

Interspecies Comparison of the Pharmacokinetics of CMX001, a Lipid Conjugated Nucleotide Analog with Broad dsDNA Antiviral Activity

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ABSTRACT

Background

CMX001 is an orally available lipid conjugate of cidofovir. CMX001 inhibits replication of all 5 families of dsDNA viruses that cause human disease. The lead indication is smallpox, with development proceeding under the Animal Efficacy Rule. Other potential indications are diseases associated with CMV, JC, and BK virus infections. CMX001 prevents morbidity and mortality in mouse and rabbit models of human smallpox but not in a monkey model. The purpose of this study was to determine whether PK parameters obtained in animals could explain differences in efficacy outcomes and be used to estimate efficacious doses in humans.

Method

CMX001 was administered orally to 80 healthy volunteers at single doses up to 2 mg/kg and multiple doses up to 1 mg/kg. Pharmacokinetic studies were conducted in mice, rabbits and monkeys, species used to model human smallpox disease. Interspecies scaling of effective doses and dose regimens from animal efficacy studies will be used to aid selection of a clinical dose for treatment of smallpox.

Results

Species	Dose (mg/kg)	CMX001		
		C _{max} (ng/mL)	AUC _{0-inf} (h*ng/mL)	DN AUC _{0-inf}
Monkey	4	13	32	8
Mouse	10	69	403	40
Rabbit	1.5	25	157	105
Human	1	176	1360	1360

PK analyses demonstrated that CMX001 was readily absorbed in mice, rabbits and humans after a single oral dose. CMX001 plasma concentrations in monkeys were 5-13 fold lower than mice or rabbits. Dose normalized AUC in humans was 13, 34 and 170-fold higher than that observed in rabbits, mice and monkeys, respectively.

Conclusion

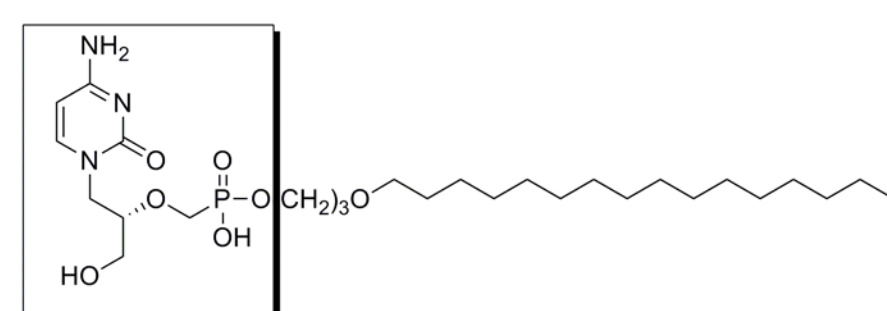
Based on plasma concentrations of CMX001 at doses that were effective in mouse and rabbit models of human smallpox, plasma concentrations in humans met or exceeded concentrations projected to be needed for treatment of smallpox and other dsDNA viruses in humans.

INTRODUCTION

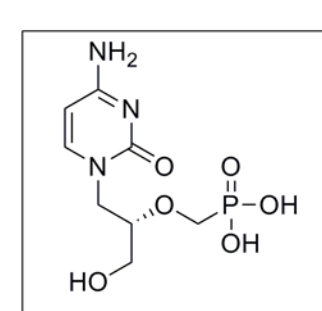
CMX001 is a lipid (1-O-hexadecyl-oxypropyl, HDP) conjugate of the acyclic nucleotide analogue cidofovir. Cidofovir, which is FDA approved for the treatment of cytomegalovirus induced retinitis in AIDS patients, is active against all five families of dsDNA viruses that cause human morbidity and mortality. The clinical utility of the drug, however, is limited by the requirement for administration by intravenous infusion and the risk of acute nephrotoxicity. Compared with cidofovir, the HDP lipid conjugate increases *in vitro* antiviral activity by up to one thousand-fold, permits oral administration and appears to eliminate nephrotoxicity¹. CMX001 is currently in development for the treatment of smallpox, and in Phase Ib/Ia development to evaluate safety and preliminary antiviral activity against BK and CMV infections in a post-transplant (solid organ and stem cell) population.

