg/kg versus placebo did not demonstrate statistical significance.

Recursive receiver operator characteristic (ROC) analysis was conducted in order to determine the optimal cut-off points for dose or exposures levels that best correlate with the survival outcome. The advantage of this analysis is that an optimal cut-off is determined based on statistical criteria that optimize sensitivity and specificity. All results converged on the same overall conclusion showing that doses of 3 mg/kg and higher, were associated with maximum survival whereas doses of less than 3 mg/kg had very low survival rate. The actual cut-off values corresponded to a dose level of 100 mg for humans of a median weight of 78.35 kg (obtained from SIGA's Phase II clinical study). This analysis strongly indicates that a dose of 400 mg of ST-246 will provide more than sufficient exposure to protect humans against OPXV disease.

4.1 SUMMARY

To summarize, SIGA has utilized a multitude of animal species, OPVXs types and variants, inoculation routes and challenge inoculums to evaluate the efficacy of ST-246. The fact of the matter remains that there is no ideal animal model that recapitulates human smallpox disease to

the letter. ST-246 is extremely safe, is taken orally once daily for two weeks and could be distributed widely, without fear of complication, in a situation that warrants availability of a countermeasure to the masses. In the absence of an NDA approval, this solution does not exist. Even approval under an Emergency Use Authorization could limit the speed and ease of getting drug to everyone that may need it.

That said, the data presented in this document support the IV MPXV model as the best representation of the course of a VARV infection in humans. The fact that treatment is withheld until a productive systemic infection is established only strengthens the model's rigor and raises the efficacy bar. Animals present with lesions and the model is almost uniformly lethal. Finally, the IV MPXV model is reproducible among multiple investigators, can be conducted under GLP and contains clearly defined endpoints. With the acceptance of this model under the animal rule pathway to U.S. NDA approval, ST-246 could be made available for use to any and all infected individuals across the United States who contracted an orthopoxvirus disease via incidental contact, or in the event of a declared emergency due to an accidental or deliberate release of an orthopoxvirus.

REFERENCES

1. Roberts K.L. and Smith G.L. 2008. Vaccinia Virus Morphogenesis and Dissemination. Trends Microbiol. 16:472-479.

2. Smith G.L., Vanderplasschen A. and Law M. 2002. The Formation and Function of Extracellular Enveloped Vaccinia Virus. J Gen Virol. 83:2915-2931.

3. Chen Y., Honeychurch K.M., Yang G., et al. 2009. Vaccinia Virus P37 Interacts With Host Proteins Associated With Le-Derived Transport Vesicle Biogenesis. Virol J. 6:44.

4. Henderson D.A., Inglesby T.V., Bartlett J.G., et al. 1999. Smallpox as a Biological Weapon: Medical and Public Health Management. Working Group on Civilian Biodefense. Jama. 281:2127-2137.

5. Smith G.L. and McFadden G. 2002. Smallpox: Anything to Declare? Nat Rev Immunol. 2:521-527.

6. Fulginiti V.A., Papier A., Lane J.M., et al. 2003. Smallpox Vaccination: A Review, Part II. Adverse Events. Clin Infect Dis. 37:251-271.

7. Kemper A.R., Davis M.M. and Freed G.L. 2002. Expected Adverse Events in a Mass Smallpox Vaccination Campaign. Eff Clin Pract. 5:84-90.

8. Fenner F., Henderson D.A., Arita I., et al. 1988. Smallpox and Its Eradication. World Health Organization, Geneva, Switzerland. whqlibdoc.who.int/smallpox/9241561106.pdf.

9. Breman J.G. and Henderson D.A. 2002. Diagnosis and Management of Smallpox. N Engl J Med. 346:1300-1308.

10. Moore Z.S., Seward J.F. and Lane J.M. 2006. Smallpox. Lancet. 367:425-435.

11. Phadke A.M., Samant N.R. and Dewal S.D. 1973. Smallpox as an Etiologic Factor in Male Infertility. Fertil Steril. 24:802-804.

12. Downie A.W. and McCarthy .K. 1958. The Antibody Response in Man Following Infection With Viruses of the Pox Group. III. Antibody Response in Smallpox. J Hyg (Lond). 56:479-487.

13. Jackson T.M., Zaman S.N. and Huq F. 1977. T and B Rosetting Lymphocytes in the Blood of Smallpox Patients. Am J Trop Med Hyg. 26:517-519.

14. Fenner F., Henderson D., Arita I., et al. 1988. The Clinical Features of Smallpox. Smallpox and its Eradication.1 - 68.

15. Martin D.B. 2002. The Cause of Death in Smallpox: An Examination of the Pathology Record. Mil Med. 167:546-551.

16. Earl P.L., B. Moss, L. S. Wyatt, et al. 1998. Generation of Recombinant Vaccinia Viruses, p. 16.17.11-16.17.19. *In* F. M. Ausubel R.B., R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current Protocols in Molecular Biology, Vol. 2. Greene Publishing Associates & Wiley Interscience, New York, N.Y.

17. Earl P.L., N. Cooper, S. Wyatt, et al. 1998. Preparation of Cell Cultures and Vaccinia Virus Stocks, p. 16.16.11-16.16.13. *In* F. M. Ausubel R.B., R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current Protocols in Molecular Biology, vol. 2. John Wiley and Sons, New York, N.Y.

18. Andrew M.E. and Coupar B.E. 1992. Biological Effects of Recombinant Vaccinia Virus-Expressed Interleukin 4. Cytokine. 4**:**281-286.

19. Henderson D.A. 1999. The Looming Threat of Bioterrorism. Science. 283**:**1279-1282.

20. Weiss M.M., Weiss P.D., Mathisen G., et al. 2004. Rethinking Smallpox. Clin Infect Dis. 39**:**1668-1673.

21. Kretzschmar M., van den Hof S., Wallinga J., et al. 2004. Ring Vaccination and Smallpox Control. Emerg Infect Dis. 10**:**832-841.

22. Kaplan E.H. 2004. Preventing Second-Generation Infections in a Smallpox Bioterror Attack. Epidemiology. 15**:**264-270.

23. Legrand J., Viboud C., Boelle P.Y., et al. 2004. Modelling Responses to a Smallpox Epidemic Taking into Account Uncertainty. Epidemiol Infect. 132**:**19-25.

24. Kaplan E.H., Craft D.L. and Wein L.M. 2002. Emergency Response to a Smallpox Attack: The Case for Mass Vaccination. Proc Natl Acad Sci U S A. 99**:**10935-10940.

25. Meltzer M.I., Damon I., LeDuc J.W., et al. 2001. Modeling Potential Responses to Smallpox as a Bioterrorist Weapon. Emerg Infect Dis. 7**:**959-969.

26. Lane J.M., Ruben F.L., Neff J.M., et al. 1970. Complications of Smallpox Vaccination, 1968: Results of Ten Statewide Surveys. J Infect Dis. 122**:**303-309.

27. Kretzschmar M., Wallinga J., Teunis P., et al. 2006. Frequency of Adverse Events After Vaccination With Different Vaccinia Strains. PLoS Med. 3**:**e272.

28. Fenner F., Henderson D., Arita I., et al. 1988. Human Monkeypox and Other Poxvirus Infections of Man., p. 1287 - 1320, Smallpox and Its Eradication. World Health Organization, Geneva.

29. Breman J.G., Kalisa R., Steniowski M.V., et al. 1980. Human Monkeypox, 1970-79. Bull World Health Organ. 58**:**165-182.

30. Di Giulio D.B. and Eckburg P.B. 2004. Human Monkeypox: An Emerging Zoonosis. Lancet Infect Dis. 4:15-25.

31. Frey S.E. and Belshe R.B. 2004. Poxvirus Zoonoses--Putting Pocks into Context. N Engl J Med. 350:324-327.

32. Nalca A., Rimoin A.W., Bavari S., et al. 2005. Reemergence of Monkeypox: Prevalence, Diagnostics, and Countermeasures. Clin Infect Dis. 41:1765-1771.

33. Reed K.D., Melski J.W., Graham M.B., et al. 2004. The Detection of Monkeypox in Humans in the Western Hemisphere. N Engl J Med. 350:342-350.

34. Chen N., Li G., Liszewski M.K., et al. 2005. Virulence Differences Between Monkeypox Virus Isolates From West Africa and the Congo Basin. Virology. 340:46-63.

35. Rimoin A.W., Mulembakani P.M., Johnston S.C., et al. 2010. Major Increase in Human Monkeypox Incidence 30 Years After Smallpox Vaccination Campaigns Cease in the Democratic Republic of Congo. Proc Natl Acad Sci U S A. 107:16262-16267.

36. Tesh R.B., Watts D.M., Sbrana E., et al. 2004. Experimental Infection of Ground Squirrels (Spermophilus Tridecemlineatus) With Monkeypox Virus. Emerg Infect Dis. 10:1563-1567.

37. Sbrana E., Jordan R., Hruby D.E., et al. 2007. Efficacy of the Antipoxvirus Compound ST-246 for Treatment of Severe Orthopoxvirus Infection. Am J Trop Med Hyg. 76:768-773.

38. Xiao S.Y., Sbrana E., Watts D.M., et al. 2005. Experimental Infection of Prairie Dogs With Monkeypox Virus. Emerg Infect Dis. 11:539-545.

39. Hutson C.L., Olson V.A., Carroll D.S., et al. 2009. A Prairie Dog Animal Model of Systemic Orthopoxvirus Disease Using West African and Congo Basin Strains of Monkeypox Virus. J Gen Virol. 90:323-333.

40. Schultz D.A., Sagartz J.E., Huso D.L., et al. 2009. Experimental Infection of an African Dormouse (Graphiurus Kelleni) With Monkeypox Virus. Virology. 383:86-92.

41. Adams M.M., Rice A.D., and Moyer R.W. 2007. Rabbitpox Virus and Vaccinia Virus Infection of Rabbits as a Model for Human Smallpox. J Virol. 81:11084-11095.

42. Nalca A., Hatkin J.M., Garza N.L., et al. 2008. Evaluation of Orally Delivered ST-246 as Postexposure Prophylactic and Antiviral Therapeutic in an Aerosolized Rabbitpox Rabbit Model. Antiviral Res. 79:121-127.

43. Roy C.J. and Voss T.G. 2010. Use of the Aerosol Rabbitpox Virus Model for Evaluation of Anti-Poxvirus Agents. Viruses. 2:2096-2107.

44. Nelson J.B. 1938. The Behavior of Pox Viruses in the Respiratory Tract : I. The Response of Mice to the Nasal Instillation of Vaccinia Virus. J Exp Med. 68:401-412.

45. Quenelle D.C., Collins D.J. and Kern E.R. 2003. Efficacy of Multiple- or Single-Dose Cidofovir against Vaccinia and Cowpox Virus Infections in Mice. Antimicrob Agents Chemother. 47:3275-3280.

46. Martinez M.J., Bray M.P. and Huggins J.W. 2000. A Mouse Model of Aerosol-Transmitted Orthopoxviral Disease: Morphology of Experimental Aerosol-Transmitted Orthopoxviral Disease in a Cowpox Virus-Balb/C Mouse System. Arch Pathol Lab Med. 124:362-377.

47. Chapman J.L., Nichols D.K., Martinez M.J., et al. 2010. Animal Models of Orthopoxvirus Infection. Vet Pathol. 47:852-870.

48. Fenner F. and Buller R. 1997. Viral Pathogenesis, p. 535-553. *In* N N. (ed.), Viral Pathogenesis. Lippincott-Raven Publishers, Philadelphia.

49. Schriewer J., Buller R.M. and Owens G. 2004. Mouse Models for Studying Orthopoxvirus Respiratory Infections. Methods Mol Biol. 269:289-308.

50. Esteban D.J. and Buller R.M. 2005. Ectromelia Virus: The Causative Agent of Mousepox. J Gen Virol. 86:2645-2659.

51. Mims C.A. 1959. The Response of Mice to Large Intravenous Injections of Ectromelia Virus. II. The Growth of Virus in the Liver. Br J Exp Pathol. 40:543-550.

52. Mims C.A. 1964. Aspects of the Pathogenesis of Virus Diseases. Bacteriol Rev. 28:30-71.

53. Buller R.M., Owens G., Schriewer J., et al. 2004. Efficacy of Oral Active Ether Lipid Analogs of Cidofovir in a Lethal Mousepox Model. Virology. 318:474-481.

54. Yang G., Pevear D.C., Davies M.H., et al. 2005. An Orally Bioavailable Antipoxvirus Compound (ST-246) Inhibits Extracellular Virus Formation and Protects Mice From Lethal Orthopoxvirus Challenge. J Virol. 79:13139-13149.

55. Jahrling P.B., Hensley L.E., Martinez M.J., et al. 2004. Exploring the Potential of Variola Virus Infection of Cynomolgus Macaques as a Model for Human Smallpox. Proc Natl Acad Sci U S A. 101:15196-15200.

56. Zaucha G.M., Jahrling P.B., Geisbert T.W., et al. 2001. The Pathology of Experimental Aerosolized Monkeypox Virus Infection in Cynomolgus Monkeys (Macaca Fascicularis). Lab Invest. 81:1581-1600.

57. Goff A.J., Chapman J., Foster C., et al. 2011. A Novel Respiratory Model of Infection With Monkeypox Virus in Cynomolgus Macaques. J Virol. 85:4898-4909.

58. Stittelaar K.J., van Amerongen G., Kondova I., et al. 2005. Modified Vaccinia Virus Ankara Protects Macaques Against Respiratory Challenge With Monkeypox Virus. J Virol. 79:7845-7851.

59. Stittelaar K.J., Neyts J., Naesens L., et al. 2006. Antiviral Treatment Is More Effective Than Smallpox Vaccination Upon Lethal Monkeypox Virus Infection. Nature. 439:745-748.

60. Saijo M., Ami Y., Suzaki Y., et al. 2006. LC16M8, a Highly Attenuated Vaccinia Virus Vaccine Lacking Expression of the Membrane Protein B5r, Protects Monkeys From Monkeypox. J Virol. 80:5179-5188.

61. Olsen R.G., Blakeslee J.R., Mathes L., et al. 1977. Preparation and Evaluation of a Noninfectious Monkey Pox Virus Vaccine. J Clin Microbiol. 6:50-54.

62. Hooper J.W., Thompson E., Wilhelmsen C., et al. 2004. Smallpox DNA Vaccine Protects Nonhuman Primates Against Lethal Monkeypox. J Virol. 78:4433-4443.

63. Earl P.L., Americo J.L., Wyatt L.S., et al. 2004. Immunogenicity of a Highly Attenuated MVA Smallpox Vaccine and Protection Against Monkeypox. Nature. 428:182-185.

64. Edghill-Smith Y., Bray M., Whitehouse C.A., et al. 2005. Smallpox Vaccine Does Not Protect Macaques With Aids From a Lethal Monkeypox Virus Challenge. J Infect Dis. 191:372-381.

65. Edghill-Smith Y., Golding H., Manischewitz J., et al. 2005. Smallpox Vaccine-Induced Antibodies Are Necessary and Sufficient for Protection Against Monkeypox Virus. Nat Med. 11:740-747.

66. Heraud J.M., Edghill-Smith Y., Ayala V., et al. 2006. Subunit Recombinant Vaccine Protects Against Monkeypox. J Immunol. 177:2552-2564.

67. Fogg C.N., Americo J.L., Lustig S., et al. 2007. Adjuvant-Enhanced Antibody Responses to Recombinant Proteins Correlates With Protection of Mice and Monkeys to Orthopoxvirus Challenges. Vaccine. 25:2787-2799.

68. Earl P.L., Americo J.L., Wyatt L.S., et al. 2007. Recombinant Modified Vaccinia Virus Ankara Provides Durable Protection Against Disease Caused by an Immunodeficiency Virus as Well as Long-Term Immunity to an Orthopoxvirus in a Non-Human Primate. Virology. 366:84-97.

69. Nigam P., Earl P.L., Americo J.L., et al. 2007. DNA/MVA HIV-1/Aids Vaccine Elicits Long-Lived Vaccinia Virus-Specific Immunity and Confers Protection Against a Lethal Monkeypox Challenge. Virology. 366:73-83.

70. Marriott K.A., Parkinson C.V., Morefield S.I., et al. 2008. Clonal Vaccinia Virus Grown in Cell Culture Fully Protects Monkeys From Lethal Monkeypox Challenge. Vaccine. 26:581-588.

71. Raymond J. and Huggins J. 2007. Evaluation of the Progression of Pathogenic Events in Cynomolgus Macaques Infected With Variola Virus.1-95.

72. Puttnam A., Marriott K., Davenport R., et al. 2007. Is the Intratracheal Challenge Route a Good Respiratory Model of Monkeypox Infection? American Society for Virology Annual Meeting. Battelle Biomedical Research Center

73. Kramski M., Matz-Rensing K., Stahl-Hennig C., et al. 2010. A Novel Highly Reproducible and Lethal Nonhuman Primate Model for Orthopox Virus Infection. PLoS One. 5**:**e10412.

74. Matz-Rensing K., Stahl-Hennig C., Kramski M., et al. 2011. The Pathology of Experimental Poxvirus Infection in Common Marmosets (Callithrix Jacchus): Further Characterization of a New Primate Model for Orthopoxvirus Infections. J Comp Pathol.

75. Duraffour S., Snoeck R., de Vos R., et al. 2007. Activity of the Anti-Orthopoxvirus Compound ST-246 Against Vaccinia, Cowpox and Camelpox Viruses in Cell Monolayers and Organotypic Raft Cultures. Antivir Ther. 12**:**1205-1216.

76. Smith S.K., Olson V.A., Karem K.L., et al. 2009. *In Vitro* Efficacy of St246 Against Smallpox and Monkeypox. Antimicrob Agents Chemother. 53**:**1007-1012.

77. Moss B. 2006. Poxvirus Entry and Membrane Fusion. Virology. 344**:**48-54.

78. Law M., Carter G.C., Roberts K.L., et al. 2006. Ligand-Induced and Nonfusogenic Dissolution of a Viral Membrane. Proc Natl Acad Sci U S A. 103**:**5989-5994.

79. Buller R.M. and Palumbo G.J. 1991. Poxvirus Pathogenesis. Microbiol Rev. 55**:**80-122.

80. Moss B. 2001. Poxviridae: The Viruses and Their Replication, p. 2849-2883. *In* D. M. Knipe and P. M. Howley E. (ed.), Fields Virology, Fourth ed, Vol. 2. Lippincott, Williams and Wilkins, Philadelphia.

81. Hruby D.E. and Byrd C.M. 2006. Less Is More: Poxvirus Proteolysis. Microbe. 1**:**70-75.

82. Berhanu A., King D.S., Mosier S., et al. 2009. ST-246 Inhibits in Vivo Poxvirus Dissemination, Virus Shedding, and Systemic Disease Manifestation. Antimicrob Agents Chemother. 53**:**4999-5009.

83. Quenelle D.C., Buller R.M., Parker S., et al. 2007. Efficacy of Delayed Treatment With ST-246 Given Orally Against Systemic Orthopoxvirus Infections in Mice. Antimicrob Agents Chemother. 51**:**689-695.

84. Grosenbach D.W., Berhanu A., King D.S., et al. 2010. Efficacy of ST-246 Versus Lethal Poxvirus Challenge in Immunodeficient Mice. Proc Natl Acad Sci U S A. 107**:**838-843.

85. Wahl-Jensen V., Cann J.A., Rubins K.H., et al. 2011. Progression of Pathogenic Events in Cynomolgus Macaques Infected With Variola Virus. Plos One. 93:1-12.

86. Bossi P., Tegnell A., Baka A., et al. 2004. Bichat Guidelines for the Clinical Management of Smallpox and Bioterrorism-Related Smallpox. Euro Surveill. 9:E7-8.

