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Supplemental Data

Acyclovir Is Activated into a HIV-1 Reverse Transcriptase Inhibitor in Herpesvirus-Infected Human Tissues

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Supplemental Experimental Procedures

Viral infections

Briefly, 9 to 27 blocks of tonsils, lymph nodes, or colorectal tissues were infected by topical application of 3–6 µl of HIV stock (0.5–1.0 ng of $p24_{gag}$). Isolates $X4_{LAI.04}$, $R5_{BaL}$, $R5_{SF162}$ and $R5_{AD8}$ were obtained from the Rush University Virology Quality Assurance Laboratory (Chicago, IL). Lamivudine resistant virus (M184V) and AZT resistant 4x were provided by Raymond Schinazi. The M184V viral stock has been prepared by electroporation of MT-2 cells with HIV-$1_{LAI.04}$ proviral DNA encoding the M184V substitution. The virus has been subsequently expanded on MT-2 cells and stored in 1mL aliquots.[1] The AZT resistant 4x contains mutations at D67N, K70R, T215Y and K219Q to express complete resistance to AZT [2,3]. For infection of cervicovaginal tissue, tissue blocks were incubated with HIV-1_{BaL} for 2 hr, washed and then individually transferred into a 96-well plate. *Ex vivo* infection of tonsillar tissue with HHV-6 was performed by inoculation of 27 tissue blocks with five microliters (approximately 2.3×10^7 DNA copies) of clarified viral stock (HHV-6B strain PL-1, kindly provided by Paolo Lusso).

MT-4 cells were infected with HHV-6B or with $X4_{LAI.04}$. Briefly, 5 x 10^6 MT-4 cells were infected with 100 µl of $X4_{LAI.04}$ stock (~20 ng of $p24_{gag}$) or with 100 µl of HHV-6B stock (~ 2×10^9 DNA copies). 2 x 10^5 HIV-1 infected cells were cultured in 2 mL of medium in 24-wells plates. HHV-6B infected cells were then added in various ratios relatively to HIV-1-infected cells (from 1:1 to 1:100). We measured HIV-1 replication by the release of $p24_{gag}$ core antigen in cell culture medium or in pooled medium bathing tissue blocks or using the HIV-1 p24 Alliance kit (PerkinElmer, Wellesley, MA). We evaluated HHV-6B viral replications by measuring the accumulation of viral DNA in culture medium by quantitative real-time PCR (see below).

[1]Schinazi RF, Lloyd RM, Nguyen MH, Cannon DL, McMillan A, Ilksoy N, Chu CK, Liotta DC, Bazmi H, Mellors JW (1993). Characteristics of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. Antimicrob Agents Chemoter, *37*, 875-881.

[2]Larder BA, Kemp SD (1989) Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science. *246*:1155-8.

[3]Arion D, Kaushik N, McCormick S, Borkow G, Parniak MA. (1998) Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase.Biochemistry *37*:15908-17.

Real-time PCR

Measurements were performed with an ABI Prism 7700 sequence detector (PE Applied Biosystems, Foster City, CA) using a real-time PCR TaqMan assay. A reference standard curve using serially diluted plasmids containing the target genes or a commercially available reference standard (Advanced Biotechnologies, Columbia, MD) was then created. The amount of cellular DNA was assessed by use of *erv-3* as a reference gene[4]. The standard curve used to determine the amount of cellular DNA was obtained with human genomic DNA (Novagen, Madison, WI) that corresponds to 8,750 – 140,000 cells (62.5 ng – 1 µg). Aliquots were prepared once by dilution of DNA in distilled water and

were stored at -20°C. All standard dilutions, controls, and samples were run in duplicate, and the average value of the copy number was used to quantify both HHV and HIV-1 DNA copies and cellular DNA. HSV-2 and the HHV-6 loads in culture medium were evaluated as the number of viral DNA copies accumulated in tissue culture medium. The DNA from 200 µL of culture medium was purified with the QIAamp kit according to the manufacturer's instructions. Quantification was performed by means of real-time PCR as described above.

[4]Yuan, C. C., Miley, W., and Waters, D. (2001). A quantification of human cells using an ERV-3 real time PCR assay. J Virol Methods *91*, 109-117.

