

Preclinical Safety Evaluation of CMX157: A Lipid-Conjugated Nucleotide Analog for Treatment of HIV

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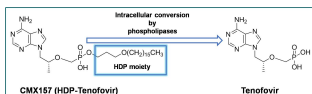
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ABSTRACT

Though widely used, tenofovir disoproxil fumarate (TDF) has reduced activity against several common HIV mutants and a risk of nephrotoxicity. CMX157 is a heptadecyloxypropyl lipid conjugate of tenofovir (TFV) with activity against wild-type and drug-resistant HIV. Unlike TDF, CMX157 is not efficiently cleaved to free TFV in the blood. This should increase the levels of active TFV-diphosphate in target cells and reduce the rate of secretion in the kidney. To support human administration, studies were conducted in rats and dogs to identify undesired pharmacological effects on the cardiovascular, CNS, respiratory, renal and gastrointestinal systems. Mitochondrial toxicity was evaluated in HepG2 cells using the Complex IV/Frattaxin ratio. Finally, *in vitro* and *in vivo* genotoxicity and 28-day rat and monkey studies were completed. A single dose of 1000 mg/kg resulted in decreased body temperature and urine volume noted for the presence of occult blood. Effects on respiration rate, urine chemistry and hemodynamic parameters were observed at 200 and 1000 mg/kg. Due to the small magnitude and/or transient nature of these changes, they were not considered biologically important. In both rats and monkeys, clear effect and NOAEL doses were identified with good exposure after oral administration. Gastric effects resulted in mortality due to dehydration at doses of 800 and 600 mg/kg/day in rats and monkeys, respectively. In monkeys, these effects reversed during a 7-day dose holiday after which dosing resumed successfully at 400 mg/kg/day. Observations included excess salivation, emesis and diarrhea. Slight elevations in ALT and BUN occurred in individual animals at doses above 200 mg/kg/day. Anatomic pathology changes were minimal and generally limited to the stomach. The NOAEL was 200 mg/kg/day in both rats and monkeys. The gastric effects occurred at doses that greatly exceed those planned for initial human studies. No important safety pharmacology effects and no mitochondrial or genotoxicity was observed. Therefore, preclinical testing supports the clinical development of CMX157.

INTRODUCTION

TFV has reduced activity against specific HIV mutants, including those with K65R, multiple thymidine analog mutations (TAMs) or multi-NRTI resistant (MNR) mutations. TFV is also associated with a risk of nephrotoxicity. Our goal is to increase the efficacy and decrease the toxicity of TFV by creating a lipid conjugate prodrug that is more stable in plasma, effectively penetrates cells and yields high intracellular concentrations of the active anabolite, TFV-diphosphate. Compared with TFV, CMX157 is > 300-fold more potent against wild-type and NRTI-resistant HIV *in vitro* and it effectively penetrates isolated human FSMCs, producing > 30-fold higher intracellular levels of the active antiviral, TFV-diphosphate.



IND-enabling preclinical safety studies of CMX157 have been completed. The studies include safety pharmacology assessments to identify undesired pharmacological activity, genetic toxicology studies, a special study to identify potential mitochondrial toxicity and multi-dose (28-day) studies to define the toxicology and toxicokinetics of CMX157 in rats and monkeys. The results of these studies, summarized below, support initial clinical studies of CMX157.

METHODS AND RESULTS

Safety Pharmacology Studies

Study	Species	Dose Levels	Major Findings
MDS 'Lead Profiling Screen'	<i>In Vitro</i> receptor binding	10 μ M	96% inhibition of adrenergic α_{2A} binding, no significant agonist or antagonist activity in a follow-up study.
HERG Channel K ⁺ Current	<i>In Vitro</i> HEK293 cells	0.1, 1, 10, 100 μ M	No effect on HERG-mediated K ⁺ current at 0.1 and 1 μ M. At 10 and 100 μ M, stable K ⁺ currents could not be established.
Neuropharmacological Profile (NPP)	Rat	0.40, 200, 1000 mg/kg	1000 mg/kg: Decreased mean body temperature at 2 hours post-dose.
Respiratory Function	Rat	0.40, 200, 1000 mg/kg	1000 mg/kg: Transient decreased respiratory frequency and tidal volume. 200 mg/kg: Transient decreased tidal volume.
Cardiovascular Assessment	Conscious-Telemetered Dog	0.40, 200, 1000 mg/kg	1000 mg/kg: Increased heart rate, decreased blood pressure. 200 mg/kg: transient decreased mean arterial and pulse pressure.
Gastrointestinal Motility	Rat	0.40, 200, 1000 mg/kg	1000 mg/kg: Slightly increased GI motility.
Renal Function	Rat	0.40, 200, 1000 mg/kg	1000 mg/kg: Small increase in ALT, decreased urine volume with occult blood, and alterations in urine chemistry.