APPENDICES

Appendix A: Small Animal Models of Poxvirus Infection

Appendix B: Expert Advisory Panel Report - Summary Evaluation of Animal Models of Variola Virus Disease and Bios of Panel Members

Appendix C: Report on Emergency Use of ST-246, May 2011

Appendix D: MMWR Report on Emergency Use of ST-246, November 2009

Appendix E: MMWR Report on Emergency Use of ST-246, May 2009

Appendix F: MMWR Report on Emergency Use of ST-246, May 2007

Appendix G: University of Chicago Account of Emergency Use of ST-246

Appendix H: Small Animal Studies With ST-246

APPENDIX A

SMALL ANIMAL MODELS OF POXVIRUS INFECTION

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1. SMALL ANIMAL MODELS OF POXVIRUS INFECTION

1.1 Monkeypox Virus Infection in Ground Squirrels, Prairie Dogs, and Dormice

Ground squirrels (*Spermophilus tridecemlineatus*) are native to North America and are susceptible to monkeypox virus. Ground squirrels infected by intraperitoneal (IP: 1.26×10^5 pfu) or intranasal (IN: 2.52×10^5 pfu) routes with MPX 2003 develop fulminant illness and all die within 6-9 days.[36] The infected animals exhibit lethargy and anorexia, virus is detectable in blood and throat swab samples, and upon necropsy relatively high titers of MPXV are detectable in liver, spleen, kidney, lung, heart, and brain. Centrilobular hepatic necrosis, moderate-to-severe splenic necrosis, and mild-to-multifocal thickening of the alveolar septa and focal lung consolidations are observed in IP-infected animals. In IN-infected animals, multifocal hepatic steatosis, diffuse hepatocytic necrosis, moderate-to-severe splenic necrosis, focal lymph node necrosis, and variable lung consolidation and interstitial inflammation are observed. A significant drawback of this model is that none of the animals exhibit detectable skin lesions that could be utilized as a therapeutic trigger and animals do not present any other obvious clinical symptoms of disease. Subcutaneous infection of ground squirrels with 100 pfu of MPXV Zaire 79 is also 100% lethal between Days 6 and 11.[37] The animals exhibit lethargy, anorexia, nosebleed, respiratory distress, apparent labored breathing, and audible wheezing. The viral load increases until death in blood, lung, liver, and spleen. White blood cell counts, levels of ALT, AST, total bilirubin, and alkaline phosphatase as well as coagulation values are elevated. Infected animals also showed pulmonary edema and hemorrhage, hepatocellular necrosis and portal inflammation, and splenic and lymph node necrosis. As with the previous routes of infection, animals do not develop a definable therapeutic trigger in the form of the classic raised pustular skin lesions associated with human smallpox disease. While this model is good for assessing improvements in percent survival, ultimately, for all three routes of infection, the clinical and pathological features of MPXV infection in ground squirrels are more similar to the severe hemorrhagic rather than the classic form of human smallpox making the MPXV ground squirrel model less than ideal in recapitulating human smallpox disease.

Prairie dogs (Cynomys ludovicianus) can be infected by IP (1.26×10^5 pfu) or IN (2.52×10^5 pfu) routes with MPX 2003 with mortality rates of 100% (Days 8-11) and 60% (Days 11-14), respectively.[38] Virus was detected in the blood, throat, and tissues of IP-infected animals and lethargy and anorexia, marked hepatic and splenic necrosis, and mild-to-moderate inflammatory changes in the lungs were observed. As with the ground squirrel model, no visible lesions developed on the skin or mucous membrane of any of these IP-infected animals demonstrating a lack of a therapeutic trigger. In all of IN-infected animals no evident hepatic lesions or splenic necrosis was observed but those animals that died exhibited marked edema, hemorrhage, and necrosis in the lungs. The temporal appearance, organ distribution, and amount of virus present in the IN-infected animals were inconsistent and variable among individual animals. Additionally, only surviving IN-infected animals developed ulcerative lesions on the lips, tongue, and buccal mucosa, along with nasal congestion and a mucopurulent nasal discharge. Similarly, in prairie dogs IN and intradermally (ID)-infected with 3.16×10^4 pfu of the MPXV-2003-358, only 25% and 50% of the infected animals die, respectively, although a generalized rash becomes apparent 9–12 days post-infection for all the animals.[39] Signs of disease in these animals included inappetence, lethargy, nasal discharge, diarrhea, and respiratory distress. Taken together, the IN and ID routes of infection in the MPXV prairie dog model produce variable mortality while the IP route of infection is useful when the primary endpoint is death, but this model clearly lacks a therapeutic trigger and does not closely resemble human smallpox disease.

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A species of African dormice, *Graphiurus kelleni*, is also susceptible to a lethal IN infection with MPXV-ZAI-79 with challenge doses of 200-2,000 pfu resulting in 100% mortality in 7-10 days.[40] Unlike the wild captured ground squirrels and prairie dogs, the benefit of using dormice is that they can be successfully propagated and maintained like laboratory mice, which allows for an accessible supply of animals with a known health status. IN challenge with 2.0×10^4 pfu, results in virus replication and dissemination to multiple organs causing severe tissue necrosis and/or hemorrhage. Signs of morbidity include decreased activity, weight loss, hunched posture, unkempt hair coat, dehydration, and conjunctivitis. However, the model lacks a therapeutic trigger in that none of these animals develop skin lesions. Additionally, this dormouse model of MPXV-infection appears to most resemble the hemorrhagic/toxic-type human smallpox and provides a more severe disease course than seen in the other rodent models of MPXV infection.

1.2 Rabbitpox Infection in Rabbits

1.2.1 INTRADERMAL VIRUS CHALLENGE MODEL

Rabbits challenged with 1000 pfu of RPXV via the ID route develop a systemic viral disease that results in 100% mortality by day 9 post-infection (p.i.).[41] Within two days p.i., a raised red swelling becomes evident at the inoculation site, develops to a pustular, black, and necrotic center by Day 5, and covers the entire thigh of the rabbit with skin up to 2 cm thick by Days 7 or 8 p.i. The appearance the red, raised swelling could possibly be considered a therapeutic trigger although it does not specifically resemble the exanthematous disease of human smallpox. Secondary lesions, due to virus spread from the inoculation site, also appear on the eyelids and other mucocutaneous tissues, such as the nose, lips, tongue, mouth, genitals, and anus. Clinical signs of disease include fever, weight loss, nasal and ocular discharge, and severe respiratory distress with open-mouth breathing. Upon necropsy, the lungs of these euthanatized rabbits appear dark and marbled with large regions of hemorrhage. This model differs from human smallpox in two aspects. First, smallpox infection occurs via the respiratory mucosa and not via the skin, although the ID-infected rabbits appear to transmit the virus to sentinel animals via aerosol transmission. Second, the severe lung damage and hemorrhage that leads to euthanasia in this model is not observed in human smallpox deaths.

1.2.2 AEROSOL RABBITPOX VIRUS CHALLENGE MODEL

Aerosol infection of rabbits with 2,000 pfu of RPXV causes 100% mortality within 6-8 days of infection.[42] This model utilizes a mechanically generated aerosol particle of ~ 1 μm in diameter that generally results in the deposition of the virus in the lower respiratory tract. Disease progression in this animal model appears similar to human smallpox in that the virus primarily replicates in the respiratory system and then quickly spreads to other organs. Infected animals start to show signs of viral disease characterized by fever, increased viral load in the blood, and facial and cervical area edema on Day 3 p.i. Clinical signs of overt disease include anorexia, lethargy, weight loss, dehydration, purulent ocular and nasal discharges, and dyspnea. Maculopapular lesions are also evident on the skin starting on Day 5 p.i., which is too late to be considered a viable therapeutic trigger since death occurs shortly thereafter. Pathological findings include multifocal proliferation and/or necrosis of the respiratory epithelium lining the bronchi, bronchioles, and other airways, and marked acute to sub-acute inflammation of pulmonary blood vessels with edema. In addition, multifocal necrosis and inflammation was observed in the liver, gonads, and other organs due to viral dissemination beyond the respiratory tract.

1.2.3 INTRANASAL RABBITPOX VIRUS CHALLENGE MODEL

To establish infection, rabbits are inoculated with 100 pfu of rabbitpox virus by intranasal administration of aerosol virus suspensions delivered to the respiratory tract.[42, 43] By Day 6 post-inoculation animals develop fever, listlessness, and purulent discharges from eyes and nose. A rash develops between Day 6 and 8 post-inoculation; however, skin lesion numbers are widely variable between individuals and range from a few scattered lesions to confluency. Thus, quantifying lesion number or severity of the rash is a subjective measure of systemic virus spread and may not be possible in all cases. One significant drawback of this model is that death in most cases usually occurs before the rash develops resulting in a lack of a therapeutic trigger, and is accompanied by a fall in body temperature to below normal levels. Since rabbits can tolerate more frequent and larger volume blood draws, blood chemistries can be measured to correlate changes in hematological status with disease progression. To quantify the level of virus spread, animals are sacrificed at selected time points post-infection and virus titers are measured in the liver, spleen, lung, kidney, and other organs. Antiviral efficacy is assessed by measuring decreased mortality, reduced virus titers in organs, and improvement of hematological status. Histological examination of tissue from sacrificed animals further defines the effects of antiviral compounds on viral pathogenesis.

1.3 Ectromelia and Vaccinia Cowpox Virus Infection in Mice

1.3.1 INTRANASAL VACCINIA/COWPOX VIRUS CHALLENGE MODEL

Vaccinia virus mouse models have been developed to measure efficacy of anti-poxvirus compounds.[44, 45] Similar models have been developed using cowpox virus as well.[45, 46] The pathogenesis of infection is dictated by the route of entry and models using intracranial, intravenous, or intranasal inoculation have been described.[47] Since intranasal infection closely mimics the natural route of smallpox infection in humans, intranasal inoculation of mice with vaccinia virus or cowpox virus produces a localized infection with replication in the nasal tissue and upper respiratory tract followed by systemic spread. This model is capable of assessing antiviral activity of compounds that inhibit multiple steps in the virus life-cycle (replication and spread). To establish infection, mice are inoculated with 10^4-10^6 pfu of vaccinia virus or cowpox virus in a small volume (20 μL). Although establishment of lethal infection requires a large inoculating dose of virus, the infection starts locally in the nasal tissue and lungs before spreading systemically through the lymph.[44, 45] Virus replicates in the reticulo-endothelial system and can be found in liver, spleen, lung, and kidney. By Day 4 post-inoculation mice begin losing weight and become lethargic. Mice continue to lose weight and their general appearance declines with most animals exhibiting signs of severe disease such as ruffled fur, hunched posture, and unsteady gate. By Day 8 post-inoculation mice are moribund and have lost as much as 30% of their initial body weight.

Mortality is the primary endpoint in this model with 100% of mice succumbing to infection by Day 10 post-inoculation. Disease progression can be monitored by measuring the change in weight during the course of infection. The change in weight correlates with systemic replication of virus in mice and is a non-invasive method of determining disease severity. The percent weight change is useful for determining disease severity when treatment protects mice from lethal infection although experiments must be tightly controlled to avoid attributing weight loss to viral infection. Thus, the efficacy of compounds that prevent mortality but do not completely inhibit virus replication and all aspects of disease progression can be assessed in this model. To quantify the level of virus spread, animals must be sacrificed at selected time points post-infection and virus titers measured in the liver, spleen, lung, kidney, and other host organs.

Antiviral efficacy is measured by the decreased mortality, inhibition of virus-induced weight loss, and reduction in viral titers in liver, spleen, lung, kidney, and other tissues. However, mice intranasally-infected with vaccinia or cowpox virus do not exhibit the extensive rash observed in smallpox cases and therefore this model lacks a definitive therapeutic trigger. In addition, unlike smallpox, it is thought that necrotizing bronchopneumonia is the cause of death in these models.

1.3.2 DERMAL/INTRANASAL ECTROMELIA VIRUS CHALLENGE MODEL

Ectromelia virus is a laboratory pathogen of mice that has been used as a model system to characterize disease pathogenesis of orthopoxvirus infections.[48, 49] The pathogenesis of ectromelia virus disease closely resembles human smallpox, with distinct stages of localized replication, systemic virus spread, and lesion formation; however, this model has considerable, shortcomings in that the time course of infection and disease progression is much shorter and death occurs due to severe liver necrosis,[48] a pathology not observed in human smallpox disease. Ectromelia virus enters through abrasions in the skin and replicates in local lymphoid cells.[50] The virus multiplies in the lymphatic endothelial cells, macrophages, and dendritic cells within the node over a period of 2-4 days. Following this latency period, the virus spreads through the lymph and enters the bloodstream to cause a primary viremia. The virus is rapidly removed by macrophages lining the sinusoids of the liver, spleen, and bone marrow.[51] Infection of the parenchymal cells of liver and lymphoid cells of spleen produces high virus titers that are released into the blood stream to cause a secondary viremia.[51, 52] In highly susceptible animals, replication in liver and spleen produces focal necrotic lesions, acute hepatitis, and multi-organ failure. In mice that are less susceptible to infection, a rash develops following the secondary viremia.[48] The rash is caused by virus replication in the perivascular cells and dermal endothelial cells and epidermis. A lethal ectromelia virus mouse model has been established to evaluate the efficacy of antiviral drugs.[49, 53] In susceptible mice as little as 1 pfu of ectromelia virus causes lethal infection.[53] A/NCR mice are inoculated with ectromelia virus either by footpad scarification, which is similar to the natural route of infection through abrasions in the skin, or intranasal delivery.[48] The virus multiplies in the lymphatic endothelial cells, macrophages, and lymphocytes within the regional lymph node over a period of 2-4 days. With high viral challenges, mice appear ill with hunched posture, ruffled coat, and increased respiration by 5-6 days post-inoculation. With lower challenge doses sickness is delayed until 8-10 days. Viral replication in liver and spleen produces focal necrotic lesions and acute hepatitis that results in death between Days 6 and 10 post-inoculation.[48]

The disease progression is similar to the intranasal vaccinia and cowpox virus models with mortality occurring in 100% of mice by Day 10 post-inoculation.[53] Disease progression can be monitored by measuring the change in weight during the course of infection. To quantify the level of virus spread, animals are sacrificed at selected time points post-infection and virus titers measured in the liver, spleen, lung, kidney, and other organs.[53, 54] Detecting virus in these tissues is an excellent measure of virus spread since viral inoculums contain 50-100 pfu, and virus is likely to be detectable only after significant levels of replication and spread. Like the intranasal vaccinia and cowpox virus models, antiviral efficacy is measured by decreased mortality, inhibition of virus-induced weight loss, and reduction in viral titers in liver, spleen, and other tissues. A major advantage of the intranasal ectromelia virus model is the low challenge dose that results in a lethal infection. This provides a large dynamic range of challenge doses that can be employed to test the robustness of a candidate therapeutic.

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APPENDIX B

EXPERT ADVISORY PANEL REPORT – SUMMARY EVALUATION OF ANIMAL MODELS OF VARIOLA VIRUS DISEASE ND BIOS OF PANEL MEMBERS

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Summary Evaluation of Animal Models of Variola Virus Disease

Focus: Non-human Primate Studies

Inger Damon, Arthur J. Goff and Grant McFadden

Disclaimer: The opinions of this report are solely those of the three authors, and not their host agencies.

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Arthur J. Goff III, Ph.D. Virologist, Viral Therapeutics - USAMRIID	Date
Grant McFadden, PhD Professor, Dept of Molecular Genetics and Microbiology U. of Florida	Apr 28/10 Date

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Issue: To support the claims by SIGA Corporation that a new drug candidate, ST-246, is a valid therapeutic for treating smallpox disease in humans, the company has provided data in a variety of animal models showing treatment efficacy and survival benefit in several models of related systemic orthopoxvirus diseases. However, smallpox is a uniquely human disease and the only surrogate model available using variola virus involves intravenous (IV) injections into nonhuman primates, at high dosages that cause lethality by a mechanism that may not actually mimic the human disease. Illness with uniform lethality, and rash illness progression similar to human smallpox, can be generated in nonhuman primates injected with monkeypox virus, but this virus is not identical to variola virus. Accordingly, an Advisory Panel was convened on March 24, 2010 in Washington, DC, to assess the strengths and weaknesses of these two animal models in terms of their likelihood of predicting efficacy and optimal dosage of ST-246 to treat cases of human smallpox disease.

Surrogate Animal Models for Smallpox: Since variola virus is a strictly human pathogen, and does not cause smallpox in any host except man in the natural setting, attempts have been made to surrogate aspects of smallpox disease with the natural strains of variola virus. Several inoculation regimes have been explored, but only IV injection of high doses of virus into nonhuman primates produces anything even resembling smallpox disease in humans, Specifically, IV injection of 10^8 infectious units of variola virus into nonhuman primates (Cynomolgus sp.) mimics certain aspects of late stage ordinary smallpox, including skin lesions and at least some mortality levels, but other key aspects of the human disease are not replicated: eg the prodomal phase of the human disease is bypassed and the progressive immune response components (both cellular and humoral) that likely affect true smallpox disease development are not induced in this model. SIGA has provided some new data showing that ST-246 can reduce the overall skin lesion count when the drug is dosed at the first onset of primary lesions, but this model has not clearly enabled measurement of protection from death because the mortality rate has been variable between studies. On the other hand, when 10^9 infectious units of variola virus are injected IV, a near-uniformly lethal syndrome is induced in nonhuman primates that bears some resemblance to late stage hemorrhagic smallpox that has been observed in a very small fraction of humans who contracted smallpox. However, this model replicates even less of the *bona fide* human smallpox disease than the 10^8 infectious unit dosage model, and death can occur prior to any lesion development, thus possibly complicating interpretation of a treatment outcome study, and certainly complicating any definitive dosing criteria. SIGA has asked the consultant team to weigh in on the merits of this latter model in evaluating ST-246 efficacy, and for potentially titrating an optimal drug dosage levels for treating the human disease. In order to address this question, the panel asked what characteristics would be required of the surrogate variola animal model in order to be capable of providing statistically compelling data on these two issues to support ST-246 licensure.

Variola Animal Model: The Ideal Scenario The ideal variola animal model should in theory provide a sufficiently accurate recapitulation of the human disease course, particularly after appearance of the first lesions, which normally would trigger ST-

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246 drug intervention. Additionally, the model should provide a sufficient kinetic approximation of human disease progression to allow for optimization of drug dosing and frequency for proposed utilization to treat disease. The model should have reproducible and defined disease severity criteria. In the opinion of the Advisory Panel, both the 10^8 and the 10^9 IV dosing models of variola in nonhuman primates have significant deficiencies, with the higher dose regime being the most deficient. Specifically, the 10^8 dosage model has the advantage of providing lesion counts but it does not induce reproducible significant mortality for reasonable statistical empowerment: in this model, ST-246 was effective when used prophylactically or at lesion onset in reducing lesion counts. In humans with true smallpox disease, the direct correlation between lesion count and mortality is an approximate estimation. On the other hand, the 10^9 dosage model cannot even be evaluated by lesion count and the mechanism of death at this dosage may correlate with some features of hemorrhagic forms of smallpox, but does not correlate at all with features of the far more prevalent "ordinary" manifestations of smallpox disease in humans. Although the rapid lethality following high dose IV injection bears some superficial clinical resemblance to end stage hemorrhagic smallpox, there many dissimilarities from this rare form of the disease, such that it would be imprudent to extrapolate any conclusions that might be derived from any ST-246 trial in this higher dose model. Accordingly, at this time, the Advisory Panel felt that the most predictive and precise efficacy and human drug dosing data for ST-246 would be from using the monkeypox model in nonhuman primates.

Monkeypox Model: When considering an appropriate animal model, one of the main considerations is production of a clinical disease that is as similar as possible to the disease being modeled in humans. An additional hurdle faced in attempting to secure FDA approval for antiviral therapeutics is that the model must produce quantifiable and reproducible results and endpoints. Without reproducible and quantifiable results, it will prove difficult to interpret efficacy data, and/or require large numbers of animals to achieve significance in interpretation of results and study endpoints. Infection of Cynomolgus macaques (Mauritius descent) intravenously with $5x10^7$ pfu of monkeypox Zaire '79 results in a systemic febrile rash followed by an orthopoxvirus disease that reasonably well mimics the acute illness phases of both Monkeypox and "ordinary" Variola infection in humans. Infected control animals developed fever around day 2 or 3 post-infection, in addition to significant lymphadenopathy. Lymphadenopathy is atypical for human smallpox but is typical of human monkeypox. Lesions appeared first in the oral cavity typically on day 4 post infection. Lesions then develop on the face, extremities, and axillary and inguinal regions similar to the distribution in human patients. Lesions follow the typical orthopoxvirus rash progression (macules/papules/-vesicles-pustules-umbilication-crust/scab) that is characteristic of the human smallpox or monkeypox disease. The time from lesion onset to scabbing lasts between 13 and 15 days. Total lesion numbers in untreated animals typically range from 800-2000. Control animals begin to succumb to disease on or about post-infection days 12-14 but occasionally survive until days 18-20. Mortality in this model is consistently >99%. In addition, viral DNAemia and lesion counts

correlate well with each other and are both excellent indicators of disease severity and mortality. The tight correlation between lesion counts, viral DNAemia, and mortality in this model creates three definable, consistent end points by which to measure efficacy and dosing optima of a particular antiviral drug. However, in this model as well, the prodomal phase of the human disease is bypassed and the progressive immune response components (both cellular and humoral) that would affect disease development are not induced in the same fashion as they would be in the prodromal phase of the human smallpox disease; this may manifest in the observed 99% mortality rate in the model.

