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A Continuous Time-Resolved Fluorescence Assay for Human Immunodeficiency Virus Reverse Transcriptase to Determine Enzymatic Mechanisms of Resistance. GB Roberts, R Ferris, KL Weaver, SA Short, L Boone, JH Chan, DJT Porter, and ES Furine; Glaxo Wellcome Inc., Research Triangle Park, NC, USA

Many non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) equilibrate with wild-type RT slowly (the rate constant for equilibration < 0.01 sec^{-1} with [NNRTI] = IC_{50}). Consequently, complete characterization of the inhibitory process requires monitoring the time-course of enzymatic activity. RT activity is typically monitored with discontinuous radioactive end-point assays. Collection of data for a complete time-course by this method is a tedious process. To circumvent this, we have developed a continuous assay to monitor primer extension that is based on time-resolved fluorescence resonance energy transfer. In this assay, RT catalyzes the incorporation of a deoxyuridine monophosphate analog labeled with Cy5 dye (Cy5-dUMP) into an europium (Eu)-labeled template primer. Primer extension is monitored by the fluorescence energy transfer from Eu (excitation 340 nm, emission 620 nm) to incorporated Cy5-dUMP (excitation 649 nm, emission 670 nm). In the absence of inhibition, the amplitude of the signal change is linearly dependent on enzyme concentration and time. With this assay one can readily monitor the steady-state rate of RT-catalyzed nucleotide incorporation and the time-course for the onset of inhibition of RT by NNRTIs. We have used this method to determine steady-state IC_{50} values for the three FDA-approved NNRTIs, nevirapine, delavirdine, and efavirenz with wild-type RT and 11 mutant RTs. The enzymatic pIC_{50} values (-log IC_{50}, M) correlate well with antiviral pIC_{50} values (R^2=0.880). Furthermore, values for association and dissociation rate constants of RT for inhibitors were determined for the slowly binding inhibitors. Resistance of mutant RTs to the NNRTIs was associated with increased values for the dissociation rate constants.

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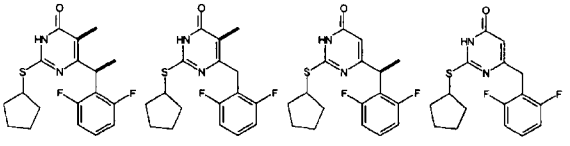
Sensitivity of mutant HIV-1 rRT to DABOs. *°Musiu C., *°Marceddu T., *°Loi A.G., ^Mai A., *Bryant M., †Sommadossi J.P. and °La Colla P. *Novirio Pharmaceuticals Inc., USA; °Dipartimento di Biologia Sperimentale, Università di Cagliari; ^Dipartimento di Studi Farmaceutici, Università "La Sapienza" di Roma; †University of Alabama at Birmingham, USA.

All of the currently available agents have shown considerable limitations, from dosing schedules, to inadequate activity and/or short- or long-term toxicity. Moreover, clinical practices are rapidly expanding the numbers of patients who have been treated with most of the available antiretroviral drugs. New agents, even of an existing class, that have proven activity against resistant virus isolates are therefore needed. In the present study we report on the activity of representative DABO derivatives against HIV-1 recombinant reverse transcriptases (rRT) containing single mutations (K103N, L100I, V106A, Y179D, Y181I, Y188C). Introduction of methyl groups both at position 5 of the pyrimidine ring and on the methylene linker was observed to lead to compounds (i.e. MC 1047) which inhibit the K103N rRT at 0.03 mM. Compounds substituted at either of the above positions (i.e. MC 922 and MC 1008) were 10-fold less active (IC_{50} = 0.3 mM), whereas unsubstituted compounds (i.e. MC 867) were 20-fold less potent (IC_{50} = 0.6 mM). Activities against mutant viruses in cell-based assays will be reported.



MC 1047 MC 922 MC1008 MC 867

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Inhibition of HIV Reverse Transcriptase Inhibitor- and Protease Inhibitor-Resistant Molecular Clones of HIV by the Biological Response Modifier, Ampligen.

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Despite the dramatic reduction of HIV load in patients on various highly active anti-retroviral therapy (HAART) regimens, recent reports of the development of drug-resistant mutants during therapy provides a challenge for long-term inhibition of HIV replication. Ampligen is a dsRNA (Poly I:PolyC_{12}U) with potent anti-HIV and immunomodulatory properties. This study examined the *in vitro* anti-HIV activity of Ampligen against molecular clones of HIV that contain specific mutations in the reverse transcriptase (M184V and JF26A7) or protease (L10R and M46I) responsible for drug resistance. An additional isolate with resistance to nevirapine was also analyzed. The fifty percent effective concentration (EC50) for lamivudine against M184V increased to >12,500 nM compared to wild-type virus (120 nM). The EC50 of zidovudine against JF26A7 increased to >20,000 nM compared to 18.3 nM for the wild-type clone. The protease resistant viruses L10R and M46I had EC50 for indinavir of 31.3 nM and 17.7 nM, respectively, compared to 6.3 nM for the wild-type clone. The EC50 of nevirapine against the nevirapine-resistant clone was >1,250 nM compared to 174 nM for the wild-type clone. All of the mutant viruses were equally sensitive to the anti-HIV effects of Ampligen with an EC50 of <5 μg/ml. This level is well below concentrations of Ampligen that are readily achieved in patients receiving twice weekly parenteral administrations. This study suggests that Ampligen will provide anti-HIV activity *in vivo* against HIV mutants that are resistant to existing anti-HIV agents.

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Mixed Dose Effect Analysis of a Biological Response Modifier (Ampligen) with 14 FDA-Approved Anti-HIV Agents.

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Poly I:PolyC_{12}U (Ampligen) is a biological response modifier, which has anti-HIV properties *in vitro* as well as the ability to augment cell-mediated immunity *in vivo*. Previous studies have demonstrated synergy between Ampligen and zidovudine (AIDS Res. Human Retrovir. 5:193-203, 1989; Antiviral Res. 17:169-177, 1992). The current study examines the cell toxicities and anti-HIV activities of all 14 FDA-approved anti-HIV agents alone and in combination with Ampligen. Mixed dose effect analyses for anti-HIV activity were performed at three drug ratios. Combination indices were determined for all drug combinations using the CalcuSyn for Windows software package. Among the nucleoside reverse transcriptase inhibitors (NRTI), abacavir sulfate, zidovudine, zalcitabine, didanosine, and stavudine demonstrated synergistic activity at all three ratios. Lamivudine exhibited synergistic activity at ratios of 1:3 and 1:1 to Ampligen but not at a 3:1 ratio. Among the three FDA approved non-nucleoside reverse transcriptase inhibitors (NNRTI), only efavirenz was synergistic at all three ratios tested. All five protease inhibitors (PI) demonstrated synergy with Ampligen; ritonavir possessed the greatest synergy and saquinavir the least synergy. The data suggest that significant reductions in the doses of anti-HIV drugs might be achieved in HAART if Ampligen were included in the cocktail, perhaps reducing HAART-related toxicities. Limited clinical trials of Ampligen in combination with existing HAART are needed to test this hypothesis.