

**POSTER 51****DEVELOPMENT OF METHOD FOR TESTING HIV-1 PROTEASE DRUG-RESISTANCE BASED ON CELL-FREE PROTEIN PRODUCTION SYSTEM**

Takashi Masaoka<sup>1,2,3</sup>, Tatsuya Sawasaki<sup>4</sup>, Wataru Sugiura<sup>1,2</sup>, Yaeta Endo<sup>4</sup>, Masashi Tatsumi<sup>2</sup>, Naoki Yamamoto<sup>2</sup>, and Akihide Ryo<sup>5</sup>

<sup>1</sup>National Hospital Organization Nagoya Medical Center; <sup>2</sup>AIDS Research Center, National Institute of Infectious Diseases, Japan; <sup>3</sup>Research resident, Japan Foundation for AIDS Prevention; <sup>4</sup>Cell-free Science and Technology Research Center, and Venture Business Laboratory, Ehime University; <sup>5</sup>Department of Microbiology and Molecular Biodefense Research, Yokohama City University School of Medicine.

Recent antiretroviral therapies using combinations of multiple drugs have improved the disease progression of the HIV/AIDS patients. However, since antiretroviral therapies must be under the long-term control, we must consider the presumable problem facing the appearance and development of resistance in the virus. In the clinical practices, the emergence of such drug-resistant HIV must be detected promptly. Principally, there are two systems to detect drug-resistant viruses, *i.e.*, genotypic and phenotypic tests. Genotypic assay is well-established and rapid to detect drug-resistant viruses by determining the nucleic acid sequences whereas phenotypic assay is expensive and quite time-consuming because of requiring cell-culture system for virus infection. However, both systems are essential to determine the fact of the drug resistance. Now, in order to improve inefficient phenotypical systems using cell cultures, here we show an alternative new system to assess drug-resistant phenotypes of patient derived HIV-1 protease (PR) rapidly.

Our systems are developed based on two technologies; 1) *in vitro* translation using wheat-germ cell-free system, which has advantage to express HIV-1 proteins at high-throughput scale with PCR-amplified DNA fragments of PR region derived from viruses in serum, 2) Amplified Luminescent Proximity Homogeneous Assay (ALPHAScreen, PerkinElmer), which allows detection of molecular interactions in solution. By using this new system, we analyzed the IC<sub>50</sub> values of various PIs for a panel of drug-resistant PRs. In addition, we obtained the IC<sub>50</sub> based on the conventional *in vitro* cleavage analysis of Pr55<sup>gag</sup>. The results demonstrated that the IC<sub>50</sub> obtained from our new system is strongly comparable to that by the conventional *in vitro* assay. Furthermore, the patterns of the PI-resistance phenotypes showed good agreement with the results of genotypical tests.

In summary, our new system could provide a rapid and reliable evaluation of drug-resistant phenotypes of PI-resistant HIV-1 and would serve as a powerful new tool in the drug discovery fields as well as in the clinical settings.