Infection of Cynomolgus macacques intravenously with 10^8 pfu of Variola strain Harper also produces a lesional disease, however the resulting disease course has not been as reproducible as the Monkeypox model. However, fewer control animals have been studied with the 10^8 pfu Variola-Harper challenge than with the monkeypox Zaire '79 challenge. Using the 10^8 pfu of Variola strain Harper IV challenge, lesion development follows a similar course and the number of lesions, within individual studies, on untreated or mock treated animals can even be similar. However, that is where the similarity between these two models ends. Mortality in the variola model in untreated animals has been variable and depending on the study, ranges from (1/7) 14% to (1/3) 33% to (2/2) 100%. Lesion counts in untreated animals display a wide range between studies (max average lesion count of ~900 in natural history serial sacrifice study vs. ~360 in untreated animals, at least for protocol 1745). Whereas in the monkeypox model viral DNAemia and lesions counts correlate well with each other and are both excellent indicators of disease severity and mortality, there is not consistent evidence across studies for correlations between these disease parameters in the 10^8 pfu of Variola- Harper IV challenge model. In natural history, or in the control arm of treatment, studies using small numbers of animals, there was an apparent correlation (in untreated animals) between viral DNAemia, rash burden and mortality. In the serial sacrifice study looking at animals through day 11 of disease, there was a correlation between viral DNAemia and rash burden. However in the recent larger (better powered) study of mock/vehicle treated animals, there was no correlation between viral DNAemia , rash burden and mortality. In review of the published literature on the 10^8 harper iv variola non human primate challenge (Huggins et.al. Nonhuman Primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246 AAC 53; 6;2620-2625 20090) there are some apparent differences between the baseline lab studies of animals challenged with monkeypox or variola (BUN,PLT, and albumen), and between the variola treatment and control arms, differences in baseline BUN, ALT, wbc, HCT and initial temperature measurements. These differences could affect disease manifestations and outcomes. If consistent results cannot be replicated in untreated control animals, then proving efficacy for a given antiviral therapeutic through this modality will be almost impossible. For these reasons, it is difficult to predict whether drug efficacy or dosing studies can be effectively designed, and whether data produced in the variola model will support smallpox antiviral efficacy assessments. At best, the variola model would produce descriptive data that suggests that the drug works in this system, and is likely to be

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efficacious in humans, but it would not be powered to generate pivotal efficacy data. The current Variola model in nonhuman primates should be considered an intriguing biological tool, however significant improvements are needed before it can be used as an animal model in which statistically significant drug efficacy can be demonstrated and quantified. Both viruses can induce a hemorrhagic form of their respective diseases if the viral IV inoculum is increased by an order of magnitude. However, the hemorrhagic manifestations in these two instances cause entirely different diseases. Animals are infected and are essentially overcome by the responses to the overwhelming amount of circulating virus before lesions, or an immune response, have the opportunity to develop. In this scenario, there is no therapeutic trigger such as onset of lesions, and therefore no reproducible therapeutic window. Results obtained in either hemorrhagic model system should thus only be considered anecdotally and not as part of a defined efficacy package.

Variola in humans and nonhuman primates: Variola in humans (smallpox) was characterized to permit epidemiologic comparisons by the WHO, and using more detailed clinical descriptions, by Dixon [Dixon CW Smallpox JA, Churchill Ltd, London 1962]. Different mortality rates, manifestation and extent of rash, degree of morbidity, levels of viremia, and hematologic parameters were observed with these different forms of disease. In Rao's summary of 3544 unvaccinated hospitalized smallpox patients [Rao AR, Smallpox, Kothari Book Depot, Bombay 1972] he obtained the following case fatality rates associated with epidemiologically distinct disease manifestations (based largely on rash phenotype). The majority of hospitalized patients had some variant of "ordinary" disease (i.e. with typical rash presentation and rash progression as described and depicted in Breman et al. [Breman JG, Henderson DA. Diagnosis and management of smallpox N Engl J Med.346 (17):1300-8, 2002]). Using these definitions, in ordinary types of smallpox, the degree of rash coalescence/burden appeared to have some correlation with observed mortality.

Type	% Total Cases	Case Fatality Rate%
Hemorrhagic early	0.7	100
Hemorrhagic late	1.7	96.8
Flat	6.7	96.5
Ordinary confluent	22.8	62.0
Ordinary Semiconfluent	23.9	37.0
Ordinary Discrete	42.1	9.3
Modified	1.7	0

Dixon used a more extensive set of clinical descriptors to define disease as either "malignant" or "benign" smallpox. Malignant forms were noted to have more systemic manifestations such as anxiety, secondary fever development after rash onset, hemorrhage, and atypical and extensive presentations (i.e. petechial or flat) of rash with extensive oropharyngeal/laryngeal involvement, and significant weight loss if survival post day 11 of illness. Malignant forms (malignant semiconfluent,

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malignant confluent, fulminating) were associated with case fatality rates of 25, 70 or 100%, respectively. Benign forms, with varying degrees of rash confluence (discrete, semiconfluent, or confluent) were associated with case fatality rates of 2, 10 or 20%, respectively. Irrespective of what type of smallpox developed, Dixon characterized the incubation period of disease to be 11-12 days. This may then suggest that mortality associated with the more "malignant" manifestations of smallpox results from an aberrant host inflammatory and immune response to the virus that results in death; if it were simply more rapid replication or accumulation of virus, perhaps due to an impaired immune system, one might expect a shorter incubation period in these forms of disease. Significant levels of viremia at or post rash onset, using inoculation of whole blood or serum on chorioallantoic membranes of embryonated hen eggs, were only routinely demonstrated in the hemorrhagic forms of smallpox disease and this viremia was observed throughout the illness course.

The current animal models of smallpox, using 10^8 pfu or 10^9 pfu IV administration of variola virus to initiate disease in nonhuman primates do not faithfully recapitulate any of these categorized descriptions of human smallpox disease. Illness onset in these nonhuman primate models is within two to three days of challenge. Rash onset in the oropharynx or skin is between days 3 and 4. Although "hemorrhagic" disease is elicited with the 10^9 pfu intravenous challenge, and appears to have a >88% case fatality rate (Jahrling et.al. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox PNAS 101(42): 15196-200; 2004), most animals die at day 3-4 (4/8), making treatment at rash onset difficult, requiring the use of large numbers of animals to demonstrate efficacy of an antiviral intervention administered at rash onset, and thus would lead to study designs where significant numbers of animals would be expected to have significant suffering. Although the illness onset to death time course, the presence of viremia throughout illness, and some hematologic parameters are consistent with descriptions of early hemorrhagic or fulminant smallpox, given the absence of an incubation period, the probable pathogenesis of "hemorrhagic" disease in this model may be different that that of human hemorrhagic/malignant disease. Using 10^8 pfu of intravenous challenge virus, varying case fatality rates have been observed (1/3 or 1/7 or 2/2), and the correlation between viral DNA levels in whole blood and rash burden as indicators of disease severity have not been reproducible across studies. The relationship of viral DNA levels to infectious virus has not been fully examined. Rash lesions do develop, between day 3 and 4, increase in number through day 7-8, and progress through "ordinary" phenotypes, with a centrifugal distribution. The extent of rash lesion development, however, has not been reproducible across studies.

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Summary: From our review of the literature and examination of the newer data provided by SIGA, the design of an animal model to faithfully reproduce "human smallpox", or a particular illness manifestation of the previously characterized human disease, has not been fully successful. Similarly, the Institute Of Medicine report "Live Variola Virus: Considerations for Continuing Research" released in 2009 recommended that the variola nonhuman primate challenge be re-evaluated for potential refinement. If variola studies, using the 10^8 pfu challenge model, were to be supportive of efficacy claims of an antiviral treatment, additional information that would be important to contrast across the various variola and monkeypox nonhuman primate studies to understand the apparent non-reproducibility of all disease parameters in the variola studies would include, but not be limited to: the animal weights, ages, sources, and baseline laboratory studies used in the various challenge studies. Ideally, the demonstration of efficacy of an antiviral against variola in an *in vivo* challenge study would be important to support efficacy of an antiviral treatment for human smallpox. CDC is working with HHS to assemble a panel of experts to review and suggest refinements to the variola challenge/non human primate model, as suggested by the IOM. It is not possible to predict when these findings will be available, nor if their recommendations will be predictive of a model with greater reproducibility, or greater similarity to human smallpox. Similarly, the timeline for WHO approval to use live variola in any new revised proposal is not possible to predict. In contrast, the monkeypox Zaire '79 $5x10^7$ infectious particle IV challenge, with initiation of treatment at lesion onset, has been reproducibly used to evaluate survival, and overall benefit/efficacy, of antiviral treatment for systemic, febrile rash during orthopoxvirus illness in nonhuman primates. Furthermore, the sensitivity of monkeypox and variola virus to ST-246 in cultured cells is very similar, consistent with the close sequence similarity between the orthologous viral protein (F13L) that is targeted by this drug. Although there are differences in manifestations of human monkeypox and human smallpox disease pathogenesis (most notably the presence of lymphadenopathy in human monkeypox, also demonstrated in the monkeypox nonhuman primate model), we conclude that the reproducibility of the monkeypox/nonhuman primate model, and its close similarity to the exanthematous phases of human monkeypox/variola disease, makes it the best currently available nonhuman primate model for evaluation of potential antiviral efficacy of ST-246 for treatment of human smallpox.

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Inger Damon, M.D., USPHS
Chief Poxvirus and Rabies Branch
Centers for Disease Control and Prevention

Dr. Damon is a leading authority on the history of smallpox and has responsibility for all research conducted using live variola virus at the BSL-4 laboratory at CDC.

Grant McFadden, Ph.D.
Professor, Departmentof Molecular Genetics & Microbiology College of Medicine,
University of Florida

Dr. McFadden is a leading authority on poxvirus pathogenesis and virus-host interactions. He is a member of the World Health Organization's Advisory Committee Variola Virus Research, including the sub-committee that reviews all proposals to conduct research using live variola virus.

Arthur J. Goff Ph.D.
Virologist
United States Army Medical Research Institute for Infectious Diseases
Virology Division, Viral Therapeutics Branch

Dr. Goff's group is actively involved with conducting BSL-3 studies using the MPX:NHP model. In addition, his team is the only group currently approved to work with live variola virus in animals and they are responsible for execution of all the variola:NHP experiments in the CDC ABSL-4 laboratories.

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APPENDIX C

REPORT ON EMERGENCY USE OF ST-246, MAY 2011

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EMERGENCY USE OF THE ANTI-ORTHOPOXVIRUS COMPOUND ST-246® IN A [redacted] (b) (6) PATIENT WITH VACCINOSIS

EIND #: 112,324

Treating Physician:

[redacted] (b) (6)

Clinical Investigational Product ST-246 Provided by:

SIGA Technologies, Inc.

4575 SW Research Way, Suite 230

Corvallis, Oregon 97333

Version Number: 1.0

August 29, 2011

Prepared by Dr. [redacted] (b) (6) **with assistance from SIGA Technologies, Inc.**

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On May 13, 2011, a possible contact vaccinia infection involving a 25 year old healthy, immunocompetent woman with a history of acne was reported to the CDC by the (b) (6) Department of Health (DoH). Approximately 10 days previously, the woman was believed to have contracted a live vaccinia virus (VACV) infection on her chin while changing a bandage covering the smallpox vaccination site of her boyfriend, a military contractor. The first lesion was noticed on May 9, with 3 more lesions developing shortly thereafter.

The Treating Physician, Dr. (b) (6), first saw the case on May 11, 2011. A primary lesion swab culture ruled out an MRSA infection, and a tentative diagnosis of VACV was made based on history and lesions, which were light pink, well-circumscribed maculae with a central umbilical region. The (b) (6) Laboratory Response Network conducted real time PCR for non-variola orthopoxvirus, and after a positive result (cycle threshold = 18), the patient was treated on May 14 with 6,000 IU/kg Vaccinia Immune Globulin Intravenous (VIG IV) at (b) (6) hospital in (b) (6) and was placed under house quarantine.

An Emergency Investigational New Drug Application (E-IND) for compassionate use was requested on May 13 and was granted to the Treating Physician (E-IND # 112,324) on May 18, 2011. After signing an Informed Consent, a daily oral dose of ST-246 (400 mg) was administered for 14 days starting on May 19. By the time treatment was initiated, lesions had grown and the primary lesion had turned black, but fever and malaise had decreased and there was minimal lymphadenopathy.

A blood sample for PCR (serum and EDTA blood) and antibody testing (serum) were taken prior to treatment initiation on May 19. Due to logistical limitations at the Medical Clinic, only blood was available for analysis at this time. Real-time PCR for non-variola orthopoxviruses was negative, but serum was ELISA-positive for VACV IgG (Table 1). However, IgM did not convert (IgM also did not convert in ELISA antibody testing on May 23 and June 1). One hypothesis for this result may be that the immune response to VACV was very low and the detected IgG may have been largely attributable to VIG IV.

Serum (3.5 hrs post treatment) was taken again on May 23 (Treatment Day 5) and June 1 (Treatment Day 14). Results were IgG positive for VACV on both these dates. Lesion swabs from the primary and satellite lesions were taken on May 31 (Treatment Day 13); all were PCR-negative (although results were deemed inconclusive for the satellite lesion swabs, as these were also negative for human DNA), and viral culture-negative after 11 days of incubation, when contamination of the culture was observed (Table 1). As all swab samples, except the first sample taken prior to treatment initiation, proved negative for VACV both by culture and PCR, drug resistance could not be evaluated.

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Table 1: Serum Antibody, Lesion Swab PCR and Viral Culture Results During Treatment

Sampling Dates	Serum IgG and IgM	PCR - Primary Lesion Swab	PCR - Satellite Lesion Swabs	Viral Culture
May 11, 2011 (Visit Day 1)	NA	Positive	NA	NA
May 19, 2011 (Treatment Day 1)	Positive for IgG only (0.55 U)	Negative (serum and EDTA blood)	NA	NA
May 23, 2011 (Treatment Day 5)	Positive for IgG only (0.60 U)	NA	NA	NA
May 31, 2011 (Treatment Day 13)	NA	Negative (pos. for human DNA)	Negative (neg. for human DNA)	Negative, at 11 days
June 1, 2011 (Treatment Day 14)	Positive for IgG only (0.57 U)	NA	NA	NA

Additional blood was taken on Days 1 (pre-treatment), 4 and 14 (both ~3.5 hours post treatment) and shipped (frozen) to SIGA Technologies, Inc. for drug concentration analysis in-house under non-GLP conditions.

Drug concentrations were determined by the LC-MS/MS method. The Day 4 sample was analyzed (single instance) upon arrival on May 23, 2011. The measured ST-246 concentration was 462 ng/mL. The second sample and a pretreatment sample arrived on June 7, 2011. Both samples were analyzed (and the Day 4 sample re-analyzed) in triplicate to confirm reproducibility of the analysis. The pretreatment sample's ST-246 levels were below the lower limit of quantitation (LLOQ), as expected. The Day 4 triplicate analysis resulted in a concentration of 440 ± 12 mg/mL, and the Day14 sample was 657 ± 17 ng/mL (Table 2).

Table 2: Serum ST-246 Concentration (3.5 Hours Post-Treatment) on Days 4 and 14

	ST-246 (ng/mL)	
	Day 4	**Day 14**
Patient Sample Concentration	462	657 ± 17
Repeat Analysis in Triplicate	440 ± 12	NA

A previous clinical study (SIGA-246-004; oral dose of 400 mg ST-246 administered daily for 14 days) indicated T_{max} occurred at approximately 4 hours post dose, but the range was from 2-6 hours, indicating that a single sample would not necessarily represent the C_{max}. In this emergency use case, only two single serum samples were obtained from the patient at 3.5 hours post treatment; one on Day 4 and one on Day 14. Although below the mean, the Day 4 and Day 14 samples were both within the range expected for that time-point, based on examination of the 2 and 4 hour concentrations, as well as the C_{max} concentrations, from the SIGA-246-004 study (Tables 3 and 4). Thus, the ST-246 exposure of the patient during the 14 day treatment was as expected and well within the range observed in previous studies of healthy volunteers.

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Table 3: Study SIGA-246-004 – Plasma ST-246 Concentrations on Days 1 and 14 (2 and 4 Hours Post Treatment)

	ST-246 (ng/mL)			
	Day 1		Day 14	
	2 Hr	4 Hr	2 Hr	4 Hr
Mean	777	1095	899	1228
Min	0	413	71.4	398
Max	1710	2560	2420	2390

Table 4: Study SIGA-246-004 - C_{max} Levels Post Treatment

	C_{max} ST-246 (ng/mL)		
Day	**Mean**	**Min**	**Max**
1	1095	413	2560
14	1228	398	2340

Throughout the treatment and post treatment period, photographs of the lesions were taken by the patient using her cell-phone (Attachment A).

The patient responded well to treatment, resuming normal activities within 5 days of treatment initiation. By this time, fever, malaise, lymph node enlargement and pain at the lesion sites were no longer present. By completion of treatment, lesions were healing well with protuberant type discoid scarring. There were no apparent side-effects of ST-246 throughout treatment.

The patient was advised not to go out into the community during treatment. Post treatment, she was informed that she could resume her part-time job provided strict hygiene measures (e.g. hand washing) were in place, and then after 14 days post treatment, she could resume all normal activities and could interview for a healthcare job. As of July 29, 2011, the patient is doing well and lesions have completely healed with minimal scarring.

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ATTACHMENT A:
PHOTOGRAPHS OF VACCINIA LESIONS

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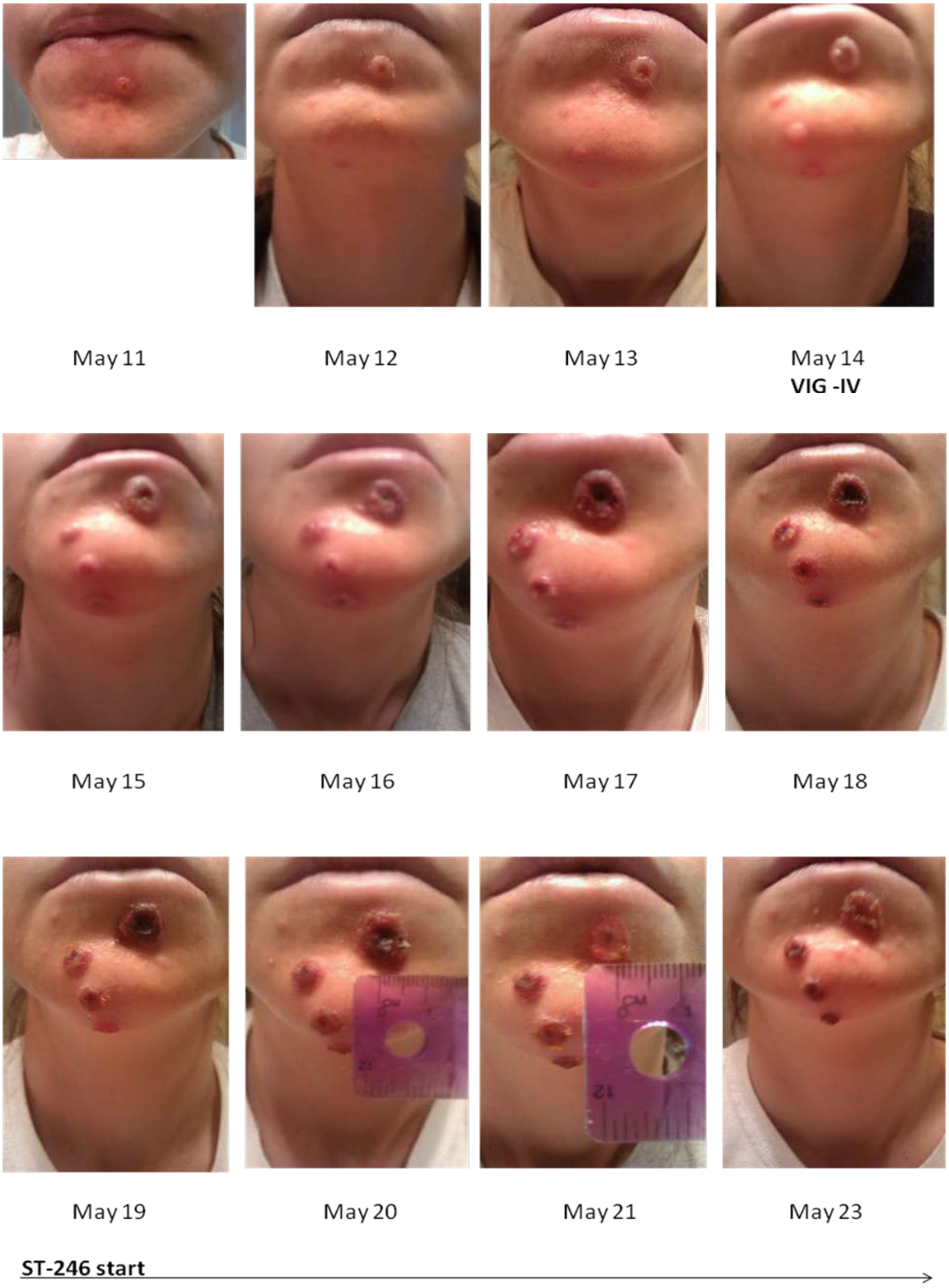
PHOTOGRAPHS OF VACCINIA LESIONS



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PHOTOGRAPHS OF VACCINIA LESIONS (continued)

May 24 May 25 May 31 June 08

ST-246 end

June 17 August 10

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APPENDIX D

MMWR REPORT ON EMERGENCY USE OF ST-246, NOVEMBER 2009

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MMWR Weekly
November 6, 2009 / 58(43);1204-1207

Human Vaccinia Infection After Contact with a Raccoon Rabies Vaccine Bait --- Pennsylvania, 2009

Since 2003, the U.S. Department of Agriculture's Wildlife Services has coordinated a multistate oral rabies vaccination (ORV) program for wildlife in a 15-state zone extending from Maine to Alabama and in Texas. The program seeks to enhance local control and prevent the spread of epizootic rabies among raccoons and, in Texas, among gray foxes and coyotes. The program uses baits containing liquid vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine. Because contact with ruptured baits can produce vaccinia virus infection in certain persons, surveillance for human and domestic animal contact with the baits is conducted, relying largely on reports from persons who find baits and call telephone numbers printed on them. In August 2009, during the autumn baiting campaign in western Pennsylvania, a woman aged 35 years who was taking immunosuppressive medication for inflammatory bowel disease contacted the Pennsylvania Department of Health (PADOH) after handling a ruptured bait, which had leaked liquid rabies vaccine onto a patch of abraded skin on her right hand. The patient subsequently developed vaccinia virus infection and was treated with human vaccinia immune globulin intravenous (VIGIV) and an investigational antiviral agent. This report describes this case, which was the second case of human vaccinia infection related to the ORV program. Public health agencies should educate the public, and particularly pet owners, regarding potential hazards associated with handling wildlife rabies vaccine baits and should provide guidance for persons exposed to this vaccine.

