



## **CMX001 FOR THE TREATMENT OF SMALLPOX**

### **SPONSOR:**

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## LIST OF ABBREVIATIONS

Abbreviation or specialist term	Explanation
AdV	adenovirus
AUC	area under the curve
BKV	BK virus
BMT	bone marrow transplant
BSL	biosafety level
CDC	Centers for Disease Control and Prevention
CDV	cidofovir
CDV-PP	cidofovir diphosphate
C <sub>max</sub>	concentration maximum
CMX001	phosphonic acid, [[(S)-2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl)ethoxy)methyl]mono[3-(hexadecyloxy)propyl] ester
CMV	human cytomegalovirus
CPXV	cowpox virus
dsDNA	double stranded DNA
EBV	Epstein-Barr virus
EC <sub>50</sub>	effective concentration 50%
ECTV	ectromelia virus
EIND	Emergency Investigational New Drug Application
GLP	Good Laboratory Practice
HHV	human herpes virus
HPV	human papillomavirus
HSCT	hematopoietic stem cell transplant
HSV	herpes simplex virus
JCV	JC virus
LD <sub>50</sub>	lethal dose 50%
LD <sub>100</sub>	lethal dose 100%
LAC	Lipid-Antiviral-Conjugate
MPXV	monkeypox virus
NHP	non-human primate
PBMC	peripheral blood mononuclear cell
PFU	plaque-forming units
PK	pharmacokinetic(s)
PV	progressive vaccinia
RES	reticuloendothelial system

<b>Abbreviation or specialist term</b>	<b>Explanation</b>
RPXV	rabbitpox virus
SRI	Southern Research Institute
Tmax	time to maximum concentration
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
VACV	vaccinia virus
VARV	variola virus
VZV	varicella-zoster virus

## 1. EXECUTIVE SUMMARY

CMX001 is a lipid conjugate of the nucleotide analog cidofovir. CMX001 efficiently enters cells, where the lipid moiety is cleaved to form cidofovir. Cidofovir is then phosphorylated to its diphosphate form which mimics a nucleoside triphosphate and acts as a selective inhibitor of DNA polymerases. CMX001 has in vitro activity against all five families of dsDNA viruses that cause human disease, including variola, the causative agent of smallpox. Chimerix is seeking a licensed indication for CMX001 for the treatment of smallpox under the “Animal Rule.” In addition, Chimerix is developing CMX001 for other potential indications (e.g., prevention of cytomegalovirus (CMV) disease and pre-emption of adenovirus (AdV) disease in hematopoietic cell transplant recipients) using more traditional regulatory pathways to approval that are based on the demonstration of safety and efficacy of the compound in the intended indication, rather than in surrogate models. Given the regulatory and scientific complexities of the “Animal Rule” which will be discussed as part of this Advisory Committee meeting, the considerable human clinical data that have been and will continue to be generated by Chimerix for these other indications (e.g., CMV and AdV) will provide important supportive clinical safety and efficacy data for CMX001 for submission in a New Drug Application.

### **CMX001 is in advanced development for the treatment of smallpox under the “Animal Rule” (21 CFR Part 314 Subpart I).**

Because smallpox has been eradicated, the effectiveness of anti-variola (VARV) agents cannot be demonstrated in human clinical trials.

According to the Animal Rule, “FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- (1) there is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
- (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;
- (3) the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity;
- (4) the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.”

### **“There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product.”**

#### **The pathophysiological mechanism of variola virus toxicity is reasonably well-understood.**

Smallpox is a highly infectious disease of humans with a significant rate of mortality. The etiological agent of smallpox is variola virus. Infection occurs via the upper respiratory tract from oropharyngeal secretions or other viral shedding of an infected individual. Following infection, limited viral replication during the incubation period produces a primary viremia

which infects cells of the reticuloendothelial system. Viral replication in these cells produces a secondary viremia that results in onset of clinical signs and symptoms including fever beginning approximately 12 days after infection (Bremant-2002). During the secondary viremia, macrophage-associated virus is deposited in the capillaries of epithelial tissues where local viral replication and immune response result in lesions in the respiratory tract and skin. Oral lesions typically begin to develop 1 to 2 days after fever with skin lesions following approximately 1 day later. Death in fatal cases of ordinary smallpox occurs approximately 22 to 28 days after infection.

**CMX001 and Cidofovir deliver the same active antiviral agent, cidofovir-diphosphate.**

CMX001 is a Lipid-Antiviral-Conjugate (LAC) that delivers high intracellular concentrations of the active antiviral agent, cidofovir diphosphate (CDV-PP). In contrast to cidofovir, CMX001 is given orally and due to its differing pharmacokinetic profile, there is no indication of cidofovir-like, dose-limiting renal toxicity based on data obtained from more than 500 patients treated to date. CMX001 is not a prodrug because it remains intact in plasma resulting in different absorption and distribution profiles compared with cidofovir.

**The mechanism of the prevention of variola virus toxicity by CMX001 is known.**

CMX001 is substantially more active in vitro than CDV against many dsDNA viruses including herpesviruses, adenoviruses, polyomaviruses and orthopoxviruses. For orthopoxviruses, the enhancement in activity (determined as the ratio of the  $EC_{50}$  CDV/ $EC_{50}$  CMX001) ranges from 24-fold for ectromelia virus to 271-fold for variola virus. The increased activity of CMX001 relative to CDV is attributable to the more efficient cellular uptake of CMX001 facilitated by the lipid chain in combination with conversion to the active antiviral cidofovir-diphosphate (CDV-PP). The net effect is that more active antiviral is produced intracellularly with lower systemic exposure to parent drug. The broad spectrum activity of CMX001 against various species of orthopoxviruses was anticipated based on the mechanism of action of the drug (inhibition of the virally encoded polymerase by CDV-PP) and the high level of homology for this enzyme seen within the family. The amino acid sequences of the catalytic subunit of the polymerase for orthopoxviruses have sequence identity ranging from 98.2% to 99.1%. Given the conservation of this region, it is not surprising that resistance to CDV-PP is slow to develop for orthopoxviruses, requires multiple mutations for high level resistance, and is associated with decreased viral fitness.

**“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.”**

**The RPXV and ECTV models are robust, reproducible, naturally permissive host models of smallpox.**

To date, CMX001 has been administered to 128 rabbits in 9 studies in the rabbitpox (RPXV) model, 813 mice in 21 studies in the ectromelia (ECTV) model, and 18 cynomolgus monkeys in 2 studies in the monkeypox (MPXV) model. Cidofovir has also been used in MPXV and VARV studies and these are relevant to CMX001 because both are analogized to the same active antiviral. Seventeen cynomolgus monkeys in 4 studies were administered CDV for treatment of

MPXV and 9 monkeys in 1 study were given CDV for treatment of variola (VARV), and based on these data, CDV is currently in the Strategic National Stockpile.

Rabbits and mice are naturally permissive hosts for RPXV and ECTV, respectively, developing severe disease resulting in a high rate of mortality following infection with an inoculum that approximates natural infection. These models are well-characterized and reproducible, producing robust data that are appropriate for studying the efficacy of potential smallpox therapeutics. In particular, studies in the RPXV model conducted at the University of Florida (Richard Moyer, Investigator) were well powered for meaningful statistical analysis of pre-determined endpoints and were randomized, blinded and placebo-controlled, providing the most robust assessment possible of the efficacy of CMX001 in an animal model of smallpox. This model is proven reproducible, having recently been successfully transferred from the University of Florida to Battelle Memorial Institute (Battelle) in Columbus, OH. A study at Battelle confirmed the lethal inoculum of RPXV stock obtained from the Moyer lab and showed clear dose-dependent mortality of a new GLP-compliant viral stock prepared by Southern Research Institute (SRI) from virus obtained from the Moyer lab. The data show the model is robust and reproducible at different sites in experiments conducted by independent study personnel.

The viruses used in the RPXV and ECTV models are highly similar to VARV. All of the polymerase subunits of RPXV, ECTV, VACV and VARV are 1005 residues in length, completely overlap, and have sequence identity ranging from 98.2% to 99.1%. This level of similarity in the target enzyme of CMX001 supports the choice of these viruses for the animal model studies required under the “Animal Rule” to establish the efficacy of CMX001 for the treatment of smallpox.

**The MPXV and VARV cynomolgus monkey models are not naturally permissive host models, and due to species-specific metabolism, monkeys are not a relevant species for evaluating the efficacy of CMX001.**

There are two models of smallpox utilizing cynomolgus monkeys: One using intravenous infection with MPXV and one using intravenous infection with VARV. The pathological manifestations of the disease produced when monkeypox (MPXV) or VARV are intravenously inoculated in cynomolgus monkeys includes fever, dermal erythema, and centrifugally distributed lesions ([Huggins-2008](#), [Jahriling-2004](#)). These models differ from smallpox by the route of infection, the high inoculum required to produce lesional disease and, most importantly, the abbreviated prodromal period since the large inoculums are placed intravenously, essentially bypassing the primary viremia stage of human disease. Because these models bypass the primary viremia stage of human smallpox, they are not ideal for evaluating smallpox antivirals whose mechanism of action is through inhibition of viral replication, such as CMX001.

Another reason why these two models are not suitable for evaluating the efficacy of CMX001 is the uniquely high degree of metabolism of CMX001 in monkeys relative to humans and other laboratory animal species. In two studies conducted at USAMRIID using the MPXV model, all animals infected with MPXV died and there was no survival benefit associated with treatment with CMX001. Therefore, because of the rapid and extensive metabolism of CMX001 in monkeys, cidofovir (CDV) will be used as a surrogate for studies conducted in monkeys. This is possible, and appropriate, because CMX001 and cidofovir are anabolized in target cells to the same active antiviral, cidofovir-diphosphate. Because CDV is administered intravenously and

excreted unchanged, the studies cannot be confounded by species-related differences in absorption or metabolism. Importantly, in these controlled laboratory experiments, measures can be undertaken to minimize the impact of potential nephrotoxicity. Because the viral kinetics of infection caused by introducing a large quantity of virus by the intravenous route differ so greatly from natural infection, the fact that the pharmacokinetics of drug delivered intravenously differ from that delivered orally is not expected to have a meaningful impact on the interpretation of the results or the relevance to treatment of smallpox using orally-administered CMX001.

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

**CMX001 prevents mortality in the RPXV and ECTV models of smallpox; successful treatment with CMX001 can be initiated at detection of lesions in rabbits.**

An Integrated Summary of Efficacy (ISE), requested by FDA, was prepared to assess the homogeneity of treatment effect (i.e., survival proportion in the CMX001 treatment arm minus survival proportion in the placebo arm) across studies in a given set of treatment arms, as well as the overall mean treatment effect. Because of the disparate nature of the animal models (i.e., differing pathogenesis) separate meta-analyses were conducted for each model. In order to harmonize data obtained using different treatment regimens within each model, two dose subgroups, “low” and “high” were identified based on a standardized dose (total amount of drug administered in the regimen divided by the number of days in the longest treatment regimen). Similarly, two “time to treatment initiation” subgroups were identified for each model, early ( $\leq 3$  days post-infection) and late ( $> 3$  days post-infection). “Early” treatment in the models was generally at a time prior to the appearance of signs of infection, while “late” treatment initiation corresponds with treatment initiating after the outward appearance of clinical signs, such as lesions, fever and/or weight loss.

The major findings from this analysis were that, for both RPXV and ECTV, both low and high dose CMX001 subgroups displayed a survival benefit, a relevant primary endpoint under the “Animal Rule,” compared with placebo whether treatment was initiated “early” or “late”. The data revealed that an unadjusted initial dose of 20 mg/kg beginning after onset of clinical signs of disease provided a survival benefit in both the ECTV and RPXV models, with or without subsequent doses (although the survival benefit improved when subsequent doses were administered).

Comparing CMX001 pharmacokinetic parameters determined using plasma concentration data from mice, rabbits and humans, anticipated human exposures at or below doses presently under study for other dsDNA viruses (CMV and AdV) exceed the exposures at effective doses in the ECTV and RPXV models. The systemic exposure (AUC) following administration of a successful 20 mg/kg dose in mice and rabbits was 50% or less than the exposure in humans administered a dose of 1.5 mg/kg, or approximately 100 mg in a 70 kg adult. This dose has shown antiviral activity against AdV and CMV in patients and is currently being evaluated for treatment of a range of dsDNA viruses in clinical studies including randomized, placebo controlled trials.

