# Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients.

Monidarin Chou, Julie Bertrand, Olivier Segeral, Céline Verstuyft, Laurence Borand, Emmanuelle Comets, Clotilde Le Tiec, Laurent Becquemont, Vara Ouk, France Mentre, et al.

AAC Accepts, published online ahead of print on 9 August 2010
Antimicrob. Agents Chemother. doi:10.1128/AAC.00512-10






# Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients (ANRS12154)

Monidarin CHOU[1], Julie BERTRAND[2], Olivier SEGERAL[3], Céline VERSTUYFT[4], Laurence BORAND[5], Emmanuelle COMETS[2], Clotilde LE TIEC[6], Laurent BECQUEMONT[4], Vara OUK[7], France MENTRE[2], Anne-Marie TABURET[6]

[1]Rodolphe Mérieux Laboratory, Faculty of Pharmacy University of Health Sciences, Phnom Penh, Cambodia, [2] INSERM UMR 738 and Paris Diderot University, [3]Assistance Publique Hôpitaux de Paris, Hôpital Bicêtre, Internal Medicine Department, Paris, France, [4]Assistance Publique Hôpitaux de Paris, Hôpital Bicêtre, Molecular Genetic, Pharmacogenetic Hormonology department and EA2706 Univ Paris Sud, France, [5]Epidemiology and Public Health Unit, Institut Pasteur in Cambodia Phnom Penh, Cambodia, [6]Assistance Publique Hôpitaux de Paris, Hôpital Bicêtre, Clinical Pharmacy, France and [7]Hospital Calmette Phnom Penh, Cambodia

Text 3668 words

Abstract 250 words

3 figures and 2 tables

**Short title**: Nevirapine population pharmacokinetics

**Correspondance to**: Dr Anne-Marie Taburet

Clinical Pharmacy Department

University Hospital Bicêtre

78 rue du Général Leclerc

94270 Kremlin Bicêtre France

Fax +33 1 45 21 28 60 Phone +33 1 45 21 28 60

Data presented previously in part at 16th CROI meeting, Montreal 2009 (abstract#691)

## Abstract

The aims of this open-label, single-center, multiple-dose pharmacokinetic study were to characterize nevirapine pharmacokinetics in a Cambodian population of HIV-infected patients and to identify environmental and genetic factors of variability focusing on the *CYP2B6*, *CYP3A5* and *ABCB1* (*MDR1)* genes.

170 Cambodian HIV-infected patients were included. Nevirapine trough concentrations were measured after 18 and 36 months of starting antiretroviral treatment and in samples drawn during a dosing interval in a subset of ten patients. All data were analyzed by nonlinear mixed effect modelling. The effect of covariates was investigated using the population pharmacokinetic model.

Patients carrying homozygous loss of function alleles of *CYP3A5 6986A>G*, *CYP2B6 516G>T*, *CYP2B6 1459C>T* and *ABCB1 3435C>T* represent 42.4%, 9.2%, 0% and 18% of the population, respectively.

The median nevirapine trough concentrations did not differ after 18 and 36 months of treatment (5705 (≤50 – 13871) ng/mL and 5709 (≤50 – 15422) ng/mL respectively). Interpatient and intrapatient variabilities of nevirapine apparent clearance were 28% and 17%, respectively. *CYP2B6 516G>T* and creatinine clearance were found to significantly affect nevirapine apparent clearance. Estimated nevirapine apparent clearance was 2.95 L/h, 2.62 L/h and 1.86 L/h for *CYP2B6 516GG, 516GT* and *516TT* genotype, respectively. Impact of creatinine clearance is small.

This study demonstrates that 95% of the patients had a sustained nevirapine exposure well above the 3000 ng/mL threshold. Nevirapine clearance was shown to be affected by

*CYP2B6 516G>T* genetic polymorphism and creatinine clearance, although this explained only part of the interpatient variability which remains low compared to other antiretroviral drugs.

**Key words**: nevirapine, Cambodia, population pharmacokinetics, pharmacogenetics,

## Introduction

In resource-limited settings, noncompetitive HIV-1 reverse transcriptase inhibitors (NNRTI) are the WHO recommended backbone of first-line antiretroviral therapy. Nevirapine in combination with two nucleoside analog inhibitors of reverse transcriptase such as stavudine, or zidovudine, in addition to lamivudine was, at the time of the study, the recommended antiretroviral regimen in treatment-naïve patients, mainly because of the availability of WHO prequalified low-cost generic fixed-dose combination (7, 27). In Cambodia, the prevalence of HIV infection among the general population aged between 15 and 49 years peaked at 2% in 1998 and had declined to 0.9% in 2006. This decrease has been attributed to many deaths among people infected during the early years of the epidemic before implementation of the continuum of care and the scaling-up of HIV prevention, care and treatment programs. At the end of 2009, it is estimated that about 37000 patients were on antiretroviral drug regimens and 69.5% were on a nevirapine backbone regimen (NCHADS source at http://www.nchads.org/).

Therefore, worldwide, most patients living with AIDS and who need antiretroviral treatment are on a nevirapine-based antiretroviral regimen. However, data on factors influencing its pharmacokinetics and exposure in different populations are lacking. Nevirapine pharmacokinetics is characterized by a long half-life, 60% binding to plasma proteins and elimination mainly through oxidative metabolism involving CYP3A and CYP2B6 (14). Both CYP3A4 and CYP3A5 share substrates and their role in nevirapine metabolism is not clearly defined. The importance of *CYP2B6* genetic polymorphism in efavirenz metabolism is now well established, but its influence on nevirapine metabolism is less clear (21). One study suggests that nevirapine could be a weak substrate of the P-

glycoprotein efflux transporter (1). *CYP3A5, CYP2B6* and *ABCB1* (*MDR1*, which endcodes for P-glycoprotein) are known to be highly polymorphic (http://www.cypalleles.ki.se/, (46)). The following genetic polymorphisms were therefore studied. The *CYP3A5*3* allele (G at position 6986) creates a cryptic splice site creating aberrant mRNA, with a premature stop codon. Individuals with at least one A allele (*CYP3A5*1*) produce high levels of full-length CYP3A5 mRNA and express an active CYP3A5 enzyme, while those carrying the *CYP3A5 6986 GG (CYP3A*3)* genotype have very low or even undetectable hepatic CYP3A5 protein content. The two most relevant SNPs of *CYP2B6 (CYP2B6 G516T* and *C1459T)* were demonstrated to result in a significant decrease in protein expression. *ABCB1 3435 C>T* was associated with decreased transport function. Consequently homozygous *CYP3A5 6986GG*, *CYP2B6 516TT* or *1459TT* and *ABCB1 3435TT* alleles are associated with loss of function protein.

