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1 **Population pharmacokinetic-pharmacogenetic study of**
2 **nevirapine in HIV-infected Cambodian patients**
3 **(ANRS12154)**

4 Monidarin CHOU¹, Julie BERTRAND², Olivier SEGERAL³, Céline VERSTUYFT⁴,
5 Laurence BORAND⁵, Emmanuelle COMETS², Clotilde LE TIEC⁶,
6 Laurent BECQUEMONT⁴, Vara OUK⁷, France MENTRE², Anne-Marie TABURET⁶

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

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

22 Clinical Pharmacy Department

23 University Hospital Bicêtre

24 78 rue du Général Leclerc

25 94270 Kremlin Bicêtre France

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

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1

2 **Abstract**

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

7 170 Cambodian HIV-infected patients were included. Nevirapine trough concentrations
8 were measured after 18 and 36 months of starting antiretroviral treatment and in samples
9 drawn during a dosing interval in a subset of ten patients. All data were analyzed by
10 nonlinear mixed effect modelling. The effect of covariates was investigated using the
11 population pharmacokinetic model.

12 Patients carrying homozygous loss of function alleles of *CYP3A5 6986A>G*, *CYP2B6*
13 *516G>T*, *CYP2B6 1459C>T* and *ABCB1 3435C>T* represent 42.4%, 9.2%, 0% and 18% of
14 the population, respectively.

15 The median nevirapine trough concentrations did not differ after 18 and 36 months of
16 treatment (5705 (≤ 50 – 13871) ng/mL and 5709 (≤ 50 – 15422) ng/mL respectively).

17 Interpatient and inpatient variabilities of nevirapine apparent clearance were 28% and
18 17%, respectively. *CYP2B6 516G>T* and creatinine clearance were found to significantly
19 affect nevirapine apparent clearance. Estimated nevirapine apparent clearance was 2.95
20 L/h, 2.62 L/h and 1.86 L/h for *CYP2B6 516GG*, *516GT* and *516TT* genotype, respectively.

21 Impact of creatinine clearance is small.

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

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

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

5

1 Introduction

2 In resource-limited settings, noncompetitive HIV-1 reverse transcriptase inhibitors
3 (NNRTI) are the WHO recommended backbone of first-line antiretroviral therapy.
4 Nevirapine in combination with two nucleoside analog inhibitors of reverse transcriptase
5 such as stavudine, or zidovudine, in addition to lamivudine was, at the time of the study,
6 the recommended antiretroviral regimen in treatment-naïve patients, mainly because of the
7 availability of WHO prequalified low-cost generic fixed-dose combination (7, 27). In
8 Cambodia, the prevalence of HIV infection among the general population aged between 15
9 and 49 years peaked at 2% in 1998 and had declined to 0.9% in 2006. This decrease has
10 been attributed to many deaths among people infected during the early years of the
11 epidemic before implementation of the continuum of care and the scaling-up of HIV
12 prevention, care and treatment programs. At the end of 2009, it is estimated that about
13 37000 patients were on antiretroviral drug regimens and 69.5% were on a nevirapine
14 backbone regimen (NCHADS source at <http://www.nchads.org/>).

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

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

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

18 **Methods**

19 ***Patients and study design***

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

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

1

2 **Genotyping**

3 DNA was extracted from patient blood by using the QUIamp® DNA Mini Kit according to
4 the protocol of the manufacturer (Qiagen). Genotyping for *CYP3A5* 6986A>G (rs776746),
5 *CYP2B6* 516G>T (rs3745274), *CYP2B6* 1459C>T (rs3211371), and *ABCB1* 3435C>T
6 exon26 (rs1045642) was performed using the TaqMan allelic discrimination assay (ABI
7 prism 7000, Applied Biosystems, Courtaboeuf, France). Primers and probes used for
8 *ABCB1*, *CYP3A5* SNPs detection have been described previously (10, 39). *CYP2B6*
9 genotyping was performed with the use of TaqMan validated SNP assays
10 (C__7817765_60 C__30634242_40) with the 7000HT Sequence Detection System
11 (Applied Biosystems). Reactions were carried out as described previously (10, 39).

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

14

15 **Assay of nevirapine in plasma**

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

22

1 **Population pharmacokinetic analysis**

2 Population pharmacokinetic modeling was performed using MONOLIX software version
3 2.4 (<http://software.monolix.org/>). A one-compartment model at steady state with first-
4 order absorption and elimination parameterized in apparent volume of distribution (V/F)
5 and clearance (CL/F) was used to describe the nevirapine concentrations. Data below the
6 limit of quantification (50 ng/mL) were discarded from the analysis. Given the expected
7 concentration levels, a patient with a concentration below this limit might be assumed not
8 to have taken his pills.

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

18 In order to assess to what extent a model parameter is likely to be under the influence of
19 genetic polymorphisms, the genetic component of the variability R_{GC} was computed as

20 described by Ozdemir et al (35): $R_{GC} = 1 - \frac{\gamma^2}{\omega^2}$ which gets closer to one as the parameter is

21 likely to be influenced by genetic polymorphisms.