The ORV program uses an orally delivered animal vaccine, Raboral V-RG (Merial, Inc., Athens, Georgia) (*1*). This vaccine consists of a partially attenuated recombinant vaccinia virus (Copenhagen strain) with a gene encoding for rabies virus glycoprotein (*2*). Persons with immunosuppressive conditions or exfoliative dermatologic conditions are at greater risk for complications (e.g., progressive vaccinia or eczema vaccinatum) when exposed to the vaccinia virus contained in Raboral V-RG vaccine. Currently, 15 states distribute ORV baits for raccoons, and Texas distributes baits for gray foxes and coyotes. The V-RG vaccine is delivered via a bait made from a fishmeal block (or a dog food block for coyotes) encasing a sealed plastic packet containing approximately 2 mL of vaccine (Figure 1) (*1*). During August-September 2009, ORV bait distribution was conducted in nonresidential areas by airplane and in rural communities by hand, in parts of western Pennsylvania, Ohio, and West Virginia.

Case Report

In early August 2009, a woman aged 35 years was picking blackberries in a rural area of Pennsylvania where ORV baits recently had been distributed. Her dog picked up a bait in his mouth and punctured the bait with his teeth. After the dog dropped the bait, the woman picked it up, and V-RG vaccine dripped from the bait onto her right hand and wrist, including sites that had been abraded by blackberry thorns. Approximately 30 minutes passed before the woman was able to wash her hands. She telephoned the ORV helpline number printed on the bait, and her call was referred to PADOH. The woman had a history of inflammatory bowel disease, for which she was taking multiple immunosuppressive medications. These medications included a suppressor of T and B cell proliferation (taken daily) and a tumor necrosis factor-alpha (TNF-alpha) blocker (taken once every 6 weeks, with the last dose on July 13); she had no smallpox vaccination scar and reported no history of smallpox vaccination.

On day 1 after her exposure to the V-RG vaccine, her physician obtained a baseline serum specimen for analysis of rabies virus neutralizing antibody (by direct virus neutralization) and orthopoxirus (vaccinia) immunoglobulin IgG

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and immunoglobulin IgM antibodies (by enzyme-linked immunosorbent assay). In addition, photos were taken of her right hand abrasions, and she was instructed to watch for signs of infection in her hand. On day 4, she reported several red papules on her right hand. She visited a local hospital emergency department, where scrapings of the papules were obtained and submitted to the PADOH Bureau of Laboratories. The woman was discharged from the emergency department and advised to stop taking her immunosuppressant medications.

On day 5, a real-time polymerase chain reaction (PCR) assay of the scrapings tested positive for nonvariola *Orthopoxvirus* DNA. Subsequent testing performed at CDC confirmed the presence of vaccinia virus DNA and rabies virus G protein DNA in papule material and serologic evidence of rabies virus neutralizing antibodies. Neither *Orthopoxvirus* IgG nor IgM antibodies were detected.

On day 6, the papules had increased in number and size, and the patient was hospitalized. Physical examination on admission showed multiple papules on erythematous bases on the patient's right hand, wrist, and arm, including three that appeared to be early vesicles. Because of the patient's immune suppression and concerns about progressive vaccinia, she was administered a single dose (6,000 IU/kg) of VIGIV (Cangene Corporation, Winnipeg, Canada) provided by CDC. Contact precautions were utilized while the patient was hospitalized.

On day 9, she had 26 classic vaccinia virus lesions, including one on a site on her right arm that might not have been in contact with the vaccine initially. The patient was afebrile and not experiencing symptoms related to her inflammatory bowel disease. However, on day 11, she experienced myalgia and headache; she had pronounced redness and edema in her right hand (Figure 2), accompanied by right axillary adenopathy. The patient remained afebrile, but was taking an antipyretic pain medication. At that time, her physician decided that to prevent exacerbation of her inflammatory bowel disease the patient soon would need to restart her immunosuppressive medications. On day 12, in an attempt to reduce viral replication and prevent progressive vaccinia, a second 6,000 IU/kg dose of VIGIV was administered along with the investigational antiviral agent, ST-246 (SIGA Technologies, Corvalis, Oregon). The ST-246 was administered orally for 14 days after a compassionate use authorization was approved by the Food and Drug Administration.

On day 13, the patient began receiving phased reintroduction of her immunosuppressive medications. She was discharged on day 19. By day 28, all scabs from her lesions had separated and her underlying inflammatory bowel disease condition was stable. The patient remained *Orthopoxvirus* IgM negative throughout her illness.

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Editorial Note:

The patient described in this report is only the second person documented with human vaccinia infection associated with the ORV baiting program, and the first infected person taking immunosuppressive medications. The one previous documented human vaccinia infection occurred in September 2000 in a pregnant woman aged 28 years with eczema who was bitten while pulling a ruptured bait from her dog's mouth (*2*). The patient in the current report was of particular concern for three reasons. First, she was immunosuppressed from medications to treat her inflammatory bowel disorder. Second, she had fresh abrasions and prolonged contact with V-RG vaccine (approximately 30 minutes) before washing her hands. Third, she did not exhibit features of a normal immune response for a person previously naive for *Orthopoxvirus* infection (i.e., an IgM antibody response was not detected.) Careful monitoring and prompt diagnostic evaluation allowed for timely medical interventions, and the vaccinia virus infection resolved. Although her treatment with 2 doses of VIGIV and ST-246 might have contributed to her recovery, the role of these agents is difficult to assess.

Since 1990, approximately 100 million doses of Raboral V-RG animal vaccine have been distributed in the United States by depositing baits by airplane or by hand in areas with dense populations of target wildlife species (e.g., raccoons in the eastern United States). Human or domestic animal contact with ORV baits has been reported rarely.

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In 2008, among 16 states where 10,339,969 baits were placed, 291 vaccine-related telephone calls involving human or domestic animal contact (2.8 per 100,000 baits) were reported by public health authorities (*3*).

The ORV packets containing the V-RG vaccine are durable and typically do not leak unless punctured. Both documented instances of human vaccinia virus infections caused by V-RG occurred after baits were found and punctured by dogs. Humans interacting with domestic animals (typically dogs, but occasionally cats) that find and eat baits are one of the most common sources of potential vaccine exposures (*4*). The Pennsylvania case described in this report underscores the importance of continuing to alert the public, particularly pet owners, during baiting season to the possibility of pets finding and ingesting baits, or carrying them into the home. Raboral V-RG has been tested for safety in dogs. Owners should not attempt to remove baits from a dog's mouth. Gloves or plastic bags should be used to pick up and examine baits if necessary and additional baits that might be picked up by dogs or other pets should be removed from the immediate area. Interviews with all persons who contact the ORV program helpline printed on the baits can enable early identification of persons at high risk for vaccinia virus infection. All callers should be asked about the nature of their contact with the bait and should be counseled to report any skin lesions. Persons with eye exposure should irrigate the eye with clean water for 15 minutes and should be referred promptly for an ophthalmologic examination. In the event of a documented infection, care should be taken to avoid transmission of the virus to household and other contacts of the patient (5).

Persons with a history of atopic dermatitis or other active exfoliative skin conditions and those with immune deficiencies or immunosuppressive conditions are at greater risk for adverse outcomes (e.g., eczema vaccinatum and progressive vaccinia) after infections with nonhighly attenuated variants of vaccinia virus (6), including Raboral V-RG (*7*). Health-care practitioners should collect specimens promptly from the earliest signs of rash and small papules and submit them to an approved laboratory for testing for vaccinia virus by real-time PCR. Most state health department laboratories and others participating in the Laboratory Response Network are equipped to test clinical specimens for the presence of nonvariola *Orthopoxvirus* DNA.

References

1. US Department of Agriculture Animal and Plant Health Inspection Service. Wildlife Services: National Rabies Management Program. Oral rabies vaccination. Washington, DC: USDA; 2009. Available at http://www.aphis.usda.gov/ws/rabies/vaccine.html. Accessed October 30, 2009.
2. Rupprecht CE, Blass L, Smith K, et al. Human infection due to recombinant vaccinia-rabies glycoprotein virus. N Engl J Med 2001;345:582--6.
3. Roess AA, Rea N, Dato V, Rupprecht C; ORV Surveillance Network. National surveillance for human and pet contact with oral rabies vaccine (ORV) baits, 2008. Presented at: XX International Conference on Rabies in the Americas, Quebec City, Canada, October 17--23, 2009.
4. McGuill MW, Kreindel SM, DeMaria A, et al. Human contact with bait containing vaccine for control of rabies in wildlife. J Am Vet Med Assoc 1998;213:1413--7.
5. Sepkowitz KA. How contagious is vaccinia? N Eng J Med 2003;348:439--46.
6. CDC. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2001;50(No. RR-10).
7. Hanlon CA, Niezgoda M, Shankar V, Niu HS, Koprowski H, Rupprecht CE. A recombinant vaccinia-rabies virus in the immunocompromised host: oral innocuity, progressive parenteral infection, and therapeutics. Vaccine 1997;15:140--8.

What is already known on this topic?

Cases of human vaccinia virus infection after contact with oral rabies vaccination (ORV) program baits are rare, but pose serious risks to persons with a history of eczema, immune deficiencies, or immunosuppressive conditions.

What is added by this report?

This report describes the second documented case in 9 years of human vaccinia virus infection related to a multistate ORV program and reinforces the need to educate the public, particularly pet owners, regarding potential hazards associated with handling wildlife rabies vaccine baits.

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What are the implications for public health practice?

Public health agencies should educate persons to call the telephone number printed on any ORV bait they touch, and health-care practitioners should test such persons for vaccinia promptly if they develop signs of the disease.

FIGURE 1. Rabies vaccine delivered to raccoons via a bait consisting of a fishmeal block (left) encasing a sealed plastic packet containing the vaccine (right) --- Pennsylvania, 2009

Photo/U.S. Department of Agriculture Wildlife Services

Alternative Text: The figure above is a photograph of a gloved hand holding a raccoon rabies vaccine bait, which consists of a fishmeal block and the sealed plastic packet that is encased inside the block and contains the vaccine.

FIGURE 2. Female patient aged 35 years exhibits vaccinia virus lesions and pronounced redness and edema on her right hand on day 11 after contact with an animal rabies vaccine bait --- Pennsylvania, 2009

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Photo/C. Encarnacion, MD

Alternative Text: The figure above is a photograph showing the right hand of a woman aged 35 years with vaccinia virus lesions and pronounced redness and edema on day 11 after contact with a raccoon rabies vaccine bait in Pennsylvania in 2009.

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APPENDIX E

MMWR REPORT ON EMERGENCY USE OF ST-246, MAY 2009

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Progressive Vaccinia in a Military Smallpox Vaccinee --- United States, 2009

On May 19, this report was posted as an MMWR Early Release *on the* MMWR *website* (http://www.cdc.gov/mmwr).

Progressive vaccinia (PV), previously known as vaccinia necrosum, vaccinia gangrenosum, or disseminated vaccinia, is a rare, often fatal adverse event after vaccination with smallpox vaccine, which is made from live vaccinia virus (*1*). During recent vaccination programs potential cases of PV were investigated, but none met standard case definitions (*2*). PV has not been confirmed to have occurred in the United States since 1987 (*3*). On March 2, 2009, a U.S. Navy Hospital contacted the Poxvirus Program at CDC to report a possible case of PV in a male military smallpox vaccinee. The service member had been newly diagnosed with acute mylegenous leukemia M0 (AML M0). During evaluation for a chemotherapy-induced neutropenic fever, he was found to have an expanding and nonhealing painless vaccination site 6.5 weeks after receipt of smallpox vaccine. Clinical and laboratory investigation confirmed that the vaccinee met the Brighton Collaboration and CDC adverse event surveillance guideline case definition for PV (*4*,*5*). This report summarizes the patient's protracted clinical course and the military and civilian interagency governmental, academic, and industry public health contributions to his complex medical management. The quantities of investigational and licensed therapeutics and diagnostics used were greater than anticipated based on existing smallpox preparedness plans. To support future public health needs adequately, the estimated national supply of therapeutics and diagnostic resources required to care for smallpox vaccine adverse events should be reevaluated.

Case Description

On January 13, 2009, a healthy service member aged 20 years received a primary smallpox vaccination (ACAM2000 [Acambis, Inc., Cambridge, Massachusetts]) in accordance with the U.S. Department of Defense smallpox vaccination policy*; no other vaccinations were administered that day. Twelve days later, the patient visited a local hospital with fever and headache of 1 day's duration and was admitted for workup of leukopenia after his white blood cell count was found to be 1,400 cells/mm3. On January 28, after transfer to a U.S. Navy tertiary-care facility, he was diagnosed with AML M0. On January 30 and February 13, the patient underwent two successive rounds of induction chemotherapy with cytarabine, idarubicin, and dexamethasone. Before initial chemotherapy, the vaccination site pustule had a central crust and measured approximately 1 cm in diameter with minimal surrounding erythema. During the patient's hospital stay from the end of January to the beginning of March, his vaccination site dressing was changed daily.

On March 2, during the evaluation of neutropenic fever, the failure of the patient's vaccination site to heal was described. An annular lesion with a deep bulla, raised violaceous leading edge, and a central crust that bled with pressure was noted. The size of the lesion had progressed to approximately 4 x 4 cm with minimal surrounding erythema or induration (Figure). The patient described no pain at the site, although he reported occasional pruritus. A swab of the lesion and serum were sent to CDC for viral and serologic analysis. Viral analysis of the swab by multiple real-time polymerase chain reaction (PCR) assays for orthopoxvirus and vaccinia yielded evidence of viral DNA; viral culture was positive for orthopoxvirus. Serum showed equivocal to absent levels of anti-orthopoxvirus immunoglobulin G (IgG) and immunoglobulin M (IgM) by enzyme-linked immunosorbent assay. The results of the

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diagnostic testing combined with the patient's medical history met the PV level 1 case definition as defined by the Brighton Collaboration and the confirmed case definition as described by CDC surveillance guidelines (*4*,5). The criteria met by both case definitions were 1) a documented clinical diagnosis of a disease that is known to be associated with cell-mediated immunodeficiency (in this case AML M0), 2) the primary vaccination site's failure to resolve (in this case >6 weeks post vaccination), and 3) the laboratory confirmation of vaccinia virus as the causative agent.

On March 3, imiquimod was applied directly to the lesion. Within 24 hours of confirmation of PV on March 4, the patient received licensed Vaccinia Immune Globulin Intravenous (Human) (VIGIV) (Cangene Corporation, Winnipeg, Canada). On March 5 and March 6, oral and topical ST-246 (SIGA Technologies, Corvallis, Oregon) were administered under an Emergency Investigational New Drug (E-IND) application. The patient remained stable until the evening of March 7, when he became septic with *Pseudomonas aeruginosa*, likely from a perirectal abscess. He required intubation, maximal vasopressor support, multiple antibiotics, and stress dose corticosteroids. He then developed multiorgan failure and began continuous venovenous hemodialysis. During the next 12 days, the patient slowly stabilized. As a consequence of the duration and amount of vasopressor support, the patient required a bilateral trans-tibial amputation because of dry gangrene of his feet.

During March 6--19, the patient received additional oral and topical ST-246 and VIGIV; his ST-246 levels were noted to be lower than those achieved both in healthy subjects in phase I clinical trials and in successful treatment of nonhuman primates with systemic orthopoxvirus disease. The lesion size remained unchanged, but the central crust of the vaccination site sloughed off, followed by most of the outer "ring" flattening, leaving a shallow ulcer with healthy-appearing granulation tissue. During his steroid taper, additional satellite lesions surrounding the vaccination site appeared on March 18, and viral DNA was detected again in the blood. These lesions became vesicular in nature, and on March 26, after a second E-IND was issued, CMX001 (Chimerix, Inc., Research Triangle Park, North Carolina), a lipid conjugate of cidofovir, was administered.

From March 24 onward, the satellite and main vaccination site lesions continued to crust, the scabs separated, and underlying tissue epithelialized (Figure). Blood viral DNA levels cleared on March 29. On April 10, the borders of lesions again appeared raised; a shave biopsy grew methicillin-resistant *Staphylococcus aureus*, which responded to antibiotic therapy. The patient received intermittent granulocyte colony-stimulating factor, and his absolute neutrophil and lymphocyte count increased over time. By May 1, significant portions of the scabs/eschars had fallen off or were removed manually, revealing healthy epidermis. Numerous therapeutics with different biologic mechanisms were used to treat PV in this patient (Table).

From February 21 onward, the patient had remained in contact isolation, first for a *Clostridium difficile* infection and then for his progressive vaccinia infection. On May 5, contact precautions were discontinued because of the lack of viable virus in lesion specimens from the previous 4 weeks. No cases of contact vaccinia were identified among this patient's health-care workers or close contacts.

During March 3--May 18, nearly 200 clinical specimens (lesion and satellite swabs/crusts, ethylenediaminetetraacetic acid [EDTA] blood, bone marrow, and serum) were collected and submitted to CDC to evaluate disease progression and guide therapeutic interventions. After April 23, swabs from satellite lesions or the main vaccination site showed significantly reduced or absent levels of viral DNA, and no viable virus was detected after April 2. Oropharyngeal sampling and bone marrow biopsies from early and late March, respectively, were negative for vaccinia virus. Orthopoxvirus DNA was detected in EDTA blood at intermittent times during the course of the patient's infection; however, no viable virus was cultured from blood. As of May 12, the patient had no demonstrable IgM response to orthopoxvirus; IgG levels appeared fully reliant on VIGIV infusion.

During March 3--May 18, a total of 20 conference calls to discuss patient status and treatment options were held between the Vaccine Healthcare Centers Network, Military Vaccine Agency (MILVAX), Bureau of Medicine and Surgery of the Navy, CDC, Food and Drug Administration (FDA), National Institutes of Health (NIH), SIGA Technologies, Chimerix, Inc., and academic and health-care professionals. As of May 18, MILVAX provided 22 and the Strategic National Stockpile (SNS) provided 254 vials of VIGIV used in treatment of this case.

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Editorial Note:

Although PV is a rare adverse event (one case per million during routine vaccination during 1963--1968), its case fatality rate in primary U.S. vaccinees was 15% despite treatment with massive amounts of VIG (intramuscular) (*6*). Extensive surgical debridement was sometimes required, even necessitating disarticulation of the arm to "debulk" the amount of infectious material (*7*). Before smallpox vaccination, patients are screened for numerous contraindications (*8*). At the time of his vaccination, the patient described in this report did not have any obvious signs or symptoms that would meet any exclusion criteria for vaccination. Training in use of, and careful adherence to, screening tools can identify vaccine candidates at risk for PV and other adverse events (*2*). Despite this, vaccinees with occult immunodeficiencies might not be recognized, and therefore appropriately deferring vaccination in these persons is not always possible.

Lack of inflammation at the expanding vaccination site is the hallmark of PV. Any smallpox vaccinee who has an expanding, nonhealing, painless vaccination site without inflammation for more than 2 weeks should be evaluated for an underlying immunodeficiency, and diagnosis of and treatment for PV should be considered. Health-care providers should report suspected cases of PV or other adverse events to the Vaccine Adverse Event Reporting System (VAERS).† Suspected cases of PV also should be reported to state health officials and CDC for clinical consultation and to obtain select therapeutics available only through the SNS. State health departments should call the CDC Emergency Operations Center at 770-488-7100.

This patient's protracted clinical course is consistent with previously published cases reports and surveillance summaries. The development of progressive vaccinia, historically observed in patients with cellular immunodeficiencies, often leads to superinfection and subsequent sepsis (i.e., fungal, parasitic, and bacterial infections resulting in toxic or septicemic shock, then ultimately death). Past treatment typically included massive doses of VIG, administration of thiosemicarbazone, blood products, and supportive care for accompanying infections (*7,9*). The improvement of progressive vaccinia in this patient was associated with receipt of VIGIV (the only licensed product for treatment of vaccinia adverse events stockpiled by the SNS), ST-246, and CMX001, and an increase in lymphocyte count. The use of two antiviral agents with different mechanisms of action§ was enabled by the research and development of medical countermeasures for smallpox preparedness activities, as well as the use of the emergency IND process. As of May 18, the patient had shed nearly all of the scab material on and around the vaccination site.