The aims of this descriptive study were to characterize nevirapine pharmacokinetic parameters in a large Cambodian population of HIV-infected patients using a population approach and to identify environmental and genetic factors of variability focusing on the *CYP3A5, CYP2B6* and *ABCB1* (*MDR1)* genes. Mixed effect models were used due to their flexibility in handling balanced and unbalanced data in a unified framework (37)

## Methods

### *Patients and study design*

The patients enrolled in this open-label, single-center, multiple-dose pharmacokinetic study were HIV-infected Cambodians. They have been included in the Esther cohort at the Calmette Hospital (Phnom Penh) since 2003, when treatment and care have been provided

to patients living with AIDS in Cambodia. This additional pharmacokinetic/pharmacogenetic study was approved by the National Ethics Committee of Cambodia. All patients signed an informed consent form which was explained orally in presence of a witness for those unable to read. To be included in the study, patients have consented to have an additional blood sample drawn at the 3-year evaluation for pharmacogenetics. During the first year about 300 HIV-infected patients were included in this cohort, most of them treated with a nevirapine + lamivudine + stavudine generic fixed-dose combination. Patients were treated with nevirapine 200 mg daily for the first two weeks and 200 mg bid thereafter in addition to stavudine 30 mg bid and lamivudine 150 mg bid. After 18 months of treatment, stavudine was switched to zidovudine 300 mg bid in most patients. Patients came to the clinic monthly for medical consultation and drug refill. They had to participate to at least three specific adherence consultations by a trained nurse. All patients were routinely monitored every six months for standard liver and renal function tests and CD4 cell count (Cyflow, Partec, Germany) in blood. As part of the 18-month (M18) and 3-year (M36) visits for evaluation of treatment efficacy, in addition to standard laboratory tests, plasma HIV RNA (41) and nevirapine plasma trough concentration before morning drug intake were measured. Samples drawn 12±2h after evening drug intake were kept for pharmacokinetic analysis. Adherence to antiretroviral therapy was monitored using a validated visual analog scale (2). Some of the patients were tested for HCV and HBV. In addition to the M18 and M36 sampling, ten patients agreed to participate in an extensive pharmacokinetic substudy. They fasted under a steady-state regimen before antiretroviral drug administration and blood samples were collected at predose and at 1 h, 2 h, 4 h, 8 h after the nevirapine morning intake.

## Genotyping

DNA was extracted from patient blood by using the QUIamp® DNA Mini Kit according to the protocol of the manufacturer (Qiagen). Genotyping for *CYP3A5 6986A>G* (rs776746), *CYP2B6 516G*>T (rs3745274), *CYP2B6 1459C>T* (rs3211371), and *ABCB1 3435C>T* exon26 (rs1045642) was performed using the TaqMan allelic discrimination assay (ABI prism 7000, Applied Biosystems, Courtaboeuf, France). Primers and probes used for *ABCB1, CYP3A5* SNPs detection have been described previously (10, 39). *CYP2B6* genotyping was performed with the use of TaqMan validated SNP assays (C___7817765_60 C__30634242_40 ) with the 7000HT Sequence Detection System (Applied Biosystems). Reactions were carried out as described previously (10, 39).

For each polymorphism, departure from Hardy-Weinberg proportions was tested using a $\chi^2$ test with degrees of freedom equal to the number of observed genotypes minus 1.

## Assay of nevirapine in plasma

Plasma nevirapine concentrations were assayed in France (M18) or Cambodia (M36) by liquid chromatography with diode array detection at 240 nm according to previously validated assays (48). The lower limit of quantification was 50ng/mL. Standard curves were linear up to 10000ng/mL. The within-day and day-to-day precisions of quality control samples included in each analytical run were below 9%. Both laboratories participate in the French program of external quality controls (Asqualab).

### *Population pharmacokinetic analysis*

Population pharmacokinetic modeling was performed using MONOLIX software version 2.4 (http://software.monolix.org/). A one-compartment model at steady state with first-order absorption and elimination parameterized in apparent volume of distribution (V/F) and clearance (CL/F) was used to describe the nevirapine concentrations. Data below the limit of quantification (50 ng/mL) were discarded from the analysis. Given the expected concentration levels, a patient with a concentration below this limit might be assumed not to have taken his pills.

In a first step, the interpatient variance matrix and the residual error model were determined with data from the 10 patients of the extended pharmacokinetic study plus the M36 nevirapine trough concentrations. The Bayesian information criterion (BIC) was used to select the residual error model (combined, proportional or constant) and the non-null interpatient variances (6). In a second step, the concentrations collected at the M18 evaluation were added to the previous data set and intrapatient (e.g. interoccasion) variances ($\gamma^2$) were added to parameters with non-null interpatient variances ($\omega^2$). To model interpatient and intrapatient variabilities we used an exponential model with Gaussian random effects.

In order to assess to what extent a model parameter is likely to be under the influence of genetic polymorphisms, the genetic component of the variability $R_{GC}$ was computed as described by Ozdemir et al (35): $R_{GC} = 1 - \frac{\gamma^2}{\omega^2}$ which gets closer to one as the parameter is likely to be influenced by genetic polymorphisms.