Primers and probes used in the quantification of viral and cellular target gene by real-time PCR.

Target gene	*Forward primer*	*Reverse primer*	*Probe*
HSV-1 UL27	cgcatcaagaccacctcctc	gctcgcaccacgcga	tggcaacgcggcccaac
HSV-2 UL27	cgcatcaagaccacctcctc	gctcgcaccacgcga	cggcgatgcgccccag
HHV-3 ORF62	cggcatggcccgtctat	tcgcgtgctgcggc	attcagcaatggaaacacacgacgcc
HHV-4 LMP-2A	aacgatgaggaacgtgaat	agtcatcccgtggagagta	agagcccccaccgcctta
HHV-5 UL83	tcgcgcccgaagagg	cggccggattgtggatt	caccgaacgaggattccgacaacgt
HHV-6 U67	cgctaggttgaggatgatcga	caaagccaaattatccagagcg	cccgaaggaataacgctc
HHV-7 U67	agcggtacctgtaaaatcatcca	aacagaaacgccacctcgat	gagaacatcgctctaactggatca
HHV-8 ORF26	gtccagacgatatgtgcgc	actccaaaatatcggccgg	ttggtggtatatagatcaagttc
HIV-1 GAG	tagcaggaactagtacccttcagg	gcttgctcggctcttagagttttata	caaataggatggatgacacat
HUMAN ERV-3	aacgatgaggaacgtgaat	cccagcgagcaatacagaattt	agagcccccacccgcctta

Flow cytometry

Flow cytometry of cells isolated from tissue blocks and stained for cell surface markers with specific antibodies. Lymphocytes, identified according to their light-scattering properties, were stained with combinations of the following fluorescent-labeled monoclonal antibodies: anti-CD3-PE-Cy7, anti-CD4-PE-Cy5.5, anti-CD8-PacificBlue, anti-CD25-APC, anti-CD38-APC-Cy5.5, anti-HLA-DR-PE-Cy5, anti-CD62L-APC-Cy7,

and anti-CD45RA-PE (Caltag Laboratories, Burlingame, CA). In cultures of MT4 cells infected with HHV-6B and HIV-1, after identification and exclusion of dead cells using the LIVE/DEAD fixable Blue Dead Cell Stain kit (Invitrogen), the presence of infected cells was evaluated by intracellular staining for viral antigens using anti-HHV-6 pp41-Alexa488 (Advanced Biotechnology, Columbia, MD) and anti-HIV-1-p24-PE antibodies (Beckman Coulter, Miami, FL). Data were acquired with an LSRII flow cytometer equipped with 355-, 407-, 488-, 532-, and 638-nm LASER lines using DIVA 5.1 software (Becton Dickenson). Data were analyzed with FlowJo software (Tree Star, San Carlos, CA). Cell depletion was quantified by the addition of Trucount™ beads (Caltag Laboratories) to each tube prior to acquisition as a volumetric control and by normalizing the number of cells by tissue-block weight.

Exogenous template RT assay

The HIV-1 (pNL4-3) *gag* RNA transcript from plasmid pRB1334, described previously [5] was used as the template (40 μg/mL final concentration). Three 2'-deoxyoligonucleotides, complementary to the template (GagRev: 5′-GCT CTC GCA CCC ATC TCT CTC C-3′; HIVGagR1: 5′-CTA TCC TTT GAT GCA CAC AAT AGA G-3′; and GagRNA Rev: 5′-AGT AGT TCC TGC TAT GTC ACT TCC C-3′) were mixed and annealed to the template (8 μg/mL final concentration each) by heating at 68° C for 10 min and slow cooling to 4° C. [Methyl-^{3}H]-Thymidine-5′-triphosphate (83.4 Ci/mmol) was obtained from Perkin Elmer Life Sciences. Unlabeled dATP, dCTP, and dGTP were obtained from Promega Corp. (Madison WI). All unlabeled and tritiated dNTPs (except for dGTP, which was used at the indicated concentrations) were used at a final concentration of 3.38 μM. ACV and ACV-TP (Moravek, Brea, CA) were used at

the final concentrations indicated. 3'-Azido-3'-deoxythymidine-5'-triphosphate (AZT-TP) at the concentrations of 3.38 and 33.8 μM was used as positive control in the reactions. To initiate the reverse transcription reactions, 5 μL of HIV-1 (NL4-3; Lot: P4163; 60 ng $p24_{gag}$ equivalents per reaction) from the AIDS Vaccine Program's, Biological Products Core (NCI-Frederick, Frederick, MD) was added. Each reaction condition was performed in duplicate.