**Completed MPXV and VARV studies in monkeys demonstrated the efficacy of CDV and provide proof of concept for treatment of human smallpox with CMX001 because both are metabolized to the same active antiviral, CDV-PP.**

Multiple studies have already been completed that demonstrate the efficacy of CDV for treatment of MPXV in the cynomolgus monkey model (Huggins-2004). In a representative study, MPXV-infected monkeys were administered 3 doses of 20 mg/kg CDV on Days 1, 6 and 11 post infection. There was a statistically significant ( $p=0.01$ ) increase in survival, a relevant primary endpoint under the “Animal Rule,” in the CDV-treated group (7 of 8 survived) compared with the placebo control group (1 of 8 survived) (Huggins-2004). Lesion counts and viremia were also statistically-significantly reduced in the CDV-treated group relative to the placebo control animals. Similarly, lesion counts and viremia were reduced in the VARV model (Huggins-2004) with a similar treatment regimen initiated on Days 0, 1, or 2 post infection. These results indicate that CDV is effective in the MPXV and VARV models in cynomolgus monkeys and suggest that additional studies in these models may not be informative, or even appropriate, given current limitations associated with the MPXV and VARV models, among them:

1. Risks to laboratory personnel working with highly pathogenic VARV and MPXV viruses
2. Risks to laboratory personnel due to viruses endemic in the study animal population (e.g., Herpes B)
3. Variability in mortality necessitates the use of large numbers of non-human primates to achieve acceptable statistical power
4. Facility limits on the number of monkeys that can be accommodated with BSL-4 containment prevents the conduct of a single well-powered study, instead requiring that data from multiple studies be pooled, further weakening statistical power.
5. Difficulty ensuring data integrity (i.e., compliance with Good Laboratory Practice Regulations) in a BSL-4 laboratory environment

**“The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.”**

**CMX001 plasma concentrations achieved in humans exceed those produced by efficacious doses in multiple animal models of smallpox.**

Overall, the data obtained in mouse and rabbit models of smallpox utilizing CMX001, the data obtained in the MPXV and VARV models utilizing CDV, and the data supporting the effectiveness of CMX001 against other dsDNA viruses in humans, supports the conclusion that CMX001 is likely to be efficacious against smallpox when treatment is initiated after the appearance of clinical signs of disease. The data also show that systemic exposures (AUC), an endpoint that traditionally correlates with efficacy, produced by efficacious doses of CMX001 in the ECTV and RPXV models were lower than those achieved in humans given a dose of 100 mg. Preliminary analysis from an ongoing expanded access study of CMX001 (Study CMX001-350) demonstrated the potential of 100 mg CMX001 administered twice weekly to control CMV and AdV infection. In addition, this dose is also currently being evaluated in 2 randomized, placebo controlled clinical trials for CMV and AdV (Studies CMX001-201 and CMX001-202), which

may provide direct evidence of antiviral activity in the clinic. Taken together, the animal efficacy and human clinical data support the conclusion that a dose of 100 mg (approximately 1.5 mg/kg) administered twice weekly may be efficacious against smallpox in humans.

**The human dose of CMX001 for treatment of smallpox will be identified using data from controlled clinical studies of other dsDNA viruses and PK parameters in relevant animal models.**

Chimerix has accumulated clinical data for CMX001 in a variety of dsDNA viral infections through controlled clinical trials and open label expanded access programs. The viral infections have included adenovirus (AdV) and multiple herpesviruses (e.g., CMV, EBV, HSV), among others. Although these viruses are genetically distinct from VARV, their pathogenesis is similar to human smallpox and they all contain a viral DNA polymerase. AdV and CMV infections in particular share many features with variola that provide a reasonable basis to expect that a dose of CMX001 which is efficacious against these viruses will also be efficacious against variola as described below. Efficacy against other dsDNA viral infections provides additional confidence for dose selection for a smallpox treatment.

The antiviral target of CMX001 against smallpox is the viral DNA polymerase. The EC<sub>50</sub> for CMX001 against VARV in cell culture is 0.1 μM. Like VARV, AdV encodes a dedicated DNA polymerase that is responsible for replicating the viral genome. The antiviral target of CMX001 against AdV is the viral DNA polymerase. The median EC<sub>50</sub> value for CMX001 against AdV is 0.02 μM. CMV also encodes a dedicated DNA polymerase that is responsible for replicating the viral genome. The antiviral target of CMX001 against CMV is the viral DNA polymerase. The median EC<sub>50</sub> value for CMX001 against CMV is 0.001 μM.

As described above, systemic exposure in humans given 100 mg were higher than those produced by doses that prevented mortality in the mouse ECTV and rabbit RPXV models. As scaling based on plasma PK parameters is the conventional means employed to scale from animals to humans, these data alone would be expected to provide a reasonable estimate of the effective clinical dose for treatment of smallpox. In addition, Chimerix has now accumulated data on the treatment of multiple viral infections caused by dsDNA viruses. These data show that the likely effective and tolerable dose and regimen for treatment of this wide range of infections is highly conserved. Hence, it is reasonable to expect that the dose and regimen effective for treatment of CMV and AdV, which will be demonstrated in randomized, placebo-controlled, pivotal efficacy studies, is the optimal dose and regimen for treatment of smallpox. Finally, if necessary, Chimerix will use CDV-PP concentration data from monkeys treated with CDV to estimate the dose and regimen needed to produce similar concentration of CDV-PP in humans administered CMX001. In addition, the feasibility of other pharmacokinetic modeling techniques to scale from data obtained using CDV in the MPXV and VARV models to estimate a therapeutic dose of CMX001 for smallpox is currently being explored.

## **2. DEVELOPING SMALLPOX ANTIVIRALS UNDER THE “ANIMAL RULE”**

Because smallpox has been eradicated, the effectiveness of anti-VARV agents cannot be demonstrated in human clinical trials. Animal models of orthopoxvirus infections are the only means by which to establish the efficacy of potential treatments for smallpox. Suitable models utilizing variola virus, however, are very limited due to the narrow host range of the virus and consequently the inability to duplicate pathogenesis of smallpox disease in non-permissive host species.

The Animal Rule (21CFR Part 314 Subpart I) is the regulatory pathway to approval for drug products for which “Definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers...; and field trials to study the product’s effectiveness after an...exposure [are] not feasible.”

According to the Animal Rule, “FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- “(1) there is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
- (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;
- (3) the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity;
- (4) the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.”

Each of these points is discussed below in the context of the program to develop CMX001 under the Animal Efficacy Rule.

### **3. THERE IS A REASONABLY WELL-UNDERSTOOD PATHOPHYSIOLOGICAL MECHANISM OF THE TOXICITY OF THE SUBSTANCE AND ITS PREVENTION OR SUBSTANTIAL REDUCTION BY THE PRODUCT**

#### **3.1. Pathophysiological Mechanism of Toxicity of Variola Virus**

Smallpox is a highly infectious disease of humans with a significant rate of mortality. The etiological agent of smallpox is variola virus. Infection occurs via the upper respiratory tract from oropharyngeal secretions or other viral shedding of an infected individual. Following infection, limited viral replication during the incubation period produces a primary viremia which infects cells of the reticuloendothelial system. Viral replication in these cells produces a secondary viremia that results in onset of clinical signs and symptoms including fever beginning approximately 12 days after infection (Bremner-2002). During the secondary viremia, macrophage-associated virus is deposited in the capillaries of epithelial tissues where local viral replication and immune response result in lesions in the respiratory tract and skin. Oral lesions typically begin to develop 1 to 2 days after fever with skin lesions following approximately 1 day later. Death in fatal cases of ordinary smallpox occurs approximately 22 to 28 days after infection. The death rate is linked to the viral load as reflected by the degree of confluency of the lesions. For example, historical data show that the case-fatality rate for ordinary type smallpox was 62% when lesions were confluent, 37% when semiconfluent, and 9.3% when discrete. In addition, the highly lethal forms of smallpox, such as hemorrhagic type with a case-fatality rate of 96%, were characterized by very high levels of viremia (Fenner-1988). Therefore, the level of viral burden is most likely the key factor influencing the toxicity of VARV.

The cause of death from smallpox has been attributed to toxemia, the release of toxic substances into the blood (Fenner-1988). One review of published smallpox case series concluded that death was the result of cytopathic effects of the virus resulting in “renal failure, shock secondary to volume depletion, and difficulty with oxygenation and ventilation as a result of viral pneumonia and airway compromise” (Martin-2002). More recently, uncontrolled inflammation resulting from a “cytokine storm” has been postulated as a factor in smallpox mortality (Jahrling-2004). Therefore, variola virus toxicity is likely caused by high viral load and the resulting cytopathic effects of viral replication, release of toxic substances from viral replication and dead cells, and deleterious effects of the immune response to these events.

#### **3.2. Mechanism of Action of CMX001 to Prevent the Toxicity of Variola Virus**

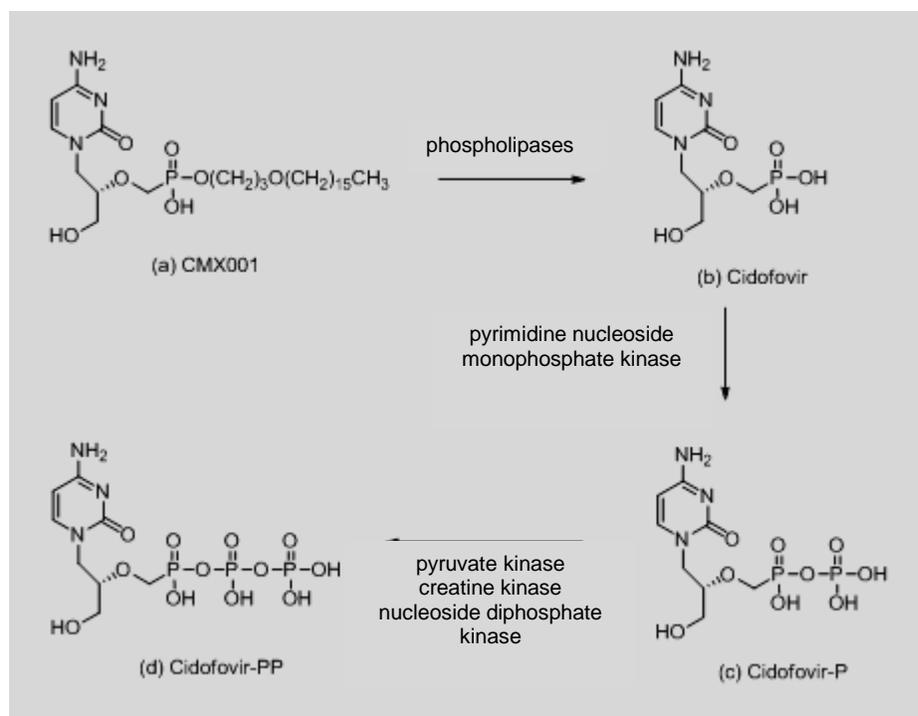
CMX001 is a lipid conjugate of the nucleotide analog CDV. CMX001 efficiently enters cells, where the lipid moiety is cleaved to form cidofovir. Cidofovir is then phosphorylated to its diphosphate form which mimics a nucleoside triphosphate and acts as a selective inhibitor of VARV DNA polymerase. By inhibiting DNA polymerization and thus viral replication, CMX001 reduces the viral burden of the host. Because the lethality of smallpox requires viral

replication to achieve a high viral load, CMX001 is expected to improve survival rates and reduce morbidity associated with smallpox through prevention of VARV replication.

### 3.2.1. Mechanism of Action: Highly Efficient Delivery of Cidofovir to the Intracellular Compartment

CMX001 (Figure 1) is formed by conjugating a lipid, 3-hexadecyloxy-1-propanol, to the phosphonate moiety of CDV. Once CMX001 is inside cells, CDV is liberated by phospholipase cleavage of the lipid ester linkage and activated by two successive phosphorylations, first to cidofovir monophosphate (CDV-P) and then to cidofovir diphosphate (CDV-PP) [Cihlar-1996]. The CDV-PP acts as a competitive, alternative substrate inhibitor of the DNA directed DNA polymerases encoded by the herpesvirus, adenovirus and orthopoxvirus families of double-stranded DNA viruses (dsDNA) [Chou-2003, Kinchington-2002, Magee-2008, Xiong-1997].

