



# Differential Evolution of Drug Resistance Mutations and Presence of M-Tropism

## In Vaginal HIV-1 Despite Advanced HIV-1 Disease

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### BACKGROUND:

HIV-1 populations in different body compartments may present unique viral dynamics. Differential evolution of the virus may be attributed to the presence of reservoirs such as resting CD4 cells and macrophages and also to the pharmacokinetics of anti-retrovirals. Differences in drug properties may cause inadequate drug concentrations in anatomical compartments such as the female genital tract. These conditions may lead to the emergence of drug resistant strains.

The virus in the female genital tract may be very important for both vertical and heterosexual transmission of HIV-1. In this study we compared the response of vaginal and plasma HIV-1 to anti-retroviral therapy and drug resistance mutations. CTL tropism and phylogenetic relationship between plasma and vaginal HIV-1 was determined by C2-V3 sequences to assess the role of vaginal tract as viral reservoir.

### METHODS:

Paired blood and vaginal swab samples collected from 45 HIV-1 infected females. Viral loads assessed with Amplicor HIV Monitor ver1.0 (Roche Diagnostics Corp). HIV-1 RNA isolated using QIAgen kit. Drug resistance mutations was assessed using TruGene Genotyping Kit (Bayer Healthcare). Reports were generated using GuideLines 6.0 (updated 2002/08/27). Envelope V3 loop were amplified using a homebrew method, then cloned using TOPO TA cloning kit (Invitrogen) and sequenced with a 7-deaza dGTP kit (Bayer Healthcare).

### Demographic and clinical description of the study population.

The mean age of the population was 40 years (range 19 to 55 years). The majority of individuals (86.6%, 36/45) reported heterosexual contact as a risk factor. Of those, 13.3% had contact with an IV drug user and 37.8% had contact with a known HIV infected male. Only two females reported IV drug use together with high-risk heterosexual contact. One of these females also reported blood transfusion as a risk factor.

Twenty percent of the individuals were receiving ART (two NRTI or combination with NNRTI) and 60% were receiving either an NRTI or NNRTI combination plus a protease inhibitor while 6.7% were not in treatment. Information on current ART was not available for 13.3% of the individuals.

CD4 T-cell counts varied from 13 to 1,278 cells/mm<sup>3</sup> (mean 399.7) with 35.5% of individuals below 200 cells/mm<sup>3</sup>, 22.2% between 200 and 400 and 42.2% above 400 cells/mm<sup>3</sup>. Plasma viral load ranged from <400 copies/ml to 750,000 copies/ml (mean 92,510 copies/ml) while vaginal viral loads ranged from <50 copies/ml to 98,940 copies/ml (mean 7,912 copies/ml). Overall, 42.2% (19/45) of the females had plasma viral loads below 1,000 copies/ml and 68.8% (31/45) had vaginal viral loads below 1,000 copies/ml. Of 21 females with detectable RNA in both plasma and vaginal secretions, 16 had levels higher than 1,000 copies/ml in both body compartments. We found only a moderate degree (Pearson's correlation of 0.502) between (log<sub>10</sub>) vaginal and plasma HIV-1 viral loads of females under ART. Vaginal viral loads were significantly lower when compared to their respective plasma pair except for two cases showing over half-log difference. Individuals' CD4 counts did not show significant association with either their plasma or vaginal viral load.

### Distribution of drug resistance in study population.

Drug resistance to one or two drug classes was detected in plasma HIV-1 of 60.7% of individuals while resistance to three drug classes was detected in 10.7%. Plasma Pol sequences showed L90M (17.1%), V82A (14.3%), and M46I/L (14.3%) as the most frequent primary resistance mutations. The most common primary RT resistance mutations were M184V & T215F (34.3%), K103N & K70R (20.0%) and D67N (22.9%). For vaginal HIV-1, the most frequent protease primary mutations were V82A (25.0%), and G48V (16.6%) while the most frequent RT resistance mutations were T215Y (33.3%) and M184V with 11.1%.

