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Resistance in the context of Mother-to-Child transmission

Mother-to-child transmission of minority HIV-1 drug resistant strains

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It is well known that drug resistant strains of human immunodeficiency virus type 1 (HIV-1) can be transmitted from an infected individual to another, compromising the efficacy of antiretroviral therapy in the latter. However, little is known about the clinical relevance of transmitted drug resistant minority viral strains, and even less about primary resistance in vertically-infected children. Two cases of primary drug resistance in vertically-infected infants who had never been subjected to antiretroviral therapy were documented. In the first case, the child’s infecting virus had the K103N reverse transcriptase (RT) mutation, but standard genotyping of the mother’s virus did not show that mutation. In the second case, the infant virus had the K101E RT mutation, again not found in the mother’s virus by standard genotyping. End-point dilution RT-PCR was conducted for the first 225 RT codons of viruses infecting both mothers, and 20-25 clones of each fragment were sequenced and analyzed for the respective mutations. Standard genotyping of the fathers’ viruses was also conducted. Phylogenetic analysis of the consensus sequences and of the individual clones was performed to evidence epidemiological linkage and to discard PCR contamination.

In the first case, none of the 20 clones analyzed from the mother’s virus showed the K103N mutation. Standard genotyping of the father’s virus, however, indicated the presence of K103N. In the second case, 1 out of 25 clones sequenced from the mother’s virus presented the K101E mutation. Sequences from each epidemiological group clustered phylogenetically as expected.

To the best of our knowledge, this is the first time mother-to-child transmission of a minority HIV-1 drug resistant strain is reported. In one case, the transmission chain most likely went from the father to the mother, and then to the child, while in the mother the frequency of the resistant variant was less than 5%. A frequency of 4% was enough to warrant vertical transmission in the second case. These results raise an important concern in the primary resistance in pediatric settings. These may compromise treatment in newly-infected infants, particularly in developing countries, where access to therapy increases at a fast pace.
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Resistance in the context of Mother-to-Child transmission

Selection of resistance mutations under nevirapine prophylaxis to prevent HIV-1 mother-to-child transmission (MTCT) in Africa

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A single dose of nevirapine can reduce significantly the transmission of HIV from MTCT. Since this strategy is simple and cheap, it has been implemented in many developing countries. However, single dose of nevirapine can induce high levels of resistance to NNRTIs, and can be related to certain subtypes. We studied NNRTI mutations after nevirapine prophylaxis in women in two African countries, Burkina Faso and Cameroon; where this prevention strategy has been implemented.

A total of 80 women, receiving single-dose nevirapine prophylaxis to prevent MTCT, were studied; 44 from Burkina Faso and 36 from Cameroon. Reverse transcriptase (RT) gene was amplified from plasma and directly sequenced. Phylogenetic analyses were performed to identify subtypes/CRFs and amino acid sequences were analyzed for presence of drug resistance mutations to NNRTIs.

All (100%) and 79.5% of samples from Cameroon and Burkina Faso were amplified and sequenced. The genetic subtype distribution is very heterogeneous: CRF02(54.2%), CRF06(23.6%), A(6.9%), G(4.2%), CRF01(2.8%), CRF13(2.8%), F2(1.3%), and unique recombinants(4.2%). Samples were collected from Burkina Faso with a median of 18 weeks[2-48] and from Cameroon with a median of 24 weeks[2-48] after nevirapine administration. Mutations associated with resistance to NNRTIs were detected in 11 women: K103N(n=5), Y181C(n=5) and K103N+Y181C(n=1). Mutations were observed in different variants : CRF02(n=4), CRF06(n=6) and CRF13(n=1). Seven women harboured resistant and wild type populations. Overall, genotypic resistances to NNRTIs were observed in 18,2% of women studied in Burkina Faso between 4 and 28 weeks after nevirapine administration and in 8.1% from Cameroon 2 and 8 weeks. Prevalence of resistance to NNRTIs after MTCT prophylaxis ranged from 8% to 18% in Cameroon and Burkina Faso, respectively. Higher rates in Burkina Faso can be explained by the fact that time between nevirapine administration and resistance testing was shorter in Burkina Faso than in Cameroon.

In our study, mutations at two RT positions were selected by nevirapine. In Ouganda, 24% of mothers selected NNRTI mutations after 8 week nevirapine administration, but only the mutation K103N was selected. The rapid selection of NNRTI resistance can comprise the further use of nevirapine in prevention of MTCT programs in developing countries.
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Drug-Drug Interactions

Nevirapine plasma concentrations in the presence of rifampicin (HIV-NAT 025)

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Background: Rifampicin may lower nevirapine plasma concentrations through induction of cytochrome P450 isoenzyme 3A4, thereby leading to suboptimal concentrations and therapy failure. Materials & Methods: Nevirapine plasma concentrations in the presence (group 1) and absence of rifampicin (group 2) were collected from patients on a generic fixed-dose combination (FDC) including stavudine and lamivudine from the therapeutic drug monitoring service at the HIV-NAT pharmacokinetic laboratory. Comparisons between groups were performed with Fisher's test or Mann-whitney test. Multivariate analysis was performed with an univariate general linear model with the nevirapine plasma concentration as dependent variable and site, age, gender, weight, height, body mass index (BMI), time after drug intake, use of rifampicin, fluconazole and co-trimoxazole as independent variables. Samples with co-mediations possibly affecting nevirapine plasma levels through CYP3A4 were excluded.

Results: In total 145 samples were collected, of which 1 was excluded. Seventy-four patients were using FDC only (group 1) and 70 patients were also on rifampicin (group 2). Groups did not differ for age (p=.192), gender (p=.940), weight (p=.112), height (p=.482) and BMI (p=.139). In group 1 the median nevirapine concentration was 8.19 mg/L (range: 1.55-25.48mg/L). Forty-five concentrations were around the trough level (median 8.17 mg/L, range: 1.55-13.58mg/L). In group 2 the median concentration was 4.56 mg/L (0.65-13.76 mg/L) of which 49 were around the trough (median 4.43 mg/L, range:0.65-13.76 mg/L). Seven concentrations were below 3.1 mg/L in group 2 and 2 in group 1 (p=0.091). In the multivariate analysis use of rifampicin (p=0.000) and time of drug intake (p=0.025) were significantly associated (R2=0.232) with nevirapine concentrations.

Conclusions: The results of this analysis show that low nevirapine concentrations can be partly explained by the use of rifampicin. More large-scale studies are needed to confirm these results,
to investigate the clinical impact of low nevirapine levels and to investigate the influence of other variables such as co-infections with hepatitis B or hepatitis C.
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Drug-Drug Interactions

Efavirenz (EFV) 600 mg is not associated with subtherapeutic EFV concentrations when given concomitantly with rifampin (RFP)

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Background: RFP is known to decrease EFV exposure 22%. In order to counter RFP CYP3A4 induction, guidelines suggest giving EFV 800 mg daily with RFP. The objective of this study was to describe EFV concentrations when patients receive either EFV 600 mg or 800 mg with RFP.

Materials & Methods: A retrospective, observational study was conducted. HIV+ patients were included if they received EFV 600 or 800 mg and RFP concomitantly and if at least one EFV level during RFP use was available in the TDM database. Patients were excluded if they received other medications known to significantly inhibit or induce EFV metabolism. Random EFV plasma concentrations were measured with a validated HPLC/UV assay. The therapeutic range for EFV was defined as 1.0 to 4.0 mg/L.

Results: Sixteen patients met the inclusion criteria. Twenty-eight EFV levels taken at a median (range) time post dose of 14.0 hours (1.0-24.0) were available while these patients received RFP. At the time of inclusion, 11 patients received EFV 800 mg and 5 EFV 600 mg. Median age and weight (range) for the sample were 34 years (21-60) and 64 kg (50-84.3), respectively. 62.5% of the sample was male (EFV 800 mg: 64%; EFV 600 mg: 60%). Median (range) EFV concentration in the 600 mg group and 800 mg group were 3.9 (1.3-10.9) and 2.9 (1.0 - 13.5) mg/L, respectively. None of the EFV concentrations were subtherapeutic. Five of the 11 patients on EFV 800 mg had toxic concentrations and the EFV dose was decreased to 600 mg in 4 of these patients. Four patients with subsequent EFV TDM levels (8 levels) while on EFV 600 mg and RFP all had toxic EFV concentrations: median (range) 5.95 (4.2-10.2) mg/L. No significant correlation existed between age, weight, and EFV dose and having a toxic EFV concentration. Toxicity data were not available.

Conclusions: Based on this small sample, EFV 600 mg with RFP does not result in subtherapeutic concentrations and may be a sufficient dose. In contrast, EFV 800mg leads to toxic EFV concentrations in almost half of the patients. We recommend EFV TDM during RFP therapy to limit the risk of resistance and toxicity.
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Drug-Drug Interactions

Pharmacokinetics of Indinavir and Ritonavir +/- Efavirenz in HIV-infected patients

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Background: The pharmacokinetic (PK) interaction of the combination indinavir (IDV) 400 mg BID, ritonavir (RTV) 400 mg BID, efavirenz (EFV) 600 mg QD with nucleoside analogues is currently unknown. Interactions affecting plasma concentration of these antiretrovirals (ARV) was anticipated. We conducted an open-label study to determine the 3-way PK interaction of IDV/RTV +/- EFV in HIV-infected.