**Figure 1: CMX001 Cleavage and Anabolism by Host Enzymes**

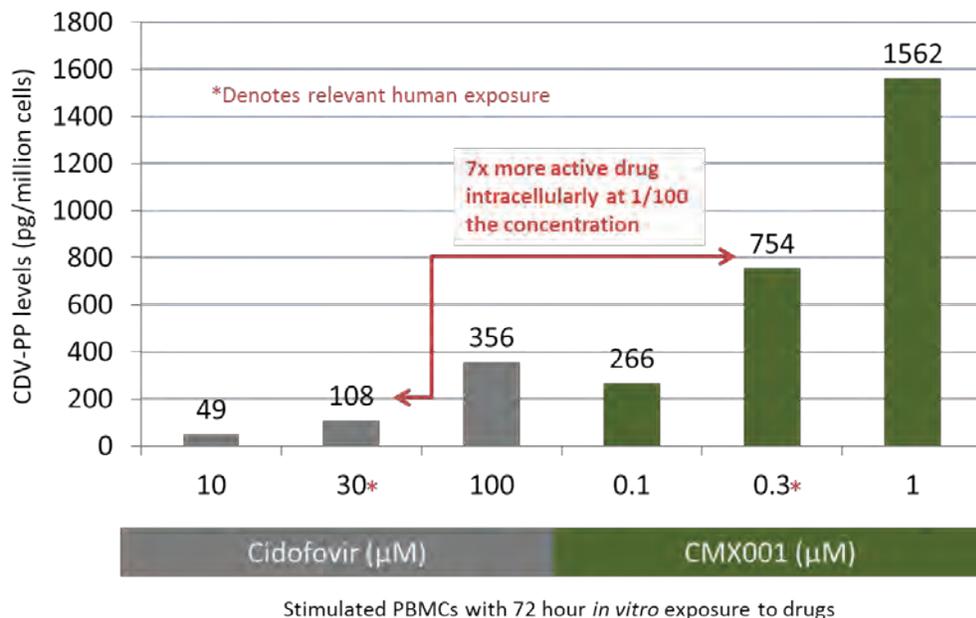


CDV is efficiently incorporated into nascent chain DNA by viral DNA polymerases resulting in significant reductions in the overall rate of viral DNA synthesis. The incorporation of a single molecule of CDV into a synthetic DNA primer by human CMV (HCMV) DNA polymerase causes the rate of DNA synthesis to decrease by 31% while the incorporation of two consecutive CDV molecules stops any further DNA elongation.

In vitro assays with purified vaccinia virus (VACV) DNA polymerase show that CDV is incorporated into nascent chain DNA opposite guanine in the template and the resultant chain is terminated at the next nucleotide ( $n + 1$ ) position (Evans-2004, Xiong-1997, Xiong-1996). Specific studies with the AdV E2 polymerase have not been conducted to date, but resistance studies with CDV have indicated this enzyme is the target for the active antiviral CDV-PP (Gordon-1996, Romanowski-2001, Kinchington-2002).

The increased antiviral efficacy of CMX001 is attributed to higher intracellular levels of the active anabolite, CDV-PP as shown in Figure 2. At physiologically relevant concentrations of CDV and CMX001 (30 and 0.3 $\mu$ M respectively), approximately 7-fold more active antiviral was produced by CMX001 despite being used at 1/100th the concentration of CDV.

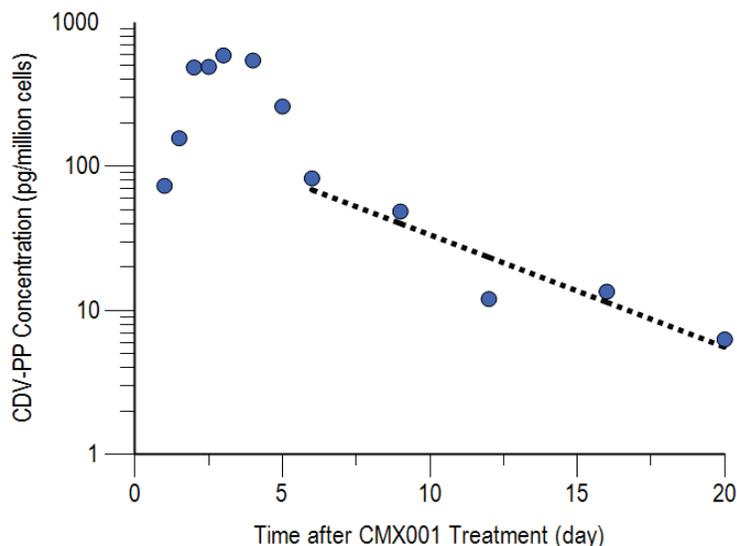
**Figure 2: Concentrations of CDV-PP ( $\mu$ M) in Human PBMCs after In Vitro Incubation with CMX001 or CDV**



The half-life of CMX001 and its metabolites, cidofovir (CDV) and cidofovir diphosphate (CDV-PP), were assessed *in vitro* using activated human peripheral blood mononuclear cells (PBMCs) in cell culture. PBMCs were isolated from healthy donors, stimulated with phytohemagglutinin (PHA)/interleukin-2 (IL-2) and incubated with CMX001 at 0.5  $\mu$ M for 1 hour or 48 hours. The PBMCs were harvested at various times to determine the intracellular concentrations of CMX001, CDV, and CDV-PP. Samples were analyzed using an LC/MS/MS method to measure intracellular concentrations of CDV-PP. The CDV-PP terminal elimination phase constant ( $\lambda_z$ ) was estimated by best-fit line (the line with highest R<sup>2</sup> value) to the last three or more time points (Figure 3).

The terminal half-life of the active metabolite, CDV-PP, was 3.9 and 6.5 days when incubation with CMX001 was for 48 hours or 1 hour, respectively.

**Figure 3: Concentration of CDV-PP in Human PBMCs after In Vitro Incubation with CMX001 for 48 Hours**



### 3.2.2. CMX001 is Active Against Viruses in All Five Families of dsDNA Viruses

CMX001 is active in vitro against a broad range of viruses from all five families of dsDNA viruses infecting humans, including multiple species of orthopoxviridae. As shown in [Table 1](#), conjugation of the lipid to the phosphonate moiety of CDV to produce CMX001 results in significant decreases in apparent EC<sub>50</sub> values relative to those observed for CDV. In the case of the orthopoxviruses, the enhancement in activity (determined as the ratio of the EC<sub>50</sub> CDV/EC<sub>50</sub> CMX001) ranges from 271-fold for variola virus to 24-fold for ectromelia virus. The increased activity of CMX001 relative to CDV is attributable to the more efficient cellular uptake of CMX001 facilitated by the lipid chain (passive diffusion and possibly flipase activity) ([Painter-2004](#)). CDV is transported into cells by a relatively inefficient method, fluid phase endocytosis ([Connelly-1993](#)).

The broad spectrum activity of CMX001 against various species of orthopoxviruses was anticipated based on the mechanism of action of the drug, inhibition of the virally encoded polymerase by CDV-PP, and the high level of homology for this enzyme seen within the orthopoxvirus family. The amino acid sequences of the catalytic subunit of the polymerase have been aligned for cowpox virus (CPXV, strain Brighton Red), ECTV (strain Moscow), MPXV, (strain Zaire 1979-005), VACV (strains 3737 and Western Reserve), RPXV (strain Utrecht) and VARV (strains Bangladesh 1975 and India 7129). Sequences were obtained from Poxvirus Bioinformatics Resource Center (Poxvirus.org) and aligned with the NCBI Protein Blast program, BLASTP 2.2.24, [Altschul 1997](#)). All of the polymerase subunits are 1005 residues in length, completely overlap, and have sequence identity ranging from 98.2% to 99.1%. This level of similarity in the target enzyme of CMX001 supports the use of surrogates for VARV in the animal model studies required under the “Animal Rule” to establish the efficacy of CMX001.

**Table 1: The In Vitro Activity of CMX001 and CDV Against Viruses in All Five Families of dsDNA Viruses Known to Cause Human Morbidity and Mortality.**

<b>Viral Class</b>	<b>Virus (ref)</b>	<b>CMX001 EC<sub>50</sub> (µM)</b>	<b>CDV EC<sub>50</sub> (µM)</b>	<b>Enhanced Activity (EC<sub>50</sub> CDV/EC<sub>50</sub> CMX001)</b>
Orthopoxvirus	VARV (Huggins 2002)	0.1	27.3	271
	VACV (Kern 2002)	0.8	46	57
	ECTV (Buller 2004)	0.5	12	24
	RPXV (Prichard 2010)	0.5	39	78
	MPXV (Huggins 2002)	0.07	4.6	65
Adenovirus	AdV 5 (Hartline 2005)	0.02	1.3	65
Herpesvirus	HSV 1 (Williams-Aziz 2005)	0.06	15	250
	HHV 6 (Williams-Aziz 2005)	0.004	0.2	50
	CMV (Williams-Aziz 2005)	0.0009	0.38	422
	VZV (Williams-Aziz 2005)	0.0004	0.5	1250
	EBV (Williams-Aziz 2005)	0.04	>170	>4250
Papillomavirus	HPV 11 (Christensen 2006)	17	200	12
Polyomavirus	BKV (Randhawa 2006)	0.13	115.1	885
	JCV (Jiang 2010)	0.045	nd	n/a

nd: not determined; n/a: not applicable;

### **3.2.3. Resistance Data**

#### **3.2.3.1. Development of Resistance to CDV in VACV Leads to a Significant Attenuation of Virulence**

In vitro experiments to generate orthopoxvirus strains resistant to CDV have been conducted and cross-resistance to CMX001 examined. Because CDV and CMX001 produce the same active antiviral intracellularly, CDV-PP, it is expected that their resistance profiles will be similar. Serial passage studies of camelpox, CPXV, MPXV and VACV viruses with CDV have all generated resistant strains ([Smeets-2002](#)). Resistant strains of VACV (strain WR) generated after 20 to 30 passages in the presence of increasing concentrations of CDV have also been shown to be cross resistant to CMX001 ([Kornbluth 2006](#)).

Twelve mutations in the polymerase domain of VACV have been associated with phenotypic resistance to CDV as shown in [Table 2](#). The specific roles of these mutations in CDV resistance have not been uniformly elucidated and some appear to be secondary; however, 11/12 of the sites involved are completely conserved among representative members of the orthopoxviruses suggesting the data obtained in VACV can be extrapolated to other members of this family, including VARV. This observation is in accord with the overall high level of sequence homology observed across the orthopoxviruses.

As shown by the data in [Table 2](#) below, the development of resistance to CDV and CMX001 in VACV leads to a significant attenuation of virulence in mice ([Andrei-2006](#)), suggesting that any mutants that might become dominant from drug pressure during treatment of smallpox would be attenuated and less virulent. No resistance to CMX001 was detected in the clinic following treatment of VACV with CMX001 in conjunction with other therapies ([Lederman-2009](#), [Lederman-2011 \[in preparation\]](#)).

In summary, resistance of VACV to CDV/CMX001:

1. Maps to E9L polymerase gene of VACV
2. Requires multiple passages
3. Requires 2 or more mutations
4. Increases EC50 8-27 fold
5. Reduces virulence in vivo 10-30 fold.

**Table 2: Mutations in the Polymerase Gene of VACV Associated with CDV Resistance.**

Mutation(s)	CDV Fold Resistance	Author	Virulence in Mice
ΔK174	3 to 4	Becker 2008	Reduced
A314T	5 to 7	Andrei 2006	Reduced
A314V	7	Becker 2008	Reduced
A684V	3 to 9	Andrei 2006; Gammon2008	Reduced
S851Y	2 to 3	Gammon 2008	Reduced
H296Y/S338F	12	Kornbluth 2006	nd
A314T/A684V	11 to 15	Andrei 2006	Reduced
A314T/T688A	11 to 17	Andrei 2006	Essentially non-virulent
A684V/S851Y	7 to 16	Gammon 2008	Reduced
ΔK174/M671I	4 to 5	Becker 2008	Reduced
A314T/A684V/ Y232H	25	Andrei 2006	nd
H296Y/A314V/ H319N/S338F/ R604S	11 to 14	Kornbluth 2006	Reduced

Amino acid numbering is according to VACV strain WR DNA polymerase (GenBank accession No. P06856).  
nd: not done

### **3.3. Conclusion Regarding Anti-Viral Activity and Resistance Profile of CMX001**

CMX001 is substantially more active in vitro than CDV against many dsDNA viruses including herpesviruses, adenoviruses, polyomaviruses and orthopoxviruses. For orthopoxviruses, the enhancement in activity (determined as the ratio of the EC50 CDV/EC50 CMX001) ranges from 271-fold for variola virus to 24-fold for ectromelia virus. The increased activity of CMX001 relative to CDV may be due to the more efficient cellular uptake of CMX001 facilitated by the lipid chain in combination with conversion to the active antiviral cidofovir-diphosphate (CDV-PP). The net effect is that more active antiviral is produced with less systemic exposure to parent drug. The broad spectrum activity of CMX001 against various species of orthopoxviruses was anticipated based on the mechanism of action of the drug (inhibition of the virally encoded polymerase by CDV-PP) and the high level of homology for this enzyme within the family. The amino acid sequences of the catalytic subunit of the polymerase for orthopoxviruses have sequence identity ranging from 98.2% to 99.1%. Given the conservation of this region it is not surprising that resistance to CDV is slow to develop for orthopoxviruses, requires multiple mutations for high level resistance, and is associated with decreased viral fitness. Therefore, the mechanism of prevention of toxicity of variola virus by CMX001 is reasonably well understood, which meets this aspect of the Animal Rule.

