

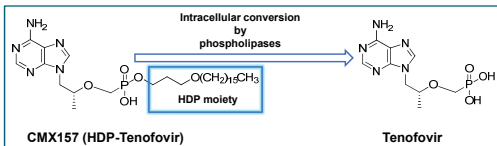
Development of Hexadecyloxypropyl Tenofovir (CMX157) for HIV: Potential for Use as a Microbicide and Therapeutic

ER Lanier, BM Lampert, LC Trost, MR Almond and GR Painter
Chimerix Inc., Durham, NC, USA

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INTRODUCTION

CMX157 is a lipid (1-O-hexadecyloxypropyl, HDP) conjugate of the acyclic nucleotide analogue tenofovir. Tenofovir disoproxil fumarate (Viread®, TDF), a prodrug of tenofovir (TFV) is one of the most widely used nucleoside/tide reverse transcriptase inhibitors (NRTIs) for treatment of HIV. However, tenofovir disoproxil fumarate is rapidly cleaved *in vivo*, leading to suboptimal uptake by target cells. In addition, the drug is ineffective against multiple HIV mutants, including those with K65R, multiple thymidine analog mutations (TAMs) or multi-NRTI resistant (MNR) mutations. Here we report the activity of a lipid conjugate of tenofovir that is stable in plasma, facilitates efficient cellular uptake and yields high intracellular levels of the active anabolite, TFV-diphosphate (TFV-PP). CMX157 has been evaluated *in vitro* to determine its primary pharmacological effects as an antiviral agent; cytotoxicity, genotoxicity, and secondary pharmacological effects of CMX157 have also been determined. Additionally, 28-day repeat-dose toxicology studies were conducted in rodent and non-rodent species *in vivo*.



METHODS

CMX157 activity against wild-type and NRTI resistant HIV was determined for a panel of 30 NRTI isolates with major NRTI mutations, including K65R +/- M184V, multiple TAM combinations +/-M184V, K70E in various combinations and MNR complexes including T69SXX and Q151M (PhenoSense™). A separate panel of 14 NRTI resistant clinical isolates and wild-type isolates from subtypes A-G, O and HIV-2 were examined in PBMCs. Additional studies determined activity in monocytic cells, cytotoxicity in dividing and non-dividing cells, tenofovir-diphosphate levels in PBMCs, and serum effects. For tenofovir anabolite analysis, PBMC samples were lysed with 70% ice cold methanol and centrifuged; supernatants were analyzed in triplicate using LC/MS/MS. TFV, TFV monophosphate and TFV diphosphate were separated by gradient, reverse phase, ion-pairing chromatography and detected by positive ion electrospray. RSDs between 2.4 and 14.2% were obtained for data shown.

To test the hypothesis that CMX157 may bind directly to HIV, purified HIV was incubated with CMX157 or TFV followed by quantification of virus-associated drug and determination of the tissue culture infectious dose (TCID₅₀). Concentrated HIV-1_{IIIb} was incubated with 500 nM CMX157 or TFV for 2 hours, pelleted to remove unbound compound and lysed with 70% methanol. Supernatants were analyzed in triplicate using LC/MS/MS; separate viral aliquots were used to determine TCID₅₀ by XTT, RT and p24 assays in CEM-S9 cells. To assess the effect of exposure time, concentrated HIV-1_{IIIb} was incubated with 500nM CMX157 for 1, 15, 30, 60, and 120 minutes prior to determination of the TCID₅₀. To determine the effect of drug dose, TCID₅₀ was determined following a 15 minute incubation of virus with eight concentrations of CMX157 ranging from 0.039 to 125 nM. HDP-acyclovir was evaluated in parallel as a control.

In vitro activity against HIV:

The *in vitro* antiviral activity profile for CMX157 was evaluated extensively for cell-type effects and HIV strain effects. It is active against all major subtypes of HIV-1 in PBMCs with IC₅₀ values ranging between 0.20 and 7.18 nanomolar (nM). By comparison, TFV IC₅₀s against HIV-1 subtypes A-G, and O ranged from 1600-4900 nM. CMX157 IC₅₀s against three isolates of HIV-2 in PBMCs were <5 nM. CMX157 was also active against HIV in monocyte derived macrophages (MDMs) with IC₅₀ between 0.56 and 4.61 nM. Extrapolating from the IC₅₀ values obtained in 0% to 40% human serum results in an estimated IC₅₀ value of 52.5 nM for 100% human serum (~77x increase). In the PhenoSense™ assay, IC₅₀s for CMX157 ranged from 0.66 nM for L74V/M184V to 57 nM for A62V/T69SVG/V51/2151; corresponding IC₅₀s for tenofovir were 227 nM and 16,959 nM. CMX157 IC₅₀s for M41L/L210W/T215Y averaged 6.3 nM without M184V and 2.2 nM with M184V (2,240 and 770 nM for tenofovir respectively). Similar data were obtained in PBMCs and data for key mutants are shown in Table 1.

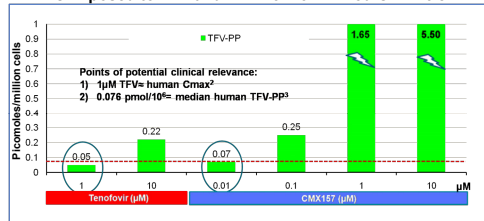
Table 1. Activity of CMX157 and Tenofovir Against HIV-1 Clinical Isolates in PBMCs

HIV RT Genotype	CMX157 IC ₅₀ (nM)	TFV IC ₅₀ (nM)
41L/67N/70R/215F/219E	3.8	6,515
41L/67N/210W/215Y/184V	3.1	5,390
41L/67N/210W/215Y	19	>8,509
75I/77L/116Y/151M/184V	5.0	>6,494
41L/210W/215Y/184V/69SSS	9.0	>6,469
65R/184V	1.8	1,036

Levels of active anabolite in human PBMCs:

Following 24 hour incubations with equimolar TFV or CMX157, human PBMCs exposed to CMX157 had higher intracellular levels of active drug (TFV-PP) as shown in Figure 1. Notably, 100 nM CMX157 produced >3x the median *in vitro* level of TFV-PP seen with standard dosing of Viread.

