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Poster presentation

Cell-based Assay for Testing Susceptibility of HIV-1 to Protease Inhibitors

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A growing emphasis on using cell-based assays for compound screening and enzyme activity is fueled by the need to decrease costs, increase success rates, and identify failures during discovery and preclinical development. Moreover, the growth in cell-based screening has led to the development of novel cell lines and technologies to increase the throughput and data output of cell-based assays. Therefore, we have also developed a new reporter system that allows monitoring of HIV-1 protease activity and susceptibility to protease inhibitors in living cells.

Our aim was to construct a cell-based assay for assessing the activity of intracellular HIV protease, use it to screen protease inhibitors and test HIV susceptibility to these drugs. Functional bioassay for resistance was constructed without the need to culture infectious HIV. These assays are based on processing of recombinant reporter proteins in mammalian cells using wild-type PR or a pool of patient-derived PR sequences. Assays not involving infectious HIV should be simpler, faster, safer, and more economic and allow implementation in clinical routine labs, which are generally not equipped for virus culture. Moreover, its application for searching inhibitors of this important enzyme will provide faster, high-throughput and reliable results.

The working hypothesis was to test if a transactivator protein conjugated to the cytoplasmic portion of a cellular receptor, via the respective cleavage peptide, could be used as an indicator of HIV protease susceptibility. This assay was constructed in a cell line that expresses an indicator protein under the inducible action of the transactivator. The cleavage of the transactivator protein by the action of the protease resulted in the expression of luciferase or β-galactosidase. In preliminary experiments, the signal obtained correlates with the intracellular activity of the protease. The optimized cleavage assay is currently being used for testing current protease inhibitors to compare the IC50 values to reference virologic assays.