Project Title:
Study on the contribution of HIV-1 CRF-01_AE specific *pol* polymorphisms to the antiretroviral drug resistance through the use of an in-house phenotypic resistance assay (Project Code: MSS 186R)

Executive Summary:

Objectives
This study has 3 major objectives:
1) Developing an in-house phenotypic drug resistance assay for measuring the drug susceptibilities of currently available PIs.
2) Verifying the phenotypic drug susceptibility on clinical HIV/AIDS patients who demonstrated clinical failure but without defined viral genotypic drug resistance mutations
3) Identifying the influence of the CRF01_AE-specific polymorphisms to the genetic barrier for the virus to develop antiretroviral drug resistance on available antretroiviral drugs in Hong Kong.

Design and Setting
Viral RNA were extracted from the 60 plasma samples. The genomic regions were amplified and then cloned into the HIV-1 vector, in order to generate different infectious HIV-1 virus with different resistance mutations. The viruses were then used for cell line transfection in the BioSafety Class 3 Laboratory. In order to compare the drug resistance level of different viruses carrying different mutations, the fold change of number of viruses under the challenge of different antiretroviral drugs at different concentrations were investigated by using the MTT testing method.

Participants
A total of 60 human plasma samples of HIV-1 infected patients visiting the Integrated Treatment Centre, Department of Health between 2011 and 2012 were included in this study. The patient background and treatment history was blinded which patient privacy will not be disturbed.

Interventions
The lack of phenotypic resistance testing protocol targeting the major circulating HIV-1 subtypes in Hong Kong makes it very difficult to determine the actual resistance level to the antiretroviral drugs.

Main outcome measures
The concordance of the phenotypic response to protease inhibitors and presence of resistance related mutations in the viral pol gene will be determined as the major outcome measure

Results

A. Development of phenotypic resistance assay for HIV-1 protease inhibitors

In this study, a standardized working protocol for estimating the phenotypic response of HIV-1 subtype B and CRF01_AE virus to different protease inhibitors was developed. This protocol required 30 days to handle up to 5 samples in a batch

with triplicate results. The protocol first PCR amplified the partial viral *pol* gene of query samples. The amplicons were then cloned into HIV-1 infectious vector for viral transfection. The infectivity and drug resistance level of the transfected viruses were then evaluated by using the $TCID_{50}$ and MTT assay.

B. Verifying the phenotypic drug susceptibility of treatment failure HIV/AIDS patients in comparing to the genotypic resistance estimation

Through the use of the phenotypic resistance assay developed in this project, the phenotypic resistance level and genotypic resistance estimation of 50 treatment naïve samples (20 subtype B and 30 CRF01_AE infected) and 10 treatment failure samples (3 subtype B and 7 CRF01_AE infected) were compared for 5 routinely used protease inhibitors (PIs) [Atazanavir (ATV), Darunavir (DRV), Indinavir (IDV), Lopinavir (LPV) and Tipranavir (TPV)].

From the treatment naïve group, we found that the average normalized phenotypic resistance level of the 5 PIs was between 0.42-2.59 fold change, which indicates the high susceptibility of these PIs. In general, these data correlates well to the Stanford HIV-1 genotypic resistance estimation. However, we observed a significant difference between the fold change of treatment naïve CRF01_AE and subtype B viruses (paired *t*-test; p=0.049). The CRF01_AE viruses were observed to be significantly more susceptible to the 5 PIs in comparing to the subtype B viruses.

From the treatment failure group, we observed that the phenotypic assay used in this study could only differentiate drug ñsensitiveò and ñresistanceò. For the 5 PIs, IC_{50} fold change >10 fold increase could be considered as phenotypically resistant.

Through this study, we found that the PI resistance interpretations of this phenotypic resistance assay were highly concordant to the Stanford HIVdb algorithm subtype B viruses. However, discrepancies between genotypic and phenotypic resistance results could be observed in 3 PIs (DRV, IDV and TPV). As the ñPI-Treatedò dataset in the Stanford HIVdb is mainly built up with subtype B viruses therefore, our study demonstrated that genotypic interpretation of non-B viruses sometimes may not truly reflect the phenotypic resistance level.

Conclusions

This project developed a working protocol for HIV-1 phenotypic resistance monitoring for a number of protease inhibitors. It can be applied to both HIV-1 subtype B and CRF01_AE viruses. The study also demonstrated that genotypic resistance interpretations could be used as PI resistance estimation for subtype B viruses but may not truly reflect the phenotypic resistance level in CRF01_AE viruses. Therefore, further exploration between the correlation between genotypic and phenotypic resistance level on non-B HIV-1 genotypes should be followed up.

Publications

The manuscript is still under preparation. It is pending to be submitted to the AIDS Research and Human Retroviruses for publication.