The rapid mobilization of military, CDC, FDA, NIH, drug manufacturer, and academic and health-care human resources to review the case's status and to provide daily, then biweekly laboratory findings that guided treatment recommendations, was enabled by smallpox public health preparedness research and training efforts. Future cases of PV likely will require similar intensive and multidisciplinary clinical consultation. Experts with background in vaccine safety, PV treatment, clinical virology, infectious disease, and immunodeficiencies should be engaged.

Continuing medical education and reinforcement of training related to the prevention, early recognition, and treatment of smallpox vaccine--related adverse events should be part of smallpox vaccination programs.¶ The patient described in this report received VIGIV in the amount originally estimated to treat 30 persons. The extraordinary amounts of VIGIV used to treat this single case of PV underscore the need to reevaluate the adequacy of the national stockpiled supply of this or other medical countermeasures (treatment or prophylactic). Such reevaluation, with additional focus on immunocompromised hosts, will aid in the smallpox vaccination program planning and overall smallpox preparedness efforts.

Acknowledgments

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Center for Drug Evaluation and Research, Food and Drug Admin; Military Vaccine Agency; Walter Reed Vaccine Healthcare Center; Bur of Medicine and Surgery of the Navy; Office of the Chief of Naval Operations; Chemical Biological Medical Systems of the US Dept of Defense; and the Emergency Operations Center and Strategic National Stockpile, CDC.

References

1. CDC. Recommendations for using smallpox vaccine in a pre-event vaccination program. Supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR 2003;52(No. RR-7).
2. Vellozzi C, Lane JM, Averhoff F, et al. Generalized vaccinia, progressive vaccinia, and eczema vaccinatum are rare following smallpox (vaccinia) vaccination: United States surveillance, 2003. Clin Infect Dis 2005;41:689--97.
3. Redfield RR, Wright DC, James WD, Jones TS, Brown C, Burke DS. Disseminated vaccinia in a military recruit with human immunodeficiency virus (HIV) disease. N Engl J Med 1987;316:673--6.
4. Nell P, Kohl KS, Graham PL, et al. Progressive vaccinia as an adverse event following exposure to vaccinia virus: case definition and guidelines of data collection, analysis, and presentation of immunization safety data. Vaccine 2007;25:5735--44.
5. CDC. Surveillance guidelines for smallpox vaccine (vaccinia) adverse reactions. MMWR 2006;55:(No. RR-1).
6. Aragón TJ, Ulrich S, Fernyak S, Rutherford GW. Risks of serious complications and death from smallpox vaccination: a systematic review of the United States experience, 1963--1968. BMC Public Health 2003;3:26.
7. Maurer DM, Harrington B, Lane JM. Smallpox vaccine: contraindications, administration, and adverse reactions. Am Fam Physician 2003;68:889--96.
8. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. Clin Infect Dis 2003;37:251--71.
9. Bray M, Wright ME. Progressive vaccinia. Clin Infect Dis 2003;36:766--74.
10. Quenelle, DC, Prichard MN, Keith KA, et al. Synergistic efficacy of the combination of ST-246 with CMX001 against orthopoxviruses. Antimicrob Agents Chemother 2007;51:4118--24.

* Information about U.S. Department of Defense policies regarding smallpox vaccination and screening before smallpox vaccination is available at http://www.smallpox.army.mil.

† Information about VAERS is available at http://vaers.hhs.gov.

§ ST-246 prevents viral egress, whereas CMX001 inhibits viral replication, and some data suggest they are synergistic in vitro (*10*).

¶ CDC's clinical evaluation tools for smallpox vaccine adverse reactions are available at http://emergency.cdc.gov/agent/smallpox/vaccination/clineval.

FIGURE. Photographs of a progressive vaccinia lesion in a military smallpox vaccinee, taken on A) March 5,* B) March 27,† and C) April 27, 2009§¶

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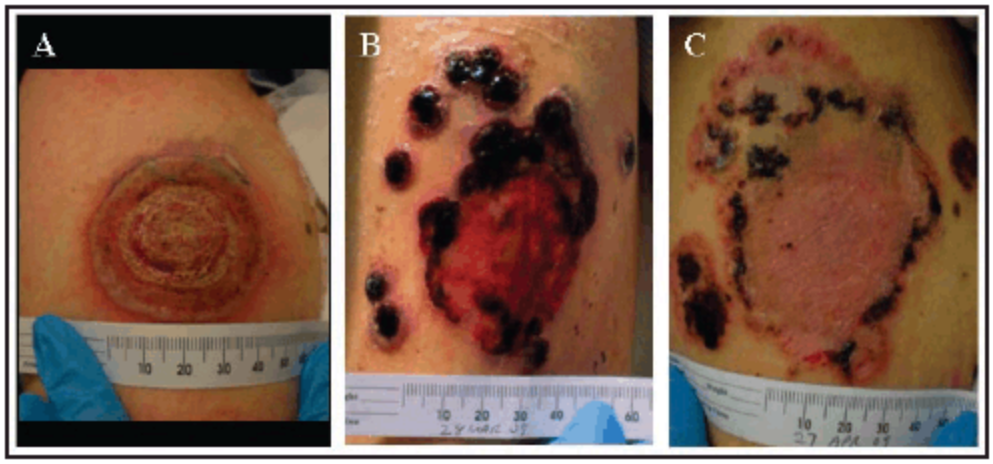

Photo/U.S. Navy

* 51 days post vaccination.

† 73 days post vaccination and 9 days post development of satellite lesions.

§ 104 days post vaccination and 40 days post development of satellite lesions.

¶ Images have been altered to remove features that might identify the patient.

Alternative Text: The figure above shows three photographic images of a progressive vaccinia lesion in a military smallpox vaccinee. The photos were taken on March 5, March 27, and April 27, 2009, respectively.

TABLE. Administration dates and dosages of therapeutics used in treatment of progressive vaccinia in a military smallpox vaccinee --- United States, March 5--May 18, 2009

Treatment*	Formulation	Dosage	Application	Administration dates
ST-246	Oral	400 mg	Once daily	March 5--19
		800 mg	Once daily	March 20--24
		1200 mg	Once daily	March 25 to present†
ST-246	Topical	1%, 0.5 mL	Once daily	March 6; April 21--May 12
		1%, 0.5 mL	Twice daily	March 7--April 20
CMX001	Oral	200 mg	Once per date	March 26
		100 mg	Once per date	April 1, 7, 13, 20, 27
Imiquimod	Topical	5%, 12.5 mg	Once daily	March 24--May 12
VIGIV§¶**	Intravenous	6,000 U/kg	Once per date	March 4, 11, 20; April 1, 3, 6, 8, 18
		18,000 U/kg	Once per date	April 9
		24,000 U/kg	Once per date	March 24; April 14, 23, 28; May 8

* In summary, the patient's dose of oral ST-246 was increased twice to obtain more optimal drug levels, CMX001 was begun, topical ST-246 and imiquimod continued, as well as periodic infusions of VIGIV at varying doses.

† As of May 18, 2009.

§ Vaccinia Immune Globulin Intravenous (Human).

¶ VIGIV is supplied as a 15 mL single dose vial containing >50,000 U/vial.

** Patient received a total of 16,740,000 U of VIGIV during March 4--May 8.

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APPENDIX F

MMWR REPORT ON EMERGENCY USE OF ST-246, MAY 2007

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MMWR Weekly
May 18, 2007 / 56(19);478-481

Household Transmission of Vaccinia Virus from Contact with a Military Smallpox Vaccinee --- Illinois and Indiana, 2007

On March 7, 2007, the Chicago Department of Public Health and the University of Chicago Pediatric Infectious Disease Service and Infection Control Program notified CDC of a child with presumed eczema vaccinatum (EV), a life-threatening complication of vaccinia virus infection (*1*). This is the first reported EV case in the United States since 1988 (*2*). This report summarizes the epidemiologic and environmental investigations conducted by local, state, and federal public health authorities in Illinois and Indiana to determine the source of exposure and to identify and monitor other persons at risk for vaccinia virus infection. This case highlights the need for clinicians to maintain a high index of suspicion when evaluating recently vaccinated patients and their family members with vesiculopustular rash.

On January 26, 2007, an active-duty U.S. service member received a first-time smallpox vaccination in preparation for overseas military deployment. He had a history of childhood atopic dermatitis (i.e., eczema) and household contact with persons with eczema (two of his three children), both of which are contraindications to vaccination. His deployment was delayed, so he made an unplanned visit home to visit his family in Indiana during February 16--20. During this period, he spent time with his son, aged 28 months, who has severe eczema and a history of failure to thrive. The father reported his vaccination site had scabbed over and that the scab had separated before the visit home; he also reported that he kept the site bandaged during the visit. His routine activities with his son included hugging, wrestling, sleeping, and bathing.

On March 3, the child was taken to a small, local Indiana hospital because of a generalized papular, vesicular rash on the face, neck, and upper extremities. Because of the severity of the illness, he was transferred to a tertiary-care facility in Chicago later that day; contact precautions were implemented at the hospital. The child's mother indicated that the boy had a fever 2 days before his hospital admission and weeping skin lesions as early as February 24. By March 7, the rash had progressed to umbilicated lesions with an erythematous base, primarily involving the child's hands, forearms, neck, chest, face, and knees and encompassing 50% of his keratinized skin (Figure). On March 8, lesion specimens were analyzed at the Illinois Department of Public Health Laboratory (IDPHL) in Chicago by real-time polymerase chain reaction (PCR) orthopoxvirus generic assay and nonvariola orthopoxvirus assay. The results of the assays were positive for orthopoxvirus DNA, supporting the clinical diagnosis of EV. The diagnosis of vaccinia was confirmed at CDC.

During March 8--28, the child was treated with a combination of immunotherapy and antivirals targeting vaccinia virus. The initial treatment included Vaccinia Immune Globulin Intravenous (Human) (VIGIV); supportive care included sedation, intubation, and mechanical ventilation. Despite these interventions, on March 10, the child's illness had progressed to hypothermia and hemodynamic instability requiring vasopressor support. Antiviral therapies with cidofovir and an investigational drug, ST-246 (SIGA Technologies, Corvallis, Oregon) under an Emergency Investigational Drug application, were initiated sequentially,* and additional infusions of VIGIV were administered. After approximately 1 week of interventions, the child began to improve. On April 19, the child was discharged home after 48 days of hospitalization; he has no known sequelae other than possible scarring of the skin.

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Clinical specimens (e.g., lesion material, blood, and serum) collected during the patient's hospitalization were analyzed in the CDC Poxvirus Laboratory. All specimens collected during the first 10 days of his hospitalization were positive for orthopoxvirus DNA using a real-time PCR assay. Before VIGIV administration, serum was positive for antiorthopoxvirus immunoglobulin M (IgM) and negative for immunoglobulin G (IgG) by enzyme-linked immunosorbent assay.

On March 6, the child's third hospital day, hospital staff members noticed that the patient's mother had approximately six vesicular lesions on her face; additional lesions subsequently developed on her right index finger and near her eyelid. The mother had a history of facial acne flare-ups and reported that she had rested her cheek on the child's abdomen while he was being treated in the hospital. Lesion material was analyzed by IDPHL and found to contain orthopoxvirus DNA signatures. The mother was isolated voluntarily in the same room as her son; on March 10, she received VIGIV treatment. Within 72 hours of the initiation of VIGIV treatment, her lesions began to scab over. Evaluation of serum collected from the mother on March 8 indicated that she had not yet developed an antiorthopoxvirus humoral immune response (IgG and IgM negative).

The couple has two other children, one with a history of eczema. Both children left the family residence at the time of the child's hospitalization and were cared for by their grandparents. Neither child had symptoms of vaccinia infection at the time of this report.

Public health and infection-control professionals interviewed community contacts, family members, and hospital staff members to identify persons who might have had physical contact (i.e., skin-to-skin) with the ill child after February 23 (the day before the child's first possible skin eruption) or the father while he was home on leave during February 16--20. Twenty-three family contacts, including the two siblings, and 73 health-care worker contacts were identified. Persons were monitored daily for the onset of contact vaccinia symptoms for 21 days after their last potential vaccinia exposure. During this period, one person had a rash, and one had fever; neither person had vaccinia virus infection. All other potential contacts remained healthy throughout the follow-up period; no nosocomial transmission occurred. Hospital and public health officials recommended that the mother and child remain isolated until they had no more vaccinia scabs.

Because the child had a rash before being hospitalized, an environmental assessment of the family home was conducted on March 13 to determine whether viable vaccinia virus was still present. Multiple swab samples obtained from the home (e.g., from a bathroom washcloth, a slipper, a toy drum, a night stand, a booster seat, and an ointment container) and from items brought to the child's hospital room (e.g., an infant drinking cup and a car seat) were positive for vaccinia virus DNA by real-time PCR assay. Cell culture of samples collected from three of these items (booster seat, toy drum, and slipper) contained viable virus. Disinfection procedures were completed on March 23 and included steam cleaning of carpeted areas, disinfection of household surfaces with phenolics, and hot washing of clothing and linens after a phenolic presoak.

Reported by: *J Marcinak, MD, S Vora, MD, S Weber, MD, K Thomson, PhD, S Garcia-Houchins, Univ of Chicago Comer Children's Hospital; S Gerber, Chicago Dept of Public Health; C Conover, MD, J Nawrocki, PhD, K Hunt, Illinois Dept of Public Health. R Panares, MD, B Suter, K Siegfried, Hammond Health Dept; R Teclaw, DVM, C Graves, MD, W Staggs, MS, D Allen, MS, K Buffin, MS, P Pontones, MA, Indiana State Dept of Health. V Fulginiti, MD, Univ of Arizona and Univ of Colorado. L Collins, MD, Walter Reed Vaccine Healthcare Center. D Scott, MD, Center for Biologics Evaluation and Research, P Patel, RPh, K Chan-Tack, MD, J DiGiacinto, PharmD, Div of Antiviral Products, Food and Drug Admin. I Damon, MD, M Reynolds, PhD, R Regnery, PhD, E Belay, MD, K Karem, PhD, V Olson, PhD, Y Li, PhD, S Smith, MS, Z Braden, C Hughes, MPH, Div of Viral and Rickettsial Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases; A Fleischauer, PhD, P Diaz, MD, L Rotz, MD, N Pesik, MD, J Barson, DO, W Bower, MD, Div of Bioterrorism Preparedness and Response, National Center for Preparedness, Detection, and Control of Infectious Diseases; J Openshaw, MS, CDC Experience Fellow; R Miramontes, PA-C, E Lederman, MD, EIS officers, CDC.*

Editorial Note:

This report describes the first documented case of EV in the United States since 1988 (*2*). The epidemiologic investigation and clinical history indicated that secondary transmission of vaccinia virus occurred between the father and child. The stage of healing of the father's vaccination site during the exposure period was reported by the father

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and was not clinically confirmed, nor was consistent use of a bandage. Serologic evidence and clinical history further suggests that tertiary transmission might have occurred between the child and mother. In addition, the possibility of transmission by fomites (i.e., contaminated objects such as toys and towels) cannot be excluded; the targeted environmental assessment detected infectious virus more than 1 week after the ill child had left the home.

The World Health Organization declared smallpox eradicated in 1979. However, smallpox vaccination was required for U.S. military personnel until 1990, when it was discontinued. After the September 11, 2001, terrorist attacks and the 2001 anthrax cases, the U.S. government reinstated smallpox vaccination for military personnel and selected health-care workers. The U.S. Department of Defense had vaccinated approximately 1.2 million persons as of March 2007.[†]

The smallpox vaccine contains live vaccinia virus, which confers protection against infection from variola virus, the cause of smallpox. Vaccinia virus can be transmitted from a vaccine recipient to other persons through direct (skin-to-skin) contact via material from the unhealed vaccination site or through indirect contact by means of fomites (*4*--*6*). Vaccinia virus can be cultured from the site of primary vaccination beginning at the time of development of a papule (i.e., 2--5 days after vaccination). Generally, a scab forms at the vaccination site by day 14 and falls off by day 21 (*7*). Until the vaccination scab falls off, a person who has been vaccinated can transmit vaccinia virus to others. Persons who are infected through contact with a person who has received smallpox vaccination are at risk for the same adverse reactions to smallpox vaccination as the vaccine recipient.

EV is a rare but serious reaction to smallpox vaccine. A history of eczema, atopic dermatitis (regardless of disease severity or activity), or Darier's disease is a risk factor for EV, both for vaccine recipients and their close contacts; having household contacts with any of these conditions also is a contraindication Although no data exist to predict the risk for EV among such persons, before 1990, the incidence rate for EV after smallpox vaccination was approximately eight to 80 cases per 1 million vaccinations (*8*). The introduction of intramuscularly administered vaccinia immune globulin treatment was estimated to have reduced EV-associated mortality from 30%--40% to 7% (*9*). Licensed in 2005, VIGIV is the only product available that is approved by the Food and Drug Administration for treating patients with EV (*8*).

Consistent with current Advisory Committee on Immunization Practices guidelines to prevent transmission of vaccinia from vaccinated persons to close personal contacts, persons who have been vaccinated should wear long-sleeved clothing and cover the vaccination site with gauze or a similar semipermeable dressing until the scab separates from the skin independently (i.e., without assistance from the person) (*3*). Vaccinated persons should not share towels or clothing with others and should wash their hands with warm, soapy water or a hand-rub solution containing ≥60% alcohol immediately after they touch their vaccination site or change their vaccination-site bandages (*3*). Contraindications to smallpox vaccination should be considered before the administration of vaccine; these include pregnancy, immune-compromising conditions (e.g., human immunodeficiency virus infection), or a chronic skin disease such as eczema. Having household contacts with any of these conditions also is a contraindication. Agencies whose health-care providers administer smallpox vaccine should periodically assess the effectiveness of vaccine-related education for these providers and for the vaccine recipients.

The administration of smallpox vaccine to this service member and his subsequent contact with his family are under investigation by the U.S. military, which will determine whether screening and education practices need to be modified (*10*). Health-care workers treating patients with EV, generalized vaccinia infection, or progressive vaccinia infection should follow contact precautions until patients' scabs have separated. Clinicians should maintain a high index of suspicion for vaccinia when evaluating vesiculopapular rashes in patients who have been vaccinated recently and in their close contacts. Suspected cases of vaccinia should be reported to state or local health departments and to the Vaccine Adverse Events Reporting System online (http://vaers.hhs.gov) or by telephone (800-822-7967). Laboratories that are part of the Laboratory Response Network (LRN) (http://www.bt.cdc.gov/lrn) have the ability to assess clinical specimens for the presence of orthopoxvirus DNA signatures. Specimens from the LRN can be forwarded to the CDC Poxvirus Laboratory for species confirmation.

References

1. Engler RJ, Kenner J, Leung DY. Smallpox vaccination: risk considerations for patients with atopic dermatitis.

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J Allergy Clin Immunol 2002;110:357--65.
2. Wien TM, Benton FR, Guill MA. Eczema vaccinatum in an 18-month-old male. Mil Med 1988;153:397--9.
3. CDC. Smallpox vaccination and adverse reactions. Guidance for clinicians. MMWR 2003;52(No. RR-4).
4. CDC. Secondary and tertiary transfer of vaccinia virus among U.S. military personnel -- United States and worldwide, 2002_2004. MMWR 2004;53:103--5.
5. Neff JM, Lane JM, Fulginiti VA, Henderson DA. Contact vaccinia -- transmission of vaccinia from smallpox vaccination. JAMA 2002;288:1901--5.
6. Sepkowitz KA. How contagious is vaccinia? N Engl J Med 2003;348: 439--46.
7. CDC. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. MMWR 2001;50(No. RR-10).
8. Wittek R. Vaccinia immune globulin: current policies, preparedness, and product safety and efficacy. Int J Infect Dis 2006;10:193--201.
9. Kempe CH. Studies smallpox and complications of smallpox vaccination. Pediatrics 1960;26:176--89.
10. Grabenstein JD, Winkenwerder W Jr. US military smallpox vaccination program experience. JAMA 2003;289:3278--82.

* Cidofovir is administered as a weekly dose as clinically indicated and reserved as second-line therapy after VIGIV in the treatment of eczema vaccinatum (*3*). ST-246 is a smallpox drug candidate with specific antiorthopoxvirus activity inhibiting virus maturation.

† US Army Center for Health Promotion and Preventive Medicine. Defense manpower data center statistical immunization reporting system; 2007.

Figure

FIGURE. Abdomen and chest of a boy aged 28 months with a rash of umbilicated lesions caused by eczema vaccinatum — United States, 2007

Photo/John Marcinak

Return to top.