Figure 1. Intracellular Levels of TFV-PP in Activated Human PBMCs Exposed to TFV and CMX157 for 24 Hours *In Vitro*

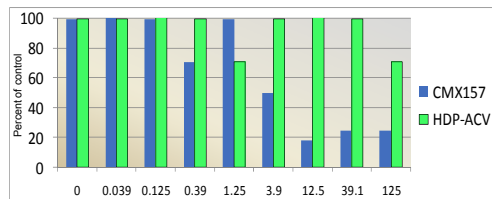


RESULTS

CMX157 associates directly with virus:

Analysis of purified HIV pellets following incubation with 500 nM drug showed >30,000 molecules of CMX157 were associated with each virion versus ≈100 molecules/virion for TFV. The effect on TCID₅₀ is shown below.

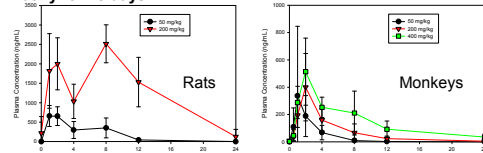
Figure 2. TCID₅₀ Effects of Pre-incubating HIV_{IIIb} with CMX157 or a Lipid Control (HDP-ACV)



As shown in Fig 2, concentrations of CMX157 down to 12.5 nM resulted in 4 fold decreases in TCID₅₀ while the lipid control bearing the same alkyl modification as CMX157, HDP-acyclovir, showed no consistent effect. TFV did not reduce the infectivity of pre-incubated virus on untreated cells even at 1000 nM (data not shown). 500nM CMX157 had a similar effect when virus was exposed for a very short time period (1 minute) or 2 hours. Data shown are for p24; similar data were obtained using XTT or RT endpoints. Incubation of CMX157 with media followed by pelleting, supernatant removal and addition of virus had no effect on TCID₅₀.

CMX157 in rats and monkeys

Figure 3. CMX157 Levels in Rats and Monkeys Given CMX157 daily for 28 days



Plasma concentrations of CMX157 and TFV were higher in rats than monkeys. This is likely due, at least in part, to emesis observed in monkeys, particularly at doses of 200 and 600 mg/kg/day. At a dose of 50 mg/kg/day, C_{max} and AUC_{0-t} for CMX157 on Day 28 (presumed steady state) were 658 ng/mL and 4268 ng*hr/mL in rats and 384 ng/mL and 838 ng*hr/mL in monkeys. Gastric toxicity resulted in mortality at a dose of 800 mg/kg/day in rats and 600 mg/kg/day in monkeys. In monkeys, the toxicity reversed during a 7-day dosing holiday after which dosing resumed successfully at 400 mg/kg/day for the remainder of the study. Frequent clinical signs in both rats and monkeys included excess salivation, emesis and diarrhea. Small changes in clinical pathology values were limited to slight elevations in ALT and BUN in individual animals at doses exceeding 200 mg/kg/day.

SUMMARY

In nonclinical studies, CMX157:

- Demonstrated *in vitro* activity against a wide range of wild-type and antiretroviral drug-resistant HIV viruses in different cell systems;
- Produced approximately 30-fold higher intracellular levels of the active anabolite tenofovir-diphosphate (TFV-PP) in human PBMCs *in vitro* as compared to TFV at physiologically relevant concentrations (TFV C_{max});
- Associated directly with HIV and subsequently reduced viral production in untreated target cells, suggesting virus exposed to CMX157 will carry the antiviral into diverse cellular and anatomical compartments;
- Displayed no antagonism in combination with any approved antiretroviral (DNS*);
- Showed no cytotoxicity in a panel of ten primary and transformed human cell types up to 10,000 nM (DNS);
- Was similar to TFV and less toxic than AZT in colony forming assays using human bone marrow progenitor cells (DNS);
- Did not produce any important undesirable pharmacological effects in safety pharmacology assays (DNS);
- Produced dose-limiting gastric toxicity in 28-day rat and cynomolgus monkey studies with NOAELs of 200 mg/kg/day;
- Was not mutagenic or clastogenic in 3 standard *in vitro* and *in vivo* genetic toxicology studies (DNS);
- Demonstrated systemic exposure to CMX157 and TFV following oral administration of CMX157 in both rats and cynomolgus monkeys.

These results suggest CMX157 may be administered to humans at doses that could effectively treat wild-type and multi-NRTI resistant HIV and that CMX157 has properties that may be desirable for a topical HIV microbicide.

CONCLUSIONS

- 1) **CMX157 is >300 fold more potent than TFV *in vitro* against wt HIV and against clinically relevant HIV mutants**
- 2) **The amount of active drug produced in human PBMCs is much higher following equimolar exposure to CMX157 vs TFV**
- 3) **CMX157 associates directly with HIV virions and subsequently reduces replication in untreated target cells**
- 4) **High plasma exposures to CMX157 were attained with minimal toxicity in two animal species**

REFERENCES

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- *DNS=data not shown for space; available on request

