Short communication

HIV-1 viral load and resistance in genital secretions in patients taking protease-inhibitor-based second-line therapy in Africa

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ABSTRACT

Background: HIV is transmitted primarily through sexual intercourse, and the objective of this study was therefore to assess whether there is occult viral replication and resistance in genital secretions in patients on protease inhibitor (PI)-based second-line therapy.

Methods: HIV-infected adults taking ritonavir-boosted lopinavir with either two NRTIs, raltegravir, or as monotherapy for 96 weeks were enrolled at seven clinical sites in Uganda. Viral load (VL) was measured in cervico-vaginal secretions or semen and in a corresponding plasma sample. Genotypic resistance was assessed in genital secretion samples and plasma samples. Results were compared between compartments and with the plasma resistance profile at first-line failure.

Results: Of the 111 participants enrolled (91 female, 20 male), 16 (14%) and 30 (27%) had VL >1000 and >40 copies/ml respectively in plasma; 3 (3%) and 23 (21%) had VL >1000 copies/ml and >40 copies/ml respectively in genital secretions. There was 74% agreement between plasma and genital secretion VL classification above/below 40 copies/ml threshold (kappa-statistic=0.29; p=0.001). RT mutations (both NRTI and NNRTI) were detected in genital secretions in 4 patients (similar profile to corresponding plasma sample at first-line failure) and PI mutations were detected in 2 (1 polymorphism with no impact on resistance; 1 with high-level PI resistance).

Conclusions: High level (>1000 copies/ml) viral replication and development of new RT or PI resistance in the genital compartment were rare. The risks of transmission arising from resistance evolution in the genital compartment are likely to be low on PI-based second-line therapy.

Running head: HIV-1 viral load and resistance in genital secretions

INTRODUCTION

HIV is transmitted primarily through vaginal and anal intercourse, and a high concentration of HIV in semen and cervico-vaginal fluid has been shown to be an independent risk factor for HIV transmission after adjustment for plasma VL (1). The genital tract and blood are separate immunologic compartments and HIV may replicate and evolve differently in the two resulting in different levels of infectivity of blood and genital secretions, and in a different spectrum of drug resistance-associated mutations (2-5). Therefore, it is important that antiretroviral therapy suppresses HIV replication in both blood and genital secretions.

Antiretroviral drugs differ in genital compartment penetration. Nucleoside-reverse-transcriptase-inhibitors (NRTIs) are concentrated in genital secretions, and non-NRTIs (NNRTIs) and raltegravir both reach similar concentrations in genital secretions and plasma (6-9). However, ritonavir-boosted protease inhibitors (bPIs) do not penetrate well into cervico-vaginal fluid (6, 7, 10) or semen (11, 12).

This study aimed to estimate the prevalence of viral replication and the prevalence and profile of resistance mutations in genital secretions compared to plasma in patients taking PI-based second-line therapy in a large clinical trial of several regimen options administered using the public health approach.

METHODS

This was a sub-study of EARNEST, an open-label, randomised second-line therapy trial conducted in sub-Saharan Africa. In this trial, patients failing first-line NRTI/NNRTI treatment by WHO criteria were randomised to one of three different treatment regimens containing ritonavir-boosted lopinavir with either two NRTIs (PI/NRTI), raltegravir (PI/RAL), or alone as monotherapy after a 12-week induction period with raltegravir (PI-mono) (13).

Adult Ugandan EARNEST participants were offered participation in this sub-study, at a single-visit 96 weeks after starting second-line therapy. Cervico-vaginal secretions were collected by self-swab or routine cervical smear (swab kit: eNAT, Copan Diagnostics, Murrieta, CA, US). Male participants provided a fresh semen sample. Plasma was collected at week 96 in the EARNEST trial for retrospective viral load (VL) testing and genotyping.

VL in genital secretions was measured at the Istituto Superiore di Sanità (ISS), Rome, Italy using the Versant kPCR 1.0 assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Samples with detectable VL (≥40 copies/ml) were tested for the presence of drug resistance using the TruGene Genotyping kit (Siemens Healthcare Diagnostics).

VL in plasma was measured at the Joint Clinical Research Centre (JCRC), Kampala, Uganda using the Abbott RealTime HIV-1 assay. Genotypic resistance testing of week 96 plasma samples (with VL>400 copies/ml) was performed at Janssen Diagnostics, Michelen, Belgium. Genotypic testing (reverse transcriptase (RT) only) was performed on baseline samples (PI/NRTI and PI/RAL arms only) at the JCRC using in-house primers. Drug-susceptibility predictions were made using the Stanford algorithm v7.

The association between viral suppression in genital secretions and second-line treatment regimen, sex, age, plasma VL and CD4 cell count at second-line therapy initiation (baseline), and CD4 cell count at week 96 was assessed using Fisher's exact test for binary and exact-logistic regression for continuous variables. The Kappa-statistic was used to assess concordance between suppression in plasma and genital secretions at week 96. The sub-study was approved by the Ugandan Ethics Committee and all participants provided written informed consent.

RESULTS

We enrolled 111 participants (91 female; 20 male; median age 36 years) at 7 Ugandan sites (34 on PI/NRTI, 49 on PI/RAL and 28 on PI-mono; the PI was ritonavir-boosted lopinavir in all cases). Of these, 16/111 (14%) and 30/111 (27%) had plasma VL>1000 copies/ml and >40 copies/ml respectively at week 96.