Chimerix's lipid conjugate technology employs covalent coupling of a synthetic lipid to drug via a phosphonate moiety². The conjugates are designed to resemble natural lipids that are readily absorbed from the small intestine via natural lipid pathways and distributed to tissues in plasma and/or lymph. Once in the cell cidofovir is cleaved from the lipid carrier by hydrolytic action of phospholipases on the phosphorous-O-alkyl bond³. A two-step phosphorylation catalyzed by anabolic kinases produces cidofovir-diphosphate, the active antiviral agent. CMX001 produces much higher intracellular cidofovir-diphosphate levels in cells compared with cidofovir.

**Figure 1. Proprietary Technology to Increase Oral Bioavailability
CMX001: A Lipid Conjugate of Cidofovir**



CMX001



Cidofovir

RESULTS

First-Time-In-Human Study: CMX001-102

CMX001-102 was a randomized, placebo-controlled, double-blind, dose escalation study evaluating single and multiple doses of CMX001 in healthy human volunteers.

	Overview	
	Single Dose Phase	Repeat Dose Phase
Objectives	To evaluate the safety and pharmacokinetics of a single oral dose of CMX001 in healthy volunteers	To evaluate the safety and pharmacokinetics of multiple oral doses of CMX001 in healthy volunteers
Study Design	54 healthy volunteers in 9 cohorts - 36 received active drug (4/cohort) - 18 received placebo (2/cohort)	30 healthy volunteers in 5 cohorts - 20 received active drug (4/cohort) - 10 received placebo (2/cohort)
Regimen	Single oral dose	Subjects received 3 doses of CMX001 on study days 0, 6 and 12
Doses	0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0, 1.5 and 2.0 mg/kg	0.1, 0.2, 0.4, 0.6 and 1.0 mg/kg
Analytes	Concentrations of Cidofovir and CMX001 were measured in plasma.	Concentrations of Cidofovir and CMX001 were measured in plasma on Days 0 and 12.
Pharmacokinetic Analysis	Noncompartmental pharmacokinetic analyses were conducted using WinNonlin (Versions 5.1 and 6.0) (Pharsight Corporation, Mountain View CA).	

Single Dose Phase

Figure 2. Mean Plasma Concentration of CMX001 and Cidofovir Following a Single Oral Administration of CMX001 in Healthy Human Volunteers (n=4/cohort)