Materials & Methods: Eight HIV-infected, ARV-experienced patients initiating IDV 400 BID + RTV 400 BID with 2 nucleoside analogs were included. Patients had their 1st PK visit after 15 days of receiving this ARV combination. EFV was added and patients returned on day 30 for their 2nd PK visit. All patients underwent intensive pharmacokinetic sampling at steady-state for IDV and RTV at visit 1 and 2 and for EFV at visit 2. PK parameters were determined using noncompartmental methods (WinNonlin version 4.0 Pharshight Corp., Mountain view, C.A.). IDV and RTV PK parameters were compared before and after initiation of EFV ( Wilcoxon Signed Rank test.)

Results: Of the 8 patients evaluated, 5 were Blacks and 3 were Whites. The median (range) age [years], viral load [copies/mL] and CD4 [cell/mm3] were 42.5 (34-51), 42,284 (1,999-256,361), 81 (37-596), respectively. The median (range) IDV Cmax, AUC12, and C12h, were 2.9 (2.0, 8.8) mg/L, 17.6 (9.5, 56.7) mg*h/L, and 0.5 (0.04, 2.5) mg/L, respectively. The IDV PK parameters were not significantly changed when EFV was added; the median (range) IDV Cmax, AUC12, and C12h, were 2.6 (1.4, 4.7) mg/L, (4.8, 37.8)mg*h/L, and 0.4 (0.05, 1.8) mg/L, respectively. The median (range) RTV Cmax, AUC12, and C12h, were 6.6 (3.4, 22.9) mg/L, 42.8 (23.8, 195.8) mg*h/L, and 1.7(0.4, 13.3) mg/L, respectively. The RTV PK parameters were not significantly changed when EFV was added; the median (range) RTV Cmax, AUC12, and C12h, were 5.0 (2.4, 10.5) mg/L, 37.7(13.1, 98.1) mg*h/L, and 1.7 (0.2, 6.0) mg/L, respectively. The pharmacokinetic profiles of IDV, RTV and EFV were comparable to historical data.

Conclusions: The PK profiles of IDV 400 mg and RTV 400 mg, both given BID with or without EFV 600 mg qd were not significantly different in HIV infected patients.
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Drug-Drug Interactions

Possible interaction between tenofovir and boosted Lopinavir; analysis at the intracellular level in HIV infected patients

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Background: An interaction between lopinavir/ritonavir (LPV/r) and tenofovir disoproxil fumarate (TDF) leading to a significant increase in tenofovir (TFV) plasma concentrations (increase of Cthrough (+51%), AUCsteady-state (+32%), and Cmax (+15%), whereas t1/2 of elimination and tmax were not affected) has been described (BP Kearney, et al., 43rd ICAAC., abstr. A-1617, 2003). Since, the active form of TFV is the corresponding intracellular di-phosphate (TFV-DP) which is produced within cells, the objective of this work was to measure TFV-DP intracellular levels in patients under TDF therapy with a regimen including or not Kaletra®.

Materials & Methods: 15 patients taking both TDF (300 mg QD) and LPV/r (400/100 mg QD) and 14 patients taking TDF without LPV/r as part as HAART (didanosine excluded) were included in a sub-study inside a more general trial aiming to study the interaction of TFV with other drugs. Blood samples were taken at baseline and at 1, 2 and 4 hours after dosing. Intracellular TVF-DP measurements were performed using a validated direct LC/MS/MS assay.

Results: Because of the very long IC TFV-DP half-life (A. Pruvost et al., AAC, 2005, in press), no significant differences were observed in IC TFV-DP levels between sampling times and concentrations were expressed as the mean of the four measurements performed on each patient. The mean CV% for intra-subject variability is 16.9% and 25.9% when TFV was given with and without the combination LPV/r, respectively. Mean (median) TFV-DP concentration reaches 280.3 (199.2); SD: 181.8; CV%: 64.9 and 181.4 (157.0); SD: 80.1; CV %: 44.1 fmol/106 cells with LPV/r and without LPV/r, respectively, exhibiting a ratio of 1.55 (1.27); P=0.144.

Conclusions: There is a trend for an increase of IC TFV-DP concentrations when used in combination with LPV/r with a quantitative incidence similar to that was reported at the plasma levels. However, due to important inter patient variability this trend does not reach statistical significance. More powerful analysis should be likely obtained in longitudinal studies. Since there is not yet therapeutic index based on IC TFV-DP concentrations, the possible consequences of this increase remain unknown.
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Pharmacokinetics Special Populations and Toxicity

Unexpected hepatotoxicity observed in a healthy volunteer study on the effects of multiple dose rifampicin on the steady-state pharmacokinetics of ritonavir boosted saquinavir and vice versa

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Background: Rifampicin is a potent CYP3A4 inducer, and co-administration with unboosted saquinavir (SQV), predominantly metabolized by CYP3A4, reduced exposure to SQV by 70%. This study was performed to determine whether ritonavir (RTV) boosting of saquinavir could overcome this interaction. Objective: To investigate the effects of multiple dose rifampicin on the steady-state pharmacokinetics of ritonavir boosted saquinavir and vice versa.

Materials & Methods: 28 healthy volunteers were randomized 1:1 to two arms of a two-period crossover study. Arm 1: SQV/RTV 1000/100mg bid for 14 days, followed by SQV/RTV with rifampicin 600mg qd for 14 days. Arm 2: rifampicin 600mg qd for 14 days, followed by rifampicin with SQV/RTV for 14 days. Subjects were monitored for adverse events and laboratory test abnormalities. Pharmacokinetic assessments were planned for: SQV and RTV on days 11.14 (Arm 1) and days 25.28 (Arms 1 and 2); rifampicin on days 11.14 (Arm 2) and days 25.28 (Arms 1 and 2).

Results: Steady-state concentrations of SQV, RTV, rifampicin and its metabolite desacetyl-rifampicin were within expected ranges by day 14. Two Arm 2 subjects experienced grade 1 elevated transaminase levels by day 14. 17/28 subjects were dosed beyond day 14 until the study was discontinued prematurely owing to unexpected hepatic adverse events in 11 subjects. Elevated transaminase levels were observed in two Arm 1 subjects (grades 2 and 3) and nine Arm 2 subjects (grades 3 and 4) following co-administration of rifampicin and SQV/RTV. Clinical symptoms and laboratory abnormalities were generally more common and severe for subjects in Arm 2 compared with Arm 1. The limited data available suggests that there was a trend for increased desacetyl-rifampicin concentrations and that there could be a possible causal relationship between these concentrations and elevated transaminase levels. Clinical symptoms abated and transaminase levels started normalizing following discontinuation of study drugs.

Conclusions: The inter-arm distribution of adverse events and the pharmacokinetic data suggest that the observed hepatotoxicity in subjects dosed with rifampicin and SQV/RTV was mediated by the direct and/or indirect effects of rifampicin exposure, although limited data warrants cautious interpretation. Rifampicin should not be administered to patients also receiving SQV/RTV.
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Pharmacokinetics of Existing Drugs

CD4 cell count declines among patients with non-detectable viral load measurements receiving non-Didanosine (ddI) containing, Tenofovir DF (TDF) based HAART

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Background: TDF has been widely used in clinical practice among both treatment naive and experienced patients. Recent data have suggested declines of CD4+ cells among patients receiving both TDF and ddI possibly as a result of adenosine-TP and ddI-TP accumulation from PNP inhibition by TDF. In theory, accumulation of naturally occurring purines, with subsequent CD4 declines could occur with TDF therapy without ddI. We have observed CD4 declines among patients with persistent non-detectable (ND) viral load (VL) measurements receiving TDF based HAART without ddI.

Materials & Methods: Observational, single site retrospective review. Subjects receiving non-ddI containing TDF HAART > 6 months with ND VL at most recent visit and > 2 consecutive documented measurements prior (<400 cps/ml) and with > 2 documented CD4 declines > 3 months apart were included. Prior receipt of TDF and ddI was allowed if off ddI for > 6 months with continued CD4 declines. Subjects were excluded if receiving known bone marrow suppressing agents. The absolute changes of CD4 from BL and from peak to nadir are described. All values are reported as median (range).

Results: 103 subjects were receiving TDF without ddI with ND VL; 18 (17%) met criteria (12 males (67%), 8 African American (44%), 7 Caucasian (38%); months of TDF receipt and ND VL were 23 (10-39) and 20 (6-37). 10 (56%) were receiving a boosted PI regimen, 5 (28%) on triple/quad NRTI alone). BL, peak and nadir CD4 were 395 (211-1259), 732 (265-1259) and 396 (139-702) for a delta of -253 cells (-88 to -901); time from peak to nadir was 12 months (6-34). 5 subjects had no CD4 increases after TDF start and had their CD4 decline from 807 (360-1259) to 430 (201-759) after 16 months of treatment.

