



## Session 6

### CONTINUING MEDICAL EDUCATION

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# ANTIVIRAL THERAPY

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**SESSION 6**  
**Clinical Implications of Resistance**



**ABSTRACT 135***Antiviral Therapy* 2004; **9**:S151.**Risk of development of drug resistance in patients starting antiretroviral therapy with three or more drugs in routine clinical practice**

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**BACKGROUND:** At a population level, durability of the benefit received from the currently available classes of antiretrovirals depends largely on the rate with which resistance mutations occur in patients in routine practice who started antiretroviral (ART) therapy with  $\geq 3$  drugs.

**METHODS:** We assembled information on resistance tests performed as part of routine care on patients starting ART with  $\geq 3$  drugs who were seen in one of six clinics in London/Brighton.

**RESULTS:** 4496 patients started ART with  $\geq 3$  drugs 1996–2003. 56% started with an NNRTI, 41% with a protease inhibitor. The cumulative risk of virological failure (two viral load  $>1000$  copies/ml after 24 weeks from start of ART, unless during interruption) was 24% by 2 years, 34% by 4 years and 42% by 6 years. 707 patients (16%) had a resistance test result at some time after start of ART. 559 (47%) of the patients with virological failure had a resistance result (314 [56%] by 6 months after first virological failure), while 148 (4%) of those not fulfilling the virological failure definition had a resistance result. Risk of  $\geq 1$  major IAS USA mutation among the whole 4496 patients was 9% by 2 years, 21% by 4 years and 30% by 6 years. Risks by 6 years for class-specific mutations were: M184V/I 18%,

$\geq 1$  TAM 15%, NNRTI 17% (25% when restricted to those who started with NN), major protease mutation 8% (10% when restricted to those who started with a PI). Corresponding figures for accumulating  $\geq 1$  mutation from *each* of the three main drug classes were 1% by 2 years, 2.5% by 4 years and 3.5% by 6 years. These are lower limit estimates as test results were not available for many with virological failure, and resistance below sensitivity limits of assays will be missed. Factors associated with higher/lower risk of virological failure and resistance will be presented.

**CONCLUSION:** In routine practice, rates of virological failure and of resistance development in patients who started ART with three or more drugs are appreciable, emphasizing the need for new antiretrovirals over the coming years.

**ABSTRACT 136***Antiviral Therapy* 2004; **9**:S152.**Absence of selection of resistant variants during the early phase of therapy with lopinavir/ritonavir (LPV/r) and efavirenz (EFV) (BIKS study)***V Ferré, C Allavena, E André-Garnier, C Rabreau, F Raffi and the BIKS Study Group*

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**BACKGROUND:** Early virological failure to TDF-containing triple nucleoside reverse transcriptase inhibitors (NRTI) combinations has been attributed to low genetic barrier to resistance and synergistic selective pressure. We evaluated whether resistant mutants were selected during the first weeks of treatment with the combination of efavirenz (EFV) and LPV/r.

**METHODS:** BIKS is a 48 weeks (W) study of LPV/r BID and EFV in 86 HIV-1 infected patients, NNRTI naive and, if PI-experienced, susceptible to LPV/r. In 19 naive patients with a slow virological response (SVR), i.e. plasma viral load (pVL) >200 copies/ml at W8 or W16 and <200 at W24, viral genotypes were performed at baseline and at W8 or W16. All reverse transcriptase (RT) and protease (P) mutations, previously described or not, were considered for the analysis. Trough plasma concentrations of LPV and EFV were measured at W4.

**RESULTS:** Baseline median pVL was 5.1 log<sub>10</sub> copies/ml, and the median decrease in pVL was 2.4 and 3.1 log<sub>10</sub> copies/ml at W8 and W16, respectively. P mutations were evidenced at baseline at positions 10 (*n*=2), 20 (*n*=8), 36 (*n*=10) and 63 (*n*=8). No patients selected new mutations associated with reduced susceptibility to LPV/r, either at W8 or W16. In seven patients, P sequences differed at W8 or W16 as compared to baseline, with the presence or more polymorphic mutations. No patients had baseline nor selected at W8 or W16 mutations associated with NNRTI resistance. Isolated A98S was present at baseline and subsequent samples in two patients. New RT polymorphic mutations were demonstrated in seven patients, including D177E in three cases and R211K in. SVR was related to higher baseline pVL (*P*<0.05) and lower EFV and LPV concentration trough at W4 (*P*<0.01). SVR nor P or RT genotypic profile at baseline or W8/W16 did not affect durability of virological

response, all 19 patients having a pVL <200 copies/ml at W48 of follow-up.

**CONCLUSION:** Despite a low genetic barrier to resistance, EFV does not select NNRTI-resistance mutations in patients having a SVR on treatment with EFV and LPV/r.

**ABSTRACT 137***Antiviral Therapy* 2004; **9**:S153.**Kinetics of the plasma viral load and resistance mutations in naive patients after initiation of NNRTI or boosted PI-containing HAART***C Torti*<sup>1</sup>, *E Quiros-Roldan*<sup>1</sup>, *F Gargiulo*<sup>2</sup>, *N Manca*<sup>2</sup>, *F Moretti*<sup>1</sup>, *A Patroni*<sup>1</sup>, *V Tirelli*<sup>1</sup>, *P Nasta*<sup>1</sup>, *S Casari*<sup>1</sup> and *G Carosi*<sup>1</sup> for the *Si.S.Ther. Study Group of the MASTER Cohort*

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**BACKGROUND:** Decrement of the plasma viral load (pVL) at week 1 has been correlated with virological efficacy after 6 months of HAART. We hypothesized that this phenomenon is due to precocious emergence of drug resistance mutations during the initial slope of the pVL. Hybridization assay is a more sensitive method for detection of HIV resistance mutations as compared with standard sequencing techniques.

**METHODS:** A subset of patients included in a prospective study of NNRTI (TDF+3TC+EFV; arm A) vs PI (ZDV+3TC+LPV/r; arm B) HAART (Simplified Sequencing THERapy, Si.S.Ther. study) have been monitored for pVL at baseline and at day +1, +3, +5, +7, +15, +30 after therapy initiation (b-DNA, Chiron Inc.). Genotype resistance testing was performed using Versant™ HIV-1 RT resistance assay (LiPA) at baseline, as well as in samples with detectable pVL during the initial follow-up.

**RESULTS:** Ten patients in arm A and nine in arm B have been considered. Mean baseline pVL was 4.40 log<sub>10</sub> copies/ml (SD: 0.80) and 4.35 log<sub>10</sub> copies/ml (SD: 0.92) in the two arms, respectively. Mean pVL decrement in arm A ranged from -0.14 (95%CI= -0.77 to -0.49) at day +1 to -2.32 (95%CI= -2.95 to -1.68) at day +30 (*P*<0.0001). In arm B, mean pVL variation was 0.12 (95%CI= -0.66 to 0.92) at day +1, and -2.07 (95%CI= -2.86 to -1.27) at day +30 (*P*<0.0001), with no significant difference with respect to arm A at linear regression analysis (*P*=0.14). In arm A, three patients, showed resistance mutations emerging during the pVL slope, which were not detected at baseline (184I, 215F and 151M+181C, respectively). In contrast, no mutations emerged in arm B. Despite these results, all patients but one (who has been followed-up only until

week 16) reached undetectable pVL (<50 copies/ml) by 28 week follow-up.

**CONCLUSION:** In this pilot study, despite similar initial potency of the two regimens, resistance mutations were observed during the initial phase of the pVL decrement using sensitive method for minority subspecies. If *de-novo* emergence of these mutations is confirmed by more specific methods (e.g. real time PCR), their impact on the virological outcome needs to be ascertained over long-term follow-up.

**ABSTRACT 138***Antiviral Therapy* 2004; 9:S154.**Estimation of phenotypic clinical cutoffs for *VirtualPhenotype*<sup>TM</sup> through meta analyses of clinical trial and cohort data***LT Bachelier*<sup>1</sup>, *B Winters*<sup>2</sup>, *D Nauwelaers*<sup>1</sup>, *A Rinehart*<sup>2</sup>, *M McGregor*<sup>3</sup>, *R Harrigan*<sup>4</sup>, *M Perez-Elias*<sup>5</sup>, *M Miller*<sup>6</sup>, *S Emery*<sup>7</sup>, *F van Leth*<sup>8</sup>, *P Robinson*<sup>9</sup>, *JD Baxter*<sup>10</sup> and *A Pozniak*<sup>11</sup>

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**BACKGROUND:** The clinical utility of phenotypic resistance data can be enhanced by evaluation of the levels of resistance associated with reduced treatment response. To define clinical cutoffs (CCO) for multiple drugs in a consistent manner, we collected resistance, treatment, and virological response data for patients treated with combination regimens. Quantitative phenotypic resistance levels were predicted by *VirtualPhenotype*<sup>TM</sup> analysis of viral genotype.

**METHODS:** Data from 11 clinical trials and two cohorts were utilized. Models of virological response as a function of baseline fold change in IC<sub>50</sub> (FC) were constructed using linear (change in viral load from baseline), and logistic regression (treatment response rate). Phenotypic susceptibility scores (PSS) of the background regimens, baseline viral load, and FC were included in each model.

**RESULTS:** Records for >13 000 patients yielded ~3150 regimens with the required baseline and outcome variables, ranging from 60 regimens including unboosted saquinavir soft gel to 1546 including lamivudine. Median log baseline viral load ranged from 3.8 (regimens with tenofovir) to 4.7 (regimens with boosted indinavir). Median PSS of background regimens was 2 (range 0–7). In a preliminary linear regression analysis, FC values (95% confidence interval) associated with a 20% diminution of virological response at 8 weeks

compared to the maximal response were: zidovudine 1.8 (1.5–2.5), lamivudine 1.1 (1.1–1.2), stavudine 1.3 (1.2–1.4), didanosine (extended release) 1.3 (1.2–1.9), abacavir 1.6 (1.1–2.6), tenofovir 1.2 (1.1–1.5), indinavir 1.2 (1.1–1.9), indinavir/r 3.5 (1.1–8.4), amprenavir 1.2 (1.1–2.4), amprenavir/r 1.5 (1.2–2.6), nelfinavir 1.1 (1.1–1.3), saquinavir 1.1 (1.1–2.1), saquinavir/r 1.6 (1.3–4.8), and lopinavir/r 6.9 (2.1–17.4). FC values associated with an 80% diminution of response were: zidovudine 17 (10–25), lamivudine 2.6 (1.9–4.6), stavudine 3.4 (3.1–3.6), didanosine(extended release) 3.6 (2.8–4.9), abacavir 5.8 (1.7–7.4), tenofovir 2.5 (1.7–3.8), indinavir 3.4 (1.9–16.4), indinavir/r 25 (1.8–31), amprenavir 3.4 (1.7–10.2), amprenavir/r 6.8 (3.6–10.5), nelfinavir 2.2 (1.7–5.3), saquinavir 2.0 (1.7–18), saquinavir/r 12.3 (5.8–27), lopinavir/r 56 (29–67). Similar values were determined in logistic regression models. While the magnitude of the virological response for individual patients is affected by covariates such as viral load and PSS, FC values associated with fractions of the effect range are not.

