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**Clinical applications of in vitro susceptibility testing of HIV-1 to Zidovudine (AZT). B. Conway, D. Ko, N. Hawley-Foss, L.G. Filion, D.W. Cameron and F.J. Diaz-Mitoma, Department of Microbiology and Immunology, University of Ottawa, Ottawa, Canada.**

In vitro susceptibility of HIV-1 to AZT was evaluated in 54 patients. Mononuclear cells were cultured with 10 μM AZT for 72 hours, harvested for PCR, and processed using a quantitative microtiter plate assay for the detection of HIV-1 proviral sequences. The reduction (%) in proviral load in AZT-containing cultures was taken as a measure of AZT resistance. We have studied 25 patients on AZT and 29 patients off AZT. Our results are shown below:

| viral load reduction (%) | <10 | 10-24 | ≥25 |
|---|---|---|---|
| AZT therapy (n=25) | 13(52%) | 2 (8%) | 10(40%) |
| no AZT therapy (n=29) | 3 (10%) | 2 (7%) | 24(83%) |

There is an association ($p<.001$) between ongoing AZT therapy and resistance to AZT. In patients on therapy followed for ≥ 6 months, 7/8 with high-grade resistance (<10% viral load reduction) did not subsequently respond clinically to AZT. In patients who had discontinued AZT therapy, 8/10 carried sensitive virus (>25% viral load reduction), suggesting that in some cases, sensitivity to AZT may reappear when the drug is discontinued. This rapid, quantitative PCR-based assay may be a useful tool in the clinical management of antiretroviral therapy.

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HIV Reverse Transcriptase: Kinetics of Incorporation of Nucleotide Analogs into Heteropolymeric Template-Primers. J. E. Reardon. Wellcome Research Labs, Research Triangle Park, NC.

Reverse transcriptase (RT, EC 2.7.7.49) encoded by the human immunodeficiency virus (HIV) is a primary target for development of therapeutic agents for treatment of acquired immunodeficiency syndrome (AIDS). The parent nucleosides of many obligate chain-terminating nucleotide analog inhibitors of RT are effective inhibitors of HIV *in vitro*. The substrate kinetic constants of a series of nucleotide analogs have been determined using heteropolymeric template-primers. The assays did not require radiolabelled nucleotide analogs. Values of $k_{cat}$ and $K_m$ for the four natural 2'-deoxynucleoside 5'-triphosphates were similar, indicating that the different DNA primed-RNA templates and nucleotide substrates were utilized equally by reverse transcriptase (RT). Further, the kinetic constants for 3'-azido-3'-deoxythymidine 5'-triphosphate (AZTTP) were similar to those of dTTP indicating that AZTTP is as efficient a substrate as dTTP for the enzyme. In contrast, the four 2', 3'-dideoxynucleoside 5'-triphosphates (ddNTP), 2', 3'-dideoxy-2', 3'-didehydrothymidine 5'-triphosphate (d4TTP) and 2', 3'-dideoxy-3'-fluoroguanosine 5'-triphosphate (FdGTP) were 4 -10-fold less efficient substrates of the enzyme. Finally, the phosphonate analog of AZTTP (3'-azido-3', 5'-dideoxythmidine 5'-methylphosphonic acid diphosphate, AZTCPDP) was a 3000-fold less efficient substrate compared to dTTP and AZTTP. The reduced substrate efficiency of AZTCPDP was due to a change in rate-determining step from dissociation of the RT•chain-terminated template-primer complex to presumably, the catalytic step.