[5]Thomas, JA., Gagliardi, TD., Alvord, WG., Lubomirski, M., Bosche, WJ., Gorelick, RJ. (2006). Human immunodeficiency virus type 1 nucleocapsid zinc-finger mutations cause defects in reverse transcription and integration. Virology *353*, 41-51.

Steady-state kinetics

Heterodimeric RT p66/p51 was expressed and purified essentially as described [6]. The following sequences were used as template and primer, respectively. The primer binding site is underlined.

T50A6: 5'-

CCAATATTCACCATCAAGGCTTGATGAAACTTCACTCCACTATACCACTC-3'

P1: 5'-GAGTGGTATAGTGGAGTGAA-3' (20 mer).

The DNA primer was 5'-labelled with [γ-^{32}P] ATP and heat-annealed with the template. Primer/template at 150 nM was incubated with 50 nM HIV-1 RT in a buffer containing 50 mM Tris-HCl (pH 7.8), and 50 mM NaCl, in the presence of increasing concentrations of dGTP or ACV-TP. Nucleotide incorporation was initiated by the addition of $MgCl_2$ to a final concentration of 10 mM, and reactions were allowed to proceed for 3 min. Reaction products were separated on 18% denaturing polyacrylamide gels followed by phosphorimaging. We determined the kinetic parameters k_{cat} and K_m by fitting the datum points to the Michaelis-Menten equation using GraphPad Prism (version 4.0). k_{cat} was

defined as the maximal rate of single nucleotide incorporation. K_m was defined as the concentration of dNTP substrate at which the rate of single nucleotide incorporation was equal to the half-maximal rate.

[6]Le Grice, SF., and Gruninger-Leitch, F. (1990). Rapid purification of homodimer and heterodimer HIV-1 reverse transcriptase by metal chelate affinity chromatography. Eur J Biochem *187*, 307-314.

ATP-dependent excision

The DNA template T50A6 was heat annealed with 5'-labelled DNA primers that were terminated with 2',3'-dideoxyguanosine 5'-monophosphate (ddGMP) and ACV-MP, respectively. Primer/template at 50 nM was then incubated with 750 nM HIV-1 RT in a buffer containing 50 mM Tris-HCl (pH 7.8), 50 mM NaCl, 10 mM $MgCl_2$, and 3.5 mM ATP as a pyrophosphate donor. Excess of HIV-1 RT yields a favorable signal to noise ratio. Aliquots were taken at different time points. Inhibition of excision in the presence of the next nucleotide substrate, a process referred to as dead-end complex formation, was studied in the presence of 20 μM dGTP (the normal counterpart of the terminating drug ACV-TP or ddGTP) and increasing concentrations of ddTTP (a chain-terminator version of the next nucleotide). The reactions were allowed to proceed for 60 min and were processed and analyzed as outlined above. Here, the datum points were fitted to a sigmoidal dose-response (variable slope) equation to determine the concentration of the next nucleotide that causes 50% inhibition of excision and rescue of DNA synthesis (IC_{50}).

Site-specific footprinting

The 5'-labeled DNA template T50A6 was heat-annealed with DNA primers that were terminated with ddGMP and ACV-MP, respectively. DNA/DNA hybrid at 25 nM

was incubated with 750 nM RT in a buffer containing sodium cacodylate at pH 7 (120 mM), NaCl (20 mM), DTT (0.5 mM) and $MgCl_2$ (10 mM). Increasing concentrations of dTTP were added to the reaction, and then the complex was treated with 0.1 mM ammonium iron (II) sulphate hexahydrate. Divalent Fe^{2+} ions bind to the ribonuclease H domain of HIV-1 RT and cause highly specific oxidative cleavage on the template. The footprinting reactions were conducted and analyzed as previously described [7,8].