The continuous covariates investigated were age, weight, ALAT, plasma creatinine, creatinine clearance, plasma HIV RNA, CD4 count and adherence (assessed using a visual analog scale) along with sex, co-treatment (stavudine or zidovudine), plasma HIV RNA above 400 copies/mL, HCV coinfection, HBV coinfection and genotype for the *CYP3A5 6986A>G*, *2B6 516G>T*, *2B6 1459C>T* and *ABCB1 3435C>T* polymorphisms as for categorical covariates.

Covariate model building was performed using an ascendant approach based on Wald tests on the effect of coefficient estimates of the population analysis. Screening of individual empirical Bayes estimates was not performed as with such a sparse design shrinkage is important (5). For the univariate analyses, no imputation of the missing covariates was performed and a 0.1 significance level was used. For final model building, the significance level was set to 0.05 and missing covariates with exception of the genotypes were imputed to the value obtained at the closest evaluation otherwise to the median. A permutation approach was then performed to assess the p-values associated with the covariates remaining in the final model. Permutation tests correct for the Wald test type I error inflation that has been shown to occur in such designs (5). One thousand permutations were performed to insure the nominal level of 0.05.

The average nevirapine clearance for each patient was computed as the mean over the empirical estimates at the different occasions. Simulations based on the final pharmacokinetic estimates were performed with R software v2.9.1 (http://cran.r-project.org/) using 250 data sets to calculate the predicted 90% interval and median which were overlaid on the observed data on a visual predictive check plot. These simulations

were also used to compute normalized prediction discrepancies using the R package (http://www.npde.biostat.fr/) to be plotted versus time.

# Results

## *Characteristics of the study population*

170 patients of the Esther cohort who were on nevirapine therapy and signed the informed consent form were included in this study. The median (range) age of the population was 36.5 (21-64) years and median weight was 55 (36-82) kg. 145 patients participated in the M18 evaluation, 161 in the M36 evaluation and 139 in both the M18 and M36 evaluations in addition to the pharmacogenetic study. In addition, 10 patients (5 men) participated in the extensive pharmacokinetic substudy and only 3 did not participate in the M18 or M36 evaluation. The patient's demographic and laboratory data are listed in Table I. An undetectable viral load (HIV RNA < 250 copies/mL) was achieved in 81% of the patients at M18 and in 94% of patients at M36. Patients with undetectable plasma viral load or lack of resistance mutation in the case of increased viral load at M18 stayed on nevirapine and 91% of them had still undetectable plasma HIV RNA at the M36 evaluation. Adherence was high in this population as 98% and 99% of the patients reported a visual analog scale ≥ 8 at the M18 and M36 evaluations.

## *Frequency of genetic polymorphism*

Loss of function alleles of *CYP3A5 6986A>G*, *CYP2B6 516G>T*, *CYP2B6 1459C>T* and *ABCB1 3435C>T* represent 65%, 35%, 1% and 38% of the population, respectively. The test for Hardy-Weinberg proportions was non significant for all four polymorphisms.

### *Nevirapine exposure*

Four patients had concentrations measured at M18, M36 and in the extensive pharmacokinetic substudy, 136 patients had concentrations at both M18 and M36 and 29 patients had concentrations at only one of these evaluations. At M18 one patient was excluded from the analysis as the only concentration was below the limit of quantification (LOQ), three other concentrations were below the LOQ, two at M18 and one at M36. Figure 1 represents the nevirapine concentrations observed at each occasion. The median nevirapine trough concentrations were 5705 ng/mL (≤50 – 13871 ng/mL) and 5709 ng/mL (≤50 – 15422 ng/mL) at M18 and M36, respectively. Note that 3.4% and 5.6% of the patients had nevirapine trough concentrations below 3000 ng/mL at M18 and M36, respectively.

### *Population pharmacokinetics of nevirapine*

Nevirapine concentrations were adequately described by a one-compartment model with first order absorption and elimination. With the basic model, the apparent clearance of nevirapine was estimated to be 2.67 L/h with an interpatient variability of 28% and an intrapatient variability of 17%. The absorption constant and the apparent volume of distribution were 1.64 /h and 213 L (on average 3.9 L/kg), respectively. Adding interpatient variabilities to these parameters did not improve the model. A constant residual error model was selected, with an estimated standard deviation of 519 ng/mL. The estimates from the basic model as well as their relative estimation error (%) are given in Table II.

The genetic component of variability, $R_{GC}$, for nevirapine clearance was 63.1%. After the first step of univariate covariate selection, *CYP2B6 516G>T* polymorphism (P=0.02 and

$3.10^{-10}$ for the GT and TT genotypes; respectively; compared with GG), creatinine clearance (P=0.07) and HCV coinfected status (P=0.04) were significantly associated with the nevirapine apparent clearance (at the 0.1 level). Interestingly, in liver function tests ALAT was not found to be a significant covariate.

Following the ascendant procedure based on the Wald test, only the effect of the *CYP2B6 516G>T* genetic polymorphism and the creatinine clearance remained in the model so that the apparent clearance of subject i at occasion k is predicted as

$$Cl_{ik} = Cl \times e^{\beta_i} \times \left( \frac{CLCR_{ik}}{median(CLCR)} \right)^{0.23}$$

where $\beta_i$ =0, -0.12 or -0.46 if patient i is GG, GT or TT for the *CYP2B6 516G>T* genetic polymorphism, and $CLCR_{ik}$ is his creatinine clearance at occasion k.

P-values of the permutation test were 0.01 for GT versus GG, 0.001 for TT versus GG and 0.007 for creatinine clearance. Estimates from the final model and their 95% confidence interval derived from the standard errors are given in Table II. The population mean clearance was estimated to be 2.95 L/h, 2.62 L/h and 1.86 L/h for patients carrying GG, GT and TT genotypes for the *CYP2B6 516G>T* polymorphism, which corresponds to 11% and 37% decreases in clearance from the GG to the GT and TT genotype, respectively. The lowest value of creatinine clearance was associated with a 14% decrease in CL/F, whereas the highest value of creatinine clearance was associated with a 16% increase in CL/F. The addition of the polymorphism and the creatinine clearance to the model lowered the interpatient variability by 3.1 and 0.3%, respectively.

