Project Title:
Establishment of a rapid, high-throughput, cost-effective HIV-1 drug resistance testing system (Project Code: MSS 183R)

Executive Summary:

Objectives
To establish a rapid, high-throughput and cost effective HIV drug resistance testing system for detecting drug resistance mutations against reverse transcriptase inhibitors in patients mainly infected with HIV-1 subtype AE, B and BC based on the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Design and setting
To establish the assay, *pol* gene fragment was prepared from the plasma viral RNA of the patients by nested PCR and the presence of drug resistance mutations (M41L, K65R, K101E/Q/P, K103N/S, M184I/V, G190A/S, L210W and T215F/Y) were subsequently analyzed by MALDI-TOF mass spectrometry (MALDI-TOF MS) using Sequenom MassARRAY® system. The sensitivity of the assay for detecting the mutant virus in a mixed viral population was evaluated by subjecting the *pol* gene fragments amplified from mixtures of plasmids containing a wild-type genotype (M184 in the RT region) and a mutant genotype (V184) at different ratios to the MALDI-TOF MS. The MS results were validated by comparing with the drug resistance (DR) profiles generated by submitting the sequences of *pol* gene fragments obtained from conventional DNA sequencing to Stanford University HIV DR database and the sequence chromatogram.

Participants
159 plasma samples were collected between 2011 and 2013 from HAART experienced or treatment naïve HIV-1 infected patients at the AIDS clinic of Shenzhen Third peopleôs Hospital after informed consent was obtained from each participant. The drug resistance status of all samples is undetermined at the time of collection. This study was conducted in compliance with the Declaration of Helsinki and was approved by the ethics review committee of Shenzhen Third peopleôs Hospital.

Results
Among the drug resistance mutations detected, the call rate was greater than 97% for M41L, K65R, M184I/V and G190A/S, 90.5% for T215F/Y, 91.2% for K101E/Q/P and 90.6% for K103N/S, respectively. Our results also revealed 95.4% AE patients, 95.7% BC patients and 80% B patients had a call rate per sample greater than 80%. Importantly, HIV-DR profiles generated from conventional sequence analysis were completely concordant to the MS results after excluding the failed calls. Using plasmid templates, the assay was sensitive to detect drug resistant variants at level about 20% of the circulating viral population. The capability for this assay to detect mixed viral populations was further verified in two different patient samples.

Outcome and conclusion
In conclusion, this multiplex assay represents a potential efficient approach for monitoring HIV drug resistance against the reverse transcriptase inhibitors in China.

Publication

Ka-Wai Cheung, Qiaoli Peng, Liufen He, Kanru Cai, Qiang Jiang, Sai Fan Ho, Boping Zhou, Li Liu, Zhiwei Chen, Hui Wang. Rapid and simultaneous detection of major drug resistance mutations in reverse transcriptase genes for HIV-1 Subtype AE, BC and B in China using Sequenom MassARRAY® system *BMC Infectious Diseases* (under review)