**4. 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**

One of the key criteria for the application of this rule is that the effect is demonstrated in more than one 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. However, variola virus naturally infects only humans, and experimental infection of other species (including monkeys, which is the only model available utilizing variola virus infection) is forced. Animal models using related orthopoxviruses produce diseases with similarities to smallpox, but the pathogenesis varies depending on the animal (host) species, the characteristics of the infecting virus, and the route of infection. Consequently, it is clear that efficacy will have to be examined in multiple models.

Efficacy studies in the RPXV and ECTV models are particularly relevant to human disease because these hosts are naturally permissive for the respective viruses much like humans are permissive for variola virus. In the RPXV and ECTV models, a low inoculum ( $\leq 100$  PFU) results in severe disease and high incidence of mortality. Similarly, smallpox is thought to result from a low inoculum, on the order of 100 PFU (Franz-2001). Results of CMX001 testing in these models are presented below.

Since 2003, CMX001 has been evaluated in 53 studies in animal models of smallpox including 12 RPXV studies in New Zealand White rabbits, 22 ECTV studies in strain A/NCr mice, 3 ECTV studies in strain SKH-1 mice, 1 ECTV study in C57BL/6 mice, 9 cowpox virus studies in BALB/c mice, 3 VACV studies in BALB/c mice, 1 MPXV study in dormice, and 2 MPXV studies in cynomolgus monkeys. In addition, the efficacy of CDV has been studied in both the MPXV and VARV models in cynomolgus monkeys and these studies are relevant to CMX001 because both drugs are analogized to the same active antiviral. Hence, 4 MPXV and 1 VARV studies in cynomolgus monkeys have also been conducted.

In 2011, FDA requested an integrated analysis and summary of efficacy for all studies of CMX001 completed in the mouse ECTV and VACV models and the rabbit RPXV model of human smallpox. The resulting meta-analysis examined data from 1165 animals in 33 studies administered CMX001 for treatment of poxvirus infection. This included, 128 rabbits in 9 studies in the RPXV model in the laboratory of Dr. Richard Moyer at the University of Florida, 813 mice in 21 studies in the ECTV model in the laboratory of Dr. Mark Buller at St. Louis University, and 224 mice in 3 studies in the VACV model conducted in the laboratory of Dr. Earl Kern at The University of Alabama at Birmingham.

Although the MPXV and VARV models have the limitations described in Sections 4.3.1 and 4.3.2 (specifically, the requirement for a very high inoculum to achieve severe disease and high mortality, and species-specific metabolism of CMX001), we also present results of testing CMX001 and CDV in MPXV and VARV models.

A brief description of each model follows and the key features of these models as related to human smallpox are shown in Table 3 below.

**Table 3: Characteristics of Proposed Animal Models of Smallpox Versus Human Disease**

	<b>Mousepox</b>	<b>Rabbitpox</b>	<b>Smallpox</b>	<b>NHP Models</b>
Naturally permissive host	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>No</b>
Estimated LD50 (PFU)	<b>~ 0.3 PFU</b>	<b>~ 10 PFU</b>	<b>~ 100 PFU<sup>1</sup></b>	<b>10<sup>6</sup>-10<sup>9</sup> PFU</b>
Route of infection	respiratory (intranasal, aerosol)	intradermal	respiratory	intravenous
Typical endpoints; signs and symptoms	<ul style="list-style-type: none"> <li>• mortality</li> <li>• viremia</li> <li>• viral load in tissues</li> <li>• weight loss/gain</li> </ul>	<ul style="list-style-type: none"> <li>• mortality</li> <li>• fever</li> <li>• lesions</li> <li>• viremia</li> <li>• respiratory distress</li> <li>• ocular/nasal secretions</li> </ul>	<ul style="list-style-type: none"> <li>• mortality</li> <li>• fever</li> <li>• lesions</li> <li>• viremia</li> </ul>	<ul style="list-style-type: none"> <li>• mortality</li> <li>• fever</li> <li>• lesions</li> <li>• viremia</li> </ul>
Major body systems/organs involved <sup>2</sup>	<ul style="list-style-type: none"> <li>• respiratory tract</li> <li>• lymphoid</li> <li>• skin</li> <li>• liver</li> <li>• spleen</li> </ul>	<ul style="list-style-type: none"> <li>• respiratory tract</li> <li>• lymphoid</li> <li>• skin</li> <li>• liver</li> <li>• spleen</li> <li>• kidney</li> <li>• gonads</li> </ul>	<ul style="list-style-type: none"> <li>• respiratory tract</li> <li>• lymphoid</li> <li>• skin</li> <li>• liver</li> <li>• spleen</li> <li>• kidney</li> <li>• testes</li> </ul>	<ul style="list-style-type: none"> <li>• respiratory tract</li> <li>• lymphoid</li> <li>• skin</li> <li>• liver</li> <li>• spleen</li> <li>• kidney</li> <li>• testes</li> </ul>
Lethality (average day of death)	~100% (6 – 12 days);	~ 100% (8 days)	~ 30% (22 – 28 days)	MPXV ~ 100% (~ 12 days) VARV ~ 30% (~ 12 days)

<sup>1</sup> An LD50 is not known for smallpox. The infective dose is estimated to be 10 to 100 PFU and the historical mortality rate is typically cited as 30%, but varied considerably. The mortality rate in a naive population may be much higher.

<sup>2</sup> Virus is detected in virtually all tissues. Examples of the most affected tissues are listed.

## 4.1. Rabbitpox Virus Model

### 4.1.1. RPXV Infection in Rabbits

The rabbitpox model is a recognized, well characterized model of smallpox (Adams-2007). The model employs intradermal inoculation of New Zealand White rabbits with RPXV and

reproduces numerous disease characteristics of smallpox including a low titer viral inoculum to achieve severe disease, fever, pox lesions, respiratory distress, and a high rate of mortality.

Both VARV, the causative agent of smallpox, and RPXV are orthopoxviruses that share many characteristics. RPXV is a host adapted subspecies of vaccinia virus which causes a disease in rabbits that mimics smallpox in humans.

New Zealand White (NZW) rabbits are highly sensitive to intradermal RPXV challenges (LD50 estimated at 10 PFU) (Adams-2007). Infection with a virus titer of approximately 100 PFU produces a very high mortality rate with a mean day of death approximately Day 8. As with other orthopoxviruses, the virus initially replicates at the infection site and then infects the lymphoid tissues (reticuloendothelial system). A secondary viremia, then further disseminates the infection. Even with intradermal inoculation, the respiratory tract is highly involved in the disease, and like smallpox in humans, rabbits infected with RPXV transmit the infection to uninfected animals via aerosolized respiratory secretions, the only model of smallpox in which this is known to occur. Infectivity in rabbits occurs synchronously with rash, similar to human smallpox.

Intradermal inoculation was chosen because it results in a high mortality rate with a low inoculum and a more reliable occurrence of ear lesions than intranasal inoculation. In some studies, the onset of ear lesions was used as a trigger for treatment initiation, thus reliable ear lesions was an advantage. Disease progression is characterized by swelling of the primary inoculation site on Day 2. Other constitutional signs of disease including fever and secondary lesions (remote from the inoculation site) begin to appear from Day 3 to 5 post-infection, typically with small lesions appearing first on the ears but also in other mucocutaneous tissues (e.g. eyelids, nose, mouth, genitalia). Lesions also occur on the neck and body of some animals (especially with high inoculum levels), although obscured by fur. By day 7-8 most animals have substantially decreased resting respiration, labored breathing and nasal discharge. Necropsy reveals lung hemorrhages over large areas (Adams-2007). Animal welfare act regulations require establishment and adherence to predetermined euthanasia criteria, therefore, unlike studies in mice, rabbits cannot be allowed to die naturally in this model. Hence, Day 8 is the mean day of euthanasia when inoculated with 100 PFU of RPXV (approximately 10-fold the LD50).

The pathological manifestations of the disease produced when RPXV is intradermally inoculated in New Zealand White rabbits resembles human smallpox in several important aspects, including an incubation phase, constitutional signs, fever, secondary lesions, respiratory involvement, and generalized viremia. The most obvious difference between RPXV in rabbits and smallpox in humans is the shorter duration of RPXV disease in rabbits. In humans with ordinary smallpox, the onset of constitutional signs and lesions typically occurs near the midpoint of the disease (Day 12 for fever and Day 14 for lesions) and death (approximately 30% historically) occurs typically between 22 and 28 days post infection.

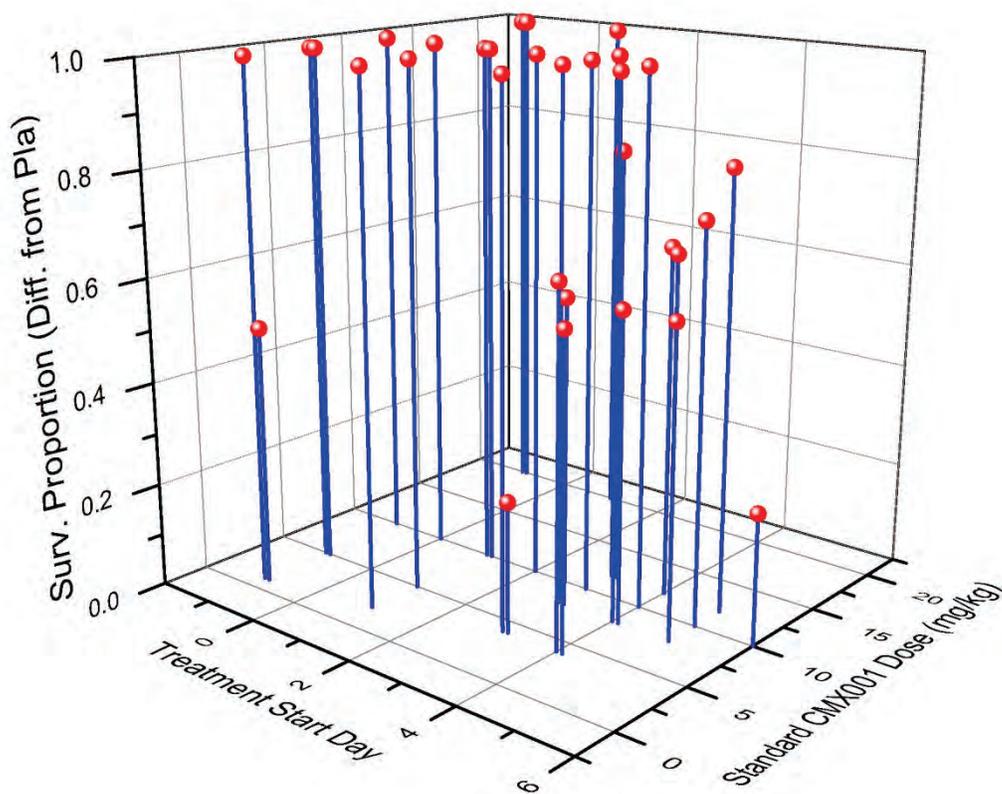
In fact, unlike models in cynomolgus monkeys, described below, infection with a virus titer of approximately 100 PFU consistently produces lesional disease with high mortality. The disease course of RPXV mimics smallpox in that both diseases have a distinct incubation period during which limited viral replications produces a primary asymptomatic viremia which infects cells of

the RES followed by a secondary viremia which is the result of viral replication in the RES. The natural history of RPXV in the rabbit has been described in detail (Adams-2007).

#### 4.1.2. CMX001 Prevents Mortality Due to Rabbitpox Virus Infection in Rabbits

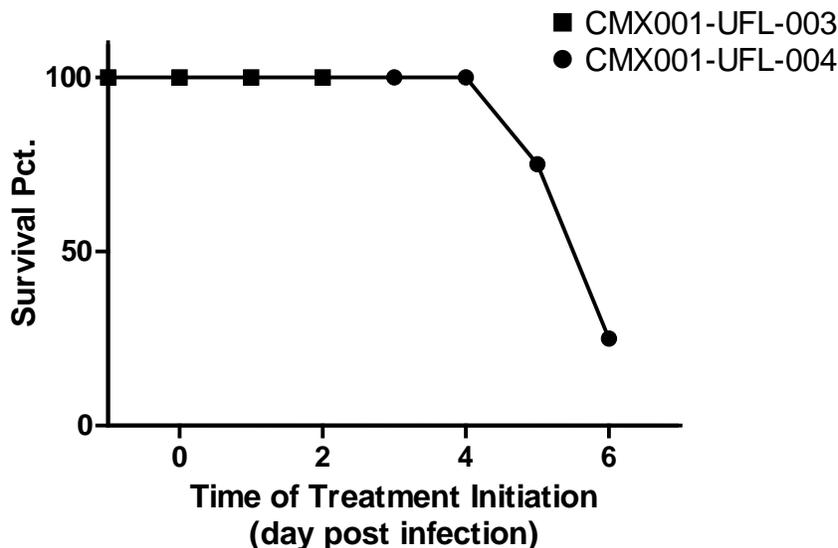
As mentioned previously, a meta-analysis was conducted for studies of CMX001 completed in the RPXV model of human smallpox, including 128 rabbits in 9 studies. Figure 4 displays the treatment effect for the RPXV model, cross-classified according to standardized CMX001 dose and treatment start day (relative to infection). There is a trend suggesting that the treatment benefit declines as treatment initiation is delayed relative to the time of infection. There is also a slight increase in treatment benefit with increasing standardized dose.