Figure 2 represents the effect of the *CYP2B6 516G>T* polymorphism and of creatinine clearance on individual nevirapine apparent clearances. Evaluation graphs, sorted by genotype for the *CYP2B6 516G>T* polymorphism, with the visual predictive check plot and the normalized prediction discrepancies versus time plot are shown in Figure 3. The predictions from the model adequately describe the observations within each genotype.

## Discussion

These are the first results on frequencies of genetic polymorphism of major drug metabolizing enzymes and transporters reported to be involved in NNRTI disposition in a large Cambodian population. Most Caucasian expressed the *CYP3A5 6986GG* genotype associated with a small amount of translated CYP3A5 protein with a G allele frequency ranging from 0.87 to 0.94 in various Caucasian populations (22, 29). In contrast, in various Asian populations G allele frequencies were lower ranging from 0.59 in Indians to 0.65 in Cambodians as demonstrated in this study, 0.67 in Vietnamese and 0.74-0.78 in Japanese, Chinese and Koreans (23, 29). The frequency is even lower in patients of African descent (0.36) (22). Higher expression of CYP3A5 protein will lead to an increase in clearance of CYP3A substrate drugs such as HIV-1 protease inhibitors. Lower saquinavir, atazanavir or indinavir concentrations (3, 24, 44) were demonstrated in patients who express CYP3A5, although disposition of lopinavir combined with ritonavir, which inhibits both CYP3A4 and CYP3A5, remains unaffected (15). This is of importance as lopinavir/ritonavir is the antiretroviral drug recommended by WHO for patients in whom a first-line NNRTI regimen fails.

The frequency of the *CYP2B6 516G>T* mutant allele associated with loss of catalytic activity varies greatly according to the study population, with the following average values: 0.14-0.18 (18, 23, 25) in Koreans and Japanese, 0.21 in Han Chinese (20), 0.22-0.25 in Caucasians (22, 25), 0.27 in Vietnamese (49), 0.32 in Thai (9, 38), 0.28-0.38 in African-Americans (22, 25), 0.42 in West Africans and up to 0.62 in Papua New Guinea (32). Not surprisingly, the frequency of 0.35 in our Cambodian population is close to that reported for people living in border countries such as Thailand and Vietnam. The T allele frequency of *CYP2B6 1459C>T* is very low in our Cambodian population as described for other East Asian populations (25, 49). The importance of the P-glycoprotein, an efflux transporter, in drug disposition has been reviewed (46). The *T* allele frequency of *ABCB1 3435C>T* in a Cambodian population is close to what was reported in Vietnamese (49), but is lower than in other Asian populations (4) or European Americans (46). All these data indicate marked differences in SNP frequencies between Cambodian and other Asian populations such as Han Chinese or Caucasian and African populations. They are in agreement with genome-wide association studies, which show the genetic substructure between different East Asian groups and low level of differentiation between Cambodian and Vietnamese (47).

The population pharmacokinetics of nevirapine was studied in a Cambodian HIV-infected population after long-term administration of nevirapine as backbone antiretroviral first-line therapy. The impressive efficacy of this antiretroviral drug regimen is in keeping with previous studies (7, 27). Such a positive virological outcome has already been pointed out in another Cambodian cohort with an efavirenz-based regimen (16) was related to high adherence to cART, as noted by Spire et al (45). In the present study most patients (99%) reported an adherence greater than or equal to 8 on a 10-point visual analog scale. It should

be stressed that in both cohorts antiretroviral therapy was provided free through Global Funds and NCHADS programs and that educational programs were implemented on a regular basis.

Although nevirapine is the antiretroviral drug of choice in low income countries, little is known of between- and within-patient variability. Our data show that after more than one year, under steady-state conditions, intraindividual variability in trough nevirapine concentrations is quite low, in agreement with previous data as Nettles et al indicated a within-patient variability of 25% in one patient who received nevirapine, which is well below what has been reported for HIV-protease inhibitors (19, 34). This is in keeping with nevirapine pharmacokinetic properties, with an absolute bioavailability reported to be 90% after single-dose administration (26). Half-life at steady state is longer than the dosing interval in most patients despite autoinducing properties, which means that delaying drug intake or missing a dose will have little influence on steady-state concentrations. Interpatient variability is also quite low, most likely because absorption variability can be ruled out. Interestingly, Manosuthi et al (30) recently reported that interpatient variability in the efavirenz group was 2.3-fold greater than in the nevirapine group, although these patients received concomitant use of rifampicin which could alter variability.

Estimation of nevirapine Cl/F calculated at steady state in our population is in the range, albeit somewhat lower, of values in previous studies including different populations (2.95 to 3.35 L/h, (11-13, 17, 33, 42, 50) and is roughly twice the apparent clearance reported after single-dose administration (21, 31), which clearly shows the importance of the autoinducing effect either on first-pass effect and bioavailability or total clearance. The 95% confidence interval for the apparent volume of distribution is large (111 – 446 L) as

the estimation error of this parameter is high. Therefore comparison with other studies reporting somewhat lower values is difficult (21, 31). Interpatient variability in V/F and $k_a$ could also not be estimated. This and the large standard error in V/F are related to the study design, since in most patients only one trough concentration was measured at each evaluation, giving mostly information on apparent clearance. This is the one of the few studies demonstrating that *CYP2B6 516G>T* genetic polymorphism and creatinine clearance affect nevirapine clearance, but explains only 3.1% and 0.3% of the interpatient variability, respectively. Apparent clearance is decreased by 37% in homozygous patients carrying the loss of function allele compared with the homozygous wild-type allele, which leads to an increased half-life estimated to be 52 h (range 28 – 96h) for GG, 59 h (29 – 120h) for GT and 83 h (38 – 178h) for TT patients. In 126 children, Saitoh et al demonstrated a 30% decrease in nevirapine clearance in children with the TT genotype compared with the GG genotype (43). Similarly, higher nevirapine concentrations have been reported in patients with the *CYP2B6 516TT* genotype (28, 36, 40), although the relationship is unclear after single-dose administration (9, 21). Such a discrepancy could be related to the autoinduction of CYP2B6 by repeated administration of nevirapine. Interestingly, genetic polymorphism was not found to affect the volume, ruling out a large inducing effect on bioavailability and first-pass effect. A relationship between nevirapine clearance and creatinine clearance was unexpected as nevirapine is eliminated mostly by biotransformations. Such a relationship was noted by Gandhi et al (17) in a cohort of HIV-infected women and they suggested that the effect of uremic toxins on relevant hepatic transporters and metabolizing enzymes may explain the influence of renal insufficiency on nevirapine clearance. However, the clinical relevance of this phenomenon is small as the

