Abstract

Sequence analysis from HIV-1 (human immunodeficiency virus type 1) subtype B and more recently subtype C infected patients has revealed that mutations in the HIV-1 protease region that confer drug resistance to boosted protease inhibitor (PIs) are rarely detected at the time of virological failure. Mutations in the HIV-1 subtype B *gag-pol* cleavage sites are thought to be compensatory mutations which arise as a result of PI use. This study investigated the presence of compensatory mutations in the HIV-1 subtype C *gag-pol* cleavage sites and matched *pol* genotypes from South African patients failing a boosted PI-based regimen, as compared to antiretroviral drug naïve patients.

A new amplification protocol encompassing the near full-length *gag*, PR and partial RT was established and used to sequence the HIV-1 *gag-pol* cleavage sites from 23 proviral DNA samples (p24 antigen cultured peripheral blood mononuclear cells; PBMCs), and 51 patient samples (23 antiretroviral drug-naïve, 26 failing second-line lopinavir/ritonavir containing regimens), all attending the Charlotte Maxeke Johannesburg Hospital. Nucleotide sequences were aligned and codon positions S373Q, A431V, I437T/V, L449P or P453L associated with known *gag-pol* cleavage site mutations were analysed and compared. The *pol* genotypes were established using an in house assay. Antiretroviral drug resistant primary virus isolates were grown from samples from patients enrolled on the CIPRA-SA study, and propagated in co-culture with PHA-activated, IL-2 stimulated PBMCs. HIV-1 *gag-pol* cleavage sites and *pol* genotypes for all primary virus isolates were established as described above.

Fifty one of 74 patient samples, used to establish the in-house *gag-pol* cleavage site assay, were successfully amplified and sequenced. Detailed analysis of the five known *gag-pol* cleavage sites revealed that 5 patient samples (4 PI-exposed, 1 unknown regimen) encoded for the previously described mutations that impact on *gag-pol* cleavage in the absence of any major PR mutations. A further five samples from patients on the failing PI-based regimen had major PR mutations. No known mutations in the *gag-pol* region were identified in patients failing a first line regimen. The *pol* mutations described in this study were similar to the findings reported for treatment failures in South African HIV-1 subtype C infected patients. Primary virus was grown from only 25 of the 91 PBMC CIPRA samples. None of the 25

CIPRA-SA primary virus isolates had *gag-pol* cleavage site mutations, and only 9 harboured known RT antiretroviral drug resistant mutations.

Overall, the presence of HIV-1 *gag-pol* cleavage site mutations may account for virological treatment failure in 5 of the South African patient samples analysed. Although the *gag-pol* cleavage site mutations detected in the current study are only present in a small proportion of treatment-experienced South African patients, this may increase due to more patients accessing second line PI-containing regimens. Thus, future genotyping work incorporating the analysis of the *gag-pol* cleavage sites in addition to the PR and RT regions is warranted. The antiretroviral drug resistant primary viruses obtained provide valuable reagents for future phenotyping studies.