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APPENDIX G

UNIVERSITY OF CHICAGO ACCOUNT OF EMERGENCY USE OF ST-246

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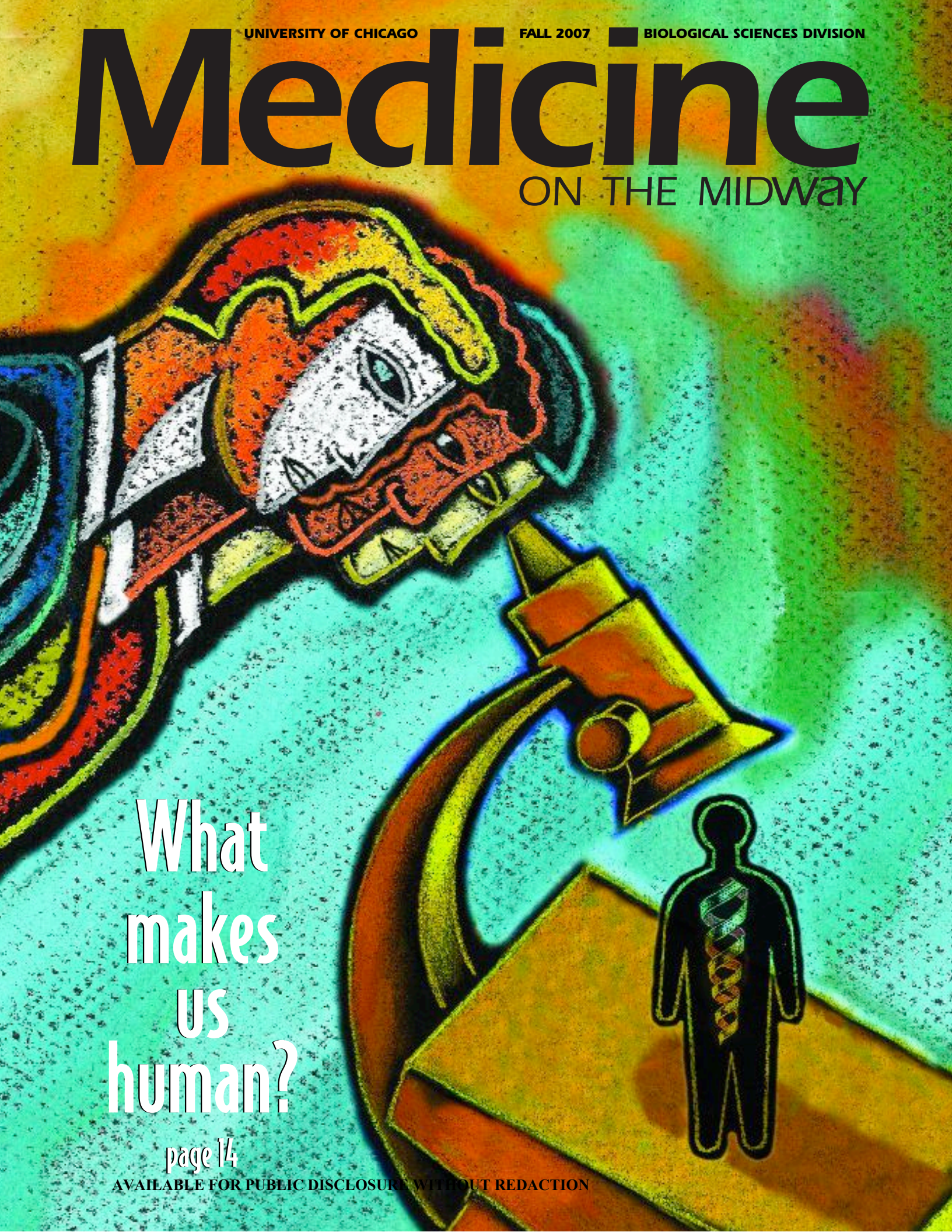
UNIVERSITY OF CHICAGO FALL 2007 BIOLOGICAL SCIENCES DIVISION

Medicine ON THE MIDWAY



What makes us human?

page 14

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CLOSE ENCOUNTER

A disease not seen since the early 1990s brings a 2-year-old boy to within inches of death

by Katie Scarlett Brandt

She felt no fear, Madelyn Kahana said, as she peered down at the tiny boy barely conscious and wrapped in blankets in his pediatric ICU bed. Despite the pustules covering his face, arms, legs and hands—teeming like hundreds of bees on a keeper—Kahana, MD, chief of pediatric critical care, and nurse Jamie Harrison remained focused on their patient rather than the potential catastrophe his infection threatened.

"Right or wrong," she said, "we had no fear."

But they had reason to fear.

This boy would be the University of Chicago Medical Center's first chance to manage what could have turned into a widespread, national medical emergency. His case mobilized the medical community: dozens of physicians, nurses, pharmacists, environmental services personnel, food services and infection control staff, social workers, the Centers for Disease Control and Prevention—and even the Department of Defense.

When Kahana and Harrison first met the boy, he'd been in the hospital for five days, after being transferred from another

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hospital and admitted to the general pediatrics floor with a flare of eczema on Saturday, March 3. He wouldn't leave the medical center for 48 days. Most of that time he spent intubated, drugged and on a ventilator. For a typical case of eczema, this would have been extreme, but the rash consuming the boy's skin was anything but typical.

On his first day in Comer Children's Hospital, the 2-year-old had cuddled up to his mother, then pulled away when doctors and nurses came too close. When pediatric infectious disease specialists John Marcinak, MD, and Surabhi Vora, MD, first checked in on him, the boy was sitting up in his crib, pointing at a cup just out of reach through the crib's bars. "Cup, cup, cup," he chanted. At this point, Marcinak said, the patient had no IV for fluids; it was clear that the virus ravishing the boy's body was already dehydrating him.

That first weekend, hospitalist Barret Fromme, MD, saw the boy. Pediatric dermatologist Sarah Stein, MD, met him on Monday, March 5. She agreed with the working diagnosis of eczema herpeticum and recommended typical antimicrobial and skin care for such cases. Despite such care, his condition worsened—the rash growing in complexity and extent, his awareness levels dropping—as physicians struggled to decipher the cause.

By Wednesday the lesions covering his arms and legs and the skin around his mouth had changed. Juicy pus oozed from them, and they now resembled craters—red blisters with indentations in their centers that resembled some form of pox infection. Stein and Fromme reassessed the situation.

"What are we missing?" they asked each other. Their treatments were failing; the boy clearly was growing sicker, seemingly each time they looked at him. That was when something occurred to Fromme and Stein: Had the boy been in contact with a member of the military? Could he have contracted a virus from someone's small pox vaccination, which is required of people in the service?

A LIVE VIRUS

Why yes, his mother said. The boy's father was in the military, recently deployed to Iraq, and had been vaccinated at the end of January.

Fromme and Stein knew that the chances of the boy having smallpox were slim to none. They also knew that the smallpox vaccination used a live-virus vaccine, and that people with eczema or compromised immune systems—those with HIV, for instance, or who have undergone organ transplants—are especially prone to contracting eczema vaccinatum, a rare smallpox-like infection, if they came into contact with the virus.

The physicians suddenly realized what they were facing. "We were fired up when we made the diagnosis. Now we knew: That's why he's not getting better," Stein said. "But then the reality set in of what this meant for the patient."

Smallpox is a vicious, deadly and extremely contagious virus that by 1980 had been eradicated by universal vaccinations; however, small stockpiles of the virus still exist in government labs, and many physicians fear that it could someday re-emerge as a bio-terrorism agent.

Kahana's first thought when she saw the boy was a drawn out, "Oh-my-God," she said. "It's no wonder so many people died. [The boy's rash] looked exactly like textbook pictures of smallpox." His hands surprised her most. "He had so many lesions, mounds and mounds. The density spread his fingers," she said.

Though the boy's father was supposed to ship out to Iraq soon after the vaccination, his deployment was delayed, and he went home for a brief visit in February to see his wife and three kids. "Last time we saw him was in July of last year," the boy's mother said in an interview with the *Northwest Indiana Times*, the only interview she granted. "Of course we wanted to see him before he left [for Iraq] because you never know what's going to happen."

The father had no intention of exposing his family to his vaccination. He kept that part of his skin—his upper left arm—completely covered and wouldn't even show it to his wife when she asked.

Pediatric infectious disease specialist John Marcinak was a key player in a team of more than 50 health care providers that saved a 2-year-old boy from dying from eczema vaccinatum. *Photo by Dan Dry*

***Opposite page:* the vaccinia virus.** *Photo by Eye of Science/Photo Researchers Inc.*

"This dad had no idea of the risk," Kahana said, citing Page 4 of a Defense Department document about the vaccine that didn't go any further than mentioning a "risk for people with eczema."

Two weeks after the father left, the boy's skin broke out in a rash, and his mother took him to St. Catherine's Hospital in East Chicago, Ind. "The doctor at St. Catherine's said he'd never seen anything like it before. Over three to four days, lesions spread over most of his body," the boy's mother told the newspaper.

From there, the boy and his mother traveled to the University of Chicago, not knowing that they wouldn't return home for almost seven weeks.

NO RELIEF

By the time the *Chicago Tribune* broke the story on March 17, the boy was in critical condition. He was unconscious and quarantined in a special negative pressure room at Comer. Hospital planners included the room for situations exactly like this one. Its design kept the virus contained, allowing hospital air to flow into the room, but not back out. The boy was extremely ill, on the brink of death, and contagious. And because nobody had seen a case of eczema vaccinatum since at least 1990, they took every precaution.

Once it occurred to the doctors that the boy could have eczema vaccinatum, caused by the vaccinia virus, they sent both his and his mother's tissue samples to the Illinois Department of Public Health and lesion photographs to the Department of Defense and the CDC. Both the IDPH and CDC confirmed the vaccinia virus infection.

The inner right leg (top), and abdomen and chest of the 2-year-old boy with a rash of dimpled lesions caused by eczema vaccinatum– a rare severe adverse reaction caused by exposure to the vaccinia virus. *Photos courtesy of John Marcinak*

Each day it seemed like there was more precautions to comply with before entering the boy's room. Stein said she wasn't scared, but slightly perplexed. First she wore only gloves; then gloves and a mask; then gloves, a mask and a gown. "Each time it was like, 'But what about yesterday? We didn't have to do that yesterday,'" she said.

Before long, physicians discovered that the boy's mother also was suffering from the vaccinia infection, though a considerably milder form. She'd contracted it through close contact with her son. A dozen lesions surrounded her mouth. "It started like a headache, flu-like symptoms as if I was coming down with a real bad cold I might get over in a few days…but I didn't expect this in a million years," she said.

The mother was confined to her son's hospital room, unable to see her other children. "She had to be there the whole time because she was infected too," Stein said. "She was remarkably calm, never antagonistic."

WITHOUT A MAP OR A GUIDE

Nobody is quite sure how, but a reporter at the St. Paul/Minneapolis's *Star Tribune* found out about the story. His editor, seeing no local angle, killed it. But the reporter called a colleague at the *Chicago Tribune*, and a Page One story the next morning drew nationwide attention. The media in general handled the story appropriately, emphasizing the clever detective work, and being careful not to alarm their readers. As other news outlets picked up the story, reporters dutifully explained that the boy did not have smallpox and his virus was under control at the medical center, quelling public fear that the infection could spread.

Kahana's own father needed reminding that his daughter, a physician for 28 years, was not treating smallpox. "He was freaked out, but I take risks every day. If I were scared all the time, it would be paralyzing," she said, likening her job to that of soldiers who can't let a fear of gunfire keep them from the front lines. "I think a doctor has to do that. It's a little boy. He needs your help."

> The physicians suddenly realized what they were facing. "We were fired up when we made the diagnosis. Now we knew: That's why he's not getting better."
>
> Sarah Stein, MD, Assistant Professor of Medicine

And the Marcinak-Kahana team provided just that. Once the boy was officially diagnosed and transferred to the PICU, Kahana became a central member of the team. For 14 days, she and Marcinak spent several hours a day in his room. Marcinak and Vora spent many extra hours each day on the phone with colleagues at the CDC.

But there were no easy answers, despite a clear diagnosis. By the Sunday after his diagnosis, the boy had progressed to multi-system organ failure. "He was in a lot of trouble," Kahana said.

Marcinak and Vora held daily conference calls from 11 a.m. to noon with infectious disease specialists at the CDC, the Department of Defense, the Food and Drug Administration, the National Institutes of Health, pharmaceutical companies and city and state public health departments from Illinois and Indiana.

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The boy was kept in a special negative pressure room (similar to one at left) in Comer Children's Hospital, designed to keep the virus contained and allowing hospital air to flow into the room, but not out. *Photo by Dan Dry*

Those calls focused on the boy's clinical care; another series of calls took place on Mondays, Wednesdays and Fridays to trace the virus' epidemiology.

One reason for the calls was orchestrating use of a drug known as VIGIV, short for vaccinia immune globulin intravenous. Experts consider VIGIV the first-line treatment for vaccinia; the CDC keeps a stockpile in case universal smallpox vaccination becomes necessary. U.S. marshals brought it in by plane on the boy's sixth day in the hospital.

During the conference calls, experts also decided to try cidofovir, an antiviral drug recognized as a second line of treatment, and the experimental ST246, which physicians had never before used in a human. Chief of the CDC's Poxvirus Laboratory, Inger Damon, MD, PhD, convened a Saturday afternoon conference call with Marcinak and the manufacturers of ST246 to discuss its use. Only hours after physicians agreed they wanted to try it, the Food and Drug Administration approved emergency use of ST246 in this case. But ultimately the boy's parents faced the decision of whether or not to use it on their son.

"We had no guide," Marcinak said. "We had to take it one day at a time."

GOODBYES, HIGH-FIVES

The parents opted to use ST246. SIGA Technologies Inc., who developed and had begun phase-1 testing of the drug, arranged to fly it to Chicago from Oregon on Saturday via a private jet that billionaire and SIGA investor Ron Perelman paid for. It arrived at 4 a.m. Sunday and was administered to the child the same morning. By the next day, although the boy looked worse, his health was improving. He remained on a ventilator and unconscious, but the oozing pustules coating his body were beginning to scab—first red, then black.

The physicians and nurses provided treatment akin to that given to a burn victim. Nurses from the burn unit pitched in when they could, coaching the boy's PICU nurses on how to keep the skin moist. "It was unbelievable," Kahana said. "Every time we changed his wraps, I thought I was watching time-lapse photography."

The plastic surgeons, who soon would cover parts of the boy with cadaver skin, or allografts, knew they first had to debride (or "pick") the boy's scabs in order to keep his skin moist for optimum healing. "They grafted his arms, hands, chin, neck. That had never been done before," Marcinak said.

Kahana said the case was worse than any similar infection she had seen in her almost three decades as a physician. "Not even the worst case of chicken pox" looked this bad, she said, "and I saw one child die from that."

As his 30th day in the medical center approached, Kahana extubated the boy. However, he remained in the hospital for two more weeks. "We kept him here a long time after [he was well enough to go home] because we wanted to make sure he wasn't shedding the virus," Stein said.

With the tube out and a healthier, happier boy laughing with nurses and physicians as they visited, only a farewell party stood between him and his return trip home.

"He looked good. My guess is he'll grow up healthy and strong," Kahana said. "It was tremendous, the collaboration and work that went into saving this child's life."

When she last saw him, he was eating the icing from his cupcakes, and he gave Kahana a high-five. Perhaps the party served as closure, too, for all the people who took care of the boy—whether they faced their fears in doing so or didn't even recognize fright as an option.

APPENDIX H

SMALL ANIMAL STUDIES WITH ST-246

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1 SMALL ANIMAL STUDIES

1.1 Optimal Oral Dose Determination

1.1.1 COWPOX VIRUS CHALLENGE MODEL

The optimal oral dose of ST-246 was determined in an intranasal cowpox virus challenge model in mice. Weanling (3 week old) BALB/c mice (10-15 animals per dose group) were challenged with $3x10^4$ pfu (LD^{99}) of cowpox virus strain Brighton Red in 20 µL volume via intranasal inoculation. Mice were either treated with placebo (vehicle) or ST-246 at 10, 30, or 100 mg/kg (30, 90, 300 mg/m2, respectively) administered by oral gavage once per day for 14 days. A positive control group of mice were treated with 15 mg/kg (45 mg/m^2) of cidofovir (CDV) administered as a single dose by intraperitoneal (IP) injection. Mice were monitored for disease symptoms and euthanized when moribund. The results shown in Table 1.1.1-1 and [Report 246-PC-018], show that 100 mg/kg (300 mg/m2) provided maximum (89%) protection against lethal cowpox virus infection in this model.

Table 1.1.1-1: Optimal Oral Dose Determination for ST-246 – Cowpox Virus Challenge Model

Treatment[a]	Mortality No. Dead/Total (%)	P-Value	MDD	P-Value
Placebo	15/15 (100)	---	9.0	---
CDV (15 mg/kg)	0/15 (0)	< 0.001	---	
ST-246 (100 mg/kg)	1/9 (11)	< 0.001	10.0	NS
ST-246 (30 mg/kg)	5/10 (50)	< 0.01	10.2	NS
ST-246 (10 mg/kg)	11/12 (92)	NS	12.2	<0.01

[a]ST-246 was prepared as a suspension in 0.75% methyl cellulose, 1% Tween 80 and delivered in 0.2 mL oral doses. CDV was prepared in sterile saline and administered IP in a 0.1 mL dose. Treatment was initiated at 4 h post-inoculation.

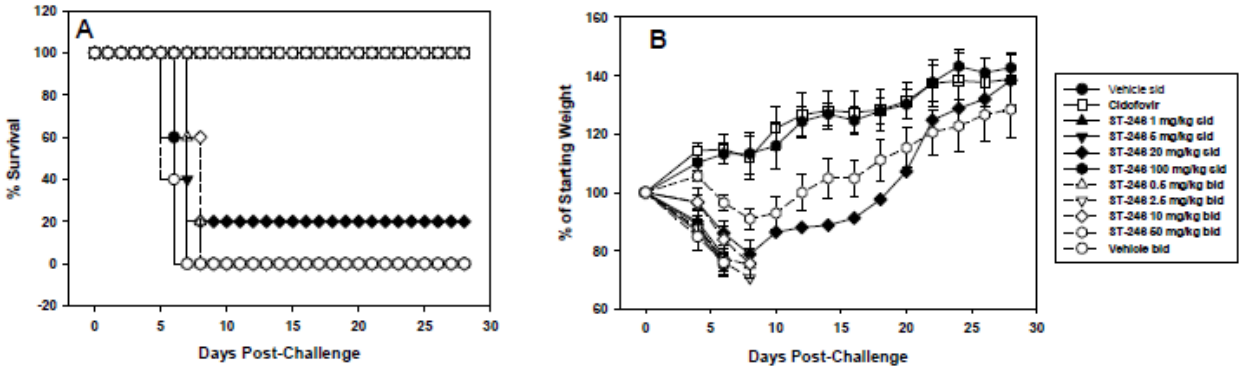
KEY: MDD = mean day of death; NS = Not significant when compared to the appropriate placebo control.

1.1.2 VACCINIA VIRUS CHALLENGE MODEL

The optimal oral dose of ST-246 was determined in an intranasal vaccinia virus challenge model in mice. Weanling (4 week old) BALB/c mice (5 animals per dose group) were treated with placebo (vehicle), ST-246, or cidofovir (positive control). ST-246 was administered once per day at 1, 5, 20, or 100 mg/kg (3, 15, 60, or 300 mg/m2, respectively) or twice per day at 0.5, 2.5, 10, or 50 mg/kg (1.5, 7.5, 30, or 150 mg/m^2, respectively). CDV was administered as a single IP injection at 50 mg/kg (150 mg/m2). One hour after the initial drug dose, mice were challenged with $4.3x10^4$ pfu ($10xLD_{50}$ as determined by titration) of vaccinia virus strain IHD-J. Virus was diluted in 1x PBS and delivered to the nares of isoflurane-anesthetized mice in a 20 µL volume. Mice were monitored for disease symptoms and euthanized when moribund or when their weights fell below 70% of their starting weight. Mice were weighed every 2 days following challenge, starting at Day 4, through day 28. The results of the dose optimization are summarized in Figure 1.1.2-1 and [Report 246-PC-013]. ST-246 at 100 mg/kg (300 mg/m^2) SID provided 100% protection against death from viral challenge (Figure 1.1.2-1A) and was equally as effective as cidofovir with respect to maintaining weight (Figure 1.1.2-1B). ST-246 at 50 mg/kg (150 mg/m^2) BID was equally as protective against death, although mice in this group experienced roughly an initial 10% weight loss and then recovered well. The only other ST-246

concentration that had any effect was 20 mg/kg (60 mg/m^2) SID, which resulted in only 20% protection.