**CONCLUSION:** The assay-specific CCO defined in this study, although preliminary, apply a consistent approach to multiple drugs and a heterogeneous population of HAART treated subjects. Cutoff definitions based on fractions of the effect range are applicable to diverse patient populations.

**ABSTRACT 139***Antiviral Therapy* 2004; **9**:S155.**Does drug class multi-resistance affect survival? Analysis from a cohort of HIV patients who experienced treatment failure***M Zaccarelli, V Tozzi, P Lorenzini, F Forbici, MP Trotta, U Visco-Comandini, C Gori, E Boumis, MC Bellocchi, R Bellagamba, R D'Arrigo, G Liuzzi, P Narciso, CF Perno and A Antinori*

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**BACKGROUND:** Drug class multi-resistance (DCMR) significantly affects virological response in treatment experienced patients and may have influence over the progression of HIV infection. Thus, the present analysis aimed at evaluating long-term survival in DCMR patients who underwent genotypic resistance test (GRT) after treatment failure.

**METHODS:** A cohort of patients experiencing treatment failure was assessed and patients were enrolled at first GRT and followed overtime. End-points of the analysis were death for any cause and AIDS-defining event or death. Drug class multi-resistance was defined according with IAS consensus. Survival analysis was performed by Cox model.

**RESULTS:** Overall, 623 patients were enrolled [main characteristics: 69.4% males; median age 38 years (IQ: 35–43); 32% IVDU; 36% previous AIDS; median CD4 count and HIV-1 RNA 302 cell/μl (IQ: 156–464) and 4.56 log<sub>10</sub> copies/ml (IQ: 3.80–5.18); median number of failures 3 (IQ: 1–4)]; 235 (37%) had DCMR to at least one drug class. Over 48 months of observation from GRT, 38 deaths and 28 new AIDS events occurred. The 36-months death proportion by Kaplan–Meier analysis was 9% among patients without DCMR, 11% with 1 DCMR, 15% with 2 DCMR and 30% in patients harboring resistance to all the three classes of drugs (*P* at log-rank <0.01). At Cox model, previous AIDS diagnosis, CD4 count (time-dependent covariate) and detection of three-class DCMR (HR: 3.5; 95% CI: 1.1–10.8) were significantly associated to increased risk of death, while use of lopinavir/r after GRT was associated to a reduced risk. The adjusted risk associated to three-class DCMR was also significant if new AIDS event or death as end

point was considered (HR: 3.4; 95% CI: 1.4–8.2) and increased if only AIDS patients were analysed (HR: 4.2; 95% CI: 1.3–14.3). A significant association with increased risk of death at Cox model was also found in patients harbouring two-class DCMR to NRTI/NNRTI (HR 2.5; 95% CI: 1.1–5.6) but not to NRTI/PI or NNRTI/PI.

**CONCLUSION:** Even in the era of highly effective antiretroviral treatments detection of drug class multi-resistance, if extended to ≥2 classes, is related to poorer clinical outcome, and represents a powerful risk-marker of disease progression and death.

**ABSTRACT 140***Antiviral Therapy* 2004; 9:S156.**Previously unclassified mutations at positions associated with antiretroviral drug resistance***SY Rhee<sup>1</sup>, L Hurley<sup>2</sup>, A Zolopa<sup>1</sup>, WJ Fessel<sup>2</sup>, DP Nguyen<sup>2</sup>, S Slome<sup>2</sup>, S Smith<sup>2</sup>, D Klein<sup>2</sup>, M Horberg<sup>2</sup>, J Flamm<sup>2</sup>, S Follansbee<sup>2</sup> and RW Shafer<sup>1</sup>*

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**BACKGROUND:** Most genotypic resistance interpretation algorithms classify a mutation as a drug resistance mutation (DRM) if it has been shown to reduce susceptibility in site-directed mutants or large numbers of clinical isolates. Mutations at ~55 protease and RT mutations have been classified as DRMs. However, many additional mutations at these positions that occur commonly in persons receiving HIV treatment have not been studied *in vitro* and are not reported by most genotypic resistance interpretation algorithms.

**METHODS:** We examined the prevalence of previously unclassified mutations in treated and untreated persons with subtype B viruses including 4400 persons from published papers and a clinic-based population. 2954 received NRTIs, 2501 received PIs, and 1125 received NNRTIs.

**RESULTS:** 36 previously unclassified mutations at 20 drug-resistance positions were significantly associated with HIV treatment. For 24 mutations, the association with treatment was strongly significant ( $P < 0.001$ , Chi-Square Test): NRTIs – E44A (1.6%), D67G (2.3%), D67E (0.9%), T69N (7.3%), T69S (3.2%), T69A (1.5%), L74I (3.7%), T215I (2.1%), T215V (1.4%), K219N (3.0%), K219R (2.5%); NNRTIs – K101Q (7.5%), K101P (2.0%), K101H (1.6%), K101N (1.0%), K103S (2.4%); PIs – K20T (3.2%), K20V (0.7%), L33I (2.3%), M36V (2.6%), M46V (0.7%), I54T (1.2%), A71I (3.0%). For 12 additional mutations, the association with treatment was weaker but also significant ( $P < 0.05$  but  $> 0.001$ ): NRTIs – T69I (0.6%), K70G (0.6%), K70E (0.3%), K219W (0.3%); NNRTIs – K101R (2.1%), Y181V (0.7%); PIs – L24F (0.7%), M36L (1.5%), G48M (0.2%), F53Y (0.5%), I54S (0.2%), N88T (0.3%). 21% of sequences from persons receiving NRTIs had  $\geq 1$  unclassified NRTI mutation. 10% of sequences from persons receiving

NNRTIs had  $\geq 1$  unclassified NNRTI mutation. 14% of sequences from persons receiving PIs had  $\geq 1$  unclassified PI mutation.

**CONCLUSION:** Previously unclassified mutations at drug resistance positions occur frequently. Further studies are needed to determine whether the phenotypic impact of these mutations is similar to the known mutations at the same positions.

**ABSTRACT 141***Antiviral Therapy* 2004; **9**:S157.**Viral load in patients failing therapy: is it the type of mutation or the total number of mutations that matter?***N Machouf, B Trottier, R Thomas, JP Routy and MA Wainberg*

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**BACKGROUND:** *In vitro* studies have shown that HIV-1 drug-resistant (DR) variants are associated with reduced fitness when compared with wild type virus and are associated with diminished replication capacity. However, compensatory mutations may develop to overcome such reduced fitness. Clinical and virological correlations in patients failing therapy are not well understood.

**OBJECTIVE:** To determine DR mutation characteristics associated with viral load (VL) in patients failing antiretroviral regimens.

**METHODS:** From November 1999 to April 2004 data from HIV-infected individuals receiving care at a large HIV community clinic (Clinique médicale l'Actuel) in Montreal were included in this study. Genotyping was performed on samples from patients failing therapy. Linear regression was used to model the simultaneous effects of each mutation and the accumulation of major and minor mutations on plasma VL. The dependent variable was the log transformed HIV RNA at time of genotyping.

**RESULTS:** 1103 isolates from 649 patients were analysed. Mean VL at time of genotyping was  $4.19 \pm 0.72$  log. Patients had a mean of 4.4 major [0–15] and 2.17 [0–8] minor protease mutations respectively. The most frequent mutations were at RT codons M184V/I (61%), T215Y/F (46%), M41L (41%), D67N (34%), K103N (24%) and at protease codons L10F/I/R/V (50%), A71V/T (37%), V77I (37%), L90M (36%), M36I (25%). We detected a decrease in VL among patients harbouring 1–4 major mutations ( $r=-0.40$ ,  $P<0.001$ ) conversely an increase in VL was observed for those having 5–12 mutations ( $r=0.27$ ,  $P<0.001$ ). This 'V shape' relationship remained significant in the adjusted model (cubic term  $\beta=0.01$ , simple term  $\beta=-0.12$ ). Furthermore, in multivariate analysis only certain mutations, i.e. M184V/I ( $\beta=-0.43$ ), K70R ( $\beta=-$

$-0.44$ ) and D30N ( $\beta=-0.24$ ) predicted a decrease in VL when controlling for the other mutations, time and number of minor protease mutations.

**CONCLUSION:** These results suggest that in addition to certain mutations such as M184V/I, K70R or D30N, the number of major mutations is an important determinant of VL in patients failing therapy. These findings have implications for switching or maintaining a suboptimal therapy in patients failing RT and protease inhibitor containing regimen.

**ABSTRACT 142***Antiviral Therapy* 2004; 9:S158.**Risk of developing selected *de novo* resistance mutations during structured therapy interruption (STI) in chronic HIV-1 infection***M Arnedo*<sup>1</sup>, *F García*<sup>2</sup>, *C Gil*<sup>1</sup>, *P Castro*<sup>2</sup>, *JL Blanco*<sup>2</sup>, *JM Miró*<sup>2</sup>, *T Pumarola*<sup>1</sup> and *JM Gatell*<sup>2</sup>

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**BACKGROUND AND OBJECTIVES:** STI may allow viral replication in the presence of drug, leading to the emergence of drug resistance mutations.

**METHODS:** 112 cycles of STI in a total of 35 patients recruited in four STI protocols were studied. Plasma samples were analysed, at baseline before starting any treatment, before starting the STI protocol and 2 weeks after STI. Proviral DNA was also assessed. All drugs were interrupted at the same time during STI. In a subgroup of eight patients genotypic and phenotypic analysis were also assessed at peak of viral load after each interruption. Analysis of genotypic resistances were performed using the TruGene HIV-1 assay (Visible Genetics). Virtual Phenotype was performed for each sample by Virco.

**RESULTS:** Resistance mutations were selected in 20 out of 112 STI cycles (18%). In 6% mutations were *de novo* and in 12% from archived mutations. Overall nine out of 35 patients (26%) selected resistance mutations during STI (14% *de novo* and 12% from archived mutations). Three out of 13 recipients of NNRTI (23%; all *de novo*) selected resistance mutations to NNRTI. Nine out of 18 recipients of lamivudine (50%; 22% *de novo* and 28% from archived mutations) selected resistance mutations to 3TC. Two out of 35 recipients of NRTI (6%; 3% *de novo* and 3% from archived mutations) selected resistance mutations to NRTI excluding the M184V mutation. Finally, no primary mutations to PI were detected. In most cases mutations were selected during first cycle of STI and did not significantly increase during successive cycles. There was good concordance between virtual phenotype and genotype.

