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# Presence of Lamivudine or Emtricitabine Is Associated with Reduced Emergence of Nonnucleoside Reverse Transcriptase Inhibitor Mutations in an Efavirenz-Based Intermittent Antiretroviral Treatment Regimen

**Stéphanie Trancart,[a] Isabelle Charreau,[b] Bruno Marchou,[c] Muriel Bocquentin,[d] Jean-Michel Molina,[e] Jacques Izopet,[f] Philippe Tangre,[b] Jean-Pierre Aboulker,[b] Anne-Marie Taburet,[d] and the ANRS 106 Study Group**

Laboratory of Pharmacology, Hospital Purpan, Toulouse, France[a]; INSERM SC10, Villejuif, France[b]; Department of Infectious Diseases, Hospital Purpan, Toulouse, France[c]; Clinical Pharmacy, Bicêtre Hospital, Assistance Publique Hôpitaux de Paris, Paris, France[d]; Department of Infectious Diseases, Saint-Louis Hospital, Assistance Publique Hôpitaux de Paris, Paris, France[e]; and Laboratory of Virology, Hospital Purpan, Toulouse, France[f]

**Efavirenz concentrations were measured in 21 patients during an interruption cycle of the ANRS 106 Window trial. The median efavirenz concentrations in the patients 12 h, 3 days, and 7 days after discontinuation of the drug were 1,962 ng/ml, 416 ng/ml, and 112 ng/ml, respectively. The half-life ranged from 27 to 136 h. No relationship between efavirenz exposure and detection of nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations was demonstrated. Patients who were treated by a lamivudine- or emtricitabine-based regimen had a lower risk of NNRTI mutation selection.**

The pharmacokinetics of efavirenz is characterized by a long half-life which raised the question of duration of monotherapy whenever an efavirenz-based antiretroviral regimen is stopped. The primary objective was to describe the rate of decrease in efavirenz concentrations during an interruption cycle in patients included in the intermittent arm of the ANRS 106 Window trial (11). Furthermore, we evaluated the relationship between these concentrations and the occurrence of new nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations.

Patients enrolled in the interruption arm and treated with efavirenz-based antiretroviral therapy (600 mg of efavirenz once a day [QD]) gave their informed consent to participate in this pharmacokinetic substudy.

Efavirenz was stopped 7 days before the other combined drugs. Blood samples were drawn 12 h and 3, 7, and 10 days following efavirenz interruption. Following an 8-week-off therapy cycle, the HIV-1 RNA genotype was determined using population sequencing with a detection cutoff of >20%.

Efavirenz was assayed by high-performance liquid chromatography (HPLC) with UV detection (lower limit of quantification [LLQ] of 50 ng/ml). Half-lives were calculated from the slope of the monoexponential decline of at least 3 graphs of log linear concentrations versus time, and the first concentration below the LLQ was set at LLQ/2. For comparison, the concentration at day 10 was extrapolated from the concentration at 12 h and the half-life, whenever the measured concentration was below the LLQ.

All data are presented as median and range. Fisher's exact test was used to compare qualitative variables, and the Wilcoxon rank sum test was used to compare continuous variables. Factors related to the selection of NNRTI mutation were identified by univariate analysis. Comparisons were made using a two-sided alpha level of 0.05. Statistical analysis was performed with the use of SAS software version 9.1 (SAS Institute Inc., Cary, NC).

Twenty-one patients (15 males with a median age of 39 years and median weight of 69 kg) participated in this substudy. The patients had a baseline median $CD4^+$ count of 649 cells/mm$^3$ (range, 435 to 1,151 cells/mm$^3$) with a median nadir of 252 cells/mm$^3$ (range, 108 to 547 cells/mm$^3$) after a median duration of NNRTI therapy of 2.6 years (range, 1.9 to 3.1 years). At the beginning of this study, the 21 patients had <400 copies of HIV-1 RNA per ml and 19 (90%) had <50 copies of HIV-1 RNA per ml. Combined antiretroviral therapy included zidovudine ($n = 1$), didanosine ($n = 8$), stavudine ($n = 9$), lamivudine or emtricitabine ($n = 16$ or 1). None of the patients was coinfected by hepatitis C or B virus. The median (range) concentrations 12 h, 3 days, and 7 days ($n = 21$) after efavirenz was stopped were 1,962 ng/ml (728 to 4,146 ng/ml), 416 ng/ml (95 to 1,390 ng/ml), and 112 ng/ml (50 to 749 ng/ml), respectively. At day 10, 11 patients (52%) presented a concentration below 50 ng/ml, and the median extrapolated concentration was 47 ng/ml (range, 6 to 598 ng/ml). The median elimination half-life of efavirenz was 49 h (range, 27 to 136 h). NNRTI mutations emerged in 7 patients: K103N ($n = 6$), G190G/A ($n = 2$) which were not present or could not be detected in the DNA genotype at the beginning of the study (Table 1); one patient developed a G190G/A mutation and then a K103K/N mutation.

At each sample time, efavirenz concentrations were not significantly higher in NNRTI mutation-positive patients than in mutation-negative patients. The relationships between the presence of NNRTI viral mutations and efavirenz pharmacokinetic characteristics or combined antiretroviral regimen are listed in Table 2. The presence of lamivudine or emtricitabine in the antiretroviral regimen was the only factor associated with the absence of NNRTI mutation ($P < 0.01$).

This substudy primarily evaluated the impact of the efavirenz

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Address correspondence to Stéphanie Trancart, trancart.s@chu-toulouse.fr.