**Figure 4: Rabbitpox Virus – Survival Benefit of CMX001 Versus Placebo Relative to Day of Treatment Initiation and Dose of CMX001**



The combined results of RPXV studies CMX001-UFL-003 and CMX001-UFL-004 clearly illustrate the effect of treatment initiation time on survival [Figure 5](#). Study CMX001-UFL-003 examined treatment initiation on Days -1, 0, 1, and 2 post-infection, while study CMX001-UFL-004 examined treatment initiation on Days 3, 4, 5, and 6 post-infection. Treatment with a standardized dose of 10 mg/kg/day (5 mg/kg bid for 5 days) began to lose efficacy when treatment was delayed until Day 5. As the mean day of death in the RPXV model is approximately Day 8, Day 4 post-infection represents the midpoint of disease. At this time, rabbits begin to show signs of systemic disease, including lesions and weight loss. Therefore, in the RPXV model, successful treatment is possible even when initiated after systemic disease has been established and clinical signs are apparent.

**Figure 5: Change in Survival Benefit with Delayed Time to Treatment Post-Infection in RPXV Studies CMX001-UFL-003 and CMX001-UFL-004**



Among all the RPXV studies, the 3 that were conducted later in development (Chimerix studies CMX001-UFL-010, CMX001-UFL-011, and CMX001-UFL-012) were blinded, therefore providing more robust data for analysis. For each study, 24 NZW rabbits were randomized to 2 groups of 12 rabbits each (6 male and 6 female per group). On Day 0 the hind flanks of each animal were shaved and the animal was immediately inoculated intradermally on each flank with 0.1 ml of PBS containing 50 PFU RPXV/flank for a total of 100 PFU RPXV per animal (more than 10-fold higher than the previously determined LD50). This inoculum, without intervention, is typically lethal within 9 days post-infection. In these studies, treatment was delayed until signs of systemic disease were evident, specifically until the first observation of lesions in the ears (Day 3 to 5 post-infection). Animals were individually randomized to blinded CMX001 (20 mg/kg) or placebo at the first sign of lesions in each animal individually. The dose formulation was prepared by Chimerix in 10% sucrose (placebo) and administered orally.

In Study CMX001-UFL-010, animals received 3 doses of 20 mg/kg CMX001 after lesions were detected in each individual animal (1 dose every other day); in study CMX001-UFL-011 animals received a single dose of 20 mg/kg CMX001; and in study CMX001-UFL-012 animals received 2 doses of 20 mg/kg CMX001 (1 dose every other day). The rabbits were monitored for disease progression through Day 14 post-infection. Animals were euthanized only after presentation of previously established clinical signs that are indicative of severe disease. In each study, a statistically significant survival benefit ( $P < 0.05$ ) in animals treated with CMX001 compared with placebo was observed. A single dose of 20 mg/kg CMX001 resulted in a survival benefit of 7/12 compared to 1/12 for placebo; 2 doses of 20 mg/kg CMX001 resulted in a survival benefit of 8/12 compared to 1/12 for placebo; and 3 doses of CMX001 resulted in a survival benefit of 11/12 compared to 2/12 for placebo. There was also a trend, albeit not statistically significant, for improved survival with a higher standardized dose of CMX001. As in studies of ECTV in mice, successful treatment was possible when treatment was initiated after appearance of clinical

signs of systemic disease, at about midpoint between infection and the mean day of death in the model.

**Table 4: Results of Randomized, Blinded, Placebo-Controlled RPXV Studies**

Study Number	Treatment Regimen	Standardized CMX001 Dose	CMX001 Survivors	Placebo Survivors	P Value <sup>1</sup>
CMX001-UFL-010	3 doses of 20 mg/kg CMX001 (1 dose every other day starting at onset of lesions, total 60 mg/kg)	12 mg/kg/day	11/12	2/12	0.0006
CMX001-UFL-012	2 doses of 20 mg/kg CMX001 (1 dose every other day starting at onset of lesions, total 40 mg/kg)	8 mg/kg/day	8/12	1/12	0.0049
CMX001-UFL-011	1 dose of 20 mg/kg CMX001 at onset of lesions, total 20 mg/kg)	4 mg/kg/day	7/12	1/12	0.0272

<sup>1</sup>two-sided Fisher's exact test

Conclusions from three randomized, blinded, placebo-controlled studies of CMX001 in the lethal rabbitpox model are:

- The rabbitpox model is a consistent model of smallpox useful for evaluation of potential therapeutic interventions. Animals infected with RPXV develop clinical signs of disease after inoculation with a relevant dose of virus that provides a window of opportunity for studying potential therapeutic agents. The resulting infection has a sufficient duration to allow appearance of clinical signs of disease to serve as a reliable trigger for initiation of treatment. The model is associated with a high level of lethality.
- The RPXV model is reproducible. A consistent number of placebo treated animals died in each of 3 placebo-controlled studies. All staff were blinded to the treatment assignment of each animal eliminating the possibility of confirmation bias in administering protocol established euthanasia criteria.
- Treatment with CMX001 provided a statistically significant survival benefit compared to vehicle, even when administration was delayed until appearance of clinical signs of systemic disease.
- The model responded predictably with a dose-related increase in survival as the number of doses of CMX001 increased. A single dose of 20 mg/kg CMX001 (standardized dose of 4 mg/kg) was sufficient to provide a statistically significant ( $P < 0.05$ ) survival benefit and additional administrations increased the survival benefit of treatment with CMX001.

## 4.2. Mouse Models of Orthopoxvirus Infection

### 4.2.1. Ectromelia Virus and Vaccinia Virus Infection in Mice

The A/NCr mouse strain is highly sensitive to intranasal ECTV challenge ( $LD_{50} = 0.3$  PFU) with complete mortality by approximately 8 days post-infection using an inoculum  $\geq 50$  times the  $LD_{50}$  (Parker-2008b). Virus initially replicates in the nasal cavity. By 3-4 days post-infection, infectious virus can be detected by plaque assay in the liver and spleen; by 6 days post-

infection these tissues contain a high titer of ECTV. Lower viral titers are detected in the kidneys from Days 3 to 7 post-infection and in the lungs on Days 1 and 2, suggesting that the lung might be directly seeded by the intranasal challenge.

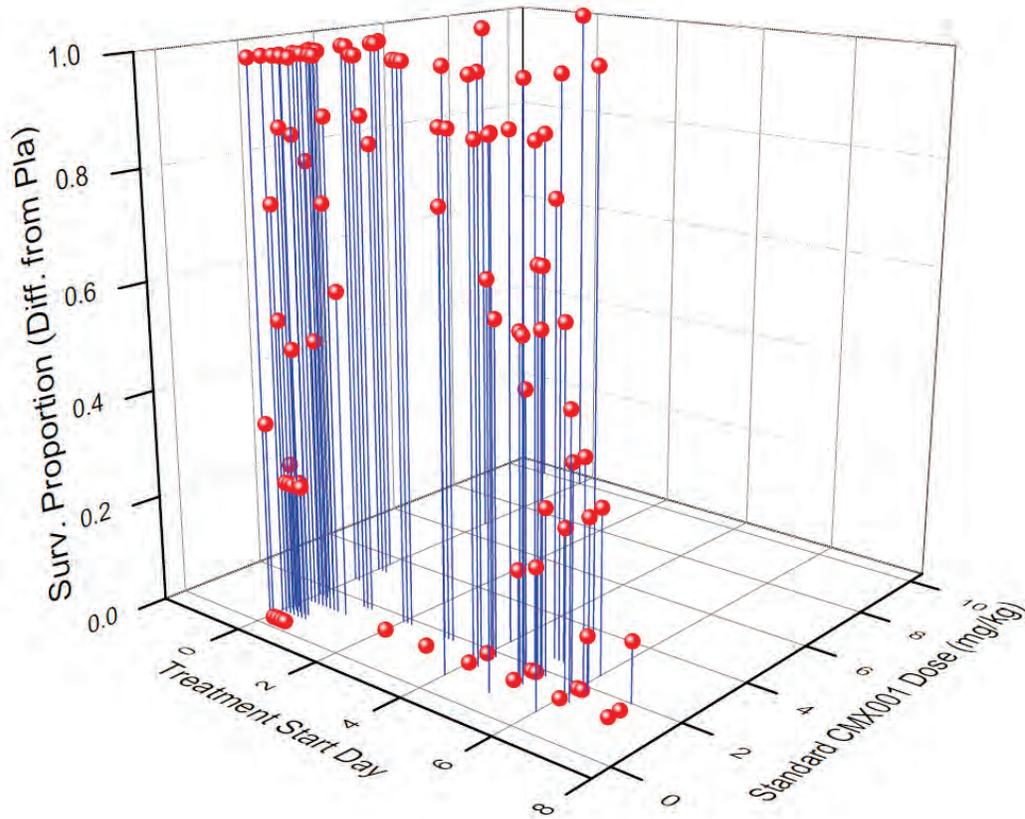
Disease progression is characterized by decreased body weight and activity beginning approximately 3 to 4 days post-infection. Up to 3 days before death, mice present with scruffy and ruffled coats and may develop conjunctivitis. They do not experience internal body temperature changes, but do have difficulty regulating temperature homeostasis that naturally fluctuates in a circadian manner. Viral DNA can be detected and quantified in whole blood samples from Day 5, and levels continue to increase until the day of death. Significant increases in ALT and AST can be detected as early as Day 6 post-infection. At this time point, serum neutrophilia develops and increases consistently over the course of the infection. Small increases in serum IL-13 and IL-9 levels are detected in the first 6 hours post-infection, but much larger and significant increases in serum IFN- $\gamma$  levels are detected by Day 4 post-infection (Parker-2008a). Given that death occurs typically by Day 8 post-infection at an inoculum of about 15 PFU, clinical signs become apparent at about the midpoint of the disease.

The ECTV model in mice resembles human smallpox in several important aspects, including respiratory route of infection, an incubation phase preceding signs of disease, generalized viremia, and a high mortality rate. Another similarity between ECTV and smallpox is the detection of virus in respiratory gases during the pre-exanthem period (Esteban-2005). The most obvious difference in the ECTV model compared to smallpox is in the shorter duration of disease in the model. In ordinary smallpox, the onset of clinical signs including lesions typically occurs near the midpoint of the disease (Day 12 for fever and Day 14 for lesions) and death occurs typically between Days 22 and 28 post-infection. Other differences include lack of fever and the higher impact of ECTV infection on the liver in mice and that A/NCr mice tend to die prior to the onset of frank lesional disease (Buller-1991).

Ectromelia virus (mousepox) infection of mice has been used extensively as a descriptive model of orthopoxvirus pathogenesis and as a model for testing the efficacy of anti-orthopoxvirus therapeutics (Parker-2010). Ectromelia virus is a natural pathogen of mice, and depending upon the mouse strain and route of inoculation, lethal infection can be initiated with a low titer inoculum as anticipated for a host-adapted virus, similar to human smallpox. Infection of mice with vaccinia (VACV) has also been used to assess the efficacy of antiviral agents against orthopoxviruses that can infect humans (Parker-2010).

Figure 6 displays the treatment effect for the ECTV model, cross-classified according to standardized CMX001 dose and treatment start day (relative to infection). Note that each data point represents the results of one study arm.