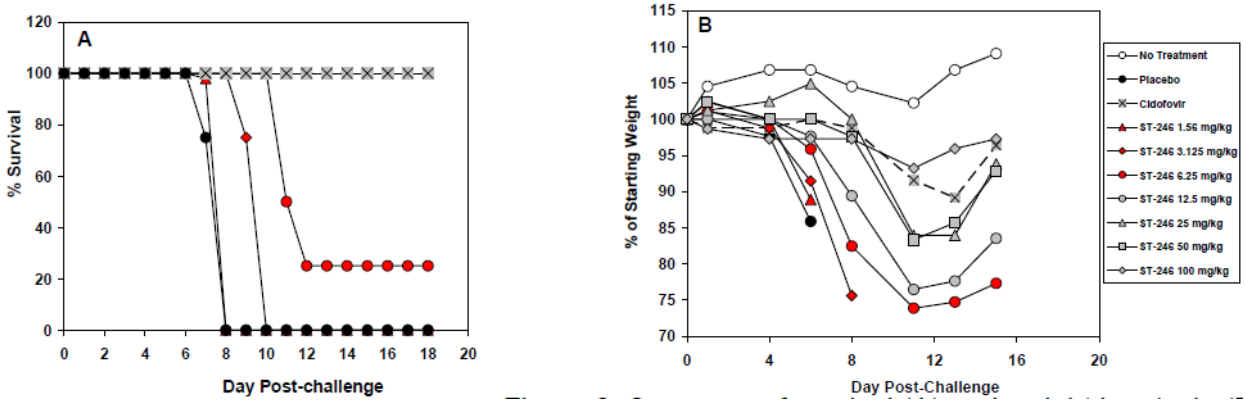
Figure 1.1.2-1: Summary of Survival (A) and Weight Loss/Gain (B) of BALB/c Mice Administered ST-246 at Various Dosage Levels Followed by Challenge at Day 0 With 10xLD$_{50}$ of Vaccinia Virus Strain IHD-J



1.1.3 ECTROMELIA VIRUS CHALLENGE MODEL

The optimal oral dose of ST-246 was determined in an intranasal ectromelia virus challenge model in mice. A/NCR mice (10 week old; 4 animals per dose group) were treated with placebo (vehicle), ST-246, or cidofovir (positive control). ST-246 was administered 4 h post virus inoculation and once per day at 1.56, 3.13, 6.25, 12.5, 25, 100 mg/kg (300 mg/m^2) for a period of 14 days. Mice were challenged with 100 pfu (10 x LD$_{50}$ as determined by titration) of ectromelia virus strain Moscow. Virus was diluted in 1x PBS and delivered to the nares of isoflurane-anesthetized mice in a 20 µL volume. Mice were monitored for disease symptoms and euthanized when moribund. Mice were weighed every 2 days following challenge, starting at Day 4 through day 28. The results of the dose optimization for ST-246 are summarized in Figure 1.1.3-1 and [Report 246-PC-024]. ST-246 at 12.5 mg/kg (37.5 mg/m^2) or above provided 100% protection against death from viral challenge (Figure 1.1.3-1A). ST-246 at 100 mg/kg (300 mg/m^2) provided 100% protection against death from viral challenge and was equally as effective as cidofovir with respect to maintaining weight (Figure 1.1.3-1B).

Figure 1.1.3-1: Summary of Survival (A) and Weight Loss/Gain (B) of A/NCR Mice Administered ST-246 at Various Dosage Levels Followed by Challenge at Day 0 With 10xLD_{50} of Ectromelia Virus



NOTE: Symbols shown in red indicate that deaths occurred in the dose group. CDV was administered as a single IP injection at 15 mg/mg (45 mg/m^2).

1.2 Optimal Oral Dose Duration Determination

1.2.1 Intranasal Cowpox Virus Challenge Model

The optimal oral dose of ST-246 for protection against lethal cowpox virus infection was used to determine the optimal dose duration in the mouse cowpox virus challenge model. BALB/c mice (4 to 6 weeks old) were challenged intranasally with $3.3x10^4$ pfu (LD_{99}) of cowpox virus strain Brighton Red. At 4 h post challenge, ST-246 was administered once per day at 100 mg/kg (300 mg/m^2) for 5, 7, 10, or 14 consecutive days. Mice were monitored daily for disease symptoms (ruffled fur, hunched posture, unsteady gait, and separation from cage mates) and euthanized when moribund. The results show that treatment of mice with ST-246 at 100 mg/kg (300 mg/m^2) for dose durations of 7 days or greater provided maximum protection against lethal cowpox virus infection. The results are summarized in Table 1.2.3-1 and [Report 246-PC-016].

1.2.2 Intranasal Vaccinia Virus Challenge Model

The optimal oral dose of ST-246 for protection against lethal vaccinia virus infection was used to determine the optimal dose duration in the mouse vaccinia virus challenge model. ST-246 was administered to BALB/c mice (5 per dose group) once per day at 100 mg/kg (300 mg/m^2) for 5, 7, 10, or 14 consecutive days starting at one hour prior to inoculation with $4.3x10^4$ pfu of vaccinia virus strain IHD-J (10xLD_{50} as measured by virus titration). Mice were monitored daily for disease symptoms (ruffled fur, hunched posture, unsteady gait, and separation from cage mates) and euthanized when moribund. Mice were weighed every 2 days following challenge, starting at Day 4. The results show that treatment of mice with ST-246 at 100 mg/kg (300 mg/m^2) for dose durations of 5 days or greater provided maximum (100%) protection against lethal vaccinia virus infection. The results are summarized in Table 1.2.3-1 and [Report 246-PC-014].

1.2.3 Intranasal Ectromelia Virus Challenge Model

The optimal oral dose of ST-246 for protection against lethal ectromelia virus infection was used to determine the optimal dose duration in the mouse ectromelia virus challenge model. A/NCR mice were inoculated intranasally with 700 pfu (1400xLD_{50} as measured by virus titration) of ectromelia virus strain Moscow. At 4 h post inoculation ST-246 was administered by oral

gavage to mice (4 animals per dose group) once per day at 100 mg/kg (300 mg/m^2) for 5, 7, 10, or 14 consecutive days. Mice were monitored daily for disease symptoms (ruffled fur, hunched posture, unsteady gait, and separation from cage mates) and euthanized when moribund. Mice were weighed every 2 days following challenge, starting at Day 4. The results show that treatment of mice with ST-246 at 100 mg/kg (300 mg/m2) for dose durations of 5 days or greater provided 100% protection against lethal ectromelia virus infection. The results are summarized in Table 1.2.3-1 and [Report 246-PC-025].

Table 1.2.3-1: Optimal Dose Duration for ST-246 in Mouse Models of Orthopoxvirus Infection

Mortality, No. Dead/Total (%)						
	CPX		VAC		ECTV	
Treatment[a]	ST-246	Placebo	ST-246	Placebo	ST-246	Placebo
ST-246 (5 day)	13/15 (87)	15/15 (100)	0/5 (0)	5/5 (100)	0/4 (0)	4/4 (100)
ST-246 (7 day)	1/15 (7)	15/15 (100)	0/5 (0)	5/5 (100)	0/4 (0)	4/4 (100)
ST-246 (10 day)	4/15 (27)	15/15 (100)	0/5 (0)	5/5 (100)	0/4 (0)	4/4 (100)
ST-246 (14 day)	1/15 (7)	14/15 (93)	0/5 (0)	5/5 (100)	0/4 (0)	4/4 (100)

[a]ST-246 was prepared as a suspension in 0.75% methyl cellulose, 1% Tween 80 and delivered in 0.2 mL oral doses. Treatment was initiated at 4 h post-inoculation.

1.3 Optimal Time of Treatment Post-Exposure

1.3.1 INTRANASAL COWPOX VIRUS CHALLENGE MODEL

To determine the time post-exposure when administration of ST-246 no longer protected against cowpox virus-induced disease, BALB/c mice were infected with 3.3x10^4 pfu/animal (LD_{99} as measured by titration) of cowpox virus strain Brighton Red. Mice were treated with ST-246 at 100 mg/kg (300 mg/m^2), once per day beginning at 4, 24, 48, or 72 hours post-exposure to cowpox virus. CDV was administered as a single IP injection at 15 mg/kg (45 mg/m^2). The animals were monitored for disease symptoms and euthanized when moribund. The results summarized in Table 1.3.1-1 and [Report 246-PC-018] show that ST-246 administered once per day at 100 mg/kg (300 mg/m^2) for 14 days reduced mortality associated with lethal intranasal cowpox virus challenge when dosing was initiated at 4, 24, 48, or 72 h post-inoculation. The antiviral effect was diminished when compound was administered at 72 h post-inoculation with mortality observed in 40% of the mice in this dose group.

Table 1.3.1-1: Post-Exposure Treatment of Cowpox/Ectromelia Virus Infection With ST-246

Mortality, No. Dead/Total (%)		
Treatment[a]	**CPX**	**ECTV**
Placebo	15/15 (100)	4/4 (100)
CDV (15 mg/kg)	0/15 (0)	0/4 (0)
ST-246 (4 h.p.i.)	1/9 (11)	0/4 (0)
ST-246 (24 h.p.i.)	4/15 (27)	0/4 (0)
ST-246 (48 h.p.i.)	1/15 (7)	0/4 (0)
ST-246 (72 h.p.i.)	6/15 (40)	0/4 (0)

[a]ST-246 was prepared as a suspension in 0.75% methyl cellulose, 1% Tween 80 and delivered in 0.2 mL oral doses. CDV was prepared in sterile saline and administered i.p. in a 0.1 mL dose at 72 h post-inoculation. Placebo treatment resulted in 15/15 (100%) deaths in all dose groups for CPX infections and 4/4 (100%) for ECTV infection.

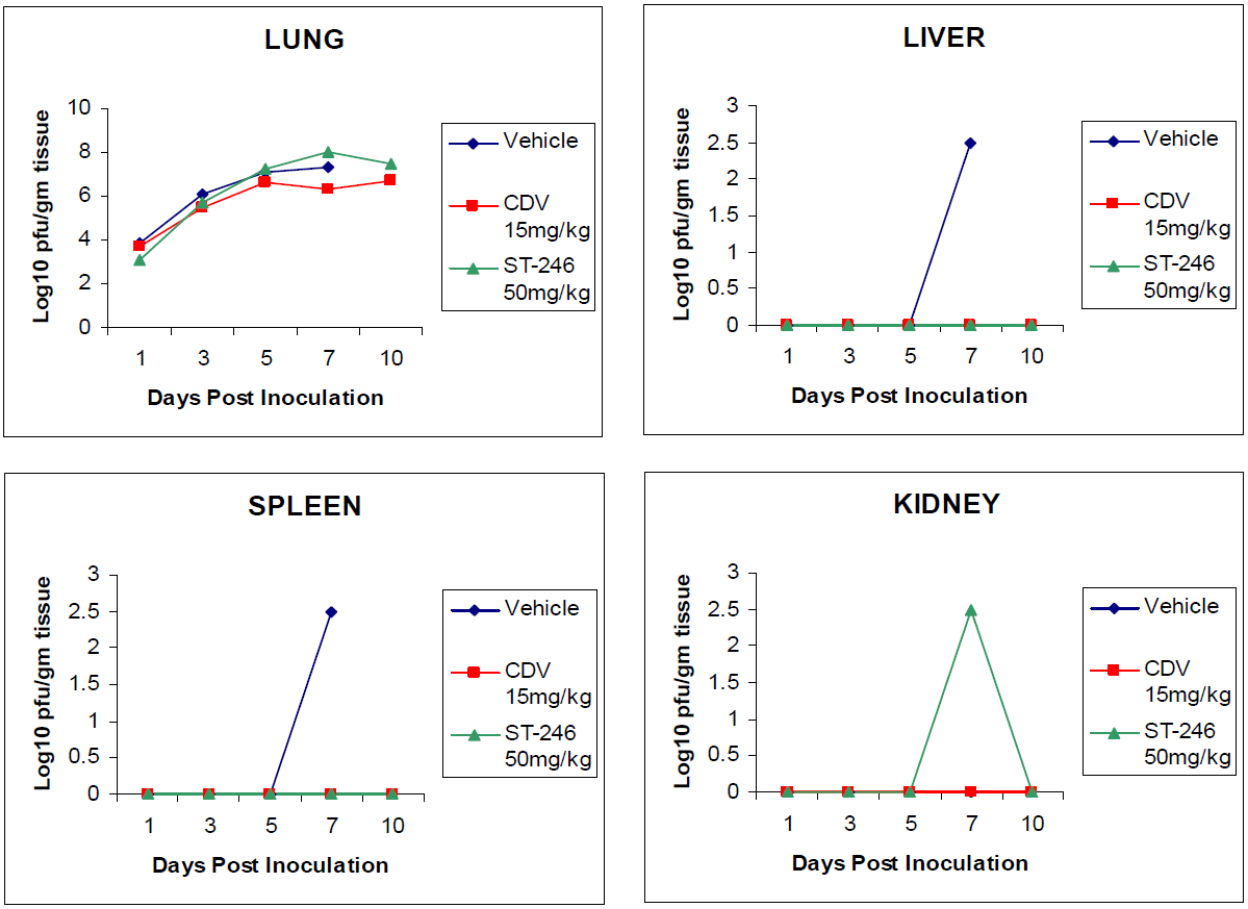
1.3.2 Intranasal Vaccinia Virus Challenge Model

To determine the time post-exposure when administration of ST-246 no longer protected against vaccinia virus-induced disease, survival was monitored in mice that were administered ST-246 treatment immediately before intranasal infection with 1.2×10^6 pfu ($10 \times LD_{50}$) of VV-WR (Day 0) or 1, 2, 3, or 4 days post infection.[82] ST-246 administered as late as three days post-infection protected 100% of the mice from death, while all vehicle-treated mice died by Day 6 post-infection. Compared to vehicle-treated mice, ST-246 treatment started four days post-infection delayed death by 2 to 4 days, but, ultimately, only one of five (20%) mice survived the lethal challenge.

1.3.3 Intranasal Ectromelia Virus Challenge Model

To determine the time post-exposure when administration of ST-246 no longer protected against ectromelia virus-induced disease, A/NCR mice were infected IN with 200 pfu/animal ($100 \times LD_{50}$ as measured by titration) of ectromelia virus strain Moscow. Mice were treated with ST-246 at 100 mg/kg (300 mg/m^2), once per day beginning at 4, 24, 48, or 72 h post-exposure to ectromelia virus. The animals were monitored for disease symptoms and euthanized when moribund. The results summarized in Table 1.3.1-1 and [Report 246-PC-026] show that ST-246 administered once per day at 100 mg/kg (300 mg/m^2) for 14 days reduced mortality associated with lethal intranasal ectromelia virus challenge when dosing was initiated at 4, 24, 48, or 72 h post-inoculation.

Figure 1.3.3-1: Effect of ST-246 on Tissue Infectivity Following Intranasal Cowpox Virus Infection of Mice



Tissue homogenates from selected organs were prepared and virus yields were determined by plaque assay.

1.4 Effects of ST-246 on Virus Dissemination

1.4.1 Intranasal Cowpox Virus Challenge Model

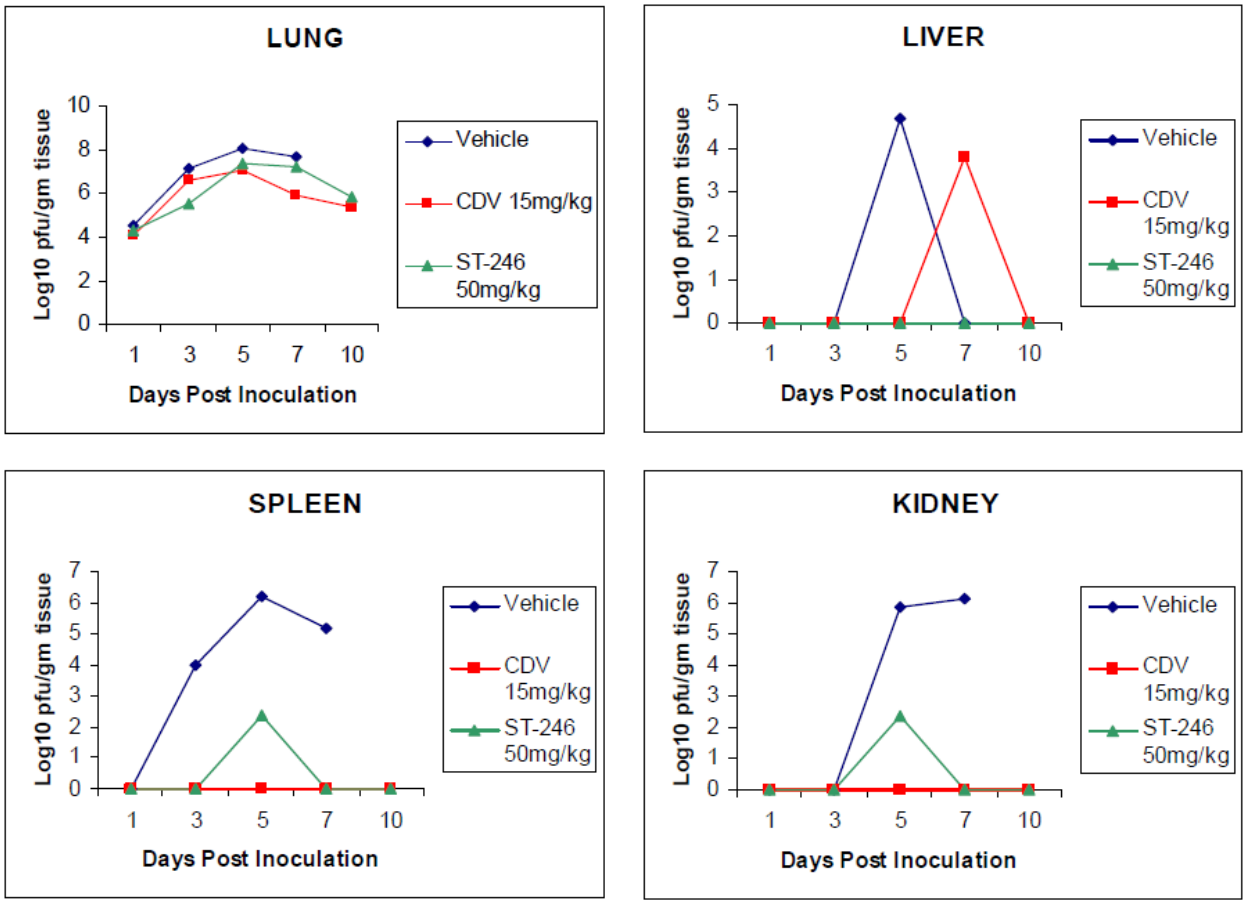
To measure the effects of ST-246 on viral dissemination, BALB/c mice were infected with a lethal dose of cowpox virus ($3.3x10^4$ pfu) via intranasal inoculation and treated with placebo or ST-246 at 50 mg/kg (150 mg/m^2) once per day beginning at 4 h post-infection. A control group was included where CDV was administered by IP injection for 9 days at 15 mg/kg (45 mg/m^2). Groups of 3 mice were sacrificed at 1, 3, 5, 7, or 10 days post-infection and viral titers and organ weights were measured in lung, liver, kidney, and spleen. Oral administration of ST-246 to BALB/c mice reduced cowpox virus titers relative to placebo-treated mice in liver and spleen but not lung (Figure 1.3.3-1). Virus was detected in kidney tissue from animals treated with ST-246 on Day 7 but not on Day 10 post-infection. These results suggest that ST-246 reduces systemic cowpox virus spread [Report 246-PC-023].

1.4.2 Intranasal Vaccinia Virus Challenge Model

To measure the effects of ST-246 on viral dissemination, BALB/c mice were infected with a lethal dose ($1x10^4$ pfu) of vaccinia virus via intranasal inoculation and treated with placebo or ST-246 at 50 mg/kg (150 mg/m^2) once per day beginning at 4 h post-infection. CDV was

administered by IP injection for 9 days at 15 mg/kg (45 mg/m^2). Groups of 3 mice were sacrificed at 1, 3, 5, 7, or 10 days post-infection and viral titers and organ weights were measured in lung, liver, kidney, and spleen. Oral administration of ST-246 to BALB/c mice reduced vaccinia virus titers relative to placebo-treated mice in liver, kidney, and spleen but not lung (Figure 1.4.2-1). These results suggest that ST-246 inhibits systemic vaccinia virus spread [Report 246-PC-019].