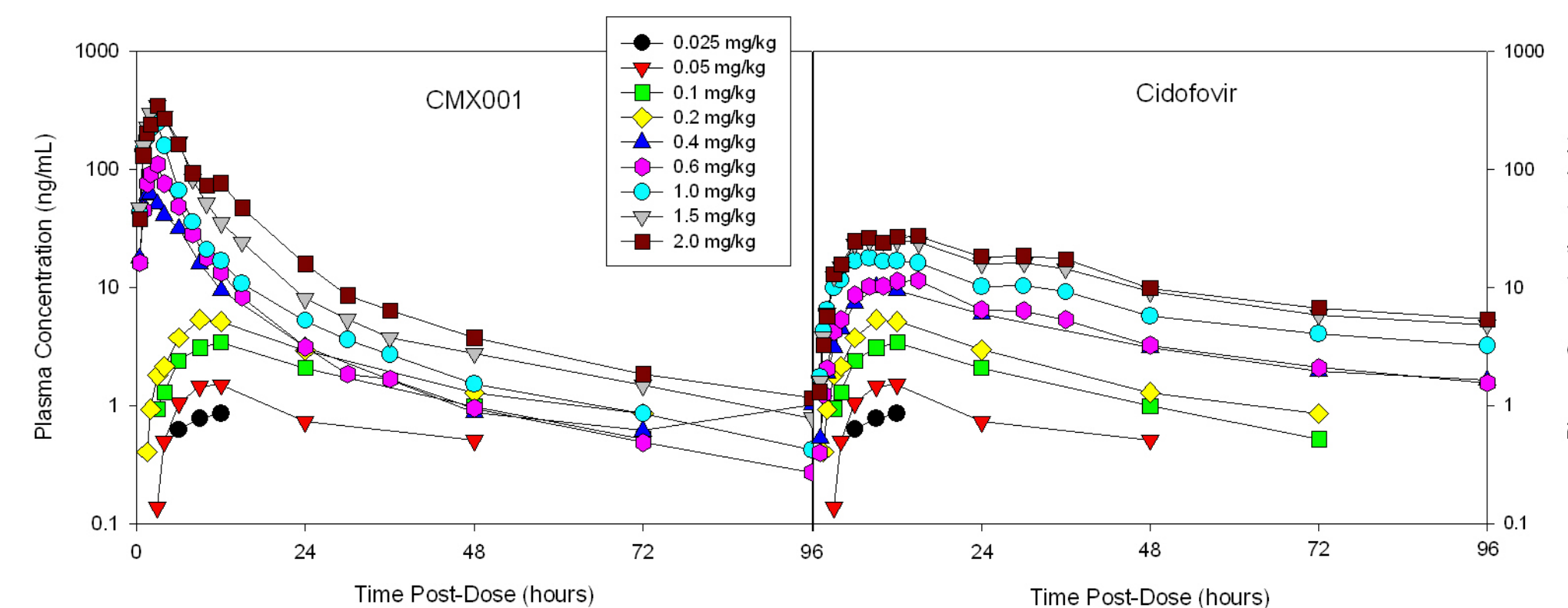


Table 1. CMX001-102 Single Dose Pharmacokinetic Parameters

PK Parameters	Cohort =1 0.025 mg/kg	Cohort =2 0.050 mg/kg	Cohort =3 0.1 mg/kg	Cohort =4 0.2 mg/kg	Cohort =5 0.4 mg/kg	Cohort =6 0.6 mg/kg	Cohort =7 1.0 mg/kg	Cohort =8 1.5 mg/kg	Cohort =9 2.0 mg/kg
CMX001									
C _{max} (ng/mL)	2.58 ± 1.40	5.28 ± 2.34	10.6 ± 4.63	24.5 ± 4.12	68.1 ± 28.0	115 ± 38.3	273 ± 131	371 ± 180	350 ± 119
T _{max} (h)	2.00 (2.00, 6.00)	2.00 (1.50, 3.00)	3.00 (3.00, 3.00)	2.03 (1.50, 3.00)	2.00 (1.50, 3.00)	2.96 (2.00, 3.00)	3.00 (2.00, 3.00)	3.00 (3.00, 12.0)	3.00 (3.00, 12.0)
t _{1/2,z} (h)	6.15 ± 0.930	7.83 ± 4.09	15.0 ± 5.02	17.6 ± 4.55	ND	25.8 ± 6.31	27.1 ± 2.80	32.7 ± 3.46	24.0 ± 0.705
AUC _{0-inf} (h*ng/mL)	21.3 ± 5.71	36.5 ± 10.7	139 ± 52.6	235 ± 48.1	ND	729 ± 253	1350 ± 646	2340 ± 1320	2650 ± 445
Cidofovir									
C _{max} (ng/mL)	ND	1.76 ± 0.481	3.44 ± 0.603	5.41 ± 0.998	10.4 ± 1.55	12.2 ± 2.39	18.3 ± 5.87	25.8 ± 2.90	31.1 ± 7.00
T _{max} (h)	ND	9.00 (9.00, 9.00)	12.0 (12.0, 12.0)	9.15 (9.00, 12.0)	9.00 (9.00, 12.0)	15.0 (12.0, 15.0)	7.00 (6.00, 15.0)	10.0 (8.00, 12.0)	11.5 (6.00, 15.0)
t _{1/2,z} (h)	ND	ND	ND	24.2 ± 1.83	45.5 ± 23.2	55.6 ± 4.04	64.5 ± 12.4	56.8 ± 4.18	63.0 ± 11.0
AUC _{0-inf} (h*ng/mL)	ND	ND	ND	195 ± 31.4	491 ± 83.1	565 ± 60.3	1030 ± 317	1510 ± 290	1740 ± 409

