POSTER 10

QUANTITATIVE ASSESSMENT OF NNRTI BARRIER TO RESISTANCE USING FACSORTING AND A NEW HIV-1 REPORTER CELL LINE

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The *in vitro* selection of HIV-1 variants resistant to antiviral compounds can provide insight into the genetic barrier and resistance profile of HIV inhibitors. Traditional low-multiplicity of infection (MOI) selection experiments identify drug resistance mutations that accumulate over time in the presence of sub-optimal concentrations of inhibitor. Alternatively, high MOI methods have been described where selection is carried out with high virus input, to maximize the genetic diversity, and fixed concentrations of inhibitor to apply high levels of selective pressure. The high MOI method provides a more standardized procedure for comparing *in vitro* characteristics of different inhibitors. Here, we used a high MOI format to develop a new quantitative method to assess the barrier to resistance and mutation profile of two non-nucleoside reverse transcriptase inhibitors (NNRTIs): etravirine (TMC125) and efavirenz (EFV). We utilized a new HIV reporter cell line designated MT4 gagGFP, in which gfp expression is regulated by expression of both HIV Tat and Rev. These cells exhibit a complete lack of gfp signal in the absence of HIV infection. Cells were infected at an MOI of > 1 in the presence of fixed concentrations of each inhibitor. Every 3-4 days cultures were monitored by FACS analysis for the appearance of gfp^+ cells. When gfp^+ cells were detected they were sorted (5-10/well) into a population of uninfected cells in equivalent concentrations of drug to allow virus outgrowth. Viral RNA was isolated from supernatants of gfp^+ cells and sequenced for changes in the reverse transcriptase gene. In two independent experiments, population sequencing revealed that the appearance of gfp signal correlated with the acquisition of known NNRTI mutations, with EFV selecting for Y188L and TMC125 selecting L100I. Further, the FACS analysis allowed a quantitative assessment of the resistance barrier of each compound by detecting both the time of initial appearance and the number of gfp^+ cells. Detectable gfp^+ cells were seen as early as day 4 for EFV and day 8 for TMC125. This method has the potential to be adapted to plate-based cytometric analysis, thus allowing rapid quantitative assessment of the resistance barrier of novel NNRTIs.