VL in the genital secretions was >1000 copies/ml and >40 copies/ml in 3/111 (3%; 2 in PI/NRTI, 1 in PI/RAL) and 23/111 (21%) respectively. There was no difference in proportion of patients >40 copies/ml between the regimens 7/34 (21%) in PI/NRTI, 10/49 (20%) in PI/RAL, 6/28 (21%) in PI-mono; P=1.00) and no difference by gender, age, plasma VL or CD4 cell count at start of second-line therapy, or CD4 cell count at week 96 (all P >0.24).

There was 74% agreement between the plasma and genital secretions classification of VL above/below 40 copy threshold at week 96. 11 (10%) participants had a VL<40 copies/ml in plasma and a VL>40 copies/ml in genital secretions, whereas 18 (16%) participants had a VL>40 copies/ml in plasma and a VL<40 copies/ml in genital secretions (kappa-statistic=0.29; p=0.001; Figure 1). VL values in genital secretions were low (maximum 1330 copies/ml) but varied more widely in plasma (maximum >1,000,000 copies/ml).

Sequences were obtained for 6/23 (26%) participants (all female) with VL>40 copies/ml in genital secretions; five had resistance mutations (Table 1). Four participants had NRTI and NNRTI mutations, mostly similar to their plasma sample at first-line failure, apart from A98G seen in 3 genital secretions samples. Two patients had PI mutations one (taking PI/NRTI) with a K20R polymorphism with no impact on PI susceptibility; the other (taking PI-mono) with M46L, I54V and V82A mutations conferring high-level resistance to LPV/r but retaining susceptibility to DRV/r. These mutations were not present in the plasma sample at first-line failure. Of the 5 participants with resistance mutations in genital secretions at week 96, only one had a corresponding plasma sequence from week 96 (no resistance mutations seen); the others had VL<400 copies/ml in plasma and were not tested.

DISCUSSION

This is the first study to our knowledge to measure VL in genital secretions in patients in sub-Saharan Africa taking a second-line PI-based regimen. Patients were managed with predominantly nurse-led care and without real-time VL monitoring, typical of the majority of settings in in which ART is delivered in sub-Saharan Africa. The proportion with detectable VL in plasma was comparable to that seen in the main EARNEST trial and other second-line trials (14, 15) (and compatible with UNAIDS targets).

ART coverage is being currently expanded in order to reduce community transmission of HIV and ultimately end the HIV epidemic. The underlying assumption that complete suppression of viral replication in plasma will result in decreased transmission is less certain for patients on PI-based second-line therapy given the known poor penetration of PIs into the genital compartment (7, 11, 12, 16), and given the potential for cross-resistance of NRTIs to the NRTIs and NNRTIs provided as part of first-line therapy in settings where failure is often detected late and where resistance testing is not performed at the time of treatment failure.

Our finding that only 3 patients (3%) had VL>1000 copies/ml in the genital secretions after 96 weeks of treatment, is therefore reassuring. A larger proportion had detectable VL>40 copies/ml, but at low levels that are unlikely to represent a substantial risk of transmission (1). Our finding that some patients with undetectable virus in plasma had detectable virus in genital secretions is consistent with an earlier study of second-line therapy in Thailand that found one third participants had a similar mismatch (17) and with the known imperfect correlation between VL in plasma and genital secretions in untreated patients (1, 18). Our low rates of viral detection in genital secretions are consistent with the 11% viral detection (40 copies/ml threshold) in semen of African men (11%) on first-line therapy (19).

As a consequence of the low level of virus present, few sequences could be generated from genital secretions. Nevertheless our data make an important contribution to the existing sparse data on resistance in this compartment in patients receiving ART in programmes following the public health approach in sub-Saharan Africa and allow several inferences. Firstly, the similarity of the resistance pattern in the 4 patients with RT mutations detected at week 96 and the pattern in their baseline plasma sample (taken after a substantial period of first-line failure), including both NNRTI and NRTI resistance mutations, suggests that the genital secretions resistance mutations are the result of persistence of resistant virus or re-emergence of archived resistance, rather than ongoing resistance evolution in this compartment. The only consistent mutation present at week 96 but not in baseline plasma (A98G, present in 3 participants), is an NNRTI mutation that is also likely to have been archived rather than evolving on second-line regimens (none contained NNRTIs). Persistent NNRTI mutations in semen after full reversion in blood were reported in a previous small study (20). As with accumulation of such resistance mutations on first-line therapy (21), the persistence of NRTI and NNRTI mutations in the genital tract on second-line therapy is of some concern in view of the potential transmission risk in any patients who later fail on second-line therapy with higher genital secretion viral loads.

Secondly, we found minimal resistance to the PI with only one patient (<1%) having resistance in the genital tract, accompanied by a moderate VL (907 copies/ml) in this compartment. This patient was on a PI monotherapy regimen which, as a result of the findings of the EARNEST trial and others, is no longer considered an appropriate treatment option for the public health approach to ART. Thus, in spite of poor penetration, overall there appears to be a low risk of transmission of PI resistance on second-line therapy in these settings.

In summary, this sub-study bolsters our confidence in the WHO recommendation of PI-based second-line therapy for the public health approach, confirming that occult

viral replication and resistance evolution in the genital tract are unlikely to represent a threat to long-term treatment outcomes and the desired goal of control of community HIV transmission.

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