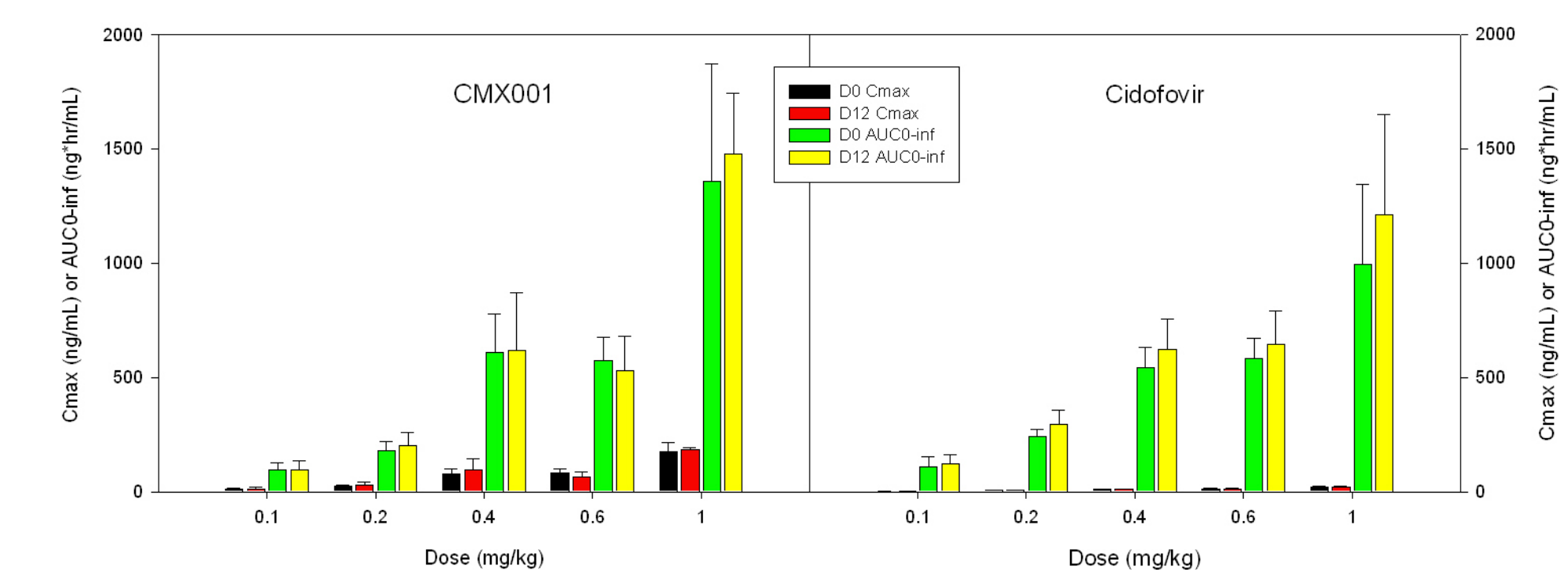
ND: Not Determined

All PK parameters are summarized by mean ± SD, except T_{max} is summarized by median (min, max).