major changes were less than 20% from the mean. In agreement with others, no relationship between nevirapine clearance and weight was evidenced (11, 22)

No modification in nevirapine pharmacokinetics was seen in patients with liver disease (8, 11) and no relationship between ALAT and nevirapine concentrations was found in the present study.

This study has a number of limitations. First, plasma HIV RNA was not measured at inclusion in the cohort as this parameter was not available in Cambodia when the Esther cohort was initiated. Therefore, no relationship between plasma HIV RNA decline and nevirapine exposure could be established. Treatment failure was only seen in a few antiretroviral-naïve patients at the first evaluation, which would have made such a relationship difficult to demonstrate. Second, patients who developed rashes and liver toxicity early after initiating treatment were switched to efavirenz, so it cannot be shown whether the frequency of occurrence of these adverse events is dependent on the *ABCB1, CYP3A5* or *CYP2B6* loss of function allele. Third, it remains to be seen whether other infrequent variants contribute to the variability in nevirapine clearance.

Despite such limitations, this study demonstrates that 95% of the patients had a sustained nevirapine exposure well above the 3000 ng/mL threshold. Nevirapine clearance was shown to be affected by *CYP2B6 516G>T* genetic polymorphism, and creatinine clearance, although this explained only part of the interpatient variability which remains low compared to other antiretroviral drugs.

## Acknowledgements

We thank all Cambodian health care providers who take care of the patients included in the ESTHER cohort. Our thanks also go to ESTHER and ANRS for their support, to Dr FX Babin and the Fondation Mérieux for supporting the Rodolphe Mérieux laboratory located in the Faculty of Pharmacy, Health Science University, Phnom Penh, Cambodia. The HIV/hepatitis laboratory of the Pasteur Institute of Phnom Penh (Dr E Nerrienet) kindly provided the HIV RNA viral load results.

## Conflict of interest/disclosure

None of the authors have conflicts of interest related to the present study

# References


1. **Almond, L. M., D. Edirisinghe, M. Dalton, A. Bonington, D. J. Back, and S. H. Khoo.** 2005. Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus-infected individuals. Clin Pharmacol Ther. **78:**132-142.
2. **Amico, K. R., W. A. Fisher, D. H. Cornman, P. A. Shuper, C. G. Redding, D. J. Konkle-Parker, W. Barta, and J. D. Fisher.** 2006. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. J Acquir Immune Defic Syndr. **42:**455-459.
3. **Anderson, P. L., C. L. Aquilante, E. M. Gardner, J. Predhomme, P. McDaneld, L. R. Bushman, J. H. Zheng, M. Ray, and S. MaWhinney.** 2009. Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors. J Antimicrob Chemother. **64:**1071-1079.
4. **Balram, C., A. Sharma, C. Sivathasan, and E. J. Lee.** 2003. Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic-genotypic correlates. Br J Clin Pharmacol. **56:**78-83.
5. **Bertrand, J., E. Comets, C. M. Laffont, M. Chenel, and F. Mentre.** 2009. Pharmacogenetics and population pharmacokinetics: impact of the design on three tests using the SAEM algorithm. J Pharmacokinet Pharmacodyn. **36:**317-339.
6. **Bertrand, J., E. Comets, and F. Mentre.** 2008. Comparison of model-based tests and selection strategies to detect genetic polymorphisms influencing pharmacokinetic parameters. J Biopharm Stat. **18:**1084-1102.
7. **Calmy, A., L. Pinoges, E. Szumilin, R. Zachariah, N. Ford, and L. Ferradini.** 2006. Generic fixed-dose combination antiretroviral treatment in resource-poor settings: multicentric observational cohort. Aids. **20:**1163-1169.
8. **Cammett, A. M., T. R. MacGregor, J. M. Wruck, F. Felizarta, P. Miailhes, J. Mallolas, and P. J. Piliero.** 2009. Pharmacokinetic assessment of nevirapine and metabolites in human immunodeficiency virus type 1-infected patients with hepatic fibrosis. Antimicrob Agents Chemother. **53:**4147-4152.
9. **Chantarangsu, S., T. R. Cressey, S. Mahasirimongkol, E. Capparelli, Y. Tawon, N. Ngo-Giang-Huong, G. Jourdain, M. Lallemant, and W. Chantratita.** 2009. Influence of CYP2B6 polymorphisms on the persistence of plasma nevirapine concentrations following a single intra-partum dose for the prevention of mother to child transmission in HIV-infected Thai women. J Antimicrob Chemother. **64:**1265-1273.
10. **Coulbault, L., M. Beaussier, C. Verstuyft, H. Weickmans, L. Dubert, D. Tregouet, C. Descot, Y. Parc, A. Lienhart, P. Jaillon, and L. Becquemont.** 2006. Environmental and genetic factors associated with morphine response in the postoperative period. Clin Pharmacol Ther. **79:**316-324.
11. **Dailly, E., E. Billaud, V. Reliquet, S. Breurec, P. Perre, S. Leautez, P. Jolliet, M. Bourin, and F. Raffi.** 2004. No relationship between high nevirapine plasma concentration and hepatotoxicity in HIV-1-infected patients naive of antiretroviral treatment or switched from protease inhibitors. Eur J Clin Pharmacol. **60:**343-348.
12. **de Maat, M. M., A. D. Huitema, J. W. Mulder, P. L. Meenhorst, E. C. van Gorp, and J. H. Beijnen.** 2002. Population pharmacokinetics of nevirapine in an unselected cohort of HIV-1-infected individuals. Br J Clin Pharmacol. **54:**378-385.