Conclusions: The long-term administration of non-ddI, TDF containing HAART substantially reduced CD4 counts among some patients with persistent ND VL measurements; many subjects were receiving a boosted PI regimen. The precise etiology, overall incidence and risk factors for this finding require further exploration.
Abstract: 86
Pharmacokinetics in Developing Countries

HIV-infection and rifampicin, isoniazid, pyrazinamide and ethambutol pharmacokinetics in a cohort of South African tuberculosis patients.

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Background: A better understanding of the pharmacokinetics of the antituberculosis drugs is especially important in developing countries where therapeutic drug monitoring is not available, many patients have HIV-infection and undernutrition, and concomitant antiretroviral therapy is increasingly available; Variable drug concentrations may be particularly problematic in those with compromised immunity, those vulnerable to adverse drug effects, and those who require treatment with additional drugs for concomitant illnesses. Evaluation of sources of pharmacokinetic variation can facilitate optimization of tuberculosis treatment regimens by identification of avoidable sources of variation and of risk factors for low or high drug concentrations in patients. Several potential determinants of drug concentration variability (including HIV-infection) are recognized, but they remain poorly characterized in tuberculosis patient populations. The aim of this study was to describe the pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol in a cohort of tuberculosis patients established on first-line treatment regimens and to evaluate determinants of pharmacokinetic variation.

Materials & Methods: Plasma concentration-time profiles were determined for each of the drugs in 142 patients with drug-sensitive pulmonary tuberculosis after 2 months of daily treatment in hospital. Pharmacokinetic measures (peak concentration, time to reach peak concentration, half-life, and area under the concentration-time curve) were described using noncompartmental analysis. Multiple linear regression was used to evaluate patient and treatment factors associated with pharmacokinetic variation.

Results: Wide variations in drug concentrations were demonstrated, especially for rifampicin. Low rifampicin levels (< 4 mg/l) were common (33%). Regression analyses demonstrated important reductions in rifampicin and ethambutol levels with HIV-infection; significantly lower rifampicin, isoniazid and pyrazinamide concentrations in men; increased isoniazid and reduced pyrazinamide levels in smokers; decreased ethambutol levels in retreatment patients; and that formulation characteristics were important determinants of rifampicin concentrations.

Conclusions: Several factors independently associated with variations in antituberculosis drug concentrations were identified. Rifampicin levels were low (even when those patients who received apparently problematic formulations were excluded) and variable. Further studies are required to assess the implications of variation in the concentrations of these drugs for the efficacy and safety of the antituberculosis agents themselves and other drugs given concomitantly.
Abstract: 87
Pharmacokinetics in Developing Countries

Preliminary data of a dual protease inhibitor regimen of nelfinavir plus indinavir boosted with ritonavir in HIV infected adult women in Thailand: efficacy, tolerability and plasma drug levels

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Background: Dual protease inhibitor (PI) antiretroviral regimens are increasingly required for patients who have extensive NRTI and NNRTI viral resistance mutations and no access to other therapeutic classes. Yet, experience is limited to regimens not readily available in resource constrained settings. We report preliminary data on the efficacy, tolerability and plasma drug trough levels of nelfinavir plus indinavir boosted with ritonavir in HIV-infected treatment experienced Thai patients.

Materials & Methods: Nelfinavir (1250 mg, bid), indinavir (400 mg, bid) and ritonavir (100 mg, bid) combination was offered to 16 HIV-infected women for whom NNRTIs and NRTIs were not an option due to resistance (N=15) or toxicity (N=1). We report steady state plasma drug trough levels measured after one month, and changes in CD4 cell counts and plasma HIV-1 RNA levels (pVL) measured after six months.

Results: At baseline, median age (range) was 30 years (24-44), weight 50 kg (31-65), CD4150 cells/mm3 (4-331), and pVL 4.63 log10 copies/mL (2.72 to 5.38). All patients had been exposed to NRTIs and NNRTIs, five also exposed to Pls. Six patients stopped this regimen (2 rash/diarrhea, 1 diarrhea, 1 loss-to-follow up, 2 withdrawals), five before 6 months. At six months, 4/11 had a VL <400 copies/mL, median pVL decrease was 1.43 log10 copies/mL (-0.7 to 2.63) and increase in CD4 count was 79 cells/mm3 (-131 to 111; N=10). Median trough indinavir, nelfinavir, M8 (nelfinavir metabolite), and ritonavir levels were 160 ng/ml (0 to 1,927; N=11), 3,465 ng/ml (0 to 11,264; N=10), 710 ng/ml (0 to 3,245; N=9), and 150 ng/ml (0 to 971; N=10), respectively. Nine of 12 patients had adequate indinavir trough levels (www.hivpharmacology.com), one of the remaining three reported non-adherence. Indinavir dosing was increased to 600 mg bid in one patient with confirmed low level. 12/12 had adequate nelfinavir levels (>800 ng/mL) but two had trough levels above 10,000 ng/ml.

Conclusions: In most patients, nelfinavir, indinavir and ritonavir plasma drug levels were adequate. When tolerated in adherent patients, this combination could suppress viral replication at 6 months in only forty percent of the patients highlighting the need for access to alternative dual PI combinations.
Abstract: 92
Pharmacokinetics of PI Boosting

The role of protease inhibitor-based antiretroviral regimens with or without boosting dose of ritonavir in developing hepatotoxicity in HIV/AIDS patients

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Background: The objective of this study was to determine the incidence of liver enzyme elevations following the initiation of PI-based antiretroviral therapy (ART) with or without pharmacokinetic boosting with RTV, and to define the role of chronic viral hepatitis on its development.

Materials & Methods: We performed prospective, cohort study and continuously monitored 429 PI naïve HIV/AIDS-patients. HIV/AIDS-patients who received RTV-boosted PIs (lopinavir, saquinavir and indinavir) as well as HIV/AIDS-patients who received unboosted PI-based ART (nelfinavir, saquinavir and indinavir) had to have at least one liver enzyme measurement before and during therapy. The incidence of grade 3 and 4 liver enzyme elevations among persons with and without hepatitis B and/or C co-infection treated with PI-based ART were compared. Multivariate Logistic Regression (MLR) was used in order to estimated the probability of developing hepatotoxicity.

Results: The incidence of grade 3 or 4 elevations among HIV/AIDS-patients was: lopinavir/RTV (133.3/33.3 mg/day) (8%), saquinavir/RTV (1000/100 mg/day) (19.3%) and indinavir/RTV (400/100 mg/day) (14.9%), nelfinavir (10%), saquinavir (13.2%), indinavir (14%). MLR shown that the relative risk (RR) of developing hepatotoxicity is 12.8 folder higher (RR=12.8, 95%CI 8.92-19.8) in patients with chronic viral hepatitis. HIV/AIDS patients co-infected with hepatitis C virus (HCV) treated with lopinavir/RTV (91%), saquinavir/RTV (71%), indinavir/RTV (88%), nelfinavir (89%) or indinavir (89%) did not develop hepatotoxicity.

Conclusions: In our study, we demonstrated that the lopinavir/RTV is not associated with a significantly increased risk of hepatotoxicity among HCV-infected and HCV-uninfected HIV/AIDS patients.
ABSTRACT 1

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Infant nevirapine resistance can be substantially reduced after single dose nevirapine by avoiding maternal nevirapine dosing and providing infants with zidovudine in addition to single dose nevirapine after birth

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BACKGROUND: The NVAZ trial in Malawi compared four regimens for prevention of HIV-1 mother-to-child transmission: Group 1: women and infants received single dose nevirapine (HIVNET 012 regimen); Group 2: women received single dose nevirapine, infants received single dose nevirapine+zidovudine twice a day for 7 days; Group 3: women (presenting in late labour) received no nevirapine, infants received single dose nevirapine; Group 4: women (presenting in late labour) received no nevirapine, infants received single dose nevirapine+zidovudine as above. The HIV-1 transmission rate was similar for Groups 1, 2 and 4, but was statistically higher for Group 3.

METHODS: We analysed nevirapine resistance 6–8 weeks after single dose nevirapine in 78 infants who became HIV-1 infected despite antiretroviral prophylaxis (23 in Group 1, 21 in Group 2, 19 in Group 3, 15 in Group 4). Infant plasma (0.1 ml) was analysed with the ViroSeq system.

RESULTS: The frequency of nevirapine resistance differed significantly among infants in Groups 1–4 (*P*=0.001). In Group 1 (HIVNET 012 regimen), 20/23=87% of infants had resistance. The frequency of resistance was lower when the mother received no nevirapine (14/19=74%, Group 3), or when the infant received nevirapine+zidovudine (12/21=57%, Group 2). The frequency of resistance was lowest when the mother received no nevirapine and the infant received nevirapine+zidovudine (4/15=27%, Group 4). In a factorial logistic regression fit using the entire data set, both avoiding nevirapine in women (*P*<0.001) and providing infants with zidovudine (*P*=0.04) independently predicted reduced nevirapine resistance. No infants had zidovudine resistance.
CONCLUSION: Nevirapine resistance in infants who became HIV-1 infected despite antiretroviral prophylaxis was significantly reduced by avoiding pre-delivery maternal nevirapine and providing infants with nevirapine+zidovudine. In NVAZ, the overall transmission rates at 6–8 weeks were not statistically different for this regimen (no maternal nevirapine, infants receive nevirapine+zidovudine, 15.3%) and the HIVNET 012 regimen (14.1%). By not exposing women to nevirapine, this regimen also prevents emergence of maternal nevirapine resistance, which could potentially compromise the efficacy of nevirapine prophylaxis in subsequent pregnancies or the efficacy of antiretroviral regimens for treatment of their HIV-1 infection.
ABSTRACT 2  
*Antiviral Therapy* 2005; 10:S4

Single dose nevirapine combined with a short course of combivir for prevention of mother to child transmission of HIV-1 can significantly decrease the subsequent development of maternal and infant resistant virus

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BACKGROUND: Single dose nevirapine (sdNVP) given at the time of delivery has decreased mother-to-child transmission (MTCT) in resource poor settings, but concerns have been raised about development of NNRTI-resistance limiting future treatment. A trial of sdNVP+short course combivir (CBV) was conducted to determine whether post-partum maternal and infant HIV-1 resistance can be reduced.