Figure 1.4.2-1: Effect of ST-246 on Tissue Infectivity Following Intranasal Vaccinia Virus Infection of Mice

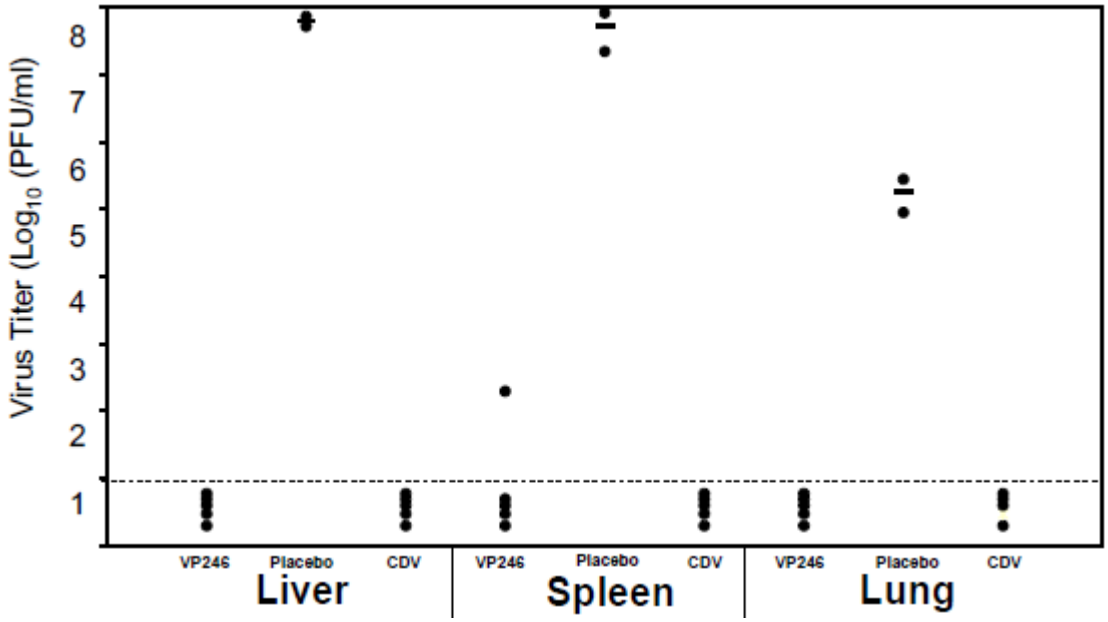


Tissue homogenates from selected organs were prepared and virus yields were determined by plaque assay.

1.4.3 Intranasal Ectromelia Virus Challenge Model

A/NCR mice were challenged with ectromelia virus at a dose of 2x10^4 pfu (40,000xLD_{50}) delivered to each naris in a volume of 20 µL. Mice were either treated with placebo (vehicle) or ST-246 administered by oral gavage at 50 mg/kg (150 mg/m^2) twice a day for 14 days. Mice were monitored for disease symptoms and sacrificed when moribund. ST-246 protected 100% of the mice from lethal challenge with ectromelia virus, while the mean time to death (MTD) for placebo-treated mice was eight days post-infection. In a separate experiment, mice were infected with 100xLD_{50} of ectromelia virus, and viral titers were measured in the liver, spleen, and lung on Day 8 post-infection. ST-246 treatment severely attenuated systemic spread with little to no virus detected in the liver, spleen, and lungs of infected animals (Figure 1.4.3-1).

Figure 1.4.3-1: ST-246 Reduces Systemic Virus Spread



Mice were inoculated with 50 pfu (100xLD_{50}) of ectromelia virus intranasal administration. Cidofovir-treated mice were injected on Day 0 with a single dose by the intraperitoneal route. ST-246 or placebo-dosed mice were treated by gavage on Day 0-14, at eight-hour intervals and twice daily. At Day 8 post-infection, five mice in each group were sacrificed and tissue infectivity measured in target organs by plaque assay.

2. RABBIT MODEL OF ORTHOPOXVIRUS INFECTION

The initial experiment was designed to determine antiviral efficacy in the rabbit model of orthopoxvirus disease. New Zealand White (NZW) rabbits (7 or 8 rabbits per dose group) were infected with 100 pfu (10xLD_{50} dose) of rabbitpox by aerosol delivery to the respiratory tract. ST-246 or placebo was administered by oral gavage at 10 or 40 mg/kg (125 or 500 mg/m^2) once per day for 14 days. Rabbits were weighed every other day and monitored for signs of disease. The results of the experiment are shown in Table 2-1 (and in Study Report 246-PC-028) and demonstrate that 40 mg/kg/day (500 mg/m^2) for 14 days provided complete protection against rabbitpox-induced disease.

Table 2-1: Antiviral Efficacy of ST-246 in a Lethal Model of Rabbitpox Infection of Rabbits

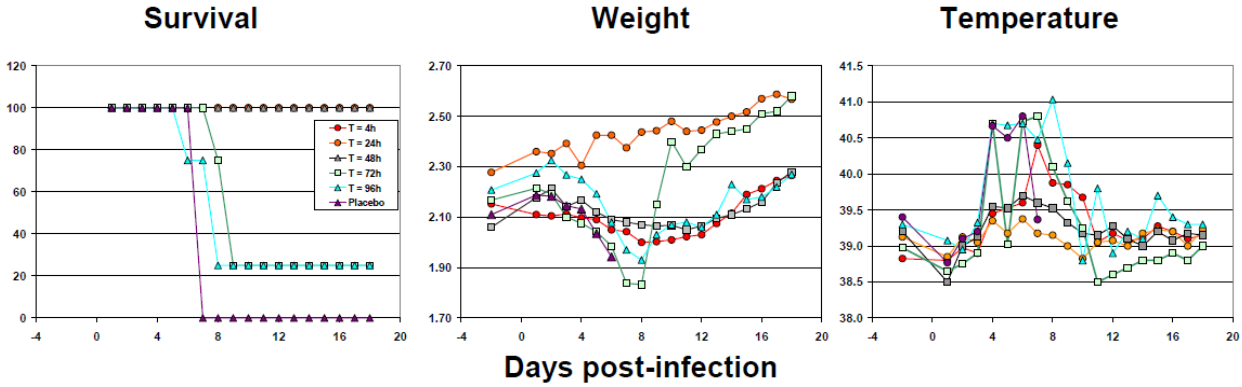
Group	Dead	Alive	% Surviving	MTD	S.D.
40 mg/kg	0	7	100	NA	-
10 mg/kg	3	5	63	7.3	1.2
Placebo	7	0	0	7.4	0.8

2.1 Optimal Time of Treatment Post-Exposure

To determine the therapeutic potential for ST-246, rabbits were administered ST-246 at 4, 24, 48, 72, and 96 hours post-exposure to rabbitpox virus. Body temperature and weights were measured on a daily basis as one measure of rabbitpox induced disease. Animals that appeared moribund were euthanized. The results show that a dose of 40 mg/kg/day (500 mg/m^2) for 14 days administered as late as 48 h post-infection provided complete protection from lethal infection with rabbitpox virus and also reduced rabbitpox virus-induced disease as measured by

the absence of a spike in body temperature (Figure 2.1-1). This data is also presented in Study Report 246-PC-029.

Figure 2.1-1: Antiviral Efficacy of ST-246 Administered at 4, 24, 48, 72, and 96 h Post-Exposure to Rabbitpox Virus to New Zealand White Rabbits



Survival, body weight, and temperature were measured daily and plotted as a function of time post-infection.

3. GROUND SQUIRREL MODEL OF ORTHOPOXVIRUS INFECTION

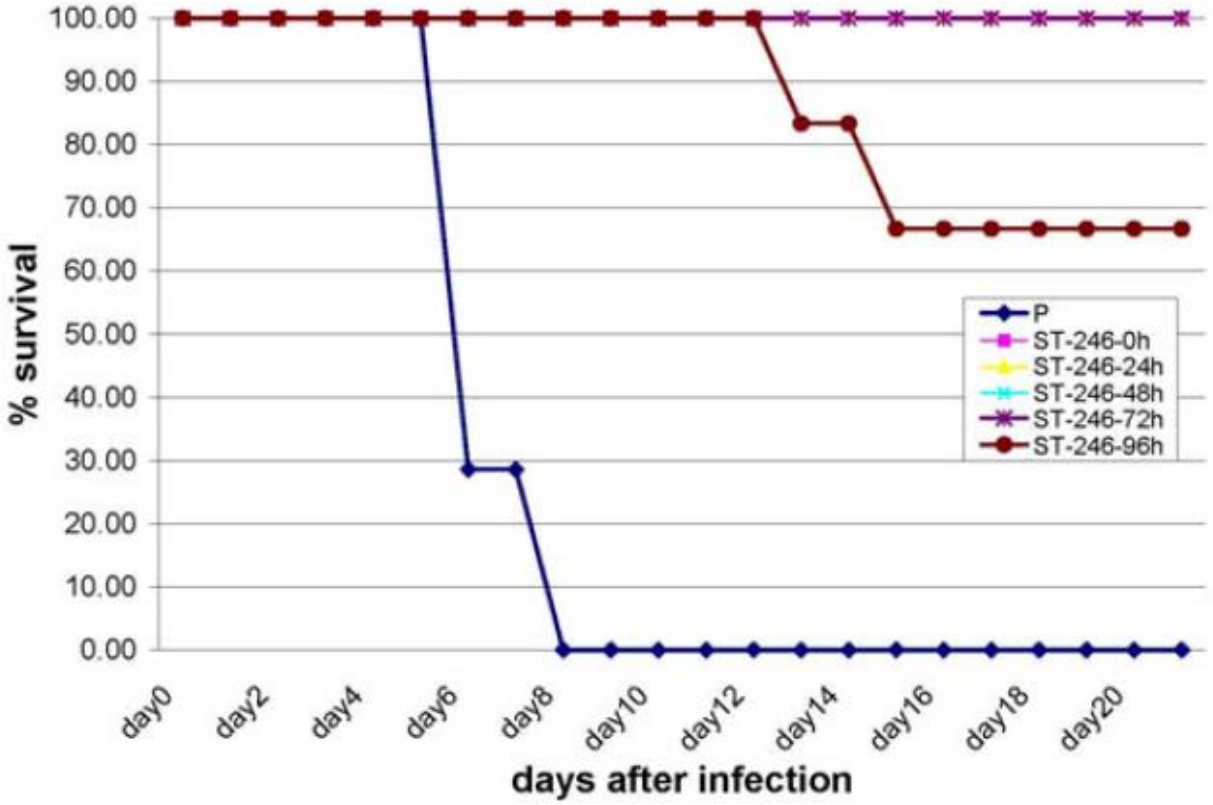
A total of 59 randomly mixed, juvenile and adult ground squirrels were divided into 8 groups. One group of 9 animals received the placebo (vehicle) plus virus (100 pfu of MPXV virus, equivalent to approximately 200xLD_{50}, given subcutaneously) and is subsequently referred to as the "placebo group." A second group of 7 uninfected and untreated ground squirrels, designated as the "normal group," was bled to obtain standard (normal) reference values for the clinical laboratory tests described below; their tissues were also used as normal controls for histopathology. The remaining 5 groups designated ST-246-0h (n = 9), ST-246-24h (n = 9), ST-246-48h (n = 9), ST-246-72h (n = 9) and ST-246-96h (n = 5), were given 100 pfu of MPX virus subcutaneously and were then treated once daily with 100 mg/kg of ST-246 beginning at approximately 0, 24, 48 72 and 96 h post-inoculation (P.I.), respectively. All treatments were continued until day 14 p.i. The animals were weighed daily and examined for signs of illness or death. Animals were monitored for signs of disease for 21 days post-infection. On Day 7 post-inoculation, 2 animals from each group were sacrificed to obtain blood and tissue samples. The appearance and reactivity of the animals were monitored throughout the course of the experiment. Physical symptoms such as anorexia, dehydration, and responsiveness to external stimuli were also measured. In addition, the animals were weighed daily. Blood chemistries from animals sacrificed at Day 7 post-infection were analyzed and coagulation assays, and hematological tests performed. In addition, liver transaminase activity was measured to assess liver function.

3.1 Morbidity and Mortality

The ground squirrels inoculated with monkeypox virus and treated orally with ST-246 immediately after challenge (0 hours) or starting at 24, 48, or 72 h post-infection all survived; none of these animals showed any apparent clinical disease. Sixty-seven percent of the animals in which treatment was started at 96 h p.i. also survived. Two of the animals in this latter group showed signs of disease beginning on Day 6 p.i., but then recovered around Day 15 p.i. and appeared well at the conclusion of the experiment. In contrast, animals infected with monkeypox virus and treated with the placebo began to show signs of illness on Day 4 p.i., and all died between Days 6 and 9 p.i (Figure 3.1-1). Likewise, there was no significant weight change

among surviving animals in the five ST-246 treatment groups or in the normal group (data not shown).

Figure 3.1-1: Survival Curves of Ground Squirrels Infected With MPXV and Treated With ST-246 or Placebo at Different Times Post-Infection



3.1.1 VIROLOGIC AND ANTIBODY DATA

None of the animals in the normal group developed detectable viremia. All of the animals inoculated with monkeypox virus and receiving placebo had high MPXV titers. None of the squirrels challenged with monkeypox virus and treated with ST-246 at 0, 24, 48 and 72 h.p.i. developed detectable viremia. The two squirrels in the ST-246-96h group (challenged with MPXV virus and treated at 96 h p.i.) that were euthanized on Day 7 p.i. had low-titer viremia (15 and 125 pfu/mL, respectively). None of the animals in the placebo group (measured at Day 6 p.i.), or in treatment arms ST-246-0h, ST-246-24h, ST-246-48h, and ST-246-72h (measured at Day 21 p.i.) developed detectable antibodies to monkeypox virus. However, the two squirrels in the ST-246-96h group that became ill and then recovered had detectable monkeypox virus antibody titers at the end of the experiment (Day 21 p.i.). These results suggest that early administration of ST-246 prevents infection and disease.

Table 3.1.1-1 shows the mean virus titers in blood and organ (liver, lung and spleen) samples taken from ground squirrels in the placebo and five ST-246 treatment groups on Day 7 p.i. of monkeypox virus. The surviving animals in the placebo group had high titers of virus in their blood and the tissues sampled. In contrast, no virus was detected in any of the animals in groups ST-246-0h, ST-246-24h, ST-246-48h or ST-246-72h. Animals in the ST-246-96h treatment group had detectable, but relatively low viral loads in the same specimens.

Table 3.1.1-1: Virus Titers From Blood and Organ Samples Taken From Ground Squirrels at Day 7 Post-Inoculation With 100 pfu of Monkeypox Virus

Treatment Group	Tissue Sample			
	Blood	Liver	Lung	Spleen
Placebo	4.9[a]	7.3	8.2	7.0
ST-246-0h	0	0	0	0
ST-246-24h	0	0	0	0
ST-246-48h	0	0	0	0
ST-246-72h	0	0	0	0
ST-246-96h	1.2	2.1	2.6	1.9

[a]Titers expressed as $\log_{10}$ pfu/mL of blood or 10% homogenate of tissue sample.

Taken together, these results demonstrate that ST-246 protects ground squirrels from a lethal monkeypox virus challenge even when compound was administered at 72 h post-exposure. Treated animals showed no evidence of virus in vital organs or in tissue samples suggesting that ST-246 inhibited virus spread. The ability of ST-246 to prevent disease was supported by hematological analysis and histopathology evaluation demonstrating that ST-246 treated animals had blood chemistries, enzyme levels, and tissue morphology similar to normal non-infected animals [Report 246-PC-022].

4. PRAIRIE DOG MODEL OF ORTHOPOXVIRUS INFECTION

4.1 Intranasal Monkeypoxvirus Challenge Model

The purpose of the study was to determine the efficacy of oral administration of the antiviral compound ST-246 in preventing monkeypox virus infection in prairie dogs. Prairie dogs are susceptible to monkeypox virus infection with a lethal disease progression resulting from intranasal infection and a mean time to death of 11 days post-inoculation. Necropsy examinations demonstrate hepatic and splenic necrosis with some inflammatory changes in the lungs. Some of the pathologic features observed in prairie dogs, including a necrotizing bronchopneumonia, have been described in cynomolgus monkeys infected experimentally by inhalation of monkeypox virus. Mortality was the primary endpoint in this study. Secondary endpoints included change in body weight, viral load, and lesion count. These endpoints were selected because they correlated most closely with the severity of orthopoxvirus disease and could be easily measured in this experimental system.

4.1.1 STUDY DESIGN

Prairie dogs were inoculated intranasally (IN) with doses between 10^3–10^5 pfu/100 g of monkeypox virus (MPXV-2003-WI-044 or MPX-2003-ROC-358) diluted in phosphate buffered saline (PBS) or sham infected with a volume of PBS equal to the volume for animals receiving virus. Inoculation was administered with a micropipettor and performed on animals while under mild anesthesia (5% isoflurane). Animals were assigned to 4 groups of 4 and one group of 2 negative control animals. The 2 negative control animals received phosphate buffered saline (PBS) to mock infection and 16 animals received monkeypox virus. ST-246 was administered under mild anesthesia to 3 groups of animals by oral gavage daily for the duration of the study. Groups received ST-246 beginning at the time of virus inoculation (Group 1), 3 days post-inoculation (Group 2), or 10 days post-inoculation (Group 3). The remaining group (Group 4) served as a positive control group and did not receive ST-246 (see Table 4.1.1-1). For 30 days post-inoculation, individual animals were observed daily for signs of fever or malaise

(inappetance, decreased activity, recumbancy with reluctance to move, etc.) and clinical lesions. Antemortem samples (lesion, ocular, oral, and anal swabs) were collected daily and performed on subjects while lightly anesthetized (5% isoflurane); body weight was measured while under anesthesia; blood was collected (for determination of possible viremia and possible antibody formation) from the lateral saphenous vein and performed under anesthesia. Blood collection was performed no more than every other day and did exceed a maximum draw volume of 7.7 mL/kg/month.

Table 4.1.1-1: ST-246 Treatment Schedule for MPXV Infected Prairie Dogs

Animal Group	Sample Number	Viral Inoculum	ST-246 Treatment
Group 1	4	1 x 10^5 pfu	Day 0-13
Group 2	4	1 x 10^5 pfu	Day 3-16
Group 3[a]	4	1 x 10^5 pfu	Day 10-23
Group 4	4	1 x 10^5 pfu	Placebo
Group 5	2	0 pfu	Day 3-16

[a]Treatment for this group was initiated upon rash onset. Rash appeared on 3 animals on Day 10 and on 1 animal on Day 24. Treatments were from Days 10 – 23 and 24 – 37, respectively.

Animals developing clinical symptoms were reported to the attending veterinarian for closer examination. Any animals losing 25% of starting body weight, and/or accruing a total morbidity score of 10, indicating severe illness, although not expected, were humanely euthanized. Prairie dogs were weighed every day for 14 days during treatment and every two days for the rest of the study.

4.1.2 RESULTS

4.1.2.1 MORBIDITY AND MORTALITY

The prairie dogs inoculated with monkeypox virus and treated orally with ST-246 immediately after challenge (Day 0) or starting 3 or 10 days post-infection all survived. None of the animals treated with ST-246 starting on Day 0 or Day 3 post-infection showed any apparent clinical disease. Animals in Group 3 were showing symptoms of disease including rash, swollen face, runny/bloody/crusty nose, lack of appetite, and weight loss prior to initiation of ST-246 treatment. All of these that were treated with ST-246 starting on Day 10-post infection also survived. Only one of the placebo treated animals survived. Both of the uninfected animals survived. See Table 4.1.2.1-1 for a summary.

Table 4.1.2.1-1: Morbidity and Mortality of Prairie Dogs

Group Number	Disease Symptoms[a]	Vesicular Rash	Survival
1	0/4	0/4	4/4
2	0/4	0/4	4/4
3	4/4	4/4	4/4
4	4/4	1/2[b]	1/4
5	0/2	0/2	2/2

[a]Symptoms included swollen face, pus or bloody discharge from nose, loss of appetite, lethargy, and difficulty in breathing.
[b]Two animals died on Day 10 prior to rash onset.

4.1.3 WEIGHT AND LESIONS

Animals were monitored daily for signs of disease including rash onset, lesion number, weight, fever, and malaise. Table 4.1.3-1 shows the summary of this data for the animals in Group 3 which were treated with ST-246 starting 10 days post-infection, and for those in Group 4 which received placebo. The rash was predominantly restricted to oral and nasal areas. A limited number of lesions were observed on the bodies of animals with the exception of Animal 3. No lesions were present on the paws. Lesions quickly dried up to form scabs, or did not develop past the macular stage.

Table 4.1.3-1: Observation of Rash Onset, Lesion Counts and Other Notable Observations

Group	Animal	Rash Onset	Lesion Number	% Weight Loss	Notable Observations
3	2	Day 10	< 10	8.9	Lesions reduced Day 16, scab Day 18, gone Day 22.
	3	Day 24	> 50	14.7	Heaviest. Lesions drying by Day 26-28, scabs Day 30.
	12	Day 10	< 5	0	Pustular lesions never developed and gone Day 14.
	14	Day 10	< 10	23.2	Swollen/bloody/pus/crusty nose. Elevated temp Day 13.
4	5	N/A		7.7	Hemorrhagic. Expired Day 10.
	6	N/A		0	Nose swollen and some blood. Mild infection.
	8	Day 10	> 10	9.9	Difficulty breathing – mouth. Expired Day 12.
	15	N/A		1.5	Only symptom was swollen nose. Exp Day 10. Ate monkey biscuit.

4.1.4 CONCLUSION

ST-246 protected prairie dogs 100% from a lethal viral challenge with monkeypox virus when administered at 0, 3, or 10 days post-infection. When administered within the first three days of exposure, ST-246 treated animals showed no signs of orthopoxvirus-induced disease as determined by rash, illness, lesions, and weight loss. ST-246, administered as late as 10 days post-infection, when the animals were already showing overt signs of disease, was able to protect 100% of the treated animals from death.

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