Small, transient changes occurred in several study endpoints, generally at the high dose of 1000 mg/kg. None of the changes were considered to be evidence of important pharmacological activity.

Genotoxicity Studies

Study	Dose Levels	Major Findings
Reverse Mutation (Ames) Assay	1 to 5,000 μ g/plate	Negative for mutagenicity
Mouse Lymphoma Assay	9.85 to 5,000 μ g/mL	Negative for mutagenicity
<i>In Vivo</i> Mouse Micronucleus Assay	0, 500, 1000 and 2000 mg/kg	Negative for clastogenicity

CMX157 was non-mutagenic and non-clastogenic in 3 genetic toxicology studies.

Mitochondrial Toxicity Study

HepG2 cells were cultured in 96 well plates for 7 days in the presence of CMX157, TFV or dDC at concentrations of 0.025, 0.1, 0.39, 1.56, 6.25, 25 and 100 μ M. Fresh compound dilutions in media were applied every 72 hours. Concentrations of Complex IV protein (COX) (mitochondrially encoded and expressed) and Frattaxin (FRA) (a nuclear-encoded, cytoplasmically translated mitochondrial protein) were measured using MitoSciences MS641 ELISA kit. The ratio of COX to FRA was calculated as described in Nadanaciva *et al.* The ratio reflects changes of mitochondrial encoded proteins in relation to nuclear encoded proteins. A value of one corresponds to no change while a ratio less than one corresponds to a specific reduction in mitochondrial biogenesis.

Figure A. Effect of CMX157 on Frattaxin and Complex IV Expression

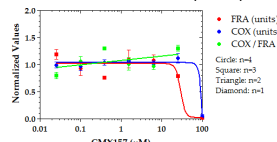


Figure B. Effect of Tenofovir on Frattaxin and Complex IV Expression

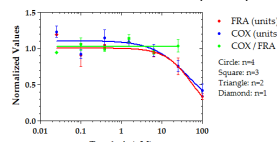
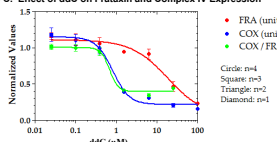


Figure C. Effect of dDC on Frattaxin and Complex IV Expression



Concentrations of CMX157 of up to 25 μ M had no effect on COX expression (Figure A). TFV had a small effect on COX at 25 μ M while dDC decreased COX levels by >75% (Figures B and C). At 25 μ M, both CMX157 and TFV had a small effect on concentrations of FRA. Neither CMX157 nor TFV had any effect on COX or FRA expression at concentrations \leq 6.25 μ M. These data suggest that CMX157, like tenofovir, has a low potential to induce mitochondrial toxicity. The expected positive response was obtained with dDC.

7 Day Toxicology Studies in Rats and Monkeys

Species	Major Findings
Rat	No effects. NOAEL was >100 mg/kg/day
Cynomolgus Monkey	1000 mg/kg: F had emesis, activity, food consumption, body wt., unkempt appearance, red cell mass and alterations in serum electrolytes associated with emesis; 1 BUN, creatinine and bilirubin; M had emesis, body wt. No gross or microscopic findings. 300 mg/kg: Emesis, no other findings. 100 mg/kg: Emesis, no other findings.

Doses of 50, 200 and 800 mg/kg/day and 50, 200, and 600 mg/kg/day were selected for 28 day studies in rats and cynomolgus monkeys, respectively.