**CONCLUSION:** 26% of patients participating in STI protocols selected resistance mutations that were already present before being recruited in about half of them. Among NNRTI and 3TC recipients the percentage was 23% and 50% and again, in both cases, selected mutations were already present before being recruited in about half of them.

**ABSTRACT 143***Antiviral Therapy* 2004; **9**:S159.**Baseline predictors and virological outcome in subjects developing mutations during intermittent HAART***L Palmisano*<sup>1</sup>, *M Giuliano*<sup>1</sup>, *MF Pirillo*<sup>1</sup>, *CM Galluzzo*<sup>1</sup>, *M Andreotti*<sup>1</sup>, *R Bucciardini*<sup>1</sup>, *E Nicastrì*<sup>2</sup>, *V Fragola*<sup>1</sup>, *M Andreoni*<sup>3</sup> and *S Vella*<sup>1</sup>

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**BACKGROUND:** We previously reported that by 21 months follow up approximately 28% of chronically infected subjects, switched from continuous to intermittent HAART, have developed mutations during treatment interruptions. We also showed that the risk of developing mutations is 10-fold higher in subjects harbouring archived mutations in proviral DNA before interrupting therapy. We here provide further data on virological outcome and baseline viral replication in these subjects, that may contribute to better understand limits and potentials of intermittent therapy in clinical practice.

**METHODS:** The relationship between plasma mutations and virologic failure (HIV RNA >400 at any time) was assessed in 108 subjects assigned to arm B of the ISS PART study, an ongoing, randomized multi-centre clinical trial comparing continuous (arm A) versus intermittent HAART in HIV+ subjects on first line HAART, all with HIV RNA <400 copies/ml and CD4 >350/mm<sup>3</sup>. Intermittent therapy consisted of four cycles 'on/off' (STIs of 1, 1, 2, 2 months, at 3-month intervals). In a subgroup of 49 subjects (24 with plasma mutations during STI), previously studied for PBMC genotype, baseline HIV RNA was measured with a modified ultrasensitive method (limit of detection: 3.5 copies/ml).

**RESULTS:** The overall prevalence rate of virologic failure in arm B was 18.4%. However, when subjects were stratified according to the presence of plasma mutations during STI, a significantly higher rate was found in those with mutations (12.4% vs 33%,  $P=0.004$ ). In the subgroup analysis, mean baseline levels of HIV RNA were significantly higher in subjects with plasma mutations, when compared to patients retaining a

wild-type virus (4.9 vs 17.5 copies/ml,  $P=0.03$ ). Baseline RNA was also correlated with the presence of mutations in proviral DNA ( $P=0.023$ ).

**CONCLUSIONS:** 1. The present results show that in subjects with 'undetectable' viraemia (measured with currently used assays), actual levels of HIV RNA, as well as the presence of mutations in proviral DNA, are predictors of resistance during STIs. 2. A correlation exists between actual levels of viraemia at baseline and presence of archived mutations. 3. Virological response to therapy reinstatement is jeopardized by the emergence of mutations during STI.

**ABSTRACT 144***Antiviral Therapy* 2004; **9**:S160.**Treatment interruption in patients with multiple failures to ARV therapy: can the controversy be solved?***D Costagliola*<sup>1</sup>, *C Duvivier*<sup>1,2</sup>, *V Calvez*<sup>3</sup>, *S Dominguez*<sup>1,2</sup>, *M Wirden*<sup>3</sup>, *J Ghosn*<sup>1</sup>, *C Delaugerre*<sup>3</sup>, *G Peytavin*<sup>4</sup> and *C Katlama*<sup>1,2</sup>

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**BACKGROUND:** Treatment interruptions (TI) remain a controversial issue in the management of patients with multiple failures.

In very advanced treatment-failing patients (VL 5.3 log<sub>10</sub>, 27 CD4), the GigHAART study has shown the benefit of an 8-week TI with a median reduction in VL of -1.91 log<sub>10</sub> versus -0.37 log<sub>10</sub> at W12 in patients with TI versus no TI (*P*= 0.008) and a benefit of +54 versus +7 CD4/mm<sup>3</sup> respectively at W24. By contrast, the Reverse study had evaluated in 23 patients (VL 5.14 log<sub>10</sub>, CD4 43/mm<sup>3</sup>) a longer duration of TI (median 170 days) with multitherapy started after reversion of resistance mutations (RM) in >2 classes and showed no benefit of TI (+0.06 log<sub>10</sub> in VL, -27 CD4/mm<sup>3</sup>) at W24.

**OBJECTIVE:** To explain this apparent discrepancy, we compared the number of RM at baseline and after TI, the number of ARV sensitive at baseline among those available and after TI among those prescribed.

**RESULTS:** In GigHAART, median baseline RM was 5, 2 and 7 for NRTI, NNRTI, PI; 5 patients (17%) had virus sensitive to <2 drugs; after TI, there was no change in the median RM; 23 patients (68%) had virus sensitive to >2 drugs.

In Reverse, the RM were 6, 2 and 9, and 17 patients (74%) had no more than one sensitive drug; TI induced a change in median RM with 0 major for PI, 0 NNRTI and 2 TAMs; 16 patients (70%) had virus sensitive to >2 drugs.

Only 1 complete shift to wild-type viruses were seen in GigHAART versus 6 complete and 7 partial shifts

(57%) in Reverse. In Reverse, the resistance profile was the same at W24 after treatment re-institution and at baseline, this was observed in most patients less than 2 months after treatment re-institution. Clonal analysis in GigHAART shown that the clones which are emerging or re-emerging are more susceptible to a multiple combination of ARV than the nearly monoclonal baseline viruses.

**CONCLUSION:** In the management of multiple failures, complete re-occurrence of wild-type viruses appears deleterious. TI should not be used unless HIV has kept some sensitivity to available drugs.

**ABSTRACT 145***Antiviral Therapy* 2004; **9**:S161.**Patterns of CD4 and HIV-1 RNA change during structured treatment interruption in patients with multidrug-resistant HIV (CPCRA 064 MDR-HIV Study)***J Lawrence<sup>1</sup>, K Huppler Hullsiek<sup>2</sup>, D Abrams<sup>1</sup>, B Schmetter<sup>3</sup>, J Baxter<sup>4</sup> and the CPCRA 064 Study Team for the Terry Beinr Community Programs for Clinical Research on AIDS (CPCRA)*

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**BACKGROUND:** Different patterns of CD4 and RNA response have been observed in patients during antiretroviral treatment interruption. We describe patterns seen at 2 months in 131 patients with multidrug-resistant (MDR) HIV who were randomized to a 4-month structured treatment interruption (STI) in the CPCRA 064 study and who had complete baseline and 2-month data.

**METHODS:** Patients were grouped by change from baseline to month 2 of the STI in two different ways: 1) by slope of CD4 where Group A (stable CD4) was above the median slope and Group B (rapid CD4 decline) was at or below the median slope and 2) by quadrant of change (above and below zero) in CD4 and HIV RNA. The baseline characteristics of the groups are compared.

**RESULTS:** The median CD4 slope (cells/month) was -22 overall, -1.5 in Group A and -50.3 in Group B. Groups A and B differed significantly ( $P < 0.001$ ) in baseline CD4 (106 vs 266) and nadir CD4 (44 vs 101), but had similar baseline HIV RNA levels (4.97 vs 4.92). Although the differences were not statistically significant, Group A (vs Group B) tended to have higher baseline resistance [number of drug resistance mutations (10.2 vs 9.5), genotypic sensitivity score (GSS; 1.9 vs 2.2), and phenotypic sensitivity score (PSS; 3.7 vs 4.7)] and less reversion of resistance during the STI [change in number of mutations (-2.5 vs -3.3) and in GSS (3.3 vs 4.7) at month 2]. When categorized by quadrants the majority (90/131) of patients showed the typical pattern of CD4 decrease and viral load (VL)

increase. However, 20 had increased CD4 and VL, 16 had decreased CD4 and VL, and 5 had CD4 increase and VL decrease.

**CONCLUSION:** Although most patients with MDR-HIV show the typical response of CD4 decline and HIV RNA rebound during treatment interruption, discordant CD4 and HIV RNA responses are not uncommon. We identified a group of patients who had relatively stable CD4 counts during treatment interruption. These patients had significantly lower baseline CD4 counts and tended to have greater baseline resistance and less reversion of resistance compared with patients who had a rapid CD4 decline.

**ABSTRACT 146***Antiviral Therapy* 2004; 9:S162.**Determinants of replication capacity (RC) in HIV-1 isolates from antiretroviral (ART)-experienced adults failing a PI-based regimen, and relationship of RC with HIV-1 RNA and CD4 counts***R Haubrich*<sup>1</sup>, *J Hernandez*<sup>2</sup>, *M Bates*<sup>3</sup>, *M Thompson*<sup>4</sup>, *D Margolis*<sup>5</sup>, *K Pappa*<sup>2</sup>, *L Yau*<sup>2</sup> and *R Schooley*<sup>6</sup>

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( $P=0.002$ ), and PR mutations at codon 82 ( $P=0.004$ ). Other factors (PSS, other mutations, duration of prior ARVs, amprenavir hypersusceptibility) were not associated with RC nor was RC predictive of week 24 CD4 or HIV RNA changes.

**CONCLUSION:** Phenotypic resistance (number of agents) and genotypic resistance (the M184V mutation, TAMs and PR mutations at 82) were associated with decreased RC in ARV-experienced subjects. RC was directly correlated with viral load and inversely correlated with CD4 counts, suggesting that decreased RC due to viral resistance may be associated with preservation of CD4 numbers.

**BACKGROUND:** Reduced replication capacity (RC) has been correlated with decreased disease progression in minimally treated HIV patients and with CD4 maintenance in antiretroviral (ARV)-experienced patients.

**METHODS:** Isolates from ARV-experienced subjects on a failing PI-based regimen entering ESS40006 were assessed using genotype, phenotype, and RC (ViroLogic) prior to an ARV switch. Pearson correlation coefficients and stepwise multiple linear regression were used to explore the relationship between RC and HIV resistance and disease status.