13. **Elsherbiny, D., K. Cohen, B. Jansson, P. Smith, H. McIlleron, and U. S. Simonsson.** 2009. Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients. Eur J Clin Pharmacol. **65:**71-80.
14. **Erickson, D. A., G. Mather, W. F. Trager, R. H. Levy, and J. J. Keirns.** 1999. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos. **27:**1488-1495.
15. **Estrela, R. C., A. B. Santoro, P. F. Barroso, M. Tuyama, and G. Suarez-Kurtz.** 2008. CYP3A5 genotype has no impact on plasma trough concentrations of lopinavir and ritonavir in HIV-infected subjects. Clin Pharmacol Ther. **84:**205-207.
16. **Ferradini, L., D. Laureillard, N. Prak, C. Ngeth, M. Fernandez, L. Pinoges, G. Puertas, A. M. Taburet, N. Ly, C. Rouzioux, S. Balkan, C. Quillet, and J. F. Delfraissy.** 2007. Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia. Aids. **21:**2293-2301.
17. **Gandhi, M., L. Z. Benet, P. Bacchetti, A. Kalinowski, K. Anastos, A. R. Wolfe, M. Young, M. Cohen, H. Minkoff, S. J. Gange, and R. M. Greenblatt.** 2009. Nonnucleoside reverse transcriptase inhibitor pharmacokinetics in a large unselected cohort of HIV-infected women. J Acquir Immune Defic Syndr. **50:**482-491.
18. **Gatanaga, H., T. Hayashida, K. Tsuchiya, M. Yoshino, T. Kuwahara, H. Tsukada, K. Fujimoto, I. Sato, M. Ueda, M. Horiba, M. Hamaguchi, M. Yamamoto, N. Takata, A. Kimura, T. Koike, F. Gejyo, S. Matsushita, T. Shirasaka, S. Kimura, and S. Oka.** 2007. Successful efavirenz dose reduction in HIV type 1-infected individuals with cytochrome P450 2B6 *6 and *26. Clin Infect Dis. **45:**1230-1237.
19. **Goujard, C., M. Legrand, X. Panhard, B. Diquet, X. Duval, G. Peytavin, I. Vincent, C. Katlama, C. Leport, B. Bonnet, D. Salmon-Ceron, F. Mentre, and A. M. Taburet.** 2005. High variability of indinavir and nelfinavir pharmacokinetics in HIV-infected patients with a sustained virological response on highly active antiretroviral therapy. Clin Pharmacokinet. **44:**1267-1278.
20. **Guan, S., M. Huang, X. Li, X. Chen, E. Chan, and S. F. Zhou.** 2006. Intra- and inter-ethnic differences in the allele frequencies of cytochrome P450 2B6 gene in Chinese. Pharm Res. **23:**1983-1990.
21. **Haas, D. W., T. Gebretsadik, G. Mayo, U. N. Menon, E. P. Acosta, A. Shintani, M. Floyd, C. M. Stein, and G. R. Wilkinson.** 2009. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African americans. J Infect Dis. **199:**872-880.
22. **Haas, D. W., H. J. Ribaudo, R. B. Kim, C. Tierney, G. R. Wilkinson, R. M. Gulick, D. B. Clifford, T. Hulgan, C. Marzolini, and E. P. Acosta.** 2004. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. Aids. **18:**2391-2400.
23. **Hiratsuka, M., Y. Takekuma, N. Endo, K. Narahara, S. I. Hamdy, Y. Kishikawa, M. Matsuura, Y. Agatsuma, T. Inoue, and M. Mizugaki.** 2002. Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. Eur J Clin Pharmacol. **58:**417-421.

24. **Josephson, F., A. Allqvist, M. Janabi, J. Sayi, E. Aklillu, M. Jande, M. Mahindi, J. Burhenne, Y. Bottiger, L. L. Gustafsson, W. E. Haefeli, and L. Bertilsson.** 2007. CYP3A5 genotype has an impact on the metabolism of the HIV protease inhibitor saquinavir. Clin Pharmacol Ther. **81:**708-712.
25. **Klein, K., T. Lang, T. Saussele, E. Barbosa-Sicard, W. H. Schunck, M. Eichelbaum, M. Schwab, and U. M. Zanger.** 2005. Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. Pharmacogenet Genomics. **15:**861-873.
26. **Lamson, M. J., J. P. Sabo, T. R. MacGregor, J. W. Pav, L. Rowland, A. Hawi, M. Cappola, and P. Robinson.** 1999. Single dose pharmacokinetics and bioavailability of nevirapine in healthy volunteers. Biopharm Drug Dispos. **20:**285-291.
27. **Laurent, C., C. Kouanfack, S. Koulla-Shiro, N. Nkoue, A. Bourgeois, A. Calmy, B. Lactuock, V. Nzeusseu, R. Mougnutou, G. Peytavin, F. Liegeois, E. Nerrienet, M. Tardy, M. Peeters, I. Andrieux-Meyer, L. Zekeng, M. Kazatchkine, E. Mpoudi-Ngole, and E. Delaporte.** 2004. Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial. Lancet. **364:**29-34.
28. **Mahungu, T., C. Smith, F. Turner, D. Egan, M. Youle, M. Johnson, S. Khoo, D. Back, and A. Owen.** 2009. Cytochrome P450 2B6 516G-->T is associated with plasma concentrations of nevirapine at both 200 mg twice daily and 400 mg once daily in an ethnically diverse population. HIV Med. **10:**310-317.
29. **Makeeva, O., V. Stepanov, V. Puzyrev, D. B. Goldstein, and I. Grossman.** 2008. Global pharmacogenetics: genetic substructure of Eurasian populations and its effect on variants of drug-metabolizing enzymes. Pharmacogenomics. **9:**847-868.
30. **Manosuthi, W., S. Sungkanuparph, P. Tantanathip, A. Lueangniyomkul, W. Mankatitham, W. Prasithsirskul, S. Burapatarawong, S. Thongyen, S. Likanonsakul, U. Thawornwa, V. Prommool, and K. Ruxrungtham.** 2009. A randomized trial comparing plasma drug concentrations and efficacies between 2 nonnucleoside reverse-transcriptase inhibitor-based regimens in HIV-infected patients receiving rifampicin: the N2R Study. Clin Infect Dis. **48:**1752-1759.
31. **Marier, J. F., M. Dimarco, R. Guilbaud, C. Dodard, G. Morelli, S. K. Tippabhotla, A. K. Singla, N. R. Thudi, and T. Monif.** 2007. Pharmacokinetics of lamivudine, zidovudine, and nevirapine administered as a fixed-dose combination formulation versus coadministration of the individual products. J Clin Pharmacol. **47:**1381-1389.
32. **Mehlotra, R. K., M. N. Ziats, M. J. Bockarie, and P. A. Zimmerman.** 2006. Prevalence of CYP2B6 alleles in malaria-endemic populations of West Africa and Papua New Guinea. Eur J Clin Pharmacol. **62:**267-275.
33. **Molto, J., M. Valle, C. Miranda, S. Cedeno, J. Miranda, J. R. Santos, E. Negredo, J. Vilaro, J. Costa, and B. Clotet.** 2008. Once- or twice-daily dosing of nevirapine in HIV-infected adults: a population pharmacokinetics approach. J Antimicrob Chemother. **62:**784-792.