[7]Gotte, M., Maier, G., Gross, H. J., and Heumann, H. (1998). Localization of the active site of HIV-1 reverse transcriptase-associated RNase H domain on a DNA template using site-specific generated hydroxyl radicals. J Biol Chem *273*, 10139-10146.

[8]Marchand, B., and Gotte, M. (2003). Site-specific footprinting reveals differences in the translocation status of HIV-1 reverse transcriptase. Implications for polymerase translocation and drug resistance. J Biol Chem *278*, 35362-35372.

Synthesis of N^2-DMF acyclovir-[1-naphthyl(methoxy-L-alaninyl)] phosphate.

To a stirring suspension of N^2-DMF ACV (0.40 g, 1.44 mmol) in anhydrous THF (15 mL) was added dropwise under an argon atmosphere ^{t}BuMgCl (1.0 M THF solution, 2.88 mL, 2.88 mmol) and the reaction mixture was stirred at room temperature for 30 min. After this period a solution of 1-Naphthyl(methoxy-L-alaninyl)-phosphorochloridate (1.41 g, 4.32 mmol) in anhydrous THF (10 mL) was added and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue purified by column chromatography gradient elution of DCM/MeOH = 96/4 then 94/6, to give a white solid (36%, 0.43 g).

^{31}P-NMR (MeOD, 202 MHz): δ 4.09, 3.99.

^{1}H-NMR (MeOD, 500 MHz): δ 8.42 (1H, s, NC*H*N$(CH_3)_2$), 8.01-7.95 (1H, m, H-8 Naph), 7.79, 7.78 (1H, 2s, H-8), 7.73-7.18 (6H, m, Naph), 5.38, 5.36 (2H, 2s, H-1'), 4.20-4.12 (2H, m, H-5'), 3.89-3.82 (1H, m, C*H*CH_3), 3.72-3.66 (2H, m, H-4'), 3.48, 3.45 (3H, 2s, $COOCH_3$), 2.94, 2.90 (6H, 2s, N$(CH_3)_2$), 1.18-1.14 (3H, s, CHCH_3).

Synthesis of acyclovir-[1-naphthyl(methoxy-L-alaninyl)]phosphoramidate (Cf2649).

A solution of N^2-DMF acyclovir-[1-naphthyl(methoxy-L-alaninyl)] phosphate (0.41 g, 0.72 mmol) in 2-propanol (15 mL) was stirred under reflux for 72 h. The solvent was then removed under reduced pressure and the residue was purified by column chromatography gradient elution of DCM/MeOH = 98/2 then 96/4 then 92/8. The product was purified by preparative TLC (gradient elution of DCM/MeOH = 98/2 then 96/4 then 95/5) then by preparative reverse phase HPLC (gradient elution of H_2O/CH_3CN= from 100/0 to 0/100 in 45 min) to give a white solid (7%, 0.028 g).

^{31}P-NMR (MeOD, 202 MHz): δ 4.07, 4.05.

^{1}H-NMR (MeOD, 500 MHz): δ 8.14-8.12 (1H, m, H-8 Naph), 7.88-7.87 (1H, m, H-6 Naph), 7.82, 7.81 (1H, 2s, H-8), 7.71-7.70 (1H, m, H-2 Naph), 7.56-7.51 (2H, m, H-5 Naph, H-7 Naph), 7.45-7.39 (2H, m, H-3 Naph, H-4 Naph), 5.44, 5.42 (2H, 2s, H-1'), 4.30-4.28 (1H, m, H-5' of one diastereoisomer), 4.27-4.24 (1H, m, H-5' of one diastereoisomer), 4.06-3.98 (1H, m, C*H*CH_3), 3.84-3.82 (1H, m, H-4' of one diastereoisomer), 3.80-3.79 (1H, m, H-4' of one diastereoisomer), 3.63, 3.59 (3H, 2s, COOC*H*$_3$), 1.34-1.30 (3H, m, CHC*H*$_3$).