- Adverse events were generally mild and not dose-dependent; there was no evidence of renal or bone marrow toxicity (based on standard clinical pathology endpoints).
- CMX001 was readily absorbed after oral administration.
- Maximum plasma concentration (C_{max}) and systemic exposure (AUC_{0-inf}) to CMX001 increased approximately in proportion to dose.
- The half-life of elimination (t_{1/2,z}) of CMX001 increased with increasing dose to range from about 24 to 32 hours in the last 4 cohorts, presumably due to better definition of the elimination phase at higher doses.
- The rate of appearance of cidofovir is dependent on the absorption rate of CMX001 and rate of conversion of CMX001 to cidofovir. Cidofovir C_{max} increased somewhat less than in proportion to dose; however AUC increased approximately in proportion to dose.
- The half-life of elimination of cidofovir increased to range from 55 to 65 hours in the last 4 cohorts.

Multi-Dose Phase

Figure 3. Mean Plasma (+/- SD) C_{max} and AUC_{0-inf} for CMX001 and Cidofovir on Day 0 and Day 12 Following Oral Administration of CMX001 on Days 0, 6 and 12 in Healthy Human Volunteers (n=4/cohort)



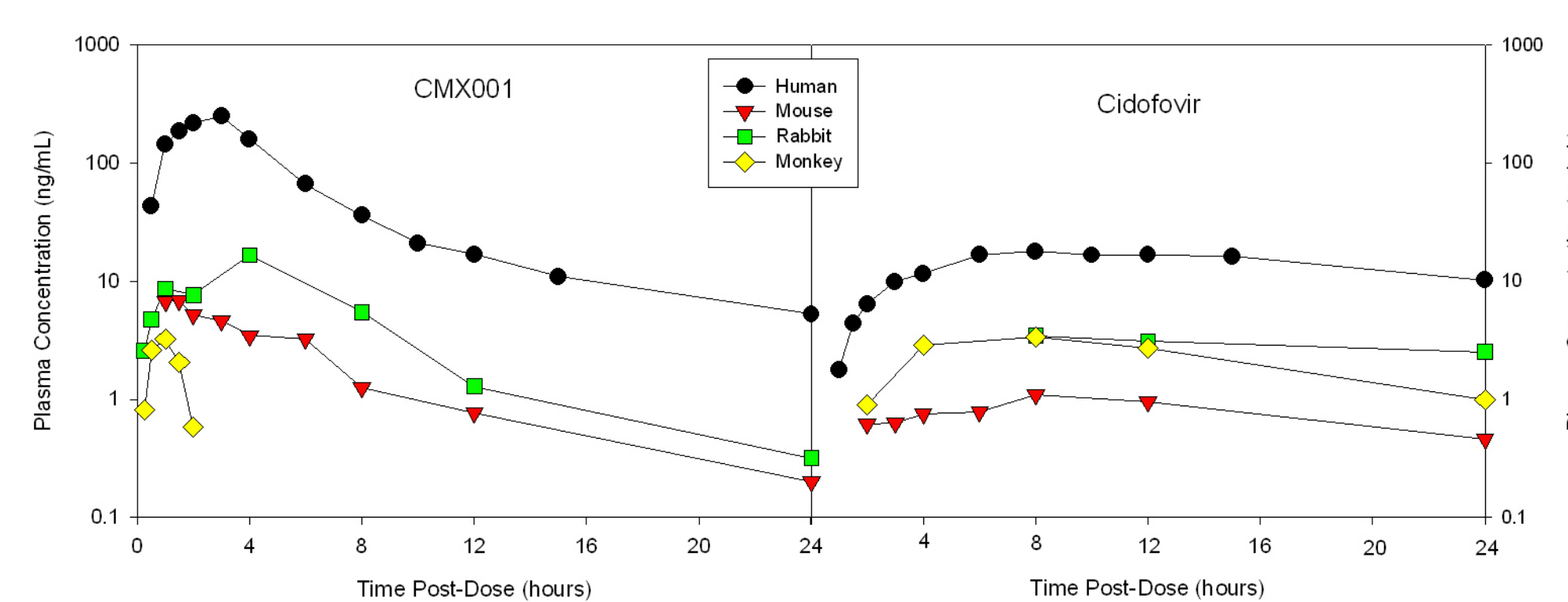
- In the multiple-dose phase of CMX001-102 there was no significant accumulation of CMX001 or CDV on Day 12 compared with Day 0.