34. **Nettles, R. E., T. L. Kieffer, T. Parsons, J. Johnson, J. Cofrancesco, Jr., J. E. Gallant, K. A. Carson, R. F. Siliciano, and C. Flexner.** 2006. Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. Clin Infect Dis. **42:**1189-1196.
35. **Ozdemir, V., W. Kalow, B. K. Tang, A. D. Paterson, S. E. Walker, L. Endrenyi, and A. D. Kashuba.** 2000. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics. **10:**373-388.
36. **Penzak, S. R., G. Kabuye, P. Mugyenyi, F. Mbamanya, V. Natarajan, R. M. Alfaro, C. Kityo, E. Formentini, and H. Masur.** 2007. Cytochrome P450 2B6 (CYP2B6) G516T influences nevirapine plasma concentrations in HIV-infected patients in Uganda. HIV Med. **8:**86-91.
37. **Pinheiro, J. C. a. D. M. B.** 2000. Mixed-Effects Models in S and S-PLUS, 1st edition ed. Springer Verlag, New York, NY.
38. **Puthanakit, T., P. Tanpaiboon, L. Aurpibul, T. R. Cressey, and V. Sirisanthana.** 2009. Plasma efavirenz concentrations and the association with CYP2B6-516G >T polymorphism in HIV-infected Thai children. Antivir Ther. **14:**315-320.
39. **Quteineh, L., C. Verstuyft, V. Furlan, A. Durrbach, A. Letierce, S. Ferlicot, A. M. Taburet, B. Charpentier, and L. Becquemont.** 2008. Influence of CYP3A5 genetic polymorphism on tacrolimus daily dose requirements and acute rejection in renal graft recipients. Basic Clin Pharmacol Toxicol. **103:**546-552.
40. **Rotger, M., S. Colombo, H. Furrer, G. Bleiber, T. Buclin, B. L. Lee, O. Keiser, J. Biollaz, L. Decosterd, and A. Telenti.** 2005. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics. **15:**1-5.
41. **Rouet, F., M. L. Chaix, E. Nerrienet, N. Ngo-Giang-Huong, J. C. Plantier, M. Burgard, M. Peeters, F. Damond, D. K. Ekouevi, P. Msellati, L. Ferradini, S. Rukobo, V. Marechal, N. Schvachsa, L. Wakrim, C. Rafalimanana, B. Rakotoambinina, J. P. Viard, J. M. Seigneurin, and C. Rouzioux.** 2007. Impact of HIV-1 genetic diversity on plasma HIV-1 RNA Quantification: usefulness of the Agence Nationale de Recherches sur le SIDA second-generation long terminal repeat-based real-time reverse transcriptase polymerase chain reaction test. J Acquir Immune Defic Syndr. **45:**380-388.
42. **Sabo, J. P., M. J. Lamson, G. Leitz, C. L. Yong, and T. R. MacGregor.** 2000. Pharmacokinetics of nevirapine and lamivudine in patients with HIV-1 infection. AAPS PharmSci. **2:**E1.
43. **Saitoh, A., E. Sarles, E. Capparelli, F. Aweeka, A. Kovacs, S. K. Burchett, A. Wiznia, S. Nachman, T. Fenton, and S. A. Spector.** 2007. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. Aids. **21:**2191-2199.
44. **Solas, C., N. Simon, M. P. Drogoul, S. Quaranta, V. Frixon-Marin, V. Bourgarel-Rey, C. Brunet, J. A. Gastaut, A. Durand, B. Lacarelle, and I. Poizot-Martin.** 2007. Minimal effect of MDR1 and CYP3A5 genetic polymorphisms on the pharmacokinetics of indinavir in HIV-infected patients. Br J Clin Pharmacol. **64:**353-362.

45. **Spire, B., P. Carrieri, P. Sopha, C. Protopopescu, N. Prak, C. Quillet, C. Ngeth, L. Ferradini, J. F. Delfraissy, and D. Laureillard.** 2008. Adherence to antiretroviral therapy in patients enrolled in a comprehensive care program in Cambodia: a 24-month follow-up assessment. Antivir Ther. **13:**697-703.

46. **Telenti, A., and U. M. Zanger.** 2008. Pharmacogenetics of anti-HIV drugs. Annu Rev Pharmacol Toxicol. **48:**227-256.