^{13}C-NMR (MeOD, 125 MHz): δ 20.35, 20.41, 20.47, 20.52 (CH*C*H_3), 51.59 (*C*HCH_3), 52.67, 52.76 (COO*C*H_3), 67.22, 67.26, 67.28, 67.32 (C-5'), 69.36, 69.42, 69.44, 69.50 (C-4'), 73.68 (C-1'), 116.23, 116.25, 116.28, 117.56, 122.69, 122.74, 125.90, 126.51, 127.42, 127.44, 127.75, 127.89, 127.94, 128.81, 128.83 (C-5, C-2 Naph, C-3 Naph, C-4 Naph, C-5 Naph, C-6 Naph, C-7 Naph, C-8 Naph, C-8a Naph), 136.26 (C-4a Naph), 139.69 (C-8), 148.00, 148.06 ('ipso' Naph), 153.38 (C-4), 155.80 (C-2), 159.57 (C-6), 175.44, 175.48, 175.58, 175.61 (*C*OOCH_3).

EI MS = 539.14 (M+Na)

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) detection of ACV-MP and ACV-TP in tonsillar tissue.

The quantification of the phosphorylated derivatives ACV-MP and ACV-TP was performed using an LC-MS/MS method. ACV-MP and ACV-TP were extracted from the pellets with 60% methanol. Internal standard, lamivudine-triphosphate (3TC-TP) was added to each tube in order to reach 100 nM after process of the samples. The pellets were vortex mixed at high speed for 15 min before centrifugation at 16,000 g for 10 min to remove cellular debris. The supernatant was evaporated to dryness under a stream of air. Dried extracts were reconstituted in 100 µL water, filtered (0.22 µm nylon centrifuge tube) by centrifugation at 16,000 x g for 10 min and analyzed immediately; 45 µL were injected on the column.

Gradient separation was performed on a Biobasic AX (50 x 1.0 mm) column for ACV-MP and on a Biobasic AX (100 x 1.0 mm) column for ACV-TP, both with a 5 µm particle size (Thermo Electron, Waltham, MA). The mobile phase consisted of: A) 20 mM ammonium acetate buffer; B) 2 mM ammonium phosphate buffer, adjusted to pH 11.0 with ammonium hydroxide and C) acetonitrile. C was kept at 20% during the entire run. 0.5 min after injection, B was increased from 0 to 80% in 8 min and maintained at 80% for -1.5 min before returning to initial conditions. The flow rate was 100 µl.min^{-1} for the first 9 min and was 200 µl min^{-1} for 14 min before returning to initial conditions. The autosampler temperature was maintained at 4°C, and the column temperature, at 20°C. The first 4.5 min and the last 10 min of the analysis were diverted to waste. The total run time was 25 min.

The mass spectrometer was operated in positive ionization mode with a spray voltage of 3.5 kV, sheath gas at 40 (arbitrary units), ion sweep gas at 0.2 (arbitrary units), auxiliary gas at 0 (arbitrary units), and a capillary temperature of 350°C. The collision cell pressure was maintained at 1.4 mTorr. Four positive ion selected reaction were monitored (with collision energy in parentheses) for ACV-MP: m/z 306 → m/z 152 (20 V), for ACV-TP: m/z 466 → m/z 152 (25 V), for ACV-TP confirmation ion: m/z 466 → m/z 135 (36 V) and for 3TC-TP: m/z 470 → m/z 112 (30 V). Thermo Xcalibur software was used to control the system and to perform data analyses.