METHODS: A prospective, randomized three-arm study compares sdNVP, sdNVP+4 days CBV and sdNVP+7 days CBV for pMTCT, conducted in South Africa; 300 mother-infant pairs were planned. The sdNVP arm was closed to accrual after an interim analysis. All 226 mothers randomized to the three arms have reached 6 weeks of follow up. SdNVP was given to the mother during active labour and to the infant within 24–72 hours after delivery. CBV BID was initiated in mother during labour and in infants soon after delivery. Maternal NNRTI resistance at weeks 2 and 6 post-partum was the primary endpoint. Infant HIV-1 transmission was determined by HIV DNA or RNA at birth, 2 and 6 weeks.

RESULTS: We evaluated 226 mothers and 228 infants with 6-week follow-up. Median entry CD4 count was 314 cells/mm3; median viral load was 4.49 log10 copies/mL. Population sequencing of 2 and 6 week specimens showed NNRTI resistance in 39/68 (57%) mothers in sdNVP; sdNVP+CBV4: 9/67 (13%); sdNVP+CBV7: 6/68 (9%). The most common maternal NNRTI resistance mutations were: K103N, Y188C, Y181C, V106M, A190G, and V106A. No NRTI mutations, including M184V, were detected. Among 228 evaluable infants, in utero transmission was 21/228 (9.2%) and at 6weeks total transmission rate was 24/228 (10.5%). NNRTI resistance was detected in 7/9 (78%) infants in sdNVP; sdNVP+CBV4: 1/8 (13%); sdNVP+CBV7: 0/7 (0%). No M184V mutations or K103N mutations were observed in sdNVP infants. The sdNVP+CBV4 infant was an in utero transmission with K103N and Y181C and her mother did not develop NNRTI resistance. CONCLUSIONS: SdNVP+CBV can significantly decrease the subsequent development of maternal and paediatric NNRTI resistant HIV-1 without risk of 3TC resistance. The optimal duration of CBV is uncertain and the two sdNVP+CBV arms remain open to accrual.
ABSTRACT 3

Antiviral Therapy 2005; 10:S5

Short-course combivir (CBV) single dose nevirapine reduces but does not eliminate the selection of nevirapine-resistant HIV-1: improved detection by allele-specific PCR

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BACKGROUND: Single-dose nevirapine (sdNVP) to prevent mother to child HIV-1 transmission (pMTCT) selects NVP resistant variants in 30–65% of mothers as detected by standard genotyping. The T.O.P.S. trial (BI 1413) is a prospective, randomized three-arm study comparing sdNVP to sdNVP +4 or 7 days of CBV for pMTCT (McIntyre et al., Int. AIDS Cong. 2004). To detect low-frequency selection of NVP-resistant variants in this study, we analysed patient samples with an allele-specific RT-PCR assay that quantifies variants encoding 103N or 181C at frequencies <0.1%.

METHODS: Samples from a subgroup of 32 women were analysed at baseline, 2 and 6 weeks post-therapy from the three treatment arms: sdNVP (10 women) or sdNVP +4 or 7 days of CBV (11 each). Plasma HIV-1 RNA was converted to cDNA, and the target region was amplified and quantified by real-time PCR. This product was used as template for a second round of real-time PCR using discriminatory primers.

RESULTS: Standard genotyping revealed NVP resistance mutations in 70% of women receiving sdNVP at week 2 and 50% at week 6. By contrast, no NVP resistance mutations at codons 103 or 181 were detected by standard genotype at week 2 or 6 among women in this study who received sdNVP +4 or 7 days of CBV. Allele-specific RT-PCR detected 103N or 181C variants in week 6 samples from 75% of women receiving sdNVP alone (mutant frequency 1–75%, median: 7%) and 6 of 22 (27%) of women receiving sdNVP +4 or 7 days of CBV (mutant frequency 0.4–8%, median: 1.4%). There was no difference between the 4 and 7 day CBV arms in the proportion of women with NVP-resistant variants.

CONCLUSIONS: Short-course CBV (4 or 7 days) reduced the selection of NVP-resistant variants following sdNVP from 75% to 27% of women as determined by allele-specific RT-PCR. The selection of low-frequency NVP-resistant variants in the CBV arms was not detected by standard genotype. The impact of these low-frequency NVP-resistant variants on future treatment options is unknown. The optimal duration of CBV to eliminate the selection of NVP-resistant needs to be established.
ABSTRACT 4
Antiviral Therapy 2005; 10:S6

Patterns of viral load and drug resistance in breast milk and blood from women treated with singledose nevirapine to reduce mother-to-child transmission of HIV-1

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BACKGROUND: The use of single-dose nevirapine (NVP) to reduce mother-to-child transmission of HIV-1 is increasing in the developing world. However, little is known regarding the effects of this regimen on viral loads in breast milk or on the temporal patterns of drug resistance that arise during the early postpartum period. These are important to determine because considerable HIV-1 transmission occurs during the first 6 weeks postpartum in breastfeeding populations. We have determined the effect of NVP on breast milk viral shedding and are examining the prevalence of NVP resistance during this early postpartum period.

METHODS: Thirty pregnant HIV-1 seropositive women in Nairobi, Kenya who planned to breastfeed were treated with the single-dose NVP regimen (HIVNET 012). Two to four breast milk samples were collected each week between delivery and 6 weeks postpartum. Breast milk HIV-1 RNA was quantified using the Gen-Probe HIV-1 viral load assay. HIV-1 DNA was extracted from blood samples collected at 1 month postpartum and was tested for NVP resistance using an allele-specific PCR assay that detects the K103N mutation.

RESULTS: Breast milk viral loads in 30 women treated with single-dose NVP were compared to breast milk viral loads from 30 women treated with the Thai-CDC short-course zidovudine regimen. A total of 404 breast milk samples from the NVP-treated women were tested, with a median of 14 samples per woman during the first 6 weeks postpartum. Between 3 and 21 days postpartum, treatment with NVP was associated with significantly greater suppression of breast milk log10 HIV-1 RNA: days 3 to 7 (1.98 vs 2.42, \(P=0.1\)); days 8 to 14 (1.78 vs 2.48, \(P=0.005\)); days 15 to 21 (1.90 vs 2.97, \(P=0.003\)). In preliminary studies, utilizing an allele-specific PCR assay, we tested one-month postpartum blood samples from the NVP-treated women and determined that 40% of the women tested to date had detectable levels of the K103N mutation. Breast milk samples are currently being tested.

CONCLUSIONS: Compared to the Thai-CDC shortcourse zidovudine regimen, single-dose NVP results in sustained suppression of breast milk viral loads during the first 3 weeks postpartum. However, the benefits of this suppression may be counterbalanced with a high prevalence of resistance.
ABSTRACT 10
Antiviral Therapy 2005; 10:S12

Resistance after single dose nevirapine prophylaxis varies by viral subtype in infants from sub-Saharan Africa

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BACKGROUND: The Ugandan HIVNET 012 trial found that administration of single dose nevirapine to women in labour and to infants after birth can prevent HIV-1 mother-to-child transmission. The NVAZ study in Malawi included women and infants who received the same single dose nevirapine regimen. A previous comparison of maternal nevirapine resistance in HIVNET 012 and NVAZ revealed that resistance was more frequent in women with subtype C HIV-1 (45/65=69%) than in women with subtype A (28/144=19%, \(P<0.0001\)) or D (35/97=36%, \(P<0.0001\)). The higher frequency of nevirapine resistance among subtype C strains was independent of baseline viral load, maternal age, parity or the time between nevirapine dosing and resistance testing. It is not known whether subtype influences emergence of nevirapine in infants who become HIV-1 infected despite nevirapine prophylaxis.

METHODS: We analysed nevirapine resistance in HIVNET 012 and NVAZ infants who became HIV-1 infected despite nevirapine prophylaxis. All of the mothers and infants in this analysis received the HIVNET 012 regimen without other antiretroviral prophylaxis. Nevirapine resistance was analysed with the ViroSeq HIV Genotyping System using 0.1 ml infant plasma samples collected 6–8 weeks after single dose nevirapine administration (6–8 weeks after birth).