47. **Tian, C., R. Kosoy, A. Lee, M. Ransom, J. W. Belmont, P. K. Gregersen, and M. F. Seldin.** 2008. Analysis of East Asia genetic substructure using genome-wide SNP arrays. PLoS One. **3:**e3862.

48. **Titier, K., F. Lagrange, F. Pehourcq, L. Edno-Mcheik, N. Moore, and M. Molimard.** 2002. High-performance liquid chromatographic method for the simultaneous determination of the six HIV-protease inhibitors and two non-nucleoside reverse transcriptase inhibitors in human plasma. Ther Drug Monit. **24:**417-424.

49. **Veiga, M. I., S. Asimus, P. E. Ferreira, J. P. Martins, I. Cavaco, V. Ribeiro, T. N. Hai, M. G. Petzold, A. Bjorkman, M. Ashton, and J. P. Gil.** 2009. Pharmacogenomics of CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP3A4, CYP3A5 and MDR1 in Vietnam. Eur J Clin Pharmacol. **65:**355-363.

50. **Zhou, X. J., L. B. Sheiner, R. T. D'Aquila, M. D. Hughes, M. S. Hirsch, M. A. Fischl, V. A. Johnson, M. Myers, and J. P. Sommadossi.** 1999. Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Antimicrob Agents Chemother. **43:**121-128.

# Figure legends

Figure 1. Plasma nevirapine concentrations versus time in 170 Cambodian HIV patients at M18 and M36 (a) and in the extensive PK substudy (b). Values below the LOQ are represented by the symbol (∗) at 0 on the y-axis.

Figure 2. Nevirapine concentrations versus time overlaid to the 90$^{th}$ interval and the median predicted from the final model (a, b and c) and normalized prediction discrepancies versus time (d, e and f), at all evaluations sorted by genotype for the *CYP2B6 516G>T* polymorphism.

Figure 3. Panel a. Mean over the individual nevirapine clearance at the different occasions (M18, M36 and the extensive PK substudy) for each of the 152 patients with an informed *CYP2B6 516G>T* genotype, sorted by genotype with the corresponding median (on a log scale). Panel b. Individual nevirapine clearance estimated at each occasions plotted versus the corresponding creatinine clearance observation. Data from each patient are connected by a segment. The solid line represents a regression spline (with y and x axis on logarithmic scale). Patients GG, GT and TT for the *CYP2B6 516G>T* polymorphism are represented with the symbols (+), (×) and (◇), respectively.

Table I. Characteristics of the patients at the 18 months and 36 months of evaluation

| | M18 (N=145) | | M36 (N=161) | |
|---|---|---|---|---|
| | Median (range) | N | Median (range) | N |
| Age (years) | 36.0 (19.0 — 56.0) | 145 | 37.0 (21.0 — 64.0) | 161 |
| Weight (kg) | 53.5 (25.0 — 79.0) | 142 | 55.0 (36.0 — 82.0) | 158 |
| ALAT (IU/mL) | 27.5 (7 — 291) | 134 | 29.0 (11.0 — 212.0) | 161 |
| Bilirubin (µmol/mL) | 7.0 (5.0 — 32.0) | 129 | 7.0 (5.0 — 37.0) | 160 |
| Creatinine (µmol/L) | 72.0 (42.0 — 108.0) | 131 | 81.0 (44.0 — 136.0) | 159 |
| Creatinine clearance (mL/min) | 89.6 (36 — 168.5) | 130 | 82.0 (44.0 — 144.2) | 156 |
| CD4 (cells/mL) | 207.0 (27.0 — 2306.0) | 145 | 299.0 (14.0 — 1054.0) | 161 |
| Plasma HIV-RNA (copies/mL) | 20.0 (20.0 — 251188.6) | 140 | 400.0 (400.0 — 190530.0) | 156 |
| | Number of patients (%) | N | Number of patients (%) | N |
| Sex (F/M) | 65 (45) / 80 (55) | 145 | 72 (45) / 89 (55) | 161 |
| Stavudine/zidovudine | 119 (90) / 13 (10) | 132 | 8 (5) / 153 (95) | 161 |
| Adherence (>8) | 128 (98) | 130 | 154 (99) | 156 |
| HIV RNA ≤ 400 copies/mL | 128 (81.0) | 140 | 147 (94.0) | 156 |
| HCV coinfection | 10 (8.0) | 125 | 11 (8.0) | 138 |
| HBV coinfection | 18 (14.0) | 127 | 20 (14.0) | 139 |
| HCV & HBV coinfection | 2 (98.0) | 125 | 2 (1.0) | 138 |

Table II. Parameter estimates and their 95% confidence intervals for the basic model (N=169) and the final model with covariates (N=152)

| Parameter | Basic model | | Final model | | |
|---|---|---|---|---|---|
| (unit) | Estimates | 95%CI | Estimates | 95%CI | P-value* |
| ka (/h) | 1.64 | (0.35 — 7.75) | 1.58 | (0.24 — 10.15) | |
| V/F (L) | 213 | (120 — 377) | 223 | (111 — 446) | |
| CL/F(L/h) | 2.67 | (2.51 — 2.84) | 2.95 | (2.70 — 3.22) | |
| $\beta_{CYP2B6\ 516GT}$ | | | -0.12 | (-0.22 — -0.02) | 0.01 |
| $\beta_{CYP2B6\ 516TT}$ | | | -0.46 | (-0.62 — -0.30) | $9.9\ 10^{-4}$ |
| $\beta_{CLCR}$ | | | 0.23 | (0.06 — 0.40) | $6.9\ 10^{-3}$ |
| $\omega_{CL/F}$ (%) | 28 | (24 — 32) | 24 | (0.20 — 0.28) | |
| $\gamma_{CL/F}$ (%) | 17 | (15 — 19) | 17 | (0.14 — 0.20) | |
| σ (ng/mL) | 519 | (408 — 630) | 580 | (454 — 716) | |

* Permutation test of covariate effect