**Figure 6: Ectromelia Virus: Survival Benefit of CMX001 Versus Placebo Relative to Day of Treatment Initiation and Dose of CMX001**

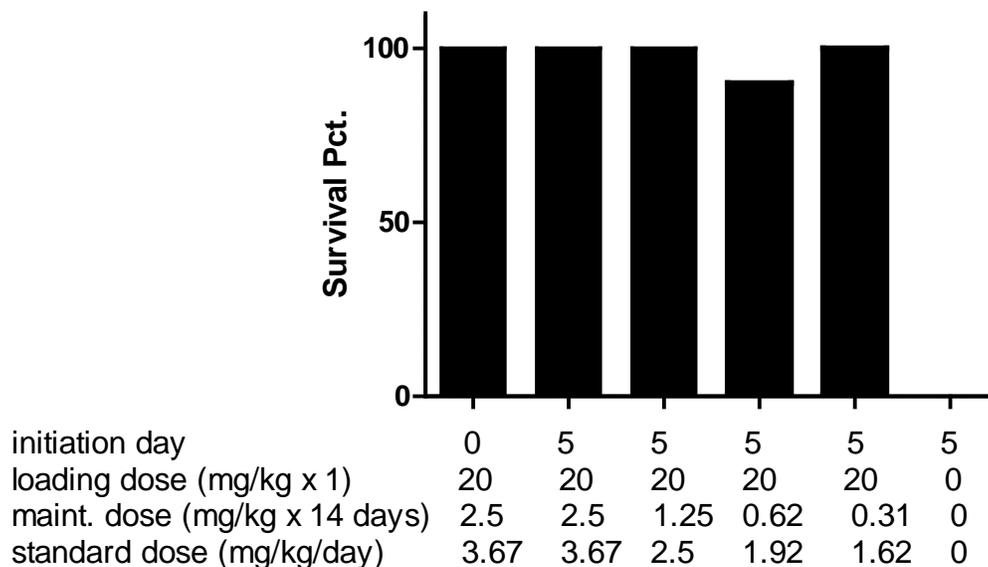


#### **4.2.2. CMX001 Prevents Mortality Due to Ectromelia Virus Infection in Mice**

As with the rabbitpox virus model, there is a general trend suggesting that the ability to produce a positive treatment effect in the ECTV model declines coincident with a delay in the time of treatment initiation post-infection.

The longest delay in treatment that still produced a survival benefit in the ECTV model was 5 days. In study C-530 (Figure 7), combining an induction dose of 20 mg/kg with daily maintenance doses of 0.31 to 2.5 mg/kg extended the ability to delay treatment for up to 5 days post-infection while retaining complete protection from disease related mortality. As the mean day of death of placebo treated animals in study C-530 was approximately 10, Day 5 represents the midpoint of the disease in this experiment. Again, this study demonstrates the utility of an induction dose and is particularly illuminating in that the survival benefit was not diminished regardless of the maintenance dose used (over the range of 0.31 to 2.5 mg/kg/day).

**Figure 7: Survival Benefit of an Induction Dose of 20 mg/kg Followed by Daily Maintenance Doses at Various Dose Levels with Treatment Initiated on Day 5 Post-Infection in ECTV Study C-530**



Conclusions from studies in the lethal ectromelia model in mice:

- The ECTV model is consistent, reproducible and uses a relevant inoculum and route of inoculation.
- CMX001 prevented mortality even when treatment was delayed to as late as Day 5 post-infection, approximately the mid-point of disease.

In the lethal ECTV mouse model, when treatment is delayed until Day 5 post-infection, when clinical signs of disease are apparent, a single (induction) dose of 20 mg/kg CMX001 (standardized dose of 1.33 mg/kg/day) at initiation of treatment provides a clear survival benefit.

### 4.3. Monkey Virus Models of Orthopoxvirus Infection

#### 4.3.1. Monkeypox and Variola Virus Infection in Monkeys

The pathological manifestations of the disease produced when MPXV is intravenously inoculated in cynomolgus monkeys includes fever, dermal erythema, poxvirus lesions with a centrifugal distribution, and high mortality (Huggins-2008). Infection with a virus inoculum of  $5 \times 10^7$  plaque-forming units (PFU) also produces lesional disease similar to human smallpox. This model differs from human smallpox, however, by the short prodromal period and high inoculum (which must be given intravenously to produce mortality) required to produce lethal infection in monkeys, which are not naturally susceptible to monkeypox virus infection. Therefore, IV infection of monkeys with MPXV and VARV essentially bypasses the primary viremia stage of human smallpox disease. The natural history of MPXV in the cynomolgus monkey has been described in detail (Osborn-2009, Huggins-2008).

The pathogenesis of the disease produced when cynomolgus monkeys are intravenously inoculated with VARV includes fever, dermal erythema, pox lesions with a centrifugal distribution, and mortality (Jahrling-2004). Infection with a virus titer of  $1 \times 10^8$  PFU (Harper strain) produces a lesional disease with limited (approximately 30%) mortality, similar to classical smallpox (Huggins-2008, Jahrling-2004). The natural history of VARV in the cynomolgus monkey has been described in detail and has many of the same limitations noted above for monkeypox (Jahrling-2004, Rubins-2004).

#### 4.3.2. Reduced Systemic Exposure to CMX001 in Monkeys

Two studies of CMX001 were conducted in the MPXV monkey model prior to knowledge of the rapid metabolism of CMX001 unique to this species. The pharmacokinetics of CMX001 have been evaluated in mice, rats, rabbits, cynomolgus monkeys and humans. CMX001 is readily absorbed in all species after oral administration, with lower systemic plasma concentrations observed in monkeys relative to other species including humans (Table 5). The dose-normalized exposure to CMX001 in humans (DN AUC<sub>0-inf</sub>) is more than 150-fold higher than that observed in monkeys.

**Table 5: Plasma C<sub>max</sub> and AUC of CMX001 after a Single Oral Administration of CMX001 to Animals and Humans**

Species	Dose (mg/kg)	CMX001		
		C <sub>max</sub> (ng/mL)	AUC <sub>0-inf</sub> (h*ng/mL)	DN <sup>1</sup> AUC <sub>0-inf</sub>
Monkey	4	13	32	8
Mouse	10	69	403	40
Rabbit	4	30	179	45
Human (Healthy)	2	350	2650	1325
Human (Patients <sup>2</sup> )	2	311	4540	2270

<sup>1</sup>dose normalized

<sup>2</sup> Mean values for 49 EIND patients

The species difference in exposure in monkeys shown in Table 5 is attributed, in part, to a higher rate of oxidative hepatic metabolism of CMX001 in monkeys. For example, after in vitro incubation with cryopreserved suspended hepatocytes, <sup>14</sup>C-CMX001 was degraded more rapidly when incubated with hepatocytes from cynomolgus monkeys compared with other species (mice, rat, rabbit and human), though the qualitative metabolite profile was similar across species (Tippin-2009). Though it is generally accepted that among laboratory animal species, monkey metabolism is most similar to human, many drugs have been shown to have dramatically different pharmacokinetic profiles in cynomolgus monkeys compared with humans, including verapamil, quinidine, tacrolimus, amitriptyline and propranolol (Akabane-2009). The differences in pharmacokinetics for these diverse drugs appear to be due, in part, to differences in cytochrome P450 expression in the gut of monkeys compared with humans.

### **4.3.3. Rationale for Use of CDV in Monkeys Rather Than CMX001**

Based on the evidence presented above, it was concluded that hepatic metabolism is a major route of elimination for CMX001, and that a higher rate of first pass metabolism in monkeys leads to lower in vivo exposure to CMX001 compared to other species, especially humans. The high degree of metabolism of CMX001 in monkeys relative to humans and other laboratory animal species negate the utility of these models for evaluating the efficacy of CMX001 against MPXV or VARV.

Thus, CDV treatment of monkeys was proposed rather than CMX001 to eliminate metabolism as a confounding factor since CDV is eliminated unchanged by the kidney in both monkeys and humans. Scaling from CDV experiments in monkeys to anticipated efficacious doses in humans would occur through the active antiviral metabolite, CDV-PP, which is common to both CDV and CMX001.

### **4.3.4. Cidofovir Produces the Same Active Antiviral as CMX001 and Prevents Mortality in the MPXV and VARV Monkey Models**

#### **4.3.4.1. Monkeypox Virus**

Prior studies have demonstrated the efficacy of CDV for treatment of MPXV infection in the cynomolgus monkey model (Huggins-2004). In a representative study, cynomolgus monkeys were infected with MPXV strain Zaire 79 by intravenous injection of 50,000,000 PFU of virus. CDV (20 mg/kg) was administered beginning on Day 1 post infection with additional doses administered on Days 6 and 11 post infection. There was a statistically significant ( $p=0.01$ ) increase in survival in the CDV-treated group (7 of 8 survived) compared with the placebo control group (1 of 8 survived) (Huggins-2004). There was also a statistically significant decrease in both lesion count and viremia in CDV treated animals compared with placebo. These results provide evidence that CDV is active against MPXV in a non-human primate model and are relevant to CMX001 because both drugs are metabolized to the same active antiviral intermediate, CDV-PP.

#### **4.3.4.2. Variola Virus**

The VARV/cynomolgus monkey model provides an opportunity to test compounds directly against the etiological agent of human smallpox. There have been no studies of CMX001 in the VARV model; however, CDV was demonstrated to be efficacious in this model (Huggins-2004). In this study, a dose of 20 mg/kg CDV was administered beginning on the day of infection, or one or two days post infection, with subsequent doses administered 5 and 10 days after the initial dose. At the inoculum of  $1 \times 10^8$  PFU used in this study, only 1 of 3 placebo treated monkeys died. Therefore, although 8 of the 9 CDV-treated animals survived (1 animal died of atypical [hemorrhagic] disease), a statistically significant survival benefit was not shown because of the small sample size and limited mortality in the control group. Importantly, CDV-treated animals had statistically significant ( $P < 0.0001$ ) reductions in lesions and DNAemia (Huggins-2004). This study demonstrated that CDV is active against VARV in the cynomolgus monkeys model and

the data obtained from this study was considered sufficient to support the acquisition of cidofovir for the Strategic National Stockpile.

#### **4.4. Relevance of Animal Models for Predicting Efficacy Against Human Smallpox**

All orthopoxviruses have similar virion morphology, are similar genetically, show extensive serological cross reactivity, and produce disease with similar pathogenesis and features in susceptible hosts. The viral DNA polymerase, which is the antiviral target of CMX001, is approximately 98% identical at the amino acid level between ECTV, RPXV, and a consensus of 48 variola virus DNA polymerases, suggesting that activity against the model viruses is predictive of activity against variola. Indeed, data from in vitro studies demonstrate that CMX001 is active and has similar EC50 values against ECTV, RPXV and variola virus of 0.5, 0.5, and 0.1  $\mu\text{M}$ , respectively. It is important to note that RPXV is considered a sub-species of VACV and that the DNA polymerases (the antiviral target of CMX001) of prototypical strains RPXV Utrecht and VACV WR are 99.9% identical.

Studies utilizing ECTV (inhalation or intranasal infection) in A/NCr mice and RPXV (intra-dermal infection) in New Zealand White rabbits are relevant animal models of smallpox because these hosts are naturally permissive for the respective virus, in the same way that humans are permissive for variola virus. In both models, a low level of inoculum ( $\leq 100$  PFU) results in severe disease and a high incidence of mortality. Similarly, smallpox is thought to result from infection with a low inoculum of variola virus, on the order of 10-100 virions. In all models, the primary endpoint is mortality since the main goal of a smallpox treatment is to prevent mortality.

The ECTV model uses a respiratory route of infection while the RPXV model utilizes intra-dermal infection. Following exposure, ECTV and RPXV infect the host and spread to regional lymph nodes. Viral replication in the lymph nodes produces a primary viremia which leads to infection of cells in the reticuloendothelial system. Further replication produces a secondary viremia and spreads the infection throughout the organism resulting in pox lesions on the skin and other manifestations specific for each virus-host combination. Smallpox is transmitted via the respiratory route and follows a similar disease progression as described above for these animal models.

Chimerix does not propose further studies in the monkeypox or variola virus model in monkeys because cidofovir, which is analogized to the same active anti-viral agent as CMX001, has already been shown to be effective in these models. Therefore, conduct of such studies with a mortality endpoint would require the exposure of a large number of NHPs and put human personnel conducting the study at risk unnecessarily. In addition, these models are not relevant models for evaluating antiviral agents whose mechanism of action is through inhibition of viral replication, such as CMX001, because the high intravenous inoculum required to produce lethal infection in monkeys bypasses the primary viremia stage of human smallpox disease. Moreover, as described in more detail in Section 4.3.2, there is a uniquely high metabolism of CMX001 in monkeys relative to humans and other laboratory animal species, making models in these species less than ideal for evaluating the efficacy of CMX001.

The activity of CMX001 against variola virus has been evaluated in vitro (EC50 =0.1 µM). Notably, this EC50 is lower than that for the permissive orthopoxvirus models described in Sections 4.2.1 and 4.2.2, of this briefing package, specifically RPXV in rabbits (EC50= 0.5 µM) and ECTV in mice (EC50 = 0.5 µM). Moreover, the EC50 for VARV is 0.1 µM, and human exposure to CMX001 after a 100 mg dose is about 6.6-fold higher than this.