Table with columns: CONCORDANT RESISTANCE, AGE (YRS), SEX, RISK FACTOR, HIV DATE, AIDS DATE, TIME ON ART, CD4 COUNT, PLASMA VL, VAGINAL VL, START TREAT, TREATMENT HISTORY, CURRENT TREATMENT. Rows include subjects 41J, 006, 025, 32A, 33B, 34B, 012, 42K, 008, 007, 014, 016, 020, 023, 034, 033, 030.

Of the studied cases 78.6% (11/14) were AIDS cases of which 54.5% (6/11) were determined either at time of first visit to clinic or shortly after. The average time to AIDS diagnosis was 2.3 years (range: 0 yr to 7 yrs). Despite treatment they did not present a sustained increase in CD4 counts and had only temporary control of plasma viremia. Treatment history of females showing "discordant" drug resistance patterns between plasma and vaginal samples was reviewed focusing on mutations M184V and T215Y. Two females had mutation T215Y absent from plasma but present in vaginal HIV-1 2 and 4 years after stopping use of zidovudine (012, & 036E). Additionally, one also had mutation M184V absent from plasma but present in vaginal HIV-1 several months after cessation of 3TC (012). A third female (041K) showing mutation M184V in vaginal HIV-1 but absent in plasma HIV-1 and the opposite for T215Y was also identified. Records for this individual noted discontinued use of zidovudine and 3TC about 4 years prior to sampling and complete cessation of treatment shortly before sampling. However, specific dates were not documented.

Table with columns: PATIENTS WITH CONCORDANT DRUG RESISTANCE, AGE (YRS), SEX, RISK FACTOR, HIV DATE, AIDS DATE, TIME ON ART, CD4 COUNT, PLASMA VL, VAGINAL VL, START TREAT, TREATMENT HISTORY, CURRENT TREATMENT. Rows include subjects 41J, 006, 025, 32A, 33B, 42K, 008, 007, 014, 016, 020, 023, 034, 033, 030.

Deduced amino acid sequence of HIV-1 Protease (top) and Reverse Transcriptase (bottom) genes in paired plasma and vaginal samples. Only those codons associated with drug resistance are shown. Were more than one amino acid is shown it represents a quasispecies of the virus.

Table with columns: PATIENTS WITH CONCORDANT DRUG RESISTANCE, AGE (YRS), SEX, RISK FACTOR, HIV DATE, AIDS DATE, TIME ON ART, CD4 COUNT, PLASMA VL, VAGINAL VL, START TREAT, TREATMENT HISTORY, CURRENT TREATMENT. Rows include subjects 41J, 006, 025, 007, 012, 014, 32A, 42K, 43L, 008, 020, 034, 033, 3E.

Table with columns: PATIENTS WITH CONCORDANT DRUG RESISTANCE, AMINO ACID POSITION AND IDENTIFICATION - PROTEASE (HIV-1 LAV1). Rows include subjects 41J, 006, 025.

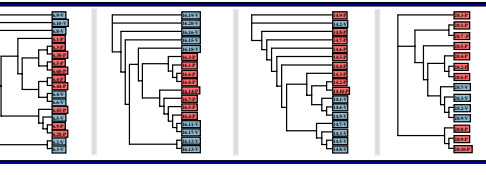
Table with columns: PATIENTS WITH CONCORDANT DRUG RESISTANCE, AMINO ACID POSITION AND IDENTIFICATION - REVERSE TRANSCRIPTASE (HIV-1 LAV1). Rows include subjects 41J, 006, 025.

Table with columns: PATIENTS WITH CONCORDANT DRUG RESISTANCE, AMINO ACID POSITION AND IDENTIFICATION - REVERSE TRANSCRIPTASE (HIV-1 LAV1). Rows include subjects 006, 025.