**RESULTS:** 59 isolates from subjects exposed to NRTIs, NNRTIs, and PIs and 26 isolates from NNRTI-naive subjects were analysed. In the NNRTI-experienced group, RC was linearly correlated with genotypic susceptibility scores (GSS, based on IAS 2003 guidelines,  $P=0.03$ ) and baseline HIV-1 RNA ( $P<0.001$ ), and inversely correlated with baseline CD4 count ( $P<0.001$ ) and the sum of fold change (FC) at baseline ( $\log_{10}$ ,  $P<0.0001$ ). The presence of the M184V mutation ( $P=0.002$ ), TAMs ( $P=0.03$ ) and PR mutations at codon 82 ( $P=0.001$ ) significantly reduced RC. Significant associations were also found between RC and the number of NRTIs, NNRTIs, PIs, or ARVs above the biological/clinical cut-off ( $P<0.03$ ). Findings were less significant in the NNRTI-naive group. In a multivariable regression model, RC was significantly associated with baseline HIV-1 RNA ( $P=0.004$ ), the presence of the M184V mutation

**ABSTRACT 147***Antiviral Therapy* 2004; **9**:S163.**Impact of three or four protease mutations at codons 33, 82, 84 and 90 on 2 week virological responses to tipranavir, lopinavir, amprenavir and saquinavir all boosted by ritonavir in Phase 2B trial BI 1182.51***DL Mayers<sup>1</sup>, J Leith<sup>1</sup>, H Valdez<sup>1</sup>, CA Boucher<sup>2</sup>, J Schapiro<sup>3</sup>, J Baxter<sup>4</sup>, S McCallister<sup>1</sup>, VM Kohlbrenner<sup>1</sup>, J Scherer<sup>1</sup> and DB Hall<sup>1</sup>*

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**BACKGROUND:** Multiple mutations at protease codons 33, 82, 84 and 90 have been previously associated with significant phenotypic resistance to amprenavir (APV), indinavir, lopinavir (LPV), ritonavir (RTV), and saquinavir (SQV), along with reduced 2 week viral load responses to RTV boosted tipranavir (TPV/r). The clinical impact of three or more protease mutations at 33, 82, 84 and 90 on subsequent antiviral responses to boosted LPV, APV or SQV remained uncertain.

**METHODS:** Treatment experienced patients with three or more protease mutations at codons 33, 82, 84 and 90 entered a randomized trial of dual-boosted protease inhibitor (PI) regimens including TPV/r to assess pharmacokinetics and safety. During the first 2 weeks of the study, 291 patients received twice daily doses of either TPV/r 500 mg/100 mg (63 patients), LPV/r 400 mg/100 mg (79 patients), APV/r 600 mg/100 mg (74 patients) or SQV/r 1000 mg/100 mg (75 patients), along with an optimized background regimen. After 2 weeks, TPV/r 500 mg/100 mg twice daily was added to the non-TPV containing regimens. Genotypic resistance was determined by population sequencing.

**RESULTS:** The median viral load reduction at 2 weeks was for APV/r 0.2 log<sub>10</sub> copies/ml, for SQV/r 0.3 log<sub>10</sub> copies/ml, for LPV/r 0.4 log<sub>10</sub> copies/ml and for TPV/r 1.2 log<sub>10</sub> copies/ml. Mutations at three and four of the codons among 33, 82, 84 or 90 were present in 83% and 17% of patients, respectively. For patients with three mutations among 33, 82, 84 and 90, the percent

of patients with a 1 log<sub>10</sub> copies/ml viral load response for APV/r, SQV/r, LPV/r, and TPV/r, were 25%, 25%, 33%, and 61%, respectively. For patients with mutations at all four codons, the percent of patients with a 1 log<sub>10</sub> copies/ml viral load response for APV/r, SQV/r, LPV/r, and TPV/r were 13%, 18%, 20%, and 42%, respectively.

**CONCLUSIONS:** In this randomized study of treatment experienced HIV-positive patients, the presence of three or four mutations at codons 33, 82, 84 and 90 was associated with median viral load responses of <0.5 log<sub>10</sub> copies/ml for ritonavir-boosted LPV, APV and SQV. TPV/r remained the most active boosted PI with a median 2 week viral load reduction of 1.2 log<sub>10</sub> copies/ml.

**ABSTRACT 148**

*Antiviral Therapy* 2004; **9**:S164.

**Minor protease inhibitor resistant variants, even at very low level, can drive the failure resistance mutations profiles of subsequent protease inhibitor-based regimen**

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**BACKGROUND:** The role of minor drug-resistant variants in antiretroviral therapy failure had been recently demonstrated for NNRTIs. Genotypic profiles linked to nelfinavir failure are mainly associated to the selection of either the D30N or the L90M mutation in the protease gene. It has been suggested that these two mutations are mutually exclusive during the evolutionary process. The aim of the study was to appreciate the prevalence of such mutated viruses in a large database of HIV-1-infected patients on treatment failure and to characterize at the clonal level the evolution of these profiles selected by nelfinavir and their evolution after subsequent PI-based regimen.

**METHODS:** We analysed 2020 HIV-1 protease sequences performed in a context of virologic failure in our center. Genotyping HIV-1 resistance testing were performed by automated population-based full-sequence analysis. Samples harboring D30N mutations were selected for further clonal analyses.

**RESULTS:** The prevalence of the D30N mutation was 3.5% and in most of cases was observed without any other major protease mutation. In the other cases, it can be associated with the M46I/L, I84V or L90M, but never with the V82A/F/T/S. In some cases, when apparently only D30N is present on standard genotype, minor species harboring only L90M at very low level (2/100 clones) can be detected. In these cases, after a subsequent PI regimen used in the context of salvage to nelfinavir, these L90M minor species can become predominant and the D30N species completely disappear under the selective pressure of the new PI-based regimen. In contrast, when only D30N species can be detected by clonal analysis, the evolution of resistance pathways occurred adding other primary PI resistance mutations, such as I84V and L90M, in addition to the D30N mutation.

**CONCLUSION:** At the time of nelfinavir failure, mutation D30N was rarely associated to other major PI resistance mutations. At the clonal level, the presence of minor species containing other PI resistance mutations, even at very low level, can explain the evolution of resistance pathways involved in the failures to subsequent other PI-based regimen.

**ABSTRACT 149***Antiviral Therapy* 2004; **9**:S165.**Virological response following switch to atazanavir/ritonavir in relation to baseline genotypic resistance pattern***S Yerly, S Vora, H Günthard, P Vernazza, H Furrer, A Zinkernagel, B Hirschel, L Perrin and the Swiss HIV Cohort Study (SHCS)*

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**BACKGROUND:** Genotypic correlates of HIV-1 resistance to atazanavir (ATV) have been described. Our aim was to assess virological response and to identify clinically relevant genotypic scores for resistance to ATV/ritonavir (RTV) in treatment experienced patients.

**METHODS:** PI-experienced patients from the SHCS who were switched to ATV/RTV (2×150 mg ATV, 100 mg RTV, QD) containing HAART with HIV-1 RNA > 1000 copies/ml at baseline were included. The impact of baseline protease mutations on virological response at 3 months was analysed.

**RESULTS:** Data of the first 25 patients (3 months follow-up) were included in this analysis. Median baseline viraemia and CD4 count were 4.88 log<sub>10</sub> copies/ml and 133/mm<sup>3</sup>, respectively. Patients had been treated with a median of three PIs (range 1–6). Reason for switch was virological failure (*n*=18), poor adherence (*n*=4) and drug intolerance (*n*=3). The median number of active drugs associated with ATV/RTV was 2 (0–4). At 3 months, the median viraemia decrease was –2.09 log<sub>10</sub> copies/ml (–4.72 to 0.39) with 19 (76%) patients presenting HIV-1 RNA <400 copies/ml and/or >1 log decrease. The median baseline number of mutations associated with PI-resistance [IAS] was 5 (range, 0 to 14) and the median number of mutations associated with ATV resistance [according to R Colonna: 10, 20, 24, 33, 36, 46, 48, 54, 63, 71, 73, 82, 84, and 90] was 3 (0 to 9). This ATV resistance score predicted 12/25 patients as resistant, but 6 (50%) of them were responders. In non-parametric univariate analyses, the protease mutations associated with a reduced virological response to ATV/RTV were 10, 20, 32, 33, 46, 47, 54, 82, 84, and 90 (*P*<0.05). There was a correlation between the number of PI mutations and virological response at 3 months (*r*=0.66, *P*<0.0001). A genotypic score derived from extended data will be presented.

**CONCLUSION:** Despite mutations associated with ATV-resistance, we have observed significant virological response in protease inhibitor and nucleoside reverse transcriptase-experienced patients who were switched to a genotype guided optimised salvage regimen containing boosted ATV.

**ABSTRACT 150***Antiviral Therapy* 2004; **9**:S166.**Reverse transcriptase mutation M184V delays the selection of thymidine-analogue mutations in children infected with subtype-C HIV-1***D Averbuch<sup>1</sup>, JM Schapiro<sup>2</sup>, D Engelhard<sup>1</sup>, S Gradstein<sup>3</sup>, G Gottesman<sup>4</sup>, E Kedem<sup>5</sup>, M Einhorn<sup>6</sup>, G Grisar-Soen<sup>7</sup>, M Ofir<sup>8</sup>, F Milguir<sup>9</sup>, H Rudich<sup>9</sup>, D Ram<sup>9</sup> and Z Grossman<sup>9</sup>*

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**BACKGROUND:** Both HIV subtype and patient's age need to be considered when making treatment decisions. We compared drug-resistance mutation selection rates in subtype-C and B children under different treatment regimens. In addition, we examined the relative prevalence and possible influence of M184V/I.

**METHODS:** Children treated in six centres in Israel with combinations of PI, NRTI and NNRTI were studied. Criteria for participation included availability of plasma samples with HIV RNA >1000 copies/ml and known treatment and clinical history. For each child, mutations found in all samples were counted only once. We compared accumulation rates of TAMs in children who were maintained on zidovudine/stavudine+lamivudine+PI/NNRTI despite loss of complete viral suppression to the rates in those treated with, or switched to, other NRTI-based regimens.

**RESULTS:** 136 samples from 59 children were genotyped (49 subtype-C, 6 subtype-B and 4 other subtypes; 0.5–18 years, median 7 years). All harboured minor PI mutations. Twenty children had consistently received only zidovudine/stavudine+lamivudine+PI (17) or NNRTI (4) over a mean of 32.7 months prior to sampling. Fourteen acquired M184V; in 12/14 it was the only RT mutation selected throughout follow up. Two children selected other mutations: one a TAM; the other, a major PI mutation. Seventeen (13 subtype C) were treated with other drugs and/or had multiple regimen switches including various NRTI combinations.

14/17 developed TAMs (2.5 per patient; sampling at 28.7 months;  $P=0.6$ ); 3/17, non-TAM RT mutations. Seven selected major PI mutations. The difference in the number of patients developing TAMs, 1/15 vs 14/17, was highly significant ( $P<0.0001$ ). Differences in HIV RNA and CD4 count at baseline, sampling time and follow-up period were not significant.

**CONCLUSION:** In this subtype C paediatric population, a treatment strategy of initiating and maintaining a thymidine analogue + lamivudine based regimen (regardless of incomplete viral suppression), compared to other initial regimens and/or frequent drug changes, resulted in reduced accumulation of TAMs. This is likely due to enhancement of a TAM-sparing effect of M184V in subtype-C infection. In small children, with antiretroviral therapy initiated soon after birth and complete adherence difficult to achieve, a conservative regimen resulting in low rates of resistance merits further investigation.