#### **4.5. Trigger for Treatment**

Although the appearance of lesions has been the preferred “trigger” for initiating treatment in animal studies designed to evaluate the efficacy of investigational products for treatment of smallpox, the use of lesions as a trigger for treatment is controversial. Notably, in humans, rash and/or lesions are not pathognomonic of smallpox. In fact, suspected cases of smallpox and monkeypox have frequently been mistaken for chickenpox (Fenner et al.- 1988; MacNeil et al.,- 2009; Meyer et al.-2002; Rimoin et al.-2007). The high variability in lesion onset, severity and association with mortality among the various orthopoxvirus models may be due to differences in the route of infection, the size of the inoculum, the local level of virus replication, the local immune response, individual immune differences and species differences in epidermal physiology. Notably, lesions may be least useful as a trigger for treatment in respiratory models, which are generally held to represent the most relevant route of infection for comparison to human VARV transmission. In contrast, a point-of-care nucleic acid test for VARV DNA (vDNA) would definitively demonstrate that the disease in question was indeed accompanied by VARV infection, and not a different rash causing agent. Data to date suggest vDNA in oropharyngeal secretions (saliva) or blood would be optimal choices for early, accurate diagnosis of VARV infection (Parker et al., 2011).

As described in (Parker-2011),“vDNA in oropharyngeal secretions during the eclipse period likely represents virus replication from the primary site of infection prior to virus replication in internal organs such as spleen liver and kidney, and as such is an early marker of virus infection. Detection of vDNA in blood represents secondary viremia, a later stage in the replication cycle and results in seeding of the cornified and mucosal epithelium - a prerequisite for rash.” The authors note that vDNA in saliva is reliably detectable in all orthopoxvirus respiratory challenge models that have been studied, including MPXV-infected prairie dogs (intranasal model), MPXV- -infected NHPs (aerosol and intrabronchiol models), RPXV- infected rabbits (intranasal model) and mousepox/ECTV-infected mice (respiratory models) (Smith - 2011; Johnson -2011; Nalca -2008; Parker-2011). Importantly, VARV is detected in saliva of smallpox patients shortly after infection (Sarkar-1974).

The increasing availability of point-of-care nucleic acid tests suggests this method for detecting VARV infection may be viable in the event of an outbreak, providing an early, specific diagnosis of the etiological agent and permitting earlier initiation of antiviral therapy when it is more effective (Niemz-2011). The use of vDNA in saliva and/or blood as a trigger for treatment initiation in animal models could provide an earlier, more relevant trigger than those currently in use, including lesions.

**5. THE ANIMAL STUDY ENDPOINT IS CLEARLY RELATED TO THE DESIRED BENEFIT IN HUMANS, GENERALLY THE ENHANCEMENT OF SURVIVAL OR PREVENTION OF MAJOR MORBIDITY**

**5.1.1. Desired Benefit in Humans**

CMX001 is being developed to treat human smallpox by preventing or reducing morbidity and mortality caused by the disease while maintaining an acceptable risk-benefit profile using a simple, convenient dose regimen and drug product in the form of a small, orally-administered, fixed dose tablet or ready-to-use pediatric formulation, stable for long term storage and distribution in large numbers of treatment courses.

**5.1.2. Animal Study Endpoints**

The primary study endpoint in all animal efficacy studies is mortality due to poxvirus disease when treatment is initiated at the first sign of lesions. Therefore, the endpoints of these animal models are the same as the desired benefit in humans, i.e. a reduction in the mortality rate associated with the disease.

## **6. THE DATA OR INFORMATION ON THE 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 pharmacokinetic data from mice, rats, rabbits, cynomolgus monkeys and humans summarized in Table 5 and discussed in Section 4.3.2 suggest that there are species differences in the first-pass elimination of CMX001 and that these differences result in the higher exposures in humans. This higher exposure in humans provides a reasonable expectation that efficacy demonstrated in animal models is relevant to treatment of human disease and that similar, or higher, exposures found to be efficacious in animals can be achieved safely in humans.

Compared with healthy adult volunteers, CMX001 exposures were similar or higher in patients treated under Emergency INDs (EINDs) with CMX001 (see data for 2 mg/kg, Table 5). Not surprisingly, due to the divergent patient population and variation in PK sampling between patients, there was greater variability in C<sub>max</sub> and AUC in EIND patients compared to data obtained in healthy volunteers (data not shown).

### **6.1. Scaling the Efficacious Dose from Animals to Humans**

For scaling, the actual effective dose rather than the standardized dose used to conduct meta-analysis across studies was used in order to allow comparison to human data. Comparison of single dose PK parameters across species is relevant and appropriate for the following reasons:

- A single dose was effective in the ECTV and RPXV models;
- There is extensive PK data in humans following a single dose; and
- Twice weekly administration in humans results in little to no accumulation of CMX001

Straightforward scaling to a human dose using exposure to CMX001 can be accomplished based on data from these animal models and pharmacokinetic studies. As noted above, a single dose of 20 mg/kg CMX001 was effective in both ECTV (mouse) and RPXV (rabbit) models. The use of the rabbitpox model, in particular, for scaling to humans is based upon the similarity of rabbitpox to variola (the viral DNA polymerases are approximately 98% identical) and because the model duplicates many key features of human smallpox disease. Furthermore, because the in vitro EC<sub>50</sub>s of CMX001 against RPXV and VARV are comparable, (0.5 μM versus 0.1 μM, respectively), it is reasonable to extrapolate efficacy against RPXV in rabbits to efficacy against VARV in humans. Similarly, data can be obtained to compare CMX001 exposure with healthy and rabbitpox-infected rabbits and healthy subjects and patients with severe dsDNA viral infections

The principal of pharmacokinetic/pharmacodynamic correlation is that the desired pharmacologic effect can be correlated with the overall extent of exposure to drug (i.e., CMX001). Therefore, absent significant differences in metabolism, it is appropriate to use plasma exposure data at efficacious doses of CMX001 in animals to estimate the anticipated

efficacious exposure in humans. As shown in Table 5, the pharmacokinetics of CMX001 are favorable in humans relative to mice and rabbits resulting in higher systemic exposure at lower, weight-based doses. In PK studies conducted by Chimerix, rabbits receiving a dose of 20 mg/kg CMX001 had a mean AUC<sub>0-inf</sub> of 1291 h•ng/mL. In healthy mice, a dose of 10 mg/kg produced an AUC<sub>0-inf</sub> of 393.5 h•ng/mL which extrapolates to an AUC<sub>0-inf</sub> of 787 h•ng/mL for a 20 mg/kg dose, assuming a proportional increase in CMX001 exposure is observed with a 2-fold increase in the dose (a dose of 20 mg/kg was not used in this study necessitating extrapolation from the 10 mg/kg dose).

Available clinical data suggest that antiviral activity in humans may be achieved against multiple dsDNA viruses with an approximate dose of 100 mg CMX001 administered twice per week. In an open label expanded access study (Study CMX001-350), a dose of 100 mg CMX001 given twice weekly was associated with a mean 10-fold reduction in viral load in patients infected with CMV and AdV. In emergency INDs (EINDs), data from critically ill and heavily pretreated patients support the use of 100 mg twice weekly in subjects with an active viral infection. In addition, the first EIND granted for administration of CMX001 was to treat progressive vaccinia (PV) in a patient with acute myelogenous leukemia who had developed PV following a smallpox vaccination (Lederman-2009). The subject was treated with an initial starting dose of 200 mg CMX001 followed by 5 subsequent doses of 100 mg given approximately once weekly. CMX001 was administered because previous antiviral therapies had not been able to contain the disease. Following the addition of CMX001 to the treatment regimen (200 mg CMX001 initial dose followed by 100 mg CMX001 weekly), poxvirus lesions continued to heal and blood viral DNA levels cleared. By the end of treatment, viable virus could not be isolated from lesions or blood.

Therefore, based on both animal model and preliminary human efficacy data, the clinical dose for smallpox is likely to be approximately 100 mg administered twice weekly for 5 doses. More than 300 patients have received doses of CMX001 equal to or exceeding this dose. In a typical 70 kg adult, a fixed dose of 100 mg equates to a dose of approximately 1.5 mg/kg. As shown in Table 6, in healthy human subjects, a dose of 1.5 mg/kg produced an AUC<sub>0-inf</sub> of 2330 h•ng/mL. Therefore, a dose of 100 mg in humans is expected to produce an AUC that exceeds the AUC that was associated with efficacious administration of CMX001 in both the ECTV and RPXV models. Although exposures in humans are compared to those in animals at the (higher) induction dose, subsequent maintenance doses in the animal models were lower, producing correspondingly lower exposures and resulting in an even larger differential compared to human exposures at the 100 mg dose.

**Table 6: Estimated CMX001 PK Parameters after a Single Dose of CMX001 in Mice and Rabbits at Efficacious Doses in Comparison to PK Parameters in Healthy Volunteers at the Anticipated Dose for Treatment of Smallpox (100 mg)**

Species	Dose (mg/kg)	Dose (mg/m2)	CMX001				
			Cmax (ng/mL)	Tmax (hr)	AUC0-last (hr*ng/mL)	T1/2 elim (hr)	AUC0-inf (hr*ng/mL)
Mouse (1)	20	60	185	2.8	759	4.7	787
Rabbit (2)	20	240	294	2.5	1288	4.9	1291
Human (3)	1.5	56	371	3.0	2330	32.7	2340

(1) Extrapolated data based on mean data from animals receiving 10 mg/kg and assuming dose proportionality.

(2) Mean data

(3) Mean data; 1.5 mg/kg single oral dose (100 mg = approximately 1.5 mg/kg in a typical 70 kg adult.)

Although one may consider these data limited by the fact that PK parameters were measured in healthy, rather than infected animals and humans, there is evidence from subjects administered CMX001 under Emergency IND (EIND) that severe disease does not result in decreased exposure to CMX001. Rather, mean CMX001 exposure (AUC) determined in more than 100 patients with severe dsDNA viral infections was the same or slightly higher compared to mean exposure in healthy volunteers receiving the same dose. PK studies in infected animals are planned to confirm this clinical finding for the relevant animal models.

Therefore, a dose of 100 mg (approximately 1.5 mg/kg) administered twice weekly is expected to be efficacious against smallpox in humans. Given the extended course of smallpox disease compared to disease in animal models, it is expected that a longer prodromal period will allow diagnosis and initiation of treatment early and, a more extended dosing period will improve efficacy relative to that achieved in the animal models which have a highly compressed window of treatment from appearance of clinical disease to death. In smallpox, the time from infection to death or recovery is approximately 3-4 weeks, thus if treatment is initiated at lesions (about 2 weeks post-infection), then a 2 or 3 week treatment course should be sufficient to prevent mortality in most patients.

## 6.2. Rationale for Use of CDV-PP in Scaling

The traditional method of scaling across species is based on a comparison of plasma exposures to the parent molecule at an efficacious dose and regimen to establish a PK/PD correlation. This method will be used to scale from rabbits to humans.

Because CMX001 will not be administered to monkeys, scaling to humans may be accomplished through comparison of intracellular CDV-PP concentrations in PBMCs if data from monkey models is determined to be critical to support a smallpox approval under the Animal Rule. CDV-PP is the active antiviral agent common to both CMX001 and CDV. CDV-PP concentrations in PBMCs are relevant to treatment of smallpox because they are expected to reflect concentrations in the cells of the reticuloendothelial system (RES), a major site of viral

(including orthopoxvirus) replication (Breman-2002). In addition, PBMCs can be easily and repeatedly obtained from both animals and humans.

There are limitations to the use of CDV-PP in PBMCs as a scaling parameter. Specifically, it may not be possible to analyze PBMC samples of VARV-infected monkeys due to constraints established by the testing facility (CDC) and World Health Organization that prohibit samples containing VARV genetic material from leaving the site. Concentrations of CDV-PP in PBMCs of MPXV infected monkeys will be used to estimate concentrations of CDV-PP associated with efficacy in infected monkeys. Further, it may not be possible to obtain sufficient PBMC samples from critically ill patients to analyze CDV-PP concentrations in humans because most severely ill patients receiving CMX001 in clinical trials are immune-compromised, often following bone marrow (BMT) or human stem cell transplant (HSCT). These patients have fewer PBMC cells in a given quantity of blood. Data from in vitro incubation of CMX001 or CDV with PBMCs may provide additional useful information (see Figure 3 of this briefing package).

As discussed previously, Chimerix does not propose further studies in the MPXV and VARV models in cynomolgus monkeys for the following reasons:

1. The efficacy of cidofovir has already been established in these models
2. CMX001 cannot be evaluated in monkey models due to the unique metabolism of CMX001 by monkeys in comparison to humans and other laboratory species
3. IV infection of monkeys with VARV or MPXV bypasses the primary viremia stage of human smallpox disease making these models unsuitable for evaluating the efficacy of antiviral therapies whose mechanism of action is to prevent viral replication, such as CMX001.

If the FDA concurs with this proposal, the use of CDV-PP for scaling from animals to humans will not be necessary. Scaling to an effective human dose will be done using rabbit to human PK parameters and considering the effective dose of CMX001 for other human viral infections (e.g., CMV and AdV).