28 Day Toxicology and Toxicokinetic Studies in Rats and Monkeys

Species	Major Findings
Rat	800 mg/kg/day: All high dose rats (20M/20F) between Days 4 and 11. Signs of toxicity included decreased activity, hunched posture, red material around mouth/nose. Necropsy findings included white pasty material in the stomach (presumed drug), red/black foci and gastric erosion and inflammation. Small spleen and thymus corresponded microscopically to lymphoid depletion. Increased incidence and severity of cardiomyopathy in females. Other microscopic findings were present in bone marrow (erythrocyte depletion) and liver (minimal to mild bile duct hyperplasia). 200 mg/kg: No mortality. Clinical signs of salivation. Slight increases (2-fold) in ALT and AST at termination. No gross or microscopic findings. NOAEL 50 mg/kg/day: Salivation
Cynomolgus Monkey	600/400 mg/kg/day: One female sacrificed on Day 8 with signs of decreased activity, thin appearance, labored respiration and dehydration. Gastric erosion was observed microscopically. After a 7-day drug holiday in remaining animals, dosing resumed at 400 mg/kg/day but was occasionally suspended due to diarrhea and dehydration. Other signs included salivation, emesis, liquid/nonformed feces and thin appearance. CMX157-related gross findings were limited to discoloration of the colon and rectum. Spleen weights were slightly reduced. There were no CMX157-related clinical pathology, ophthalmologic, electrocardiographic or microscopic findings. 200 mg/kg/day: Excess salivation and occasional emesis, diarrhea, and dehydration were the only observations. NOAEL 50 mg/kg/day: Occasional emesis.

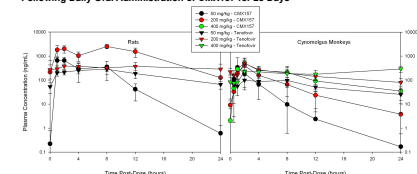
Doses of 50 and 200 mg/kg/day were well tolerated for 28 days in both rats and monkeys. Clinical signs at these doses included salivation and occasional fecal changes. Emesis was occasionally observed in monkeys. There were no important clinical or anatomic pathology findings. A dose of 200 mg/kg/day was the NOAEL in both rats and monkeys.

Toxicokinetic Results from 28 Day Studies in Rats and Monkeys

Mean Steady State Toxicokinetic Parameters in Rats and Monkeys Following Daily Oral Administration of CMX157 for 28 Days

Species	Dose (mg/kg/day)	CMX157					Tenofovir				
		T _{max} (hr)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·hr/mL)	t _{1/2} (hr)	AUC ₀₋₂₄ (ng·hr/mL)	T _{max} (hr)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·hr/mL)	t _{1/2} (hr)	AUC ₀₋₂₄ (ng·hr/mL)
Rats*	50	1.0	657.5	4270.0	2.2	4268.1	8.0	657.5	5420.7	11.6	4318.2
	200	8.0	2515.0	17600.8	3.6	31097.5	2.0	2515.0	32580.4	59.4	8104.4
Monkeys	50	1.3	384.3	870.3	3.3	838.2	4.7	384.3	2039.2	10.7	1390.3
	200	2.7	403.3	1733.7	2.8	1707.7	1.7	403.3	5726.5	12.2	4219.8
	400	2.3	524.0	3915.6	6.5	3557.0	14.6	524.0	19795.6	72.4	5026.1

Mean (±SD) Plasma Concentration versus Time Profiles for Rats and Monkeys Following Daily Oral Administration of CMX157 for 28 Days



*All 800 mg/kg/day rats died or were sacrificed moribund between Days 4 and 11.

The results of toxicokinetic analyses of plasma samples obtained from rats and monkeys demonstrated good systemic exposure to both CMX157 and CMX157 (tenofovir) after once daily oral administration of CMX157 for 28 days at doses up to 200 mg/kg/day in rats and up to 400 mg/kg/day in monkeys.

SUMMARY

- There was no important secondary pharmacological activity in a wide variety of *in vitro* and *in vivo* safety pharmacology studies.
- CMX157 was non-genotoxic.
- CMX157 did not cause mitochondrial toxicity.
- The dose-limiting toxicity in rats and monkeys following daily oral administration of doses that are anticipated to provide large multiples of human exposure was gastrointestinal.
- Clinical signs of toxicity included emesis, salivation, diarrhea and dehydration and the toxicity is expected to be fully reversible.

CONCLUSION

The preclinical safety profile of CMX157 was favorable at doses expected to produce large multiples of human exposure. Together, these data support further clinical development of CMX157.

REFERENCES

- Nadanaciva, S. Wilts, JH, Barker ML *et al.* Lateral flow immunoassay for detecting drug-induced inhibition of mitochondrial DNA replication and mtDNA-encoded protein synthesis. *J. Immunol. Methods* 2009, 343: 1-12