**ABSTRACT 151***Antiviral Therapy* 2004; **9**:S167.**Genotypic reverse transcriptase evolution in antiretroviral-experienced patients receiving tenofovir DF-containing regimens***B Masquelier<sup>1</sup>, D Descamps<sup>2</sup>, L Bocket<sup>3</sup>, C Tamalet<sup>4</sup>, M Wirden<sup>5</sup>, B Montès<sup>6</sup>, J Izopet<sup>7</sup>, P Palmer<sup>8</sup>, V Schneider<sup>9</sup>, A Ruffault<sup>10</sup>, V Ferré<sup>11</sup>, G Peytavin<sup>2</sup>, A Trylesinski<sup>12</sup>, M Miller<sup>13</sup>, F Brun-Vézinet<sup>2</sup>, D Costagliola<sup>14</sup> and the ANRS AC11 Resistance Study Group*

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**BACKGROUND:** We previously validated a genotypic score (TDF mutation score) as an independent predictive factor of the virological response to tenofovir DF (TDF)-containing regimens in antiretroviral-experienced patients (*Antiviral Therapy* 2003; **8**:S119). We set up a follow-up study to describe the reverse transcriptase genotypic evolution on TDF-containing regimens in this context of salvage therapy.

**METHODS:** Antiretroviral-experienced patients included into the TDF ATU cohort were followed for six months on TDF-including regimens. We defined a group of patients with plasma HIV-1 RNA >1000 copies/ml at month 6 (M6) on TDF ( $n=97$ ). Genotypic resistance analysis was processed from plasma sampled at baseline (M0) and at M6. The genotypic evolution was studied at each amino acid involved in resistance to nucleoside reverse transcriptase inhibitors (NRTI) and at other residues previously shown to correlate with NRTI therapy. The prevalence of mutations at baseline and at M6 were compared using the Mac Nemar test and the evolution of the number of thymidine analogue mutations (TAMs) and of the TDF mutation score using the Wilcoxon test.

**RESULTS:** 85 patients with an available genotype at M6 were included. At M0, the mean plasma HIV-1

RNA was  $5 \pm 0.6 \log_{10}$  copies/ml and the mean CD4 cell count  $196 \pm 145$  cells/ $\mu$ l. At M0, the mean number of TAMs was  $2.9 \pm 1.6$  and the mean TDF mutation score  $3.1 \pm 1.9$ . Among these non-responders, the mean plasma viral load at M6 was  $4.8 \pm 0.7 \log_{10}$  copies/ml; no significant increase in any individual mutation frequency was detected. The mean number of TAMs and the TDF mutation score were  $3.3 \pm 1.5$  and  $3.6 \pm 1.9$  at M6 (both  $P \geq 0.46$  compared to M0). One patient had virus with the RT K65R mutation at M0, and two others showed selection of this mutation at M6; at M0 one had the Q151M multi drug resistance complex, and the other had only the A62V and M184V mutations. Thus, K65R developed in 2/85 patients (2.2%).

**CONCLUSION:** In antiretroviral-experienced patients receiving TDF-containing regimens, minimal RT genotypic changes were shown despite continuous HIV-1 replication. Cross-resistance mediated by the baseline NRTI resistance mutations appears to be responsible for enabling continued replication.

**ABSTRACT 152***Antiviral Therapy* 2004; 9:S168.**Clinical and genotypic correlates of K65R mutation in an unselected cohort of HIV-infected persons naive for tenofovir***MP Trotta, S Bonfigli, F Ceccherini Silberstein, D Zinzi, R D'Arrigo, F Soldani, M Zaccarelli, P Marconi, U Visco Comandini, E Boumis, F Forbici, V Tozzi, P Narciso, CF Perno and A Antinori*

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**BACKGROUND:** Mutation RT-K65R confers resistance to tenofovir (TDF), so the identification of clinical and genotypic factors related with the occurrence of K65R could be useful in selecting patients who had greatest probability of response to TDF-based regimens.

**METHODS:** Clinical, viro-immunological and genotypic data of all persons failing HAART who underwent genotypic resistance test (GRT) during 1999–2003 were collected in a database and retrospectively analysed for this study.

**RESULTS:** Out of 1392 GRT performed on 771 patients, 12 TDF-naive patients had the K65R mutation with an overall prevalence of 1.6%, ranged between 0.59% and 2.08% without a significantly change in K65R prevalence over the study period ( $P$  at Chi-square for linear trend = 0.842). At multivariate analysis, previous AIDS (OR 4.54; 95%CI 1.04–19.82) and use of abacavir (13.50; 2.57–70.82) and of efavirenz at GRT (14.32; 3.33–61.61) were associated with a greater risk of developing K65R, while patients with longer exposure to lamivudine were less likely to present the mutation (0.94; 0.89–0.98 for each more month of therapy). In a separate multivariate logistic model, presence of M184V (0.06; 0.01–0.86) and TAMs (0.35; 0.16–0.82 for each more mutation among TAMs) seems to be protective for the emergence of K65R, while a strong positive correlation was found with the cluster of mutations associated with Q151M (3.47; 2.07–5.80). Moreover, NNRTI-induced L100I-mutation was independently associated with an higher probability of presenting K65R (16.87; 2.59–110.02).

**CONCLUSIONS:** 1) In antiretroviral-experienced patients, selection and sequencing of the best nucleoside-backbone should be based on patients' clinical history and genotypic test; 2) caution should be used in starting TDF in patients who are failing an abacavir-containing regimen; 3) longer exposure to lamivudine and presence of M184V seems protective for K65R suggesting the utility to incorporate lamivudine in TDF-based regimens; 3) K65R was inversely associated with TAMs indicating that these patterns could represent antagonistic ways of viral evolution; 4) the strong association between K65R and Q151M-complex confirm the utility of performing systematically GRT in therapy failure before initiation of TDF; 3) since nucleoside and non-nucleoside inhibitors bind to different sites on reverse transcriptase in a non-exclusive way, cross-resistance should be further investigated.

**ABSTRACT 153***Antiviral Therapy* 2004; **9**:S169.**Selection of the K65R mutation in plasma and PBMCs of HIV-2-infected patients receiving tenofovir-containing regimen***D Descamps<sup>1</sup>, F Damond<sup>1</sup>, S Matheron<sup>1</sup>, G Peytavin<sup>1</sup>, S Delarue<sup>1</sup>, P Campa<sup>1</sup>, G Collin<sup>1</sup>, S Pueyo<sup>2</sup>, G Chêne<sup>2</sup>, F Brun-Vézinet<sup>1</sup> and the French ANRS HIV-2 Cohort Study Group*

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**BACKGROUND:** The objective of our study was to determine retrospectively which substitutions in the reverse transcriptase (RT) gene are selected *in vivo* during tenofovir (TDF)-containing regimen in HIV-2-infected patients included in the French ANRS HIV-2 cohort.

**METHODS:** Seven HIV-2-infected patients, nucleoside RT inhibitors (NRTIs) experienced for a median of 55 months (range: 12–132) and having received TDF were studied. HIV-2 RT gene was amplified and sequenced from plasma samples and PBMCs before the introduction and under TDF exposure. RT sequences were compared to HIV-2 consensus sequences and mutations at position known to be associated with HIV-1 resistance were considered ([www.iasusa.org](http://www.iasusa.org)) as well as all selected mutations during TDF therapy.

**RESULTS:** TDF was associated with various NRTIs and boosted protease inhibitors (PIs) in six out of seven patients. At baseline, among the seven patients studied, three had plasma viruses with mutations at positions known to be involved in HIV-1 resistance: K70R+M184V ( $n=1$ ), Q151M ( $n=1$ ), D67N+K70T+M184V+S215Y+E219D ( $n=1$ ). Only one had a virus harbouring a K65R mutation in PBMCs not present in plasma. After a TDF median exposure of 6 months (range: 4–9), four out the seven patients, receiving only lamivudine as other NRTI, had plasma viruses harbouring the K65R substitution known to be selected in HIV-1 infected patients receiving TDF. In all cases, K65R was associated with other newly selected substitutions: A62V+N69S+M184V ( $n=1$ ), K70R ( $n=1$ ), Q151M ( $n=1$ ), M184V ( $n=1$ ). One out the three remaining patients had mutant viruses: D67G+K70K+M184V. Others mutations of unknown

impact in HIV-1 were selected: K43R, P51S, K64R, K82R, D86E, V111I, I180L, H121Y/C, S163A and F214L. In PBMCs, K65R was not detected except in the patient where this mutation was already present before TDF introduction.

**CONCLUSION:** As reported in HIV-1, K65R mutation emerges after a short TDF exposure in HIV-2-infected patients. The K65R substitution was not selected in the patient with virus harbouring thymidine analogues mutations at baseline, as also described in HIV-1. Phenotypic and clinical studies on a large number of patients are needed to determine the relevance of this substitution in HIV-2-treated patients.

**ABSTRACT 154***Antiviral Therapy* 2004; 9:S170.**Development of resistance mutations in patients receiving salvage therapy with tenofovir***J Grebely, J Raffa and B Conway*

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67 and 70 may protect against its development and may help identify a subgroup of patients in whom TDF resistance may be slower to develop after its initiation in salvage therapy.

**BACKGROUND:** Tenofovir (TDF) is often used in salvage therapy due to its activity against many NRTI-resistant strains. There are only limited data on 12 patients regarding the emergence of K65R in treatment-experienced patients receiving TDF. With this in mind, we evaluated the evolution of genotypic NRTI resistance in treatment-experienced patients receiving TDF.

**METHODS:** All patients receiving TDF-based therapy >3 months were included in this retrospective chart review. Study endpoints were virological suppression and the evolution of drug resistance mutations in the setting of virological failure.

**RESULTS:** A total of 65 patients (58 men, 7 women) were evaluated, including 15 males on a triple NRTI regimen. Baseline CD4 and plasma viral load in the latter group (230 cells/mm<sup>3</sup> and 21 800 copies/ml) compared to values of 200 cells/mm<sup>3</sup> and 87 100 copies/ml in the patients on double class regimens. At a median 52 weeks, similar increases in CD4 count were observed (+42 and +25 cells/mm<sup>3</sup>) and median plasma viral load was 102 and 2850 copies/ml in patients on double and single class regimens, respectively. Maximal virological suppression (intent-to-treat; <400 copies/ml) was achieved in 31/50 (62%) and 3/15 (20%) patients receiving double and single class regimens. The emergence of K65R (*n*=7) was more frequent (5/12, 42% vs 2/19, 11%) in patients on single class regimens. Other RT mutations were always present, most frequently Y181C (4/7) and/or M184V (4/7). Three patients had mutations at codons 67 and 70 at baseline. Two of these achieved maximal virological suppression without the development of K65R. The third received TDF-ABC alone over 16 months, maintained a viral load 3000–15 000 copies/ml without the development of the K65R mutation.