1 The continuous covariates investigated were age, weight, ALAT, plasma creatinine,
2 creatinine clearance, plasma HIV RNA, CD4 count and adherence (assessed using a visual
3 analog scale) along with sex, co-treatment (stavudine or zidovudine), plasma HIV RNA
4 above 400 copies/mL, HCV coinfection, HBV coinfection and genotype for the *CYP3A5*
5 *6986A>G*, *2B6 516G>T*, *2B6 1459C>T* and *ABCB1 3435C>T* polymorphisms as for
6 categorical covariates.

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

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

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

3 **Results**

4 ***Characteristics of the study population***

5 170 patients of the Esther cohort who were on nevirapine therapy and signed the informed
6 consent form were included in this study. The median (range) age of the population was
7 36.5 (21-64) years and median weight was 55 (36-82) kg. 145 patients participated in the
8 M18 evaluation, 161 in the M36 evaluation and 139 in both the M18 and M36 evaluations
9 in addition to the pharmacogenetic study. In addition, 10 patients (5 men) participated in
10 the extensive pharmacokinetic substudy and only 3 did not participate in the M18 or M36
11 evaluation. The patient's demographic and laboratory data are listed in Table I. An
12 undetectable viral load (HIV RNA < 250 copies/mL) was achieved in 81% of the patients
13 at M18 and in 94% of patients at M36. Patients with undetectable plasma viral load or lack
14 of resistance mutation in the case of increased viral load at M18 stayed on nevirapine and
15 91% of them had still undetectable plasma HIV RNA at the M36 evaluation. Adherence
16 was high in this population as 98% and 99% of the patients reported a visual analog scale \geq
17 8 at the M18 and M36 evaluations.

18 ***Frequency of genetic polymorphism***

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

22

1 ***Nevirapine exposure***

2 Four patients had concentrations measured at M18, M36 and in the extensive
3 pharmacokinetic substudy, 136 patients had concentrations at both M18 and M36 and 29
4 patients had concentrations at only one of these evaluations. At M18 one patient was
5 excluded from the analysis as the only concentration was below the limit of quantification
6 (LOQ), three other concentrations were below the LOQ, two at M18 and one at M36.
7 Figure 1 represents the nevirapine concentrations observed at each occasion. The median
8 nevirapine trough concentrations were 5705 ng/mL (≤ 50 – 13871 ng/mL) and 5709 ng/mL
9 (≤ 50 – 15422 ng/mL) at M18 and M36, respectively. Note that 3.4% and 5.6% of the
10 patients had nevirapine trough concentrations below 3000 ng/mL at M18 and M36,
11 respectively.

12

13 ***Population pharmacokinetics of nevirapine***

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

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

1 3.10^{-10} for the GT and TT genotypes; respectively; compared with GG), creatinine
 2 clearance (P=0.07) and HCV coinfecting status (P=0.04) were significantly associated with
 3 the nevirapine apparent clearance (at the 0.1 level). Interestingly, in liver function tests
 4 ALAT was not found to be a significant covariate.

5 Following the ascendant procedure based on the Wald test, only the effect of the *CYP2B6*
 6 *516G>T* genetic polymorphism and the creatinine clearance remained in the model so that
 7 the apparent clearance of subject *i* at occasion *k* is predicted as

$$8 \quad Cl_{ik} = Cl \times e^{\beta_i} \times \left(\frac{CLCR_{ik}}{\text{median}(CLCR)} \right)^{0.23}$$

9
 10 where $\beta_i = 0, -0.12$ or -0.46 if patient *i* is GG, GT or TT for the *CYP2B6 516G>T* genetic
 11 polymorphism, and $CLCR_{ik}$ is his creatinine clearance at occasion *k*.

12 P-values of the permutation test were 0.01 for GT versus GG, 0.001 for TT versus GG and
 13 0.007 for creatinine clearance. Estimates from the final model and their 95% confidence
 14 interval derived from the standard errors are given in [Table II](#). The population mean
 15 clearance was estimated to be 2.95 L/h, 2.62 L/h and 1.86 L/h for patients carrying GG, GT
 16 and TT genotypes for the *CYP2B6 516G>T* polymorphism, which corresponds to 11% and
 17 37% decreases in clearance from the GG to the GT and TT genotype, respectively. The
 18 lowest value of creatinine clearance was associated with a 14% decrease in CL/F, whereas
 19 the highest value of creatinine clearance was associated with a 16% increase in CL/F. The
 20 addition of the polymorphism and the creatinine clearance to the model lowered the
 21 interpatient variability by 3.1 and 0.3%, respectively.