### **6.3. Summary of Human Dose Selection for Treatment of Smallpox**

In summary, the target dose of CMX001 for smallpox treatment in humans will be estimated using the following:

- Plasma concentration data and pharmacokinetic parameters associated with efficacious treatment of rabbitpox infection in rabbits compared to human plasma pharmacokinetic parameters for CMX001.
- Plasma concentration data and pharmacokinetic parameters associated with efficacious treatment of severe life-threatening dsDNA virus infections (e.g. CMV, AdV) in immunocompromised patients may also be used to establish a dose and regimen for treatment of the life-threatening dsDNA virus infection caused by variola virus (smallpox).

- If additional monkey studies with CDV are required, CDV-PP concentrations in PBMCs associated with efficacious treatment of monkeypox virus infection using CDV in cynomolgus compared to human CDV-PP data in PBMCs.

**7. CLINICAL PLAN FOR CMX001 FOR TREATMENT OF SMALLPOX, INCLUDING DATA OBTAINED FROM STUDIES OF SUBJECTS WITH ADENOVIRUS AND CYTOMEGALOVIRUS INFECTION**

**7.1. Studies Proposed for the Clinical Development Program for CMX001 for Treatment of Smallpox**

**7.1.1. Smallpox Clinical Pharmacology Plan**

**Table 7: Smallpox Clinical Pharmacology Plan**

<b>Study Number</b>	<b>Abbreviated Title</b>
CMX001-106	An Open-Label Study to Determine the Safety and Pharmacokinetics of CMX001 in Subjects with Impaired Hepatic Function and Healthy subjects with Normal Hepatic Function
CMX001-108	A Double-Blind Randomized Crossover Trial to Define the ECG Effects of CMX001 using a Clinical and a Supratherapeutic Dose Compared to Placebo and Moxifloxacin (a Positive Control) in Healthy Men and Women: A Thorough ECG Trial
CMX001-112	A Phase 1, Mass Balance Study Following Administration of a Single, Oral Dose of <sup>14</sup> C radiolabeled CMX001 in Healthy Male Subjects
CMX001-113	Effect of CMX001 on the Pharmacokinetics of a Specific CYP3A4/5 Substrate, Midazolam, in Healthy Adults
TBD	Effect of CMX001 on the Pharmacokinetics of a P-gp Substrate Digoxin (Narrow Therapeutic Index)
CMX001-114	An Open-Label, Randomized, 2-Way Crossover, Single-Dose Study in Healthy Volunteers Comparing the Effect of Food on CMX001 Bioavailability

**7.1.2. Clinical Safety Database**

In addition to the clinical pharmacology studies listed in the previous section, it is anticipated that the clinical safety database for CMX001 will be comprised from these studies:

- Clinical Study CMX001-102 “A Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Dose-Escalation Study of the Safety and Pharmacokinetics of an Oral Formulation of CMX001 (HDP-Cidofovir Conjugate) in Healthy Adult Subjects” (Complete)
- Clinical Study CMX001-102 Addendum entitled: “A Randomized, Double-Blind, Placebo-Controlled, Limited Multi-Dose, Dose-Escalation Study of the Safety and Pharmacokinetics of CMX001 in Healthy Subjects” (Complete)

- Clinical Study CMX001-103 “An Open-Label, Randomized, 3-Way Crossover, Single-Dose Study in Healthy Volunteers Comparing the Bioavailability of CMX001 (HDP-Cidofovir Conjugate) Delivered as a Tablet Formulation vs. a Solution Formulation and the Effect of Food on CMX001 Bioavailability” (Complete)
- Clinical Study CMX001-104 “A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Study of the Safety, Tolerability and Population Pharmacokinetics of CMX001 in Post-Transplant Subjects with BK Virus Viruria” (Report in preparation)
- Clinical Study CMX001-201 “A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of the Safety, Tolerability and Ability of CMX001 to Prevent or Control CMV Infection in R+ Hematopoietic Stem Cell Transplant Recipients” (Ongoing)
  - Allogeneic stem cell transplant recipients who were CMV seropositive (R+) at the time of transplant are eligible for enrollment.
  - Dosing is initiated immediately following engraftment and continuing through week 13 post transplant
  - 239 patients enrolled across 5 treatment cohorts
  - Study completion 4Q2011
- Clinical Study CMX001-202 “A Randomized, Placebo-Controlled, Multi-Site Phase 2 Study Evaluating the Safety and Efficacy of Preemptive Treatment with CMX001 for the Prevention of Adenovirus Disease Following Hematopoietic Stem Cell Transplantation (the ADV HALT Trial)” (Ongoing)
  - Pediatric and adult recipients of an allogeneic HSCT with detectable AdV DNA in their plasma PCR
  - Age >3 months ≤ 75 years
  - 48 patients planned
- Clinical Study CMX001-350 “A Multicenter, Open-label Study of CMX001 Treatment of Serious Diseases or Conditions Caused by dsDNA Viruses” (Ongoing)
- CMX001 Investigator-held EINDs (Complete)

At the time of submission of an NDA for the treatment of smallpox indication all safety data from all completed and ongoing studies will be presented and integrated as part of the safety database including any patients dosed in studies planned for the future.

Based on the completed and planned studies listed above, it is anticipated that ~1300 subjects will be exposed to at least one dose of CMX001 at the completion of these studies in both controlled and uncontrolled studies, with more than 50% from controlled studies. These exposures are tabulated by pediatric/adult and total weekly dose in Table 8 below.

**Table 8: Overall Extent of Exposure to CMX001 at Completion of Ongoing Clinical Studies of CMX001**

	Overall	Single Dose (<100mg)	Single Dose (≥100mg)	Multiple Dose <200mg adult; <4mg/kg peds	Multiple Dose ≥200mg per week, adult; ≥4mg/kg per week, peds)	
					Controlled <sup>1</sup>	Uncontrolled <sup>1</sup>
<b>Adults</b>	~1100	35	90	100	570	350
<b>Pediatric</b>	190	NA	NA	10	30	150

<sup>1</sup>Note: all EIND patients counted as multiple dose. Doses are considered as total weekly dose

### **7.1.3. IND Protocol for Treatment of Smallpox**

In addition to the animal studies, as part of the clinical development plan, Chimerix has developed an IND protocol for CMX001 for the treatment of smallpox as a sub-study to Study 350. Data collection during a smallpox emergency could occur under this IND protocol prior to NDA approval. In the event of an approval prior to a smallpox outbreak, the protocol would be used to generate clinical safety and efficacy data that would be a post-marketing requirement for approval under the “Animal Rule.”

## **7.2. Prevention of Morbidity and Mortality in Human Infections with dsDNA Viruses**

Chimerix has accumulated clinical data for CMX001 in a variety of dsDNA viral infections through controlled clinical trials and open label expanded access programs. The viral infections have included adenovirus (AdV) and multiple herpesviruses (e.g., CMV, EBV, HSV), among others. Although these viruses are genetically distinct from variola virus, they are closely related and their pathogenesis is similar to human smallpox and they all contain a viral DNA polymerase. AdV and CMV infections in particular share many features with variola that provide a reasonable basis to expect that a dose of CMX001 which is efficacious against these viruses will also be efficacious against variola. Efficacy against other dsDNA viral infections provides additional confidence for dose selection for a smallpox treatment.

**Table 9: Similarities of Human Smallpox to AdV and CMV Disease**

<b>Disease Aspect</b>	<b>Smallpox</b>	<b>AdV</b>	<b>CMV</b>
<b>Antiviral Target (CMX001)</b>	Viral DNA polymerase	Viral DNA polymerase	Viral DNA polymerase
<b>Antiviral EC50 In Vitro</b>	0.1 µM	0.02 µM	0.001µM
<b>Route(s) of Infection</b>	Respiratory epithelium (inhalation)	Respiratory epithelium (inhalation)	Mucosal epithelium (contact)
<b>Tissue Distribution</b>	Disseminated, including: respiratory epithelium liver spleen bone marrow lymphoid tissue kidney skin other viscera	Disseminated, including: respiratory epithelium liver spleen bone marrow lymphoid tissue kidney bladder intestine heart	Disseminated, including: respiratory epithelium, liver spleen bone marrow lymphoid tissue kidney salivary glands

The antiviral target of CMX001 against smallpox, AdV, and CMV is the viral DNA polymerase encoded by each virus. The EC50 for CMX001 against variola virus in cell culture is 0.1 µM. The median EC50 value for CMX001 against AdV is 0.02 µM. The median EC50 value for CMX001 against CMV is 0.001 µM.

Preliminary analysis from an open label expanded access study of CMX001, Study CMX001-350, demonstrated the potential of 100 mg CMX001 administered twice weekly to control CMV and AdV infection. This dose regimen (100 mg CMX001 administered twice weekly) is currently being evaluated in two randomized, controlled clinical trials for CMV and AdV (Studies 201 and 202), which may provide evidence of tolerability and antiviral activity in the clinic. Blinded data regarding prevention of CMV in HCT recipients shows a dose-related decrease in failure rate across cohorts (Study 201; Complete data expected 4Q2011)

The first EIND granted for administration of CMX001 was to treat progressive vaccinia (PV) in a patient with acute myelogenous leukemia who had developed PV following a smallpox vaccination. The subject was treated with a single 200 mg dose of CMX001 followed by five 100 mg doses (q6d; approximately once weekly). CMX001 was administered as previous antiviral therapies had not been able to contain the disease. Following the addition of CMX001 to the treatment regimen, poxvirus lesions including new satellite lesions continued to heal and blood viral DNA levels cleared. By the end of treatment, viable virus could not be isolated from lesions or body fluids and a significant proportion of the scabs had fallen off. Since that initial patient, over 200 patients have been treated with CMX001 under EINDs (or foreign equivalents) for a range of dsDNA viral diseases.

### **7.3. Conclusion Regarding Relevance of Clinical Data from Human Infections with Other dsDNA Viruses**

Given the broad similarities between AdV, CMV and VARV infection, it is not surprising that doses of CMX001 that may be efficacious against AdV and CMV infection produce systemic exposures to CMX001 that are likely to be effective against smallpox based on in vitro EC50s and activity in animal models of smallpox. Each disease results in widely disseminated infections, requiring that the active metabolite of CMX001 (CDV-PP) must be widely distributed in human tissues for efficacy. While skin lesions are not a characteristic of AdV or CMV infection, doses of CMX001 that have anecdotally treated HSV lesional disease demonstrate the ability of CMX001 to treat a lesional skin disease. In aggregate, the human clinical experience with CMX001 provides strong support for establishing a dose and regimen for CMX001 against VARV infection.

The CMX001 clinical development program will provide considerable experience in using the drug across a range of populations and viral targets. Given that potentially millions of patients could receive a countermeasure during a public health emergency, this data will be an important part of the appropriate evidence for submission in a New Drug Application for smallpox. Data from well-controlled studies of the safety and efficacy of CMX001 to prevent CMV disease and pre-empt AdV disease in transplant patients is being generated. In addition, up to 200 patients may be enrolled in Study 350 which will generate additional open label safety and antiviral activity data for CMX001 for the treatment of various dsDNA infections. Proof of antiviral activity of CMX001 in humans against CMV and adenovirus may provide a good starting point for selection of a dose for treatment of smallpox in combination with efficacy data from studies in animal models of smallpox.

## 8. CONCLUSIONS

This document has summarized information relevant to the following issues for CMX001:

- CMX001 is in advanced development for the treatment of smallpox under the “Animal Rule” (21 CFR Part 314 Subpart I)
- The pathophysiological mechanism of variola virus toxicity is reasonably well understood
- CMX001 and CDV deliver the same active antiviral agent, CDV-PP
- The mechanism of prevention of variola virus toxicity by CMX001 is known
- The RPXV and ECTV models of smallpox are robust, reproducible, naturally permissive host models of smallpox which are sufficiently well characterized for predicting the response in humans to VARV
- The MPXV and VARV cynomolgus monkey models are not naturally permissive host models, and due to species specific metabolism, monkeys are not a relevant species for evaluating the efficacy of CMX001
- CMX001 prevent mortality in the RPXV and ECTV models of smallpox; successful treatment with CMX001 can be initiated after detection of lesions in rabbits
- Completed MPXV and VARV studies in monkeys demonstrated the efficacy of CDV and provide proof of concept for treatment of human smallpox with CMX001 because both are metabolized to the same active antiviral, CDV-PP
- CMX001 plasma concentrations achieved in humans exceed those produced by efficacious doses in multiple animal models of smallpox
- The human dose of CMX001 for treatment of smallpox will be identified using data from animal efficacy studies in relevant animal models of smallpox, controlled clinical studies of other dsDNA viruses, and PK parameters in relevant animal models

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