**CONCLUSIONS:** Virological response to TDF in salvage therapy was poorer in patients on single class regimens, and was associated with the development of K65R. Certain baseline mutation patterns at codons

**ABSTRACT 155***Antiviral Therapy* 2004; **9**:S171.**Dynamic of selection of the K65R and M184V/I mutations in patients enrolled in Tonus trial***C Delaunay<sup>1</sup>, D Descamps<sup>1</sup>, R Landman<sup>1</sup>, G Peytavin<sup>1</sup>, P Flandre<sup>2</sup>, A Trylesinski<sup>3</sup>, G Collin<sup>1</sup>, A Benalycherif<sup>1</sup>, F Monchecourt<sup>3</sup>, F Brun-Vézinet<sup>1</sup> and the Tonus Trial Study Group (IMEA 021)*

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**BACKGROUND:** Tonus was a pilot study where 38 naive patients received abacavir, lamivudine, and tenofovir once daily regimen. Patients demonstrated high rates of early virological failure and frequent selection of the M184V and K65R mutations (86% and 76% at week [W] 12 and/or 24, respectively) as reported in other similar studies. The objective of our study was to evaluate the dynamic of selection of K65R and M184V/I mutations in RT gene from patients enrolled in Tonus trial.

**METHODS:** Bulk sequencing of RT gene was performed on plasma HIV-1 RNA at baseline, W4 and W12 in 21 failing patients. RT nested PCR products obtained from two patients with a K65K/R mixed population at W4 were selected. Baseline and W4 plasma samples were cloned and screened for the presence or absence of the K65R mutation by a selective K65R real time PCR assay. Clones detected as K65R mutant by this selective PCR assay were sequenced.

**RESULTS:** At baseline bulk sequencing showed that the RT sequences were wild type except in one patient harbouring a T215E substitution. At W4, M184V/I was detected in 10 patients and K65K/R in two patients (in association with M184I in one case). At W12, M184V/I was found in 18/21 patients in association with the K65R in 13 cases. None of the 180 analysed clones from baseline samples for each of the two patients with K65K/R at W4, showed the presence of the K65R mutation by selective PCR. At W4, K65R mutation was present in 82/171 and 21/163 clones of these two patients by both selective PCR and sequencing while M184V/I was never present. In the patient with both K65K/R and M184I mutations detected by standard genotyping at W4, clonal sequencing showed that these mutations are present in separate clones.

**CONCLUSION:** In this population of naive patients receiving abacavir/lamivudine/tenofovir, M184V/I mutation was selected more frequently at W4 than K65R mutation. However, K65R mutation can also be detected early. These preliminary results did not show that these mutations are initially carried by the same genome. However, despite optimized PCR conditions in order to minimize recombination, this phenomenon cannot be excluded.

**ABSTRACT 156***Antiviral Therapy* 2004; 9:S172.**Early virological failure and occurrence of resistance in naive patients receiving tenofovir, didanosine and efavirenz***D Podzamczer<sup>1</sup>, E Ferrer<sup>1</sup>, JM Gatell<sup>2</sup>, J Niubo<sup>1</sup>, D Dalmau<sup>3</sup>, A Leon<sup>2</sup>, H Knobel<sup>4</sup>, D Iniguez<sup>1</sup> and I Ruiz<sup>1</sup>*

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**OBJECTIVE:** To describe the occurrence of a high early virological failure (VF) rate and development of resistance mutations in patients receiving tenofovir, didanosine and efavirenz.

**METHODS:** HIV-infected naive patients were enrolled in a pilot, randomized, intensification trial of tenofovir/didanosine (250 mg)/efavirenz with (arm A) or without (arm B) lopinavir/r for 12 weeks. Viral loads were determined at 0, 1 and 3 months, and in a subgroup of patients additionally at days 3, 7, 14 and 21. In patients with VF, genotypic resistance tests were performed at baseline and at each time point. As several cases of early VF (a drop of <2 log at month 3, or a rebound of >1 log from the nadir) were detected in arm B, an unplanned interim analysis was performed and a DSMB recommended stopping enrolment.

**RESULTS:** 36 patients were enrolled, 19 in arm A and 17 in arm B. Baseline median CD4 counts were 185 (6–490) cells/ul and median VL was 144207 (29752–>500000) copies/ml. 26 of the 36 enrolled patients completed 3 months of therapy and were included in the interim analysis. 6/14 (42.8%) patients in arm B developed VF vs 0/12 in arm A ( $P=0.017$ ). 6/6 VF pts had VL >100000 copies/ml and an advanced stage of disease (CD4 <200 plus CDC stage C or B3) vs 0/8 non-VF pts ( $P<0.001$ ). T69D/N (one patient) and T69S (one patient) were the only relevant mutations at baseline. At failure, G190S/E alone or associated with K103N and other mutations were detected in five patients, K103N/L100I/ V108I in one, L74V/I in four and K65R in two cases. Efavirenz mutations appeared since as soon as day 14 or 21 to as late as month 3; L74V/I were detected between month 1 and 3; and K65R appeared at month 3.

**CONCLUSION:** A high early virological failure rate was found in a group of naive patients with advanced

disease and high VL, treated with tenofovir/didanosine/efavirenz. A peculiar resistance pattern was detected including G190E/S and L74V/I in most patients. It should be elucidated if an interference between tenofovir and didanosine or other mechanisms, are involved.

**ABSTRACT 157***Antiviral Therapy* 2004; **9**:S173.**Baseline genotypic analysis of treatment-naïve patients taking tenofovir DF (TDF) or stavudine (d4T) in combination with lamivudine (3TC) and efavirenz (EFV)***MD Miller<sup>1</sup>, NA Margot<sup>1</sup>, JM Waters<sup>2</sup>, J Harris<sup>2</sup>, B Lu<sup>1</sup> and AK Cheng<sup>1</sup>*<sup>1</sup> Gilead Sciences, Inc., Foster City, Calif., USA; and <sup>2</sup> Gilead Sciences, Inc., Durham, NC, USA

**BACKGROUND:** Among treatment-naïve patients, baseline mutations due to transmission of resistant virus or natural HIV-1 variation may affect treatment response.

**METHODS:** Study 903 was a 144 week, randomized, double-blind study of TDF therapy in treatment-naïve patients from the US, EU and South America. Patients received either TDF ( $n=299$ ) or d4T ( $n=301$ ) with 3TC and EFV. Plasma HIV-1 was analysed genotypically at baseline ( $n=598$ ) and virological failure (VF;  $>400$  copies/ml,  $n=96$ ). Non-B HIV-1 subtypes and genetic variation within HIV-1 RT (1–400) were assessed as predictors of VF and resistance development.

**RESULTS:** VF occurred in 15.7% of patients in the TDF arm and 16.3% in the d4T arm ( $P=0.91$ ). Non-B subtypes were observed in 7.7% of patients (2.7% F, 2.3% A/AG/AE, 1.7% C, 1.0% other). In both arms, non-B subtype patients responded similarly to therapy compared to those with B-subtype ( $P>0.75$ ). Few patients had detectable primary resistance mutations to NNRTIs at baseline ( $n=2$ , both K103N), one responded through week 144 and one developed VF. More patients had detectable NRTI-associated mutations at baseline ( $n=26$ , 4.3%, excluding V118I). No patients had T215Y/F, but 15 had other T215 mutations suggesting reversion; seven of these also had M41L and/or L210W. In both arms, antiviral response through week 144 appeared unaffected by these baseline NRTI mutational patterns ( $P>0.39$ ). At each individual RT position, mutations at five locations showed moderate statistical significance (S48, V179, E248, E370, Q373;  $P<0.05$ , but  $>0.01$ , unadjusted). Of these, V179D may be clinically relevant as VF patients with V179D (and no other mutations) developed V106M

and high-level NNRTI resistance ( $>100$ -fold,  $n=3$ ), rather than developing K103N. V106M has been reported with subtype-C, however patients here were subtype-B. Mutations V118I and at I135 were not associated with VF ( $P>0.5$ ).

**CONCLUSION:** Non-B subtypes and baseline NRTI-associated mutations did not significantly impact treatment response in this study. Primary NNRTI-associated mutations were very rarely observed. A natural polymorphism of V179D was associated with development of V106M and high-level NNRTI resistance. These results suggest that in the absence of primary NNRTI-resistance, baseline NRTI-associated mutations among treatment-naïve patients have a negligible clinical impact through 144 weeks of therapy in these regimens.

**ABSTRACT 158**

*Antiviral Therapy* 2004; 9:S174.

**Pre-existing L74V is a risk factor for virological non-response and development of K65R in patients taking tenofovir DF (TDF)**

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**BACKGROUND:** Among treatment-experienced patients, prior abacavir (ABC) and/or didanosine (ddI) failure can result in development of either the L74V or K65R mutations in HIV-1 reverse transcriptase (RT). K65R shows reduced susceptibility to tenofovir *in vitro* and *in vivo*, but L74V shows full susceptibility to tenofovir *in vitro*. L74V was observed in 10% of patients at baseline in the tenofovir (TDF) intensification studies 902 and 907 and most often with multiple TAMs.

**METHODS:** Retrospective population-based and single-genome sequencing (SGS) was performed to assess low-level baseline K65R and mutation development during treatment with TDF plus other antiretrovirals.

**RESULTS:** In the absence of TAMs, baseline L74V was significantly associated with development of K65R with four/five L74V patients versus 10/106 non-L74V patients developing K65R ( $P=0.0008$ ). Patients with baseline TAMs, regardless of L74V, did not develop K65R. Baseline plasma samples were available for three of four patients with L74V at baseline who later developed K65R; all four patients were virological non-responders. SGS of 50–100 clones from each showed K65R in 2.6% and 7.7% of baseline sequences from two patients which were not detected by population sequencing. Full development of K65R had occurred by week 12 in both patients, one taking TDF+didanosine+efavirenz+saquinavir and the other TDF+abacavir+lamivudine+amprenavir. Population-based sequencing over the course of therapy showed the disappearance of L74V and the appearance of K65R in all four patients with intermediate time points having mixtures. SGS confirmed that the K65R and L74V mutations were present on independent genomes in the mixture populations.

**CONCLUSION:** As previously observed in clonal analyses of didanosine-failure, the K65R and L74V

mutations occur on distinct genomes and appear to represent an unfavoured combination for the virus *in vivo*. Prior therapy with NRTIs that can select for either L74V or K65R (abacavir, didanosine, and possibly stavudine) can result in a bulk population genotype that shows L74V but does not detect low-level K65R. Subsequent therapy with TDF, an NRTI that selects only for K65R, may result in expansion of a pre-existing K65R mutant and poor virological response. These data may have implications for how NRTIs are sequenced as part of HAART regimens.