Overview of Animal Efficacy and Pharmacokinetic Studies:

- Efficacy experiments were conducted to determine, primarily, the ability of CMX001 to prevent mortality in mouse, rabbit and monkey models of human smallpox infection in order to support development of CMX001 under "The Animal Efficacy Rule".
- In mice and rabbits, oral doses ranging from 1 to 20 mg/kg were efficacious (ie prevented disease related mortality) with variables effecting the dose dependent on:
 - When, in relation to infection, treatment was initiated
 - Prophylaxis = low dose; late stage infection = high dose
 - Regimen and cumulative dose
 - Long regimen = low dose; short regimen = high dose
 - The viral titer of the inoculum
 - Low titer inoculum = low dose; high titer inoculum = high dose
- Although there was evidence of an antiviral effect, a regimen using a maximum oral dose of 10 mg/kg did not prevent mortality in the monkeypox model.
- Pharmacokinetic studies were conducted in uninfected animals, independently, or as components of toxicology studies, to provide pharmacokinetic parameters for CMX001 and cidofovir.

To compare across different studies in which different doses were used, plasma concentration versus time plots were normalized by dividing plasma concentration by dose at each timepoint.

Figure 4. Dose Normalized Mean Plasma Concentrations of CMX001 and Cidofovir in Mice, Rabbits, Monkeys and Humans



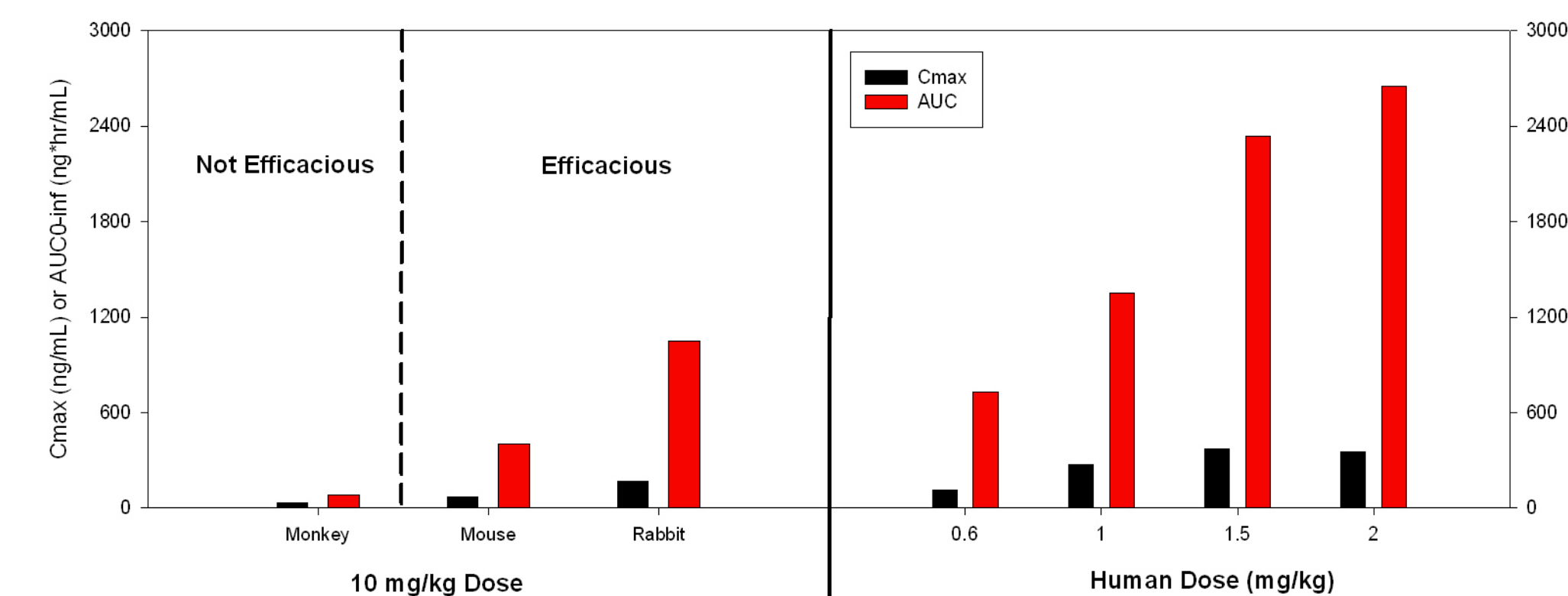
- Humans have significantly higher exposures to CMX001 and cidofovir at a normalized dose of CMX001 compared with mice, rabbits or monkeys.
- Monkeys have lower exposure to CMX001 compared with mice and rabbits.
- Monkeys have equivalent or higher exposure to cidofovir compared with mice and rabbits.