RESULTS: Genotypes were obtained for HIV-1 from 24 infants in HIVNET 012 (nine with subtype A, one with subtype C, nine with subtype D, and five with intersubtype recombinant HIV-1) and 23 infants in NVAZ (all with subtype C). Nevirapine resistance mutations were detected in HIV-1 from 11/24 (46%) infants in HIVNET 012, compared to 20/23 (87%) infants in NVAZ \((P=0.005, \text{ exact test})\).

CONCLUSION: Nevirapine resistance was more frequent in Malawian infants with subtype C than in Ugandan infants (mostly with other subtypes) 6–8 weeks after single dose nevirapine. This is consistent with the higher frequency of nevirapine resistance observed in these trials among mothers with subtype C than with A or D. Further studies are needed to evaluate whether the higher frequency of nevirapine resistance observed in Malawian infants after single dose nevirapine reflects effects of HIV subtype, or other factors.
Resistance mutations arise in the majority of women provided single-dose NVP and appear to differ in emergence and persistence

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BACKGROUND: Conventional sequence testing has shown that approximately 40% of women who receive peripartum single-dose nevirapine (SD-NVP) generate virus with resistance mutations to non-nucleoside reverse transcriptase inhibitors. However, the sensitivity limitation of sequencing prevents reliable detection of variants that comprise less than 20% of the sample virus population. Therefore, there is a need for sensitive assays that improve detection of drug-resistant virus emergence and persistence to better assess treatment implications.

METHODS: To identify low-frequency resistant variants, we developed HIV-1 subtype C real-time PCR assays for two common NVP mutations, K103N and Y181C. Assay evaluations of mutant plasmids in wildtype backgrounds established detection limits of 0.2% for K103N and 0.3% for Y181C, 40–100-times more sensitive than population-based sequencing. The real-time assays were used to reassess the emergence of drug resistance in women who received SD-NVP. We analysed genotyped matched pairs of pre-NVP and post-NVP plasma, collected 6–36 weeks postpartum, from 58 South African women. Of the post-NVP specimens, 40 had no detectable mutations, 16 had K103N, and 12 had Y181C by population-based sequencing. None of the pre-NVP specimens had evidence of resistance by sequencing.

RESULTS: Using real-time PCR analyses, neither K103N nor Y181C were detected in pre-treatment samples. The assays successfully identified the 16 post-NVP samples with sequence-detectable K103N and the 12 samples with Y181C. Of the post-NVP specimens negative for mutations by sequencing, real-time PCR testing found 16/40 (40%) were positive for K103N at 6–36 weeks post-exposure. Additionally, testing for Y181C found 3/40 (8%) samples were positive at 6–12 weeks postpartum. The K103N frequency appeared to peak <3 months post-NVP. Clonal sequencing confirmed resistance mutations in all representative samples.

CONCLUSIONS: The finding of K103N and Y181C in an additional 43% of women with previously undetectable resistance suggests that resistant viruses emerge in the majority of women receiving SD-NVP. The disproportionately high numbers of K103N and its detection up to 36 weeks post-NVP suggests that in adults with HIV-1 subtype C this mutation has a selective advantage over Y181C and persists longer. These data emphasize the importance of sensitive assays to better assess the clinical implications of drug-resistant variants.
High frequency of nevirapine resistant mutations in the HIV quasi species found in NVP-treated participants of an MTCT Ugandan cohort

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BACKGROUND AND HYPOTHESIS: The effectiveness of nevirapine (NVP) treatment and emergence of drug resistance was examined in an observational mother-to-child HIV transmission (MTCT) cohort in Uganda. NVP-resistant viruses can be transmitted or emerge independently in either the mother or infant. Given the dominance of NVP resistance after single dose in some cases, we suspect that NVP resistant clones may be at high frequencies in the HIV population in most NVP treated mothers and infants. METHODS: Mother-infant pairs from an AZT-treated (n=48), NVP-treated (n=61), and untreated group (n=90) were enrolled at postnatal clinics in Kampala, Uganda. Detailed phylogenetic analyses were performed on the mother and infant HIV-1 RT sequences. A quantitative radiolabelled oligonucleotide DNA ligation assay (OLA) was used to detect very low frequencies of drug resistance mutations (K70R, K103N, and Y181C at <1%) in the patient quasispecies. Ligation partners accommodated intersubtype variability with inosine or uracil at polymorphic sites in the DNA oligonucleotides.

RESULTS: HIV transmission rates were similar in the nevirapine (NVP) (16.4%) and AZT (16.7%) groups and offered protection as compared to the naive group (48%, P=0.0001). The subtype prevalence in the mothers was 58% A, 23% D, 3% C, and 15% recombinants (A/D, C/D) and transmission was not skewed for a particular subtype. In the 6 week post-partum sample, mothers and infants treated with NVP harbored HIV-1 with a dominant K103N (n=2) or Y181C (n=5) (16%) whereas AZT resistant mutations were absent in AZT-treated pairs. A quantitative OLA identified 181C or 103N (at 1–40%) in another 22 of 44 infected mother or infants treated with NVP (50%) and in one of 63 untreated mothers or infants. K70R was not detected in the HIV quasispecies of AZT, NVP, or untreated groups.

CONCLUSIONS: These analyses suggest that NVP resistant mutations can be detected in over 75% of the NVP-treated infected mothers and infants. Prominence of these mutations in the quasispecies of HIV-infected mothers and infants 6 weeks post-partum and single-dose NVP treatment is of concern for prevention of MTCT and future treatment of NVP-containing treatment regimens in Uganda.
ABSTRACT 13
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Increased sensitivity of detection of K103N resistance variants by real-time PCR in RNA and DNA after single-dose nevirapine

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BACKGROUND: Nevirapine resistance mutations (NVPR) are selected following single-dose treatment (sdNVP) in women (22–75% 1–2 weeks; 15–48% 6–8 weeks) and babies (39–53% 4–8 weeks). Standard genotyping fails to detect mixtures below <20% suggesting that NVPR is substantially underestimated. Real-time PCR methods improve sensitivity of detection and quantification of minority populations. The most common high-level NVPR selected in women following sdNVP is K103N in HIV-1 reverse transcriptase (RT) which confers cross-resistance to other NNRTI’s and can persist for many months. We have developed a real-time PCR assay for the detection and semi-quantification of K103N in RNA and DNA collected 6–48 weeks post-sdNVP from women enrolled in a pMTCT program.

METHOD: Viral RNA and DNA was used to amplify RT sequences by nested (RT)-PCR. Diluted amplicons were used for real-time PCR detection of wild-type (K, lysine, AAA, AAG) and mutant codons (N, asparagine, AAC, AAT) at position 103. Threshold cycles (Ct) were used to derive relative K103N frequencies. Increased sensitivity of real-time PCR detection was assessed by comparison with samples previously genotyped (“in-house”).

RESULTS: K103N was identified in 52% (n=31) of RNA samples previously genotyped and in 87% by real-time PCR (sensitivity=0.2%). Genotyping failed to detect mutants below <10%. Cross-sectional analysis of RNA collected 6–48 weeks post-sdNVP showed 87% (n=32) had K103N at 6 weeks, which declined to 67% (n=27), 35% (n=37) and 13% (n=54) at 12, 21 and 48 weeks respectively. In DNA collected at 6 and 48 weeks post-sdNVP, 53% (n=43) had K103N at 6 weeks and 4.2% (n=48) at 48 weeks. Longitudinal analysis of RNA 6–48 weeks post-sdNVP for 16 women showed similar decay rates but higher levels of K103N persisted longer.

CONCLUSION: Real-time PCR detection of K103N was more sensitive than genotyping. An additional 73% (n=15) of RNA samples previously identified as wild-type by genotyping had detectable K103N. Cross-sectional analysis showed that K103N was detectable in RNA in the majority of women 6 weeks post-sdNVP which declined over time and was present in a minority at 48 weeks. In DNA, K103N was detectable in <50% of samples at 6 weeks post-sdNVP and was essentially absent at 48 weeks.
ABSTRACT 16
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Virological response to antiretroviral therapy in the setting of the K65R mutation

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BACKGROUND: The impact of the K65R reverse transcriptase (RT) mutation on virological response to salvage therapy has not been fully defined. We performed a retrospective analysis of virological response to subsequent therapy in antiretroviral-experienced patients with K65R drawn from a large clinical cohort.

METHODS: We identified all K65R mutations in virus samples from three California clinical programs from the period 7/1/97 to 10/1/04. From 9060 isolates (6147 patients) we found 169 (144 patients) with K65R. Complete treatment histories were available for 98 patients, including 39 who had ε1 plasma HIV-1 RNA measurement following a change in therapy based on the genotype.

RESULTS: Baseline characteristics. Patients were highly treatment-experienced. Previous treatment included tenofovir (TDF, 25/39 patients), didanosine (ddl, 28/39), abacavir (18/39), and lamivudine (36/39). Nineteen patients (49%) had used both TDF and ddl. Mutations M184V, Q151M, and L74V were present in 22 (56%), 7 (18%, three of whom also had M184V), and 2 (5%) of patients, respectively. At least one TAM was present in 17 patients (44%); five patients had ε3 TAM’s, three of whom also had M184V and two with Q151M. Five patients had no other known resistance mutations.