Supplemental Table and Figures

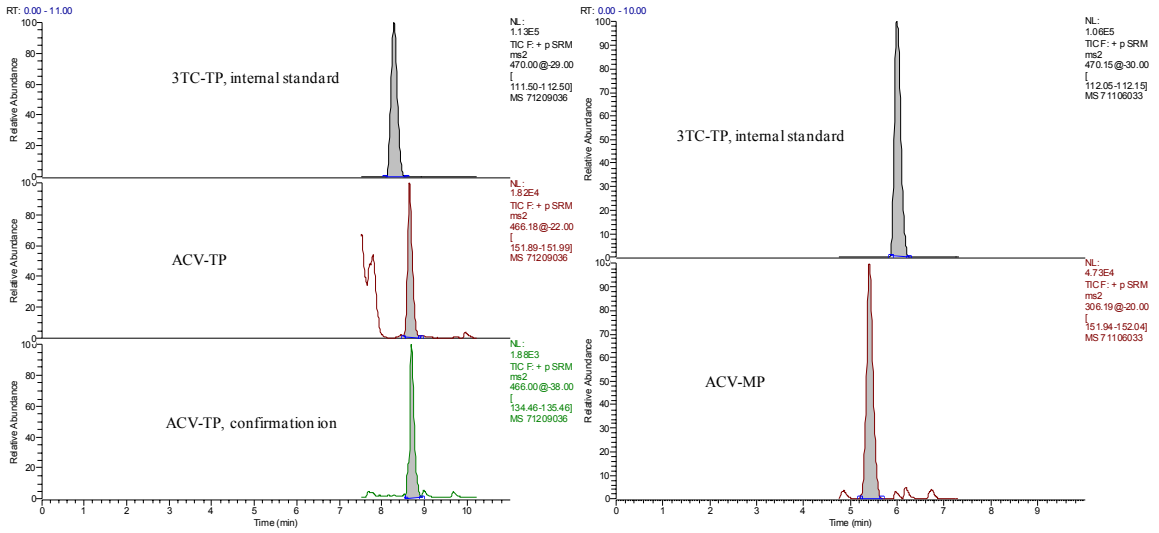
Table S1. Kinetics of incorporation of ACV-TP by HIV-1 RT

Substrate	dGTP	ACV-TP
k_{cat}, min^{-1}	0.47 ±0.02	0.63 ±0.02
K_m, µM	0.032 ±0.005	0.045 ±0.005
k_{cat} / K_m	14.7	14

We determined the kinetic parameters k_{cat} and K_m by fitting the data points to the Michaelis-Menten equation using GraphPad Prism (version 4.0). k_{cat} was defined as the maximal rate of single nucleotide incorporation. K_m was defined as the concentration of dNTP substrate at which the rate of single nucleotide incorporation was equal to the half-maximal rate. The ratio (k_{cat}/K_m) measures the efficiency of single nucleotide incorporation events under steady-state conditions.

Figure S1: Detection of ACV-MP and ACV-TP by LC-MS/MS

a. **b.**

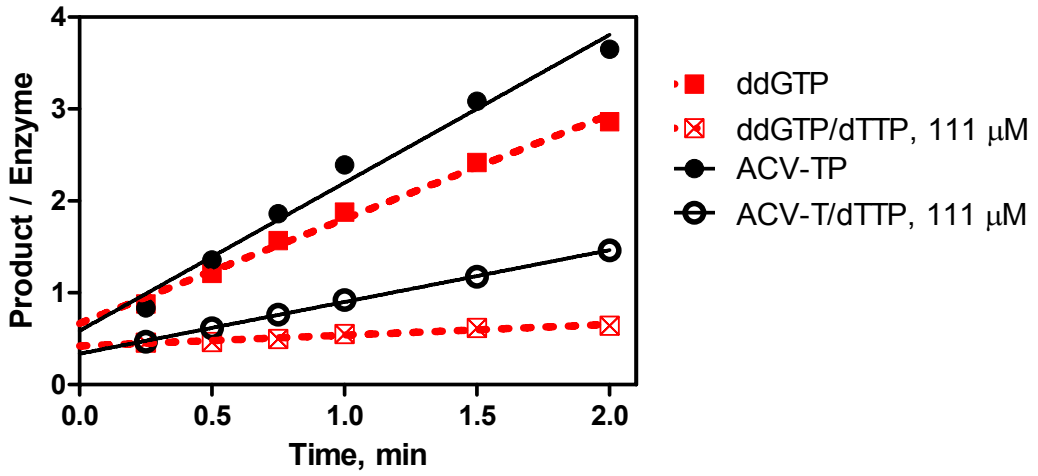


Chromatograms shown in Figure S1a and S1b were obtained from two different injections of the same sample: a long column (100*1 mm) was necessary for the separation of ACV-TP peak from the matrix and a shorter column (50*1) was sufficient to detect ACV-MP without matrix effect.

a: Typical chromatogram illustrating the presence of acyclovir-triphosphate (ACV-TP). All three Reconstituted Ion Chromatograms were obtained from a single LC-MS/MS experiment.

b: Typical chromatogram illustrating the presence of acyclovir-triphosphate (ACV-TP). The two Reconstituted Ion Chromatograms were obtained from a single LC-MS/MS experiment.