Comparison of deduced amino acid sequences in areas of Protease and RT genes not associated with drug resistance for those individuals showing identical drug resistance patterns between plasma and vaginal samples (silent mutations are shown in italics). Highlighted codons correspond to mutations located at predefined CTL epitopes in Protease and Reverse Transcriptase. The presence of mutations at these sites suggests both compartments evolve independently to select against immune pressure.

Table with columns: CTRPNNKRKSGIHIGPGAAYFATGDIEIGDIOAHCT, % change, % cons. Rows include subjects 007, 012, 014, 32A, 33B, 42K, 43L, 008, 020, 034, 033, 030.

CTL epitopes for each V3 clone was determined by the presence of the CCR5 requirement motif as described by Xiao et al (1998) and by calculating the amino acid charge of the V3 loop. In cases the RT motif could not be determined, tropism was assigned by amino acid charge (if 6-5 = R5; if -2-5 = X4). Areas inside the blue rectangle corresponds to CCR5 usage motif and the yellow area indicate difference from the reference sequence. Some sequences have frame shift mutations that were put back to the original reading frame for this analysis.



### RESULTS:

Detectable RNA in both plasma and vaginal samples was found in 16 of 45 (35.5%) cases and sequences were obtained for 15. Concordant drug resistance mutation patterns between plasma and vaginal HIV-1 were observed in 26.6% (4/15) cases.

Discordant mutation patterns between plasma and vaginal HIV-1 were observed in 80.0% (12/15) of cases. Two cases (18.2%) appeared to show delayed emergence of drug resistance in vaginal HIV-1 while 3 cases (27.3%) appeared to show delayed clearance of drug resistance from vaginal HIV-1. Treatment history of "discordant" cases showed about 18 percent of cases presented delayed emergence of resistance in vaginal HIV-1 while about 27 percent showed delayed clearance of resistant mutants from vaginal virus even 2 to 4 years after stopping use of the drug.

Concordant drug resistance patterns were observed in three cases. Further analysis of sequence in areas not related to drug resistance showed that plasma and vaginal virus presented different mutations and were distinct from each other suggesting different rates of evolution in both compartments. When these mutations were compared with known CTL epitopes we observed the majority of mutations occurred in regions that contain CTL epitopes recognized in conjunction with one or more HLA alleles.

In most cases, we observed higher variability in plasma C2-V3 and found 10-66.7% of clones to be T-tropic. In most cases, vaginal HIV-1 was M-tropic despite presence of T-tropic viruses in plasma and regardless of concordance or discordance of resistance mutations.

C2-V3 phylogenetic analysis showed plasma and vaginal sequences tended to cluster separately. Sequence homology was lower (91.7 to 97.5%) in cases with discordant patterns compared to those with concordant patterns (98.9% to 99.9%) suggesting they represent independent viral lineages.

### CONCLUSION:

Our data suggests the vaginal HIV-1 lineage may be evolving under particular population dynamics and local selective forces independently from plasma HIV-1. The presence of delayed emergence/clearance of drug resistance mutations suggests differences in viral turnover rates and other local differences that may include pharmacological factors. Additionally, immunological factors acting independently in plasma and vaginal compartments are suggested by distinct mutations at CTL epitope sites in those cases where drug resistance was identical between compartments. Mutations at critical CTL epitopes suggests drug resistant strains as well as CTL escape variants are contributing factors to virus replication in these females. The selection of different escape variants again suggests viral compartmentalization in the genital tract. When V3 loop sequences were analyzed they showed higher variability in plasma than in vaginal virus regardless of concordance or discordance of drug resistance mutations between plasma and vaginal virus. In most cases, vaginal HIV-1 remained M-tropic even in advanced HIV disease and despite the presence of T-tropic viruses in the plasma compartment. These observations suggest the vaginal tract may serve as reservoir for M-tropic drug resistant strains that may contribute to the transmission of drug resistant strains. These has important clinical implications in the risk of perinatal and heterosexual transmission of HIV-1. Further studies are needed to understand the factors affecting evolution of HIV-1 in the female genital compartment.

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