RESPONSE TO THERAPY: Thirty-nine patients changed therapy based on their genotype results; 36 (92%) were changed to a protease inhibitor-based regimen. Eleven new regimens included TDF. The median baseline viral load was 15 849 copies/ml. Overall we observed decreases in viral loads following a change in antiretroviral therapy: median changes observed: -1.3 log at 34 days, -1.7 log at 85 days, and -1.95 log at 157 days. Fourteen patients (36%) achieved plasma viral loads of <50 copies/ml. The response observed was independent of previous antiretroviral use (including TDF and the combination of TDF/ddI), or any component of the new regimen. The response was independent of the presence of other resistance mutations.

CONCLUSIONS: Among this cohort of highly treatment-experienced patients with the K65R mutation, response to subsequent antiretroviral therapy was robust in spite of the presence of multiple resistance mutations in many of these cases. This response appeared independent of specific prior RT inhibitor use or specific components of the new regimen.
ABSTRACT 17
Antiviral Therapy 2005; 10:S19

Intensification of a failing regimen with AZT may cause sustained virological suppression in the presence of the K65R mutation

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BACKGROUND: The K65R resistance mutation limits the number of NRTI options. However, it has the potential to decrease phenotypic AZT-resistance and almost never occurs concurrently with TAMS. Patients with a K65R mutation usually maintain AZT/D4T as options.

METHODS: Three patients experienced virological failure associated with the K65R and other resistance mutations. The three regimens were then intensified with AZT without changing any other drugs, despite genotypic resistance.

RESULTS: None of the patients had a past history of TAMS. Two of the three patients were taking a triple nucleoside regimen. Patient 1 was RTI–naive and then failed with ABC/ddI/3TC. Patient 2 who was ART–experienced, was never able to attain undetectable viraemia in 4 years of treatment. She failed on a triple regimen of ABC/ddI/TDF. Genotype resistance testing at VF showed K65R, L74V, M184V, and the Y115F mutations to be present in both patients. Patient 3, ART-experienced, started a regimen of TDF, 3TC, and NVP. After 18 months with viral load <50 copies/ml, he experienced a rebound above detectable limits which continued for the next 6 months. Genotype testing showed the K65R, G190S and Y181C mutations. All three patients had K65R but no TAMS. Their failing regimens were intensified with AZT and the other drugs were kept despite genotype resistance mutations. All three patients had an immediate viral load reduction to undetectable levels within 4 weeks: Patient 1 dropped more than 2 logs from 5300 copies/ml to <50 copies, patient 2 dropped greater than 3 logs from 48000 to <50 copies, and patient 3 had a 1.4 log reduction from 1130 to <50 copies/ml. Follow up time ranges from 7–16 months. All three patients have maintained their undetectable levels and all three remain on their regimens.

CONCLUSION: In these patients with the K65R mutation and no TAMS, AZT was added to regimens in which genotypic testing showed resistance to all their drugs. AZT was the only active drug, however, this addition was enough to achieve sustained viral load reductions to below 50 copies/ml.
ABSTRACT 18
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Patterns of resistance mutations in patients failing on a didanosine (ddI) and tenofovir (TNF) containing regimens

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BACKGROUND: Due to a high rate of early virological failure (VF) with selection of resistance mutations the EMEA does not recommend the coadministration of ddI and TNF unless strictly necessary.

METHODS: From our database we have evaluated the virological outcome of TNF plus ddI based-regimens (n=92). Twenty-five of them were antiretroviral naïve and 67 were virologically suppressed for at least 6 months, who had previously been exposed to a median of four regimens (from two to 10) during a median of 65 months (IQR 40–96). Patients began or switched their antiretroviral regimen to a new combination consisting on TDF once daily plus ddI EC once daily (adjusted to body weight) plus a third nucleoside (NRTI) (n=21), or a non-nucleoside (NNRTI) (n=48) or lopinavir/ritonavir (PI) (n=23). Whenever VF was detected, a genotypic resistance test was performed both at VF, at prior to any antiretroviral therapy or in previous episodes of VF if any.

RESULTS: After a median follow-up of 23 months (IQR 19–26), 19 from the 92 developed VF (21%). Seven of the 19 failing patients received a NRTI (lamivudine, n=5; abacavir, n=2), 11 a NNRTI (efavirenz, n=7; nevirapine, n=4) and 1 lopinavir/ritonavir, in addition of ddI and TNF. The genotypic analyses at VF detected resistance mutations in 18 patients (95%), and was wild type in the patient treated with PI. In these 18 patients, resistance mutations in the reverse transcriptase gene were never detected at baseline before any antiretroviral therapy and were de novo in 16 patients (84.2%) and both “the novo” and emergent of archived mutations in the remaining two. M184V was selected in all the NRTI treated patients (seven of seven). K65R was selected in 11 patients (58%) and L74V in four (22.2%). L100I, K103N/R/T, 106I/M, Y181C and G190E/Q/S were selected in all the NNRTI treated patients (11 of 11) and emerged in two additional patients (68.4%).

CONCLUSIONS: These results argue against the use of TDF-ddI plus a NRTI or a NNRTI not only in naïve patients but also in previously suppressed patients. Conversely combinations including lopinavir/ritonavir seem to be virologically safer.
Genotypic resistance in patients with persistently detectable low-level viraemia treated with triple nucleoside antiretroviral therapy

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OBJECTIVE: A significant proportion of patients failing triple nucleoside analogue combinations appears to maintain long-term low plasma viral load (VL) (<104cp/ml) in spite of HIV resistance. The virological determinants of this particular evolution profile are not known.

PATIENTS AND METHODS: We retrospectively analysed HIV reverse transcriptase (RT) genotypic profile in 27 patients receiving triple nucleoside therapy for more than 6 months with stable VL<104cp/ml (LVL group). These genotypes were compared to those in eight patients with VL>104cp/ml (HVL group). LVL patients had a median gain of 214±247 CD4/mm3 relative to pretherapy levels and maintained a median VL of 2370 cp/ml (52–9500) for a median 32 months; at the beginning of treatment they had a median VL of 105 cp/ml and 238 CD4 cells/mm3. HVL patients, received only 10 months of triple nucleoside therapy.

RESULTS: Among LVL patients, all had at least one resistance mutation in RT, including mutations of codon 215 in 21. Interestingly, while 18 patients had a T215F mutation, only three had a T215Y mutation, which was always associated with L210W; in two of them T215Y was preceded by a T215F and appeared as viral load increased significantly. In 10/18, the T215F mutation was also associated with K219E and in 16 of 18 with a combination of D67N and K70R. In HVL patients, all developed at least one RT mutation. Remarkably, seven of eight patients had a T215Y mutation. Among them, six also displayed a M41L, but only two had a combination of D67N and K70R, while one had a K219E.

CONCLUSIONS: Among patients failing triple nucleoside therapy, the T215F/K219E resistance pathway, as opposed to the T215Y/L210W pathway, appears to be highly prevalent in patients retaining low VL levels. Whether this relates to the replicative capacity or the resistance properties of these viruses needs to be further investigated. That could influence the moment when a treatment must be modified in a failing patient.
Relation between the antiretroviral activity of didanosine (ddI) and the number of reverse transcriptase (RT) mutations: dINAM study

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BACKGROUND: Didanosine may retain substantial antiretroviral activity despite the presence of RT mutations. We have investigated the effectiveness of adding ddI to a stable failing antiretroviral (ARV) therapy on plasma viral load (VL), and its relation with the baseline number of different clusters of RT mutations.

METHODS: Consecutive patients failing to an ARV regimen not containing ddI and with VL above 1000 copies/ml were eligible for the study. After a genotypic and virtual phenotype resistance testing, ddI (Videx EC 400 mg or 250 mg qd according to body weight) was added to the failing therapy. A clinical, immunological, and virological evaluation was performed at baseline and at weeks 2 and 4. Thereafter the ARV regimen was optimized according to resistance test results.

RESULTS: Forty patients were included. Mean (range) baseline PVL and CD4+ lymphocytes were 4560 copies/mL (IQR 2810–14900) and 405 cells/mm3 (IQR 290–505) respectively. At weeks 2 and 4 the median HIV RNA reduction was 0.73 and 0.67 log10 respectively. At week 4 the proportion of patients with a drop VL>0.5 log, >1 log or with PVL<200 copies/ml was 63%, 30% and 24% respectively. Mean rise in CD4+ cell count at week 4 was 53 cells/mm3. At baseline, the median number RT, nucleoside-associated mutations (NAMs) or thymidine-associated mutations (TAMs) were 4.3 and 3 respectively. Relations between number of mutations in each studied cluster (RT/NAMs/TAMs) and median log10 drop HIV RNA level were: RT mutations: 0–2: -1.37; 3–4: -0.67; 5: -0.35; and 6–7: -0.50. NAMs mutations: 0–1: -1.18; 2: 0.88; 3: -0.67; 4: 0.63; and 5–6: -0.47. TAMs mutation : 0–1: -1.18; 2: 0.88; 3: -0.67; 4: -0.35; 5: -0.11 log. We did not find differences in comparing patients with two different pathways of TAMs. VL drop was statistically significant (P<0.05) compared with baseline when the number of baseline mutations /RT, NAMs or TAMs) was less than four. Median drop of VL in the three patients harbouring 74V+65R was -0.02 log compared with -0.69 log in the remaining 37 patients (P<0.05). VL drop was similar irrespective of the pathways of TAMs (41L,210W,215Y vs 67N,70R, 215F, 219E/Q).