Figure S2. Measurement of the dissociation rate constant (k_{off}) for the enzyme/nucleic acid substrate binary complex.



	ddGTP	ddGTP/dTTP, 111 μM	ACV-TP	ACV-T/dTTP, 111 μM
Slope	1.1 ± 0.051	0.11 ± 0.0082	1.6 ± 0.11	0.51 ± 0.028

200 nM DNA/DNA template/primer hybrid (T50A6/P1) were incubated with 25 nM of HIV-1 RT in a buffer containing 50 mM Tris-HCl pH 7.8, 50 mM NaCl and 2 μM ACV-TP or ddGTP in the presence or absence of 111 μM dTTP as the next correct nucleotide to be incorporated at position n+1 following incorporation of ACV-MP or ddGMP at position n. Nucleotide incorporation was initiated by the addition of 10 mM $MgCl_2$. Aliquots were taken at different time points from 0.5 to 4 minutes and analyzed on denaturing polyacrylamide gels. The incorporation of single nucleotides was quantified as the fraction of the DNA substrate (template/primer) converted to product (template/primer+1 nucleotide) from which the concentration of reaction product was calculated.

The reaction product was normalized to the concentration of RT used in the reaction mixture and plotted *vs.* the respective time point. The data points were fitted by linear regression using GraphPad Prism (version 4.0) to determine the rate constant of the nucleotide incorporation from the slope of the linear relationship line. Slope is expressed in terms of min-1 and under the conditions of the experiment is equivalent to k_{off} for the binary complex (in the absence of dTTP) or ternary dead-end complex (DEC) (in the presence of dTTP).

Our reaction conditions are consistent with the so called "forced termination" [8]. Therefore, the rate constant of the nucleotide (ACV-MP or ddGMP) incorporation in the absence of dTTP reflects the dissociation constant for dissociation of the binary complex, while in the presence of dTTP it measures the dissociation rate for the enzyme/template/primer+ACV-MP (or ddGMP)/dTTP ternary complex immediately prior to incorporation of dTTP, since ACV-MP and ddGMP are obligate chain terminators[9].This ternary complex is referred to as a DEC.

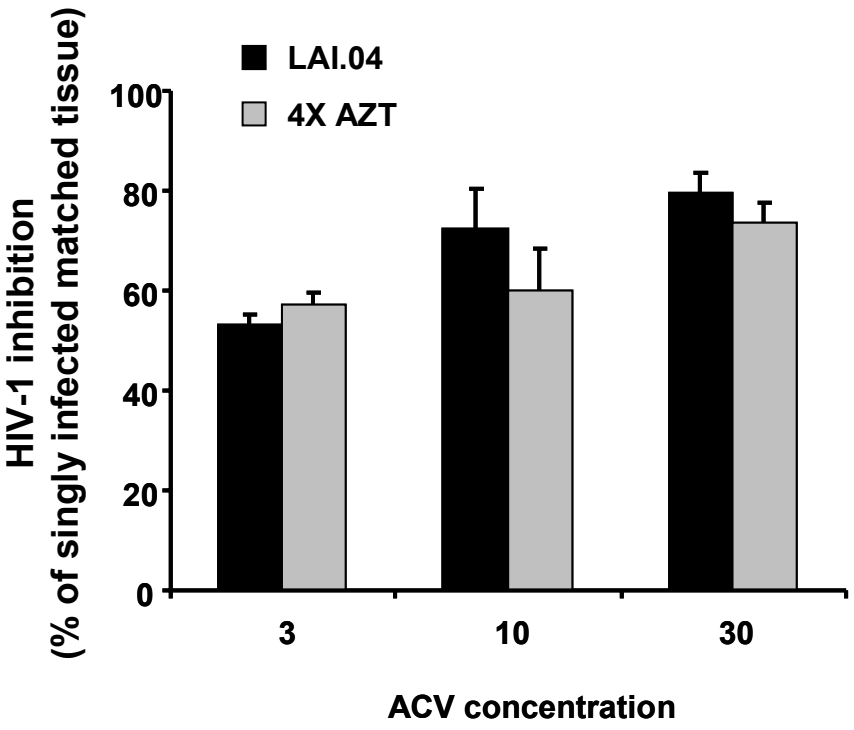
Note that the dissociation constant for ACV-terminated primers is approximately three-fold increased in the context of the ternary complex, which demonstrates that the next complementary nucleotide can bind to the complex. A 10-fold increase is measured with ddGMP-terminated primers, which shows that DEC-formation is more efficient in this context.

