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Genotypic characterization of gag-pol cleavage site mutations in HIV-1 infected patients failing HAART

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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 (PI) 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 28 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 P453A associated with known gag-pol cleavage site mutations were analysed and compared to pol genotypes. 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 2 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.