CONCLUSION: ddI retains significant antiretroviral activity when the number of RT, TAMs or NAMs is less than four except when both the 74V and 65R are present.
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Evaluation of dried blood spots for HIV-1 drug resistance testing

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BACKGROUND: Dried blood spots (DBS) are simpler to prepare, store, and transport than plasma and, therefore, represent a good alternative for drug resistance genotyping. However, the utility of DBS for drug resistance monitoring is not known. We investigated the rate of amplification and sequencing of HIV-1 protease/ reverse transcriptase (PR/RT) from DBS stored at different conditions, the nature of the amplified product (DNA/RNA), and the homology between plasma and DBS sequences.

METHODS: Two matched plasma/DBS (903 paper) panels, each consisting of 3 samples with low, medium, or high RNA virus loads (VL) were stored at -20°C (panel A) or at both -70°C and room temperature (panel B) for 5–6 years. Panels were generated using blood collected from HIV-infected donors enrolled in the Virology Quality Assessment (VQA) Program. Samples from panel A had resistance-associated mutations. Twenty-one plasma/DBS from untreated individuals from Cameroon were stored at -20°C for 2–3 years (median VL=18 000, range=830–190 000). Total nucleic acids were extracted from 50ul spots using the Nuclisens method. A 1.023 Kb PR/RT fragment was amplified by RT-nested PCR and sequenced. Amplifications were also done without RT to evaluate the contribution of proviral DNA.

RESULTS: Amplification and sequencing was successful in the 3 DBS from panel A and in 2 of the 3 DBS from panel B stored at -70°C. None of the DBS from panel B that were stored at room temperature were successfully amplified. All DBS from Cameroonian patients were amplified and sequenced including the 8 patients with low VL (830–10 000 RNA copies/ml). Proviral DNA contributed significantly to DBS sequences in 4/5 spots from panels A and B, and in 17/21 specimens from Cameroon. PR/RT sequences from plasma and DBS from panel A were generally concordant though one sample showed Y181C only in plasma.

CONCLUSIONS: Our results show the feasibility of using this DBS method for drug resistance testing, despite the need to amplify a large fragment. Longterm storage at -20°C appears to preserve nucleic acids in DBS. The frequent contribution of proviral DNA to DBS sequences highlights the need for a wider evaluation of the concordance of resistance genotypes between plasma and DBS.
Comparison of whole blood, plasma, and PBMC HIV-1 resistance genotyping and matched dried samples using SampleTanker™ a novel dried transportation matrix

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BACKGROUND: Cooperative studies worldwide are addressing the use of whole blood for biomarker analysis in developing countries. We have previously shown the utility of SampleTanker™ (Research Think Tank, Inc), an inexpensive transportation system for HIV-1 genotypic analysis of dried plasma. Herein we describe the utility of dried whole blood or peripheral blood mononuclear cells (PBMC) using SampleTanker for HIV-1 genotypic analysis.

METHOD: Twenty HIV-1 positive, ARV experienced patients had blood drawn in EDTA VACUTAINER™ and VACUTAINER CPT tubes. The CPT and all but one EDTA tube were centrifuged using standard conditions and times for plasma and PBMC collection; one unspun EDTA tube represented the whole blood sample. Each sample type was loaded on a SampleTanker and air dried in a circulating biological hood for a minimum of 5 h. Samples were extracted using QIAamp® Viral RNA Mini Kit or QIAamp DNA Blood kit (Qiagen) and NucliSens® MiniMag (BIOMERIEUX). Genotyping was performed using TRUGENE HIV-1 Genotyping Assay. Sequences derived from plasma virus were used as the reference for directly comparing sequences from whole blood and PBMC. Sequence analysis was performed using MuTanker™ Comparator software (Research Think Tank, Inc).

RESULTS: Genotypes from liquid and SampleTanker specimens were successfully obtained using TRUGENE HIV-1 Genotyping Assay. Genotypes of liquid and matched dried SampleTanker samples were highly concordant. The liquid and SampleTanker whole blood and PBMC genotypes, as compared to plasma genotypes, were consistent in overall RAM results with the exception of mixture populations detected in the PBMC fractions at some codons. Overall the liquid and dried whole blood and PBMC fraction were identical. Accuracy and reproducibility of genotypic results comparing liquid to dried specimens was ε98% at the nucleotide level, ε99% at the amino acid level and 100% concordance was observed for reported resistance associated mutations (RAM).

CONCLUSION: Accuracy and reproducibility of results from plasma, whole blood and PBMC were comparable to the TRUGENE product insert specifications for FDA approved plasma genotyping. Concordance of protease and reverse transcriptase gene RAM results between liquid and dried SampleTanker samples demonstrate potential utility for resistance testing in studies using whole blood.
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**AZT resistance: why do HIV-1 and HIV-2 choose different pathways?**

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**BACKGROUND:** Resistance to a nucleoside analog reverse transcriptase inhibitor (NRTI) implies that RT has an increased ability to discriminate between the NRTI and normal nucleosides. Discrimination can take place either at the time the NRTI is incorporated into viral DNA, or after the NRTI is incorporated (selective excision). AZT is an NRTI that is a potent inhibitor of both HIV-1 and HIV-2 replication. ATP-mediated excision is the mechanism that underlies the most common form of HIV-1 resistance to AZT. However, when HIV-2 is challenged with AZT, it usually develops resistance by reduced incorporation rather than selective excision.

**METHODS:** We compared the structure and biochemical properties of HIV-1 RT and HIV-2 RT to try to understand why excision is the preferred resistance pathway for AZT resistance for HIV-1 RT but not for HIV-2 RT. RESULTS: Although both RTs have similar levels of polymerase activity, HIV-1 RT is more susceptible to inhibition by (more readily incorporates) AZTTP than HIV-2 RT. HIV-1 RT is more efficient at carrying out the excision reaction with ATP as the pyrophosphate donor. This suggests HIV-1 RT has a better nascent ATP binding site than HIV-2 RT, making it easier for HIV-1 RT to develop a more effective ATP binding site by mutation. A comparison of the structure of the putative ATP binding sites of HIV-1 and HIV-2 RT shows numerous differences in the putative ATP binding sites that could explain why HIV-1 RT binds ATP more effectively. In contrast, HIV-2 RT, which incorporates AZTTP less efficiently than HIV-1 RT, develops resistance by acquiring mutations that further reduce AZTTP incorporation. The polymerase active sites of the two enzymes show differences that could help explain why HIV-2 RT acquires mutations that reduce the incorporation of AZTTP.

**CONCLUSIONS:** Each RT prefers a pathway for AZT resistance that extends the properties of the wild-type enzyme. Viewed in this light, the development of the excision resistance mechanism by HIV-1 RT is an unfortunate coincidence based on the existence of a nascent ATP binding site that has no clear role in normal viral replication.
Interactions between nevirapine resistance mutations and NRTI resistance mutations

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BACKGROUND: Interactions between nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations may influence the rate and pathways of resistance development in combination therapy.

METHODS: We studied resistance in reverse transcriptase sequences from 940 patients with subtypes A (220 patients), B (222), C (130), F (31), G (169) and other or unknown subtypes (168), who received nevirapine as sole NNRTI, in combination therapy. Data were derived from clinical databases and the scientific literature. In sequences with only one of the three most prevalent NNRTI mutations (103N, 181C and 190A), we determined the influence of subtype, NRTI treatment and presence of a NAM, known NRTI and major PI resistance mutations (according to IAS-USA) on the presence of these three mutations using multivariable logistic regression models (43 variables). The odds ratios with 95% confidence intervals are reported.

RESULTS: The multivariable analysis showed that independent predictors of the presence of the 103N mutation was presence of a NAM (0.39 [0.18–0.88]) and the presence of 65R (0.19 [0.04–0.92]) and 184V mutations (1.53 [0.99–2.38]), next to treatment with AZT (0.66 [0.418–1.04]) and D4T (0.53 [0.34–0.82]). Independent predictors for the presence of the 181C mutation were the presence of a NAM (4.18 [1.85–9.43]), and presence of 74V (3.1 [1.37–6.98]), 184V (0.43 [0.27–0.70]), and 215Y (0.20 [0.09–0.46]) mutations. Independent predictors for the presence of
the 190A mutation were the presence of 67N (2.39 [1.06–5.40]), 215Y (3.49 [1.2–10]), and 215F (3.25 [1.25–8.50]), next to treatment with AZT (2.88 [1.53–5.41]) and D4T (2.89 [1.62–5.16]).

CONCLUSIONS: The analysis shows a different prevalence of the three most important nevirapine resistance mutations, depending on NRTI resistance mutations and NRTI treatment. The independent influence of AZT and D4T treatment may be due to reversion of resistance mutations. The analysis suggests interactions between NNRTI and NRTI resistance pathways, and in particular an antagonistic effect between 181C and 184V, in addition to earlier reported interactions between 184V and 215Y/F, and 181C and 215Y/F.
Selection of resistance following first-line antiretroviral regimens among HIV-1 subtypes


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BACKGROUND: With increasing access to antiretroviral therapy many patients will receive WHO-recommended first-line regimens (zidovudine/stavudine+lamivudine+nevirapine/efavirenz). Assessment of drug resistance to these regimens may be relevant for initial and subsequent therapy.