[8] . Wilson JE, Aulabaugh A., Caligan B, McPherson S, Wakefield JK, Jablonski S, Morrow CD, Reardon JE, Furman PA. (1996) Human immunodeficiency virus type-1 reverse transcriptase. Contribution of Met-184 to binding of nucleoside 5'-triphosphate. J Biol Chem. *271*:13656-62.

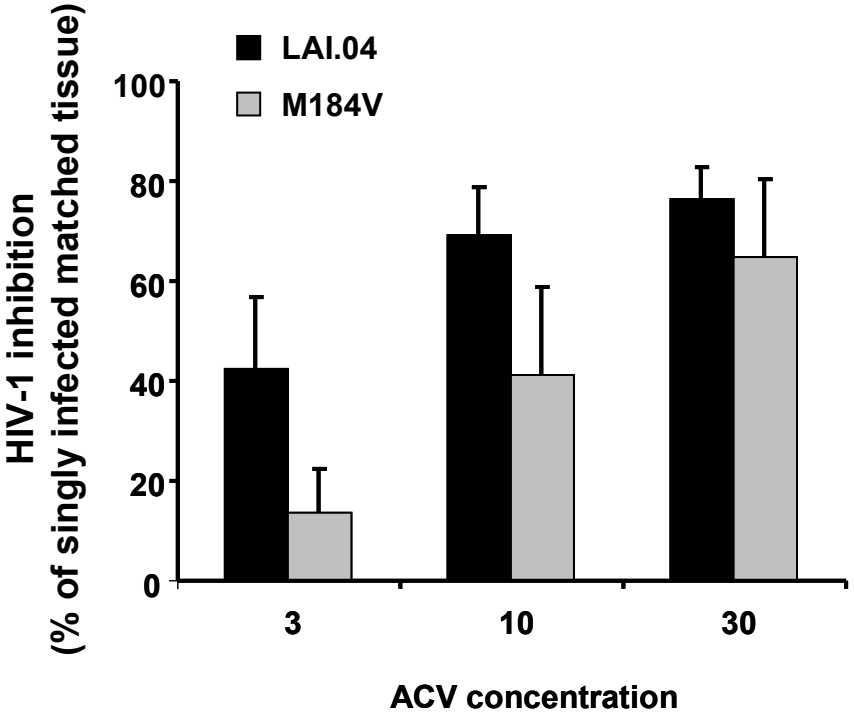
[9]. Kati WM, Johnson KA, Jerva LF, Anderson KS. (1992) Mechanism and fidelity of HIV reverse transcriptase. J Biol Chem *267*:25988-97.

Figure S3. Effect of ACV on NRTIs resistant viruses

a.



b.



a. Blocks of human lymphoid tonsillar tissues were inoculated *ex vivo* with the AZT resistant isolate AZT 4x containing mutations at D67N, K70R, T215Y and K219Q and treated with ACV (3,10, 30 μM). HIV-1 replication was monitored by measuring $p24_{gag}$ accumulated in culture media over 3 day periods. Presented are means ± SEM of the results with tonsils from 3 donors. Note that AZT-resistant HIV-1 retains full sensitivity to ACV.

b. Blocks of human lymphoid tonsillar tissues were inoculated *ex vivo* with lamivudine resistant virus (M184V) and its parental strain (LAI.04) and treated with ACV (3,10, 30 μM). HIV-1 replication was monitored by measuring $p24_{gag}$ accumulated in culture media over 3 day periods. Presented are means ± SEM of the results with tonsils from 5 donors. Note that M184V RT HIV-1 is less sensitive to ACV than its parental strain.