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Rapid Emergence of HIV-1 Resistant to Non-nucleoside Inhibitors of Reverse Transcriptase.

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Several compounds from a newly discovered class of potent and selective non-nucleoside inhibitors of human immunodeficiency virus-1 (HIV-1) reverse transcriptase (RT) are undergoing evaluation in clinical trials. We studied the potential for development of viral resistance to one of the prototype compounds BI-RG-587. HIV-1 resistant to BI-RG-587 emerged after only one cycle of in vitro infection in the presence of the drug. Resistant virus was cross-resistant to the non-nucleoside TIBO derivative R82150, but remained susceptible to dideoxynucleosides derivatives and phosphonoformate. Native (virion-associated) and recombinant RT derived from resistant virus were insensitive to both BI-RG-587 and TIBO R82150. The therapeutic efficacy of these compounds as single agents may be limited by emergence of resistant HIV-1. Their use in combination with other antivirals that do not show cross-resistance should be considered. Supported by Medical Research Service of the Dept. of Veterans Affairs (JWM) and by NIH grants CA44358 and AI25899 (Y-CC).

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Poly r(A) Reverse Transcriptase (^{3}H) SPA,a new enzyme assay system

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Amersham's reverse transcriptase enzyme assay system is a new assay incorporating the scintillation proximity assay (SPA) principle (Prod. Nb. NK 9020). This is a homogeneous assay using a DNA/RNA polynucleotide system with biotin/streptavidin bead capture. The primer DNA, containing biotin at its 5' end was in our experiments a 16 mer oligo (T) annealed to a poly r(A) template of approximately 300 bases in lenght. Incorporation of (^{3}H) TTP by reverse transcription, results in extension of the primer. Upon addition of the streptavidin SPA beads, the biotinylated extended primer binds to the beads, the incorporated tritiated nucleotides are able to stimulate the scintillant and produce a signal, it is not the situation of tritiated nucleotides unincorporated. We tested this method, first on recombinant HIV1-RT, second with HIV-1 lysed suspension and third with the Rauscher murine Leukemia Virus (R-MuLV). We have determined optimal assay conditions for recombinant HIV1-RT with this method. The (^{3}H) TTP (Amersham ref. TRK 576) at 0,043 μM, produces 70000 cpm in optimal conditions and is linear until 200 ng enzyme/assay up to 2h incubation. The assay has been used to evaluate reference compounds. With the HIV-1 lysed suspension, a modified medium gives the best activity compared with the kit medium. These conditions show a kinetic activity optimal for 3 h at 37°C. The IC_{50} values for several drugs are : PFA = 6×10^{-8}M, TIBO = 0,05 mM, AZT-TP = $1,4 \times 10^{-9}$ M and one analog of BI-RG587 inhibits 40 % at 0,04 μM. A preliminary assay on the (R-MuLV) shows that Mn Cl_2 will not substitute by $MgCl_2$ (RT-Mn sensitive). We hope to test in the future several recombinant RT: AMV-RT, M. MuLV... We postulate that, with small modifications, the Poly r (A)-RT- (^{3}H) SPA enzyme assay system can be substituted advantageously for the TCA method in the evaluation of anti-RT drugs or in assays using RT as a marker for HIV replication.