Characterization of a new use for acyclovir and tenofovir using human cervico-vaginal tissue *ex vivo*

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Background: HSV-2, one of the most common HIV-1 copathogens, establishes a synergistic interaction with HIV-1. Several clinical trials have shown that the anti herpetic drug acyclovir decreases HIV-1 viral load and delays HIV-1 disease progression. Similarly, CAPRISA004 reported that a vaginal gel containing 1% of the anti-HIV-1 drug tenofovir unexpectedly decreases the risk of HSV-2 acquisition by 51%. Using a system of cervico-vaginal tissue culture that reflects many of the *in vivo* tissue features, we evaluated the dual anti-HIV-1/HSV-2 activity of acyclovir and tenofovir.

Methods: Human tissues were infected *ex vivo* with HIV-1 (laboratory strains, primary isolates or NRTI-resistant HIV-1 variants), HSV-2 (HSV-2_G, HSV-2_{MS}) or with a combination of HIV-1 and HSV-2 variants and treated with acyclovir or tenofovir. HIV-1 and HSV-2 replication were respectively monitored by measuring HIV-1 $p24_{gag}$ or HSV-2 DNA accumulated in culture media.

Results: Acyclovir suppressed the replication of the laboratory $X4_{LAI.04}$ HIV-1 with an EC50 of 3 µM (95% Confidence Interval (CI):1.85-5.24). No statistical difference between the inhibition of primary isolates and AZT-resistant variant was observed (n=5). In contrast, the EC_{50} for the 3TC-resistant virus (M184V) was four times higher than that of the parental HIV-1 isolate (n=5). Tenofovir suppressed HSV-2 replication with respective EC_{50}s of 14 µg/ml (CI:10-163) for HSV-2_G, and 19 µg/ml (CI:27-127) for HSV-2_{MS}. Tenofovir 66 µg/ml reduced HSV-2_G and HSV-2_{MS} replication by 87±12% and 91.7±3.2%, respectively (p< 0.01). Accordingly with the *ex vivo* results, we found in a cell-free system that the triphosphate form of acyclovir was a direct inhibitor of the HIV-1 reverse transcriptase and that tenofovir-diphosphate was a direct inhibitor of the HSV DNA polymerase.

Conclusion: Using human tissue culture *ex vivo*, we showed that (i) acyclovir inhibits HIV-1 at concentrations that are clinically relevant and commonly achieved in plasma of patients treated with acyclovir or valacyclovir; (ii) tenofovir, at the concentration achieved in topical vaginal application (which is substantially higher than achieved upon systemic administration) acts as a double-targeted antiviral agent as well. These data explain the inhibition of HIV-1 in clinical trials using acyclovir and the inhibition of HSV-2 observed in the clinical trial using tenofovir (CAPRISA004).