METHODS: We compared drug exposure and mutation frequencies at 18 NRTI and 15 NNRTI resistance positions among 379 patients infected with subtypes A (26), B (175), C (61), D (15), F (14), G (32) and CRF01_AE (56), failing first-line regimens. Pairwise comparisons among all subtypes were performed using Fisher Exact tests with correction for multiple comparisons. To study mutations co-selection, we defined ‘mutation sum’ as sum of concomitant mutations at positions 41, 67, 70, 210, 215, 219 (TAMS); 184; and 103, 106, 181, 188, 190 (NNRTI); and compared sums among subtypes using Student’s t-test ($P \leq 0.01$). To study potential effects of NRTI mutations after first-line regimen failure, on recommended second line NRTIs (abacavir, tenofovir, didanosine), we defined ‘genotypical susceptibility score’ (GSS) as $[1 – (drug score)/60]$, (drug score determined by the Stanford database); and compared the three-drug GSS sums (range 0 for most resistant to 3 for least resistant) among subtypes, using Student’s t-test ($P < 0.0001$).

RESULTS: On average, patients from various subtypes were exposed to 2.2–3.0 NRTIs and 1.0–1.2 NNRTIs, with no inter-subtype differences in drug-class exposure. 90-100% had at least one RT mutation (67–100% NRTI; 85–100% NNRTI). Significant differences in specific mutation
frequency occurred in six NRTI (41, 67, 70, 75, 210, 215) and three NNRTI (103, 181, 190) non-
polyomorphic resistance positions, most of which were not explained by different drug exposure. Subtypes B, F and CRF01_AE had significantly higher mutation scores (5.0, 4.6, 4.3) than subtypes A, C, D and G (2.5, 2.9, 3.6, 2.9), suggesting more mutations following first-line regimens. Subtype B, F and CRF01_AE had significantly lower mean GSS (1.0, 1.4 and 1.1) than subtypes A, C, D and G (2.6, 2.3, 2.5, 2.2), indicating less susceptibility to secondline NRTIs.

CONCLUSIONS: While differences in resistance could be partly explained by specific drug exposure or duration, in real life data sets, HIV-1 subtypes evolved different degrees of drug resistance to first line regimens, which may affect decisions on initial and subsequent antiretroviral regimens.
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Antiretrovirals, Africa and the evolution of drug-resistant HIV: predictions for Botswana

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BACKGROUND: A major cause for concern is the belief that the planned antiretroviral roll-out in Africa will quickly lead to high rates of transmission of drug-resistant HIV strains. The World Health Organization (WHO) has therefore recommended, and drafted, surveillance guidelines to monitor the emergence of both acquired and transmitted drug resistance in Africa during the roll-out. The WHO monitoring scheme samples newly diagnosed individuals for transmitted drug resistance, and they have specified a value of 5% transmitted resistance as their detection threshold. However, no predictions have been made as to how quickly this level is likely to be reached in any African country. Here, we predict the evolution of transmitted resistance in Botswana, and we determine when the WHO threshold will be exceeded.

METHODS: Approximately 40% of adults in Botswana are HIV-infected. Currently their antiretroviral program (that began in 2002) treats 34,000 patients, with a goal of treating 85,000 patients (that is, ~30% of HIV-infected adults) by 2009. We predict the expected temporal evolution (from 2002 to 2009) of transmitted resistance in Botswana due to this high treatment rate. We present a new stochastic dynamic model of the emergence and evolution of drug resistance. We formulate a birth-death-immigration master equation, and obtain an analytical solution of the probabilistic epidemic dynamics. We use data from Botswana, and predict the emergence of transmitted resistance assuming that the drug-resistant strains that will evolve will be 25%, 50% or 100% as transmissible/fit as the wild-type strains.

RESULTS: We show that – if rates of acquired resistance are extremely high, but drug-resistant strains are only half as fit/transmissible as wild-type strains – transmitted resistance will remain low (<5% by 2009) and will be undetectable by the WHO. However, we show that the WHO threshold will be exceeded in Botswana if drug-resistant strains evolve that are as fit/transmissible as the drug-sensitive strains; under these conditions, transmitted resistance in Botswana will rise to ~10% by 2009.

CONCLUSIONS: The WHO’s surveillance system in Africa will only detect transmitted resistance by 2009 in countries like Botswana (that plan to achieve high treatment rates) if very fit/transmissible drug-resistant strains evolve.
Genotypic and phenotypic explanation for failure of triple NRTI therapy with lamivudine, didanosine and tenofovir

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BACKGROUND: The ‘Jemsek’ study (Abstract #51, CROI 2004) tested a three drug regimen containing lamivudine (3TC), didanosine (ddI), and tenofovir (TFV) in treatment naive patients. The study showed rapid virological failure in 91% (20 of 22) of patients, with 100% of failure genotypes having M184V and 50% having K65R. The reason for this rapid virological failure has not been clearly defined. Standard phenotyping showed no resistance to TFV (fold-change <1.4). To gain further insight, we investigated HIV-1 genotype and phenotype at the single genome level.

METHODS: We analysed end of study samples using single genome sequencing for nine of the 10 patients who failed with a standard genotype showing both 65R and 184V mutations. We also generated recombinant HIV clones from patient samples and picked clones with 184V alone or 65R+184V for phenotyping. Susceptibility testing of the clones to 3TC, ddI, TFV, and abacavir (ABC) was performed using a single-cycle replication assay in P4/R5 cells.

RESULTS: We analysed a total of 204 single genome sequences from the 9 failure samples. Of these, 102 (50%) had 65R+184V and 77 (38%) had 184V alone. Only 2 sequences (1%) had 65R alone. The remaining sequences consisted of 21 (10.3%) with 184I alone, 1 (0.5%) with 65R+184I, and 1 (0.5%) wild type. Phenotypic testing of recombinant HIV clones showed greater resistance for those with 65R+184V versus the 184V alone to ABC (mean 6.5-fold versus 2.5-fold, respectively), ddI (4.3-fold versus 1.9-fold), and TFV (2.6-fold versus 0.6-fold). The IC50 for 3TC was >270 μM for all clones.

CONCLUSIONS: The rapid failure of the triple NRTI regimen of 3TC+ddI+TFV results from 2 nucleotide changes in RT, which when combined, confer resistance to each drug. In virus having 184V, the 65R mutation reverses hypersusceptibility to TFV and increases resistance to ddI. Only 50% of sequences had both 65R and 184V, indicating that resistance to 3TC alone contributed to failure. The reason standard susceptibility testing of samples with 65R and184V has not shown TFV resistance is that the virus population likely consists of a mixture of TFV-hypersusceptible virus with 184V alone and TFV-resistant virus with 65R+184V.
The presence of selected mutations influence the disappearance of major protease resistance mutations during treatment interruption

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BACKGROUND: The dynamics of disappearance of resistant variants during treatment interruption (TI) varies according to the number and type of mutations. We then investigated the evolution of mutations associated with resistance to protease inhibitors (PI) during TI, and their ability to affect virus replication.

METHODS: 88 patients having at least two genotypes, one at last PI-containing regimen failure and one (or more) during TI (median, 4 months) were analysed. The association between mutations and viraemia/CD4 cell-count was assessed by median test. Chi-square test was used to detect whether specific mutations (present at TI) affect the dynamics of disappearance of other mutations. The time of disappearance (mean-life) of a specific mutation was estimated by fitting an exponential decay (F(t)=F0 exp(-t/t)) for the mutation frequency alone or in presence of other mutations.

RESULTS: Complete disappearance of major PI-resistance mutations and of recently associated-resistance mutations (K20T, K43T, Q58E, T74S, I85V, Q92K, C95F) occurred between 6 and 12 months of TI; this strongly supports their involvement in drug-resistance. By contrast, minor mutations M36I, L63P, V77I remained stable at high frequency even after TI (>75%), suggesting their limited interference with viral fitness. The disappearance of M46I and L90M was associated with higher viral load increase (P<0.001) and with greater decrease of CD4 cell-count (P=0.05 and P=0.024, respectively) compared with viral load in patients that maintained such mutations. Selected mutations influenced the disappearance of L90M and V82A. Indeed, L90M disappeared in 79% of patients without M36I, but only in 27% of patients carrying also M36I (P=0.001); V82A disappeared in 83% of patients without A71V, but only in 27% of patients carrying A71V (P=0.02). The mean-life of L90M (but not V82A) was prolonged by the presence of L10I (from 138+/−8 days to 248+/−23 without/with L10I), M36I (from 135+/−6 days to 320+/−40 without/with M36I) and A71V+V82A (from 124+/−9 days to 214+/−24 without/with A71V+V82A).

CONCLUSIONS: Minor mutations play a crucial role in the appearance and stabilization of L90M at virological failure. Overall, the characterization of mutations more detrimental for viral fitness can be used in designing clinical strategies aimed to reduce the virusrelated damage in highly drug-experienced patients.