

# 15th International HIV Drug Resistance Workshop



13-17 June 2006, Sitges, Spain

## A NOVEL ASSAY TO DETERMINE THE HIV-1 *IN VITRO* REPLICATION RATE INDEPENDENT OF VIRUS INPUT CONCENTRATION, AND ITS APPLICATION TO ANALYSE THE EVOLUTION OF THE REPLICATION RATE OF VIRUSES EMERGING FROM *IN VITRO* SELECTION WITH TMC-114

*Antivir Ther.* 2006, 11:S50 (abstract no. 42)

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**BACKGROUND:** Evaluation of the replication rate (RR) of HIV has become essential for wild-type and mutant strains. Accurate measurement of the RR is limited by the variability of viral input during the experimental procedure. In this study, a methodology combining titration and growth dynamic experimental determinations with rules-based algorithms and a simple growth dynamic mathematical model allowed for accurate and reproducible measurement of the RR of virus strains in cell culture.

**METHODS:** High throughput (eight serial dilutions of 72 viruses per assay) measurements of viral growth at six successive time-points between 24 and 86 hours are performed using MT4-cells equipped with an LTR-controlled EGFP reporter system. Data analyses take into account dynamic range, weighing of the data points, and extrapolation of the growth curves. The experimental measurements are normalized for viral concentration, by multiplying the fluorescence (F) by the viral dilution (Dv) [ $\text{Log FP} = (\text{Dv})\text{Log F} + \text{Log Dv}$ ]. Since  $\text{Log FP}$  is a linear function of time, of which RR is the slope, the absolute RR equals  $\delta\text{Log FP}/\delta t$ . This calculation results in the ability to accurately measure RR values independently of the virus input concentration. Results are normalized to an internal control (HIV-1/IIIB) and are therefore independent of inter-experimental variability. The final RR (expressed as % of HIV-1/IIIB RR) is therefore the expression of the intrinsic RR of the HIV-1 strain tested.

**RESULTS:** The influence of TMC-114 (darunavir) selective pressure on the RR of HIV-1/IIIB was determined. Data showed that loss of susceptibility to TMC-114 was

associated with a decreased RR for the selected viruses, and this correlated with the number of emerging mutations in the protease. Site-directed mutants carrying one, two or three of these protease mutations also had a decreased RR. Further analysis on the role of mutations in the gag gene that can compensate for this decrease is ongoing.

**CONCLUSIONS:** A novel assay has been designed, which accurately and reproducibly determines the RR of fully replicative viruses, independently of virus input concentration. Viruses selected under pressure of TMC-114 show a decrease in RR, which correlates with the number of mutations in the protease.

2006-06-13  
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