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**TABLE 1** Mutation "*de novo*" of NNRTI resistance mutations in patients on intermittent efavirenz-based therapy

| Patient | HIV-1 DNA RT genotype at the beginning of the study[a] | HIV-1 RNA RT genotype during the trial (wk of detection)[b] | EFV elimination $t_{1/2}$ (h)[c] | Extrapolated EFV concn at day 10 (ng/ml) |
|---|---|---|---|---|
| 1 | NA | K103N (W56) | 41 | 47 |
| 2 | NA | G190G/A (W72) | 55 | 167 |
| 3 | M41I/M<br>M184I/M<br>G190G/R | K103N (W8) | 136 | 598 |
| 4 | M184M/V | K103K/N (W8) | 47 | 25 |
| 5 | M41M/L | G190G/A (first at W40 and then at W72) | 52 | 38 |
| | T215T/F/S/I | M41L<br>K103K/N<br>T215F | | |
| 6 | WT | K101K/N<br>K103K/N<br>M184 M/V (W56) | 60 | 184 |
| 7 | K70T | K103K/N (W56) | 49 | 108 |

[a] RT, reverse transcriptase; NA, not available; WT, wild type.
[b] The week the mutation was detected is shown in parentheses (e.g., W56 for week 56).
[c] EFV elimination $t_{1/2}$:, efavirenz elimination half-life.

half-life and concentration on the emergence of NNRTI resistance mutations. The efavirenz concentration in plasma measured 12 h after the dose fell within the range of previous studies and in the 1,000- to 4,000-ng/ml range of the therapeutic window (12). Half-lives were highly variable (21, 22), and as expected, they were shorter than the half-life reported after administration of a single dose (6), as a consequence of the autoinduction property of efavirenz. A genetic polymorphism of *CYP2B6 G516T* (4, 6, 10, 14, 16, 17, 20, 23) leads to prolonged efavirenz half-lives and has been recognized as a factor that may explain the variability of efavirenz pharmacokinetics (5, 21). Unfortunately, at the time the study was designed, blood samples were not collected for pharmacogenetics, and the ethnicity of patients was not recorded.

No significant relationship was found between the emergence of NNRTI mutations and either plasma concentrations or the rate of decline of efavirenz, which is in agreement with the results of Pirillo et al. (19). Nevertheless, we noted a tendency for a longer half-life and higher efavirenz concentrations, i.e., a more prolonged exposure to efavirenz in those patients in whom NNRTI mutations emerged. At the time of the study, none of the patients had received nucleoside analogs such as tenofovir or abacavir that are known to have prolonged intracellular half-lives and that are recommended first-line nucleoside (or nucleotide) analogs in combination with NNRTI backbone. However, our data remain relevant for patients living with AIDS in resource-limited countries where efavirenz combined with zidovudine and lamivudine is increasingly being used.

Interestingly, the presence of lamivudine or emtricitabine in the antiretroviral drug regimen seemed to protect against the selection of NNRTI-resistant variants. These data indicate that lamivudine or emtricitabine have a long enough intracellular half-life for reduction of viral resistance to NNRTI (1). Previously reported data for nevirapine in the prevention to mother-to-child transmission support these findings (2, 3, 13).

There are several limitations of this substudy that have to be considered. (i) We cannot ascertain whether patient adherence remained steady throughout the trial, as pharmacokinetic determinations were not always concomitant with the time of detection of mutations. (ii) HIV mutations were detected in plasma viral RNA using population sequencing with a detection cutoff of >20%. The absence of detection of resistance mutations does not exclude the presence of a minority of resistant variants (7, 8, 9, 15, 18). (iii) Only a small number of patients were included in this substudy.

In summary, this study could not demonstrate a significant relationship between efavirenz exposure and detection of NNRTI mutations, but patients who were treated by a lamivudine- or

**TABLE 2** Factors related to the occurrence of NNRTI resistance mutations in 21 patients in the pharmacokinetic study

| Factor | Value for patients[a] | | *P* value[b] |
|---|---|---|---|
| | With NNRTI mutation ($n = 7$) | Without NNRTI mutation ($n = 14$) | |
| EFV half-life (h) | 52 (41–136) | 36 (27–94) | 0.23 |
| EFV concn (ng/ml) at the following times: | | | |
| 12 h | 2,200 (728–2,960) | 1,900 (1,319–4,146) | 0.91 |
| Day 3 | 563 (221–1,390) | 351 (95–886) | 0.31 |
| Day 7 | 114 (62–749) | 80 (50–374) | 0.20 |
| Extrapolated EFV concn at day 10 (ng/ml) | 108 (25–598) | 25 (6–455) | 0.28 |
| Prior duration of NNRTI therapy (yr) | 2.6 (0.7–3.5) | 2.1 (1.0–4.4) | 0.53 |
| No. of patients (%) with the following drug in regimen: | | | |
| Zidovudine | 1/7 (14) | 6/14 (43) | 0.34 |
| Didanosine | 4/7 (57) | 4/14 (29) | 0.35 |
| Stavudine | 4/7 (57) | 5/14 (36) | 0.35 |
| Lamivudine or emtricitabine | 3/7 (43) | 14/14 (100) | 0.0058 |

[a] The values are medians with ranges shown in parentheses unless specified otherwise.
[b] The *P* value comparing the value for patients with NNRTI mutation to the value for patients without NNRTI mutation is shown.

emtricitabine-based regimen and were therefore on a dual-long-half-life regimen had a lower risk of NNRTI mutation selection.

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