**ABSTRACT 159***Antiviral Therapy* 2004; **9**:S175.**Impact of HIV resistance mutations, drug resistance and viral fitness on antiviral activity of tenofovir/abacavir/lamivudine in the ESS30009 study***LL Ross<sup>1</sup>, P Gerondelis<sup>1</sup>, EG Rouse<sup>1</sup>, ML Lim<sup>1</sup>, Q Liao<sup>1</sup>, BC Wine<sup>1</sup>, SK Griffith<sup>1</sup>, MH St Clair<sup>1</sup>, MS Shaefer<sup>1</sup>, AE Rodriguez<sup>2</sup>, JE Gallant<sup>3</sup> and ER Lanier<sup>1</sup>*

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**BACKGROUND:** ESS30009 is an ongoing randomized trial comparing tenofovir/abacavir/lamivudine to efavirenz/abacavir/lamivudine in antiretroviral-naïve subjects. An unexpectedly high rate of early virological non-response (VNR) to tenofovir/abacavir/lamivudine resulted in early termination of this regimen.

**METHODS:** 50/102 (49%) tenofovir/abacavir/lamivudine-treated subjects experienced VNR. Plasma-derived HIV genotype/phenotype data was obtained by ViroLogic for 41 VNRs at baseline and week 12. Select early (weeks 2–8) timepoints were genotyped by GlaxoSmithKline.

**RESULTS:** At week 12, 40/41 isolates (98%) had M184I or V or mixtures, one was wild-type, 22 (54%) had K65R or K/R mixtures. Median baseline HIV RNA for all VNR was 4.8 log<sub>10</sub> and 4.1 log<sub>10</sub> at week 12. Median baseline HIV RNA for subjects who selected K65R or mixture (K65Rfull/mix, *n*=22), K65K/R mixture only (K65Rmix, *n*=17) and full K65R (*n*=5) was 4.8, 4.7, and 5.5 log<sub>10</sub>, respectively, and at week 12 was 4.2, 4.1, and 4.8 log<sub>10</sub>, respectively. Median fold-resistance (MFR) for VNR subjects (*n*=41) at baseline and week 12 was, respectively, 0.89 and 3.46 (abacavir), 0.96 and 124 (lamivudine), and 0.84 and 0.54 (tenofovir). At week 12, K65Rfull/mix, K65Rmix, and K65Rfull, respectively, had MFRs of 3.8, 3.46, and 7.78 (abacavir), 126, 126 and 126 (lamivudine), and 0.56, 0.51 and 1.39 (tenofovir). At baseline, median replicative capacity was 96% (*n*=25), and decreased slightly to 76% at week 12 (*n*=33). 184V/I and/or 65R could be detected in some subjects as early as week 2. Clonal genotypic analysis detected genomes with unlinked K65R and/or M184V, followed by enrichment for virus containing both mutations at later timepoints.

**CONCLUSION:** By week 12, 98% of tenofovir/abacavir/lamivudine VNR subjects selected for virus with M184I/V/mixtures, 54% had K65R or mixtures, with only modest changes from baseline observed in replicative capacity despite substantial rebounds in viral load. Phenotypic resistance was not as reflective of viral response as genotype. Detection of genotypic resistance at rebound or shortly afterward suggests that the low efficacy may result in part from a low genetic barrier. Clonal analysis indicates resistance arose from distinct viruses with K65R or M184V followed by selection for viruses containing both mutations.

**ABSTRACT 160***Antiviral Therapy* 2004; **9**:S176.

**Persistence of nevirapine-resistant virus and pharmacokinetic analysis in women who received intrapartum NVP associated to a short course of zidovudine (ZDV) to prevent perinatal HIV-1 transmission: the Ditrame Plus ANRS 1201/02 Study, Abidjan, Côte d'Ivoire**

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**BACKGROUND:** Ditrame Plus was an open-labelled non-randomized trial. Consenting women with HIV-1 infection started oral ZDV (300 mg twice daily)  $\geq$ 36 weeks gestation. One oral dose of 600 mg ZDV+200 mg NVP was given just before beginning of labour. Neonates were treated for 1 week with ZDV syrup (2 mg/kg/6 h) + one single dose of NVP syrup (2 mg/kg on day 2–3). 381 women were enrolled in the DitramePlus trial and the vertical transmission rate was 6.4% at week 6 post-partum (PP). 74/381 women were included in resistance substudy.

**METHODS:** Genotypic resistance analysis by sequencing reverse transcriptase gene was performed on mothers DNA-PBMC at week 4 PP when a NVP-R (resistant) mutation was detected in concomitant plasma samples. The same analysis was performed at week 4 of life, 3 months and 12 months, on DNA-PBMC of children who had detectable NVP-R in plasma samples at week 4. Mothers' NVP-plasma concentrations were determined by a validated HPLC assay at 48 h PP (LOQ 50 ng/ml).

**RESULTS:** Twenty-one (33%) out of 74 women developed a detectable NVP-R mutation at 4 week PP. NVP-R mutations were detectable in six children at wk 4 (23%). DNA samples were available at week 4 PP for

20/21 women who developed NVP-R mutations in plasma. DNA-PBMC NVP-R mutations were detected in 15/20 women (75%). Follow-up plasma and DNA-PBMC samples collected 12 months after delivery for three women lacked detectable mutation. All six children who developed NVP-R mutations in plasma sample at week 4, had detectable DNA-PBMC NVP-R mutations. Follow-up plasma and DNA-PBMC samples collected at 3 months (one child) and until 12 months (one child) revealed the persistence of NVP-R mutations. The median plasma NVP concentration normalized to 48 hours PP was 598 ng/ml [315–885] for the mothers who had not acquired NVP-R virus compared to 851 ng/ml [633–1063] for the mothers harbouring NVP-R virus ( $P=0.014$ ).

**CONCLUSION:** NVP concentration analysis showed wide inter-individual variability. Therefore, emergence of NVP-R mutations strongly correlated with a high level of NVP concentration, owing to a prolonged period of viral replication under NVP selective pressure. The clinical follow-up of the cohort confirms the archival of viral resistance. Its impact on the response to treatment for mothers and children should be evaluated.

**ABSTRACT 161***Antiviral Therapy* 2004; **9**:S177.**The V106M mutation in treatment failures from a randomized controlled trial of lamivudine and stavudine, with nevirapine and/or efavirenz***DB Hall<sup>1</sup>, F van Leth<sup>2</sup>, P Robinson<sup>1</sup>, P McKenna<sup>3</sup>, FWMN Wit<sup>2</sup> and JMA Lange<sup>2</sup>*

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**INTRODUCTION:** Understanding resistance associated with clade C HIV-1 is important for the optimization of HAART in the developing world, particularly sub-Saharan Africa. Emergence of the V106M mutation has been observed in subtype C virus in association with efavirenz (EFV) use. The 2NN trial enrolled 1216 HIV-1+ treatment-naive patients from six continents in a 48-week investigation of antiretroviral regimens containing nevirapine (NVP) and/or EFV on a background of lamivudine (3TC) and stavudine (d4T). Virological failures ( $n=123$ ) were identified and baseline and post failure plasma-derived virus reverse transcriptase gene was sequenced. In addition a random sample of 153 virological responders had baseline sequencing.

**METHODS:** The sequence data from population sequencing at VIRCO was searched, identifying all occurrences of the V106M mutation. Patterns of occurrence with respect to NNRTI treatment, HIV-1 subtype, other emergent RT mutations, and relationship to virological response are described. Compliance was assessed by pill counts.

**RESULTS:** The V106M mutation was not observed at baseline in any of the 276 samples. There were 11 virological failure patients in whom V106M emerged, all with subtype C virus. Among these 11, 10 patients had been treated with EFV or EFV/NVP-containing regimens ( $P=0.005$ ). For 9/11 patients with V106M there was at least one other NNRTI-associated mutation. Emergence of V106M followed a decline in viral load of 1.5 to 4.6  $\log_{10}$  copies/ml (median 3.1). At first documented presence of V106M, the viral load was 0.5  $\log_{10}$  copies/ml (median) below baseline and CD4 count was 120 cells (median) above baseline. In four of seven patients with follow-up after the first detection

of the V106M mutation was not sustained in four of seven patients with follow-up after the first emergence of V106M. All four with reversion to V were treatment compliant and no new resistance-associated mutations were detected.

**CONCLUSION:** The V106M mutation is observed in subtype C, almost exclusively in EFV or NVP/EFV treated patients. The mutation which emerged after virological response was associated with return of the viral load to near baseline. The V106M mutation was not associated with CD4 count decline and was not well-sustained.

**ABSTRACT 162***Antiviral Therapy* 2004; 9:S178.

**Substitutions within HIV gp41 amino acids 36–45 are identified as the primary determinants for loss of *in vitro* susceptibility to enfuvirtide: results of data mining analyses of genotypic changes in gp41 in TORO 1 and TORO 2 that associate with changes in phenotypic susceptibility to enfuvirtide**

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**BACKGROUND:** This study explored the associations between genotypic changes (GT-change) in full-length gp41 (aa 1–345) and changes in phenotypic susceptibility (PT-change) to enfuvirtide from baseline (BL) using multivariate statistical techniques. These studies could provide additional insight into the genotypic basis of phenotypic sensitivity to enfuvirtide.

**METHODS:** ANOVA models and regression tree models were used to examine the relationship between PT-change and GT-change at all gp41 aa positions for 383 patients in TORO studies (293 treated with enfuvirtide, 90 control). The PT-change was derived for each patient as the fold change of the FCIC<sub>50</sub>s (IC<sub>50</sub> normalized to reference) between virological failure and BL. GT-change was expressed as change or no change.

**RESULTS:** The previously reported primary resistance loci within gp41 (aa 36–45) accounted for greater than 90% of the variation in PT-change explained by the ANOVA and regression tree models. Other positions (18, 24, 33, 126, 256, 312 and 331) had a smaller effect ( $P < 0.05$ ) after adjusting for the effect of the primary positions. Approximately 74% of the variation in PT-change was explained by the aforementioned GT-change in primary and accessory loci. The effect of primary positions in the models can be replaced by presence versus absence of any change at loci 36, 38, 40, 42, 43 and 45. The geometric means (GM) of PT-change for patients having any change at these positions ( $n=265$ ) were 35.5- versus 1.2-fold for patients

without a change ( $P < 0.0001$ ). For patients with any change, those who also had GT-change at the other positions mentioned above were seen to have 20.6–148.1 (range of GM) additional PT-change. The regression tree, which split at gp41 positions 38, 36, 43 and 45 or at any change within 36–45, confirmed the primary resistance positions. Additional analysis indicated that GT-change at the aforementioned primary positions along with 126 and 331 were likely enfuvirtide-induced changes ( $P < 0.05$ , Fisher's exact test).