Table 2. Interspecies Comparison of Single Dose Oral Pharmacokinetic Parameters in Mice, Rabbits, Monkeys and Humans

Species	Median Efficacious Dose* (mg/kg)	CMX001				Cidofovir			
		C _{max} (ng/mL)	C _{max} /Dose	AUC _{0-inf} (hr*ng/mL)	AUC _{0-inf} /Dose	C _{max} (ng/mL)	C _{max} /Dose	AUC _{0-inf} (hr*ng/mL)	AUC _{0-inf} /Dose
Monkey	10	33	3	80	8	35	4	493	49
Mouse	10	69	7	403	40	11	1	175	18
Rabbit	10	167	17	1046	105	35	3	2207	221
Human	1	176	176	1360	1360	19	19	996	996

*Median efficacious dose in mouse and rabbit models of smallpox. A dose of 10 mg/kg was the highest dose tested in the monkey model and did not prevent mortality. A human dose of 1 mg/kg is provided for reference, the final human dose has not been identified.

- Following a dose of 1 mg/kg, humans have significantly higher peak plasma concentrations and systemic exposures to both CMX001 and cidofovir than occurred at efficacious doses in mouse and rabbit models of human smallpox.
 - Rank order of CMX001 systemic exposure (AUC_{0-inf}/Dose) following a normalized dose of CMX001:
 - Monkey < Mouse < Rabbit < Human
 - (170-fold) (5-fold) (13-fold)
 - Rank order of cidofovir systemic exposure (AUC_{0-inf}/Dose) following a normalized dose of CMX001:
 - Mouse < Monkey < Rabbit < Human
 - (55-fold) (3-fold) (12-fold)
- Monkeys have much lower systemic exposure to CMX001 than mice or rabbits which may explain the lack of efficacy in the monkey model.



CONCLUSIONS

- CMX001 was well tolerated in single and multiple dose healthy human volunteer studies with no evidence of renal or bone marrow toxicity.
- C_{max} and AUC_{0-inf} increased approximately in proportion to dose for both CMX001 and cidofovir with no significant accumulation after multiple doses.
- The long half-life of CMX001 supports infrequent administration.
- Systemic exposure to CMX001 in humans at oral doses that were safe and well tolerated exceeded those at efficacious doses in mouse and rabbit models of smallpox.
- Systemic exposure to CMX001 in monkeys was low following oral administration, possibly due to metabolism, which is likely to contribute to the lack of antiviral effect in that model.
- Plasma concentrations of CMX001 were more predictive of efficacy in animal models than concentrations of cidofovir.

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