**CONCLUSION:** Our data mining confirmed aa 36–45 as the only primary resistance predictors for losses of susceptibility to enfuvirtide. Other positions were identified as loci for accessory mutations that may increase the level of phenotypic resistance in the presence of the primary mutations.

**ABSTRACT 163***Antiviral Therapy* 2004; **9**:S179.**Low levels of adherence confers greater risk for non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance than protease inhibitor (PI) resistance***DR Bangsberg<sup>1</sup>, R Gupta<sup>2</sup>, R Harrigan<sup>3</sup>, D Guzman<sup>1</sup>, ED Riley<sup>1</sup>, R Clark and SG Deeks<sup>1</sup>*

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**BACKGROUND:** There exists a complex relationship between medication adherence and the risk of developing drug-resistance mutation. For example, resistance to single PI therapy occurs most frequently at high levels of adherence, while resistance to ritonavir-boosted PI therapy may occur at middle ranges of adherence. The relationship between adherence to the risk of developing resistance has not been well-defined for NNRTIs.

**METHODS:** All participants were enrolled in the REACH study, a population-based cohort of urban poor HIV+ individuals in San Francisco. Participants on stable NNRTI or single PI therapy for >6 months were monitored with unannounced pill counts at their usual place of residence. Genotypic drug resistance was determined in all participants with HIV RNA >50 copies/ml. Resistance was defined as one or more major resistance mutations based on recent IAS USA guidelines. Participants whose viral load remained undetectable during the observation period were assumed to have not developed drug-resistance. The association between adherence quartile and PI or NNRTI resistance was tested with chi square for trend and logistic regression.

**RESULTS:** Of 110 people eligible for study, 74 (67%) failed to achieve and maintain undetectable viral loads during the observation period. Genotypes were successful in 72 individuals for a total of 108 people in the analysis. 54 individuals received an NNRTI-based regimen and 54 received a PI-based regimen. In the lowest adherence quartile (0–53%), the prevalence of resistance was higher in NNRTI than PI treated individuals (54% vs 21%;  $P<0.01$ ). In a logistic model controlling for treatment duration, prior nucleoside exposure, and baseline CD4, the odds of NNRTI resistance declined with increasing adherence (OR=0.75 for a 10%

increase in adherence 95% CI: 0.57–0.97;  $P=0.03$ ). Conversely, there was a trend towards an increasing odds for PI resistance (OR=1.36 for a 10% increase in adherence; 95% CI: 0.98–1.9,  $P=0.07$ ).

**CONCLUSION:** Among participants with low medication adherence, resistance was more common in NNRTI than PI treated individuals. Whereas low-pill burden NNRTI regimens are often advocated for patients at risk for nonadherence, these data suggest that such individuals may be at higher risk for resistance on NNRTI based therapy than PI based therapy.

**ABSTRACT 164***Antiviral Therapy* 2004; 9:S180.**A meta-analysis of the virological efficacy of regimens containing four versus three active antiretroviral agents as initial therapy for HIV-1 infection***A Hill*

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**INTRODUCTION:** A combination of three antiretroviral agents is the current standard of care for initial HIV therapy, but suboptimal responses are seen in clinical trials and cohorts. Additional antiretrovirals may increase the likelihood or durability of a response. Non-randomized studies of three class regimens consisting of four or five agents have shown more rapid initial declines in HIV RNA, compared to two class triple therapy.

**METHODS:** Literature was searched using Cochrane methodology and investigators contacted as needed. Studies included were in treatment-naive adults, randomized and comparative of a regimens of three vs four or more antiretrovirals and had available 48–52-week HIV RNA intent-to-treat (ITT) outcomes with a cut off of 50 copies/ml. Where not available, 400 copy data (two trials) and 24 week data (one trial) was used. The method of DeSimonian and Laird was used for meta-analysis of the HIV RNA response data.

**RESULTS:** Nine studies met the inclusion criteria (SPICE, the Danish PI study, NZTA4002 ACTG 384, ACTG 388, CHARM, 2NN, C&W Quad study, Frankfurt Quad study). 1180 patients received triple therapy and 1187 patients quadruple therapy. Baseline demographics included a wide range of CD4 counts (range of means 22–365/mm<sup>3</sup>) and viral loads (range of means 4.8–5.4 log<sub>10</sub>). All individual studies had well matched for baseline characteristics. A test for heterogeneity indicated considerable variation between studies in the proportion of patients with less than 50 copies/ml after 48 weeks of treatment. The estimated overall mean difference (quadruple-triple) in percent with HIV RNA response was –2.39% (95% CI –8.2%, 3.4%) (*P*=0.42). Most trials showed trends for higher rates of Grade 3 or 4 adverse events for four drug HAART.

**CONCLUSION:** This meta-analysis of randomized controlled studies indicates that four drugs do not

improve ITT virological efficacy outcomes relative to standard three drug regimens. Four drug regimens cannot be recommended as initial therapy for HIV-1 infection. There may be an upper limit to the antiviral efficacy of HAART using the available three classes of antiretrovirals.

**ABSTRACT 165***Antiviral Therapy* 2004; **9**:S181.**Analyses of virological response and enfuvirtide resistance through 48 weeks in the TORO 1 and 2 studies***T Melby*<sup>1</sup>, *R DeMasi*<sup>1</sup>, *D Kuritzkes*<sup>2</sup>, *G Heilek-Snyder*<sup>3</sup>, *M Salgo*<sup>4</sup>, *N Cammack*<sup>3</sup>, *TJ Matthews*<sup>1</sup> and *ML Greenberg*<sup>1</sup>

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**OBJECTIVES:** To present 48 week virology analyses from TORO 1 and 2. New data are presented on patients not meeting virological failure; correlates of changes in susceptibility on treatment; genotypic and phenotypic changes observed in samples obtained after enfuvirtide discontinuation; and substitutions outside gp41 amino acids (aa) 36–45.

**METHODS:** Resistance data were generated using the PhenoSense and GeneSeq entry assays (Virologic, Inc.) for TORO 1 and 2 patients at baseline and at virological failure (VF) or at week 48 for non-VF patients with viral load (VL) >1000 copies/ml.

**RESULTS:** Virological response showed no correlation with baseline coreceptor tropism ( $P=0.5292$ ) or envelope subtype ( $P=0.6204$ ) and only a weak association with baseline  $IC_{50}$  ( $r=0.07$ ,  $P=0.0774$ ). At VF and at week 48 for non-VF patients, the geometric mean decreases in enfuvirtide susceptibility were 29.5-fold and 7.1-fold ( $P<0.0001$ ), respectively; decreases exceeding fourfold were observed for 249/277 (89.9%) and for 8/17 (47.1%;  $P<0.0001$ ) patients, respectively. Change in susceptibility to enfuvirtide on treatment was not correlated with time to VF but was positively correlated with change in VL at week 48 ( $r=0.18$ ,  $P<0.05$ ) and with substitutions in gp41 aa 36–45 ( $P<0.0001$ ). For samples obtained after discontinuation of enfuvirtide, an inverse relationship was observed between change in susceptibility from baseline and time off enfuvirtide. The estimated mean time to <fourfold change in susceptibility was 32 days (95% CI=24,41). Substitutions in gp41 aa 36–45 were seen in paired samples from 266/287 (92.7%) patients at VF and from 9/17 (52.9%) non-VF patients at week 48; V38A, N43D, G36D and V38M were most frequently observed. N126K and G129Z (any amino

acid) were the most common changes observed outside of gp41 aa 36–45.

**CONCLUSION:** Consistent with earlier reports, no correlation was seen between virological response to enfuvirtide and baseline tropism or clade but response trended with lower baseline  $IC_{50}$ . Change in susceptibility on treatment correlated significantly with viral load and substitutions in gp41 aa 36–45 and was higher for VF than non-VF patients. The relationship observed between time after discontinuation and enfuvirtide susceptibility agrees with previous findings suggesting that enfuvirtide resistance mutations impair viral fitness *in vivo*.

**ABSTRACT 166***Antiviral Therapy* 2004; **9**:S182.**Rate of virological failure and resistance profiles in patients treated with triple nucleoside regimens***P Gil, A Barrios, T García-Benayas, L Valer, L Martín-Carbonero, I Maida and V Soriano*

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**BACKGROUND:** Virological failure seems to occur more frequently with triple nucleoside combinations than with regimens based on non-nucleosides and/or protease inhibitors. A low genetic barrier to resistance and/or pharmacokinetic interactions may explain this finding.

**METHODS:** A retrospective review of all HIV-infected patients who had received triple nucleoside combinations at our institution was carried out. Virological failure was defined as lack of achievement of plasma HIV RNA <50 copies/ml after initiating therapy and/or viral rebound in subjects who previously attained it. Genotypic analyses were performed at the time of first virological failure.

**RESULTS:** Of the 260 patients assessed, 13 (5%) were drug-naive; 131 (50.4%) underwent simplification from more complex regimens having baseline VL <50 copies/ml; and 116 (44.6%) were treatment-experienced patients with detectable viral load who initiated three nucleoside in an attempt to facilitate treatment compliance. The most frequent regimens were Trizivir, Combivir+TDF, TDF+ddI+3TC, d4T+ddI+3TC and TDF+ABC+3TC.

Virological failure occurred in 77 (29.6%) patients: 3/13 (23.1%) drug-naive, 22/131 (16.8%) simplified and 52/116 (44.8%) pre-treated patients. Mean time to virological failure was 11 months (range 3–36).

Subjects receiving AZT-based regimens ( $n=123$ ) failed less frequently than the rest ( $n=137$ ): 16.3% vs 41.6% ( $P<0.001$ ). Patients who had received prior suboptimal mono-bitherapy with nucleoside analogues failed more frequently than subjects who received triple nuc combinations as first antiretroviral treatment (37.3% vs 15.9%;  $P=0.002$ ).

Genotypic results could be obtained from only 34 failing patients. Low plasma HIV RNA levels precluded to obtain results in the rest. M184V was found in 18 (53%) and K65R+M184V in seven (20.6%) cases. Interestingly, no TAMS were seen in subjects with K65R. Moreover, K65R was selected in 7/25 patients failing with nucleosides other than AZT but in 0/9 on AZT regimens ( $P=0.15$ ). All patients who developed K65R were receiving at least two of the following nucleosides: TDF, ABC or ddI.

**CONCLUSION:** Virological failure rate is frequent in patients receiving triple nucleoside combinations, particularly in drug-naive patients and rescue interventions. It is less frequent when prescribed for simplification purposes. The inclusion of AZT seems to reduce the risk of virological failure as well as the chances for selecting K65R.