1 Figure 2 represents the effect of the *CYP2B6* 516G>T polymorphism and of creatinine
2 clearance on individual nevirapine apparent clearances. Evaluation graphs, sorted by
3 genotype for the *CYP2B6* 516G>T polymorphism, with the visual predictive check plot and
4 the normalized prediction discrepancies versus time plot are shown in Figure 3. The
5 predictions from the model adequately describe the observations within each genotype.
6

7 **Discussion**

8 These are the first results on frequencies of genetic polymorphism of major drug
9 metabolizing enzymes and transporters reported to be involved in NNRTI disposition in a
10 large Cambodian population. Most Caucasian expressed the *CYP3A5* 6986GG genotype
11 associated with a small amount of translated CYP3A5 protein with a G allele frequency
12 ranging from 0.87 to 0.94 in various Caucasian populations (22, 29). In contrast, in various
13 Asian populations G allele frequencies were lower ranging from 0.59 in Indians to 0.65 in
14 Cambodians as demonstrated in this study, 0.67 in Vietnamese and 0.74-0.78 in Japanese,
15 Chinese and Koreans (23, 29). The frequency is even lower in patients of African descent
16 (0.36) (22). Higher expression of CYP3A5 protein will lead to an increase in clearance of
17 CYP3A substrate drugs such as HIV-1 protease inhibitors. Lower saquinavir, atazanavir or
18 indinavir concentrations (3, 24, 44) were demonstrated in patients who express CYP3A5,
19 although disposition of lopinavir combined with ritonavir, which inhibits both CYP3A4
20 and CYP3A5, remains unaffected (15). This is of importance as lopinavir/ritonavir is the
21 antiretroviral drug recommended by WHO for patients in whom a first-line NNRTI
22 regimen fails.

1 The frequency of the *CYP2B6* 516G>T mutant allele associated with loss of catalytic
2 activity varies greatly according to the study population, with the following average values:
3 0.14-0.18 (18, 23, 25) in Koreans and Japanese, 0.21 in Han Chinese (20), 0.22-0.25 in
4 Caucasians (22, 25), 0.27 in Vietnamese (49), 0.32 in Thai (9, 38), 0.28-0.38 in African-
5 Americans (22, 25), 0.42 in West Africans and up to 0.62 in Papua New Guinea (32). Not
6 surprisingly, the frequency of 0.35 in our Cambodian population is close to that reported
7 for people living in border countries such as Thailand and Vietnam. The T allele frequency
8 of *CYP2B6* 1459C>T is very low in our Cambodian population as described for other East
9 Asian populations (25, 49). The importance of the P-glycoprotein, an efflux transporter, in
10 drug disposition has been reviewed (46). The T allele frequency of *ABCB1* 3435C>T in a
11 Cambodian population is close to what was reported in Vietnamese (49), but is lower than
12 in other Asian populations (4) or European Americans (46). All these data indicate marked
13 differences in SNP frequencies between Cambodian and other Asian populations such as
14 Han Chinese or Caucasian and African populations. They are in agreement with genome-
15 wide association studies, which show the genetic substructure between different East Asian
16 groups and low level of differentiation between Cambodian and Vietnamese (47).

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

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

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

18 Estimation of nevirapine Cl/F calculated at steady state in our population is in the range,
19 albeit somewhat lower, of values in previous studies including different populations (2.95
20 to 3.35 L/h, (11-13, 17, 33, 42, 50) and is roughly twice the apparent clearance reported
21 after single-dose administration (21, 31), which clearly shows the importance of the
22 autoinducing effect either on first-pass effect and bioavailability or total clearance. The
23 95% confidence interval for the apparent volume of distribution is large (111 – 446 L) as

1 the estimation error of this parameter is high. Therefore comparison with other studies
2 reporting somewhat lower values is difficult (21, 31). Interpatient variability in V/F and k_a
3 could also not be estimated. This and the large standard error in V/F are related to the study
4 design, since in most patients only one trough concentration was measured at each
5 evaluation, giving mostly information on apparent clearance. This is the one of the few
6 studies demonstrating that *CYP2B6 516G>T* genetic polymorphism and creatinine
7 clearance affect nevirapine clearance, but explains only 3.1% and 0.3% of the interpatient
8 variability, respectively. Apparent clearance is decreased by 37% in homozygous patients
9 carrying the loss of function allele compared with the homozygous wild-type allele, which
10 leads to an increased half-life estimated to be 52 h (range 28 – 96h) for GG, 59 h (29 –
11 120h) for GT and 83 h (38 – 178h) for TT patients. In 126 children, Saitoh et al
12 demonstrated a 30% decrease in nevirapine clearance in children with the TT genotype
13 compared with the GG genotype (43). Similarly, higher nevirapine concentrations have
14 been reported in patients with the *CYP2B6 516TT* genotype (28, 36, 40), although the
15 relationship is unclear after single-dose administration (9, 21). Such a discrepancy could be
16 related to the autoinduction of *CYP2B6* by repeated administration of nevirapine.
17 Interestingly, genetic polymorphism was not found to affect the volume, ruling out a large
18 inducing effect on bioavailability and first-pass effect. A relationship between nevirapine
19 clearance and creatinine clearance was unexpected as nevirapine is eliminated mostly by
20 biotransformations. Such a relationship was noted by Gandhi et al (17) in a cohort of HIV-
21 infected women and they suggested that the effect of uremic toxins on relevant hepatic
22 transporters and metabolizing enzymes may explain the influence of renal insufficiency on
23 nevirapine clearance. However, the clinical relevance of this phenomenon is small as the

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

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

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

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

1

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3

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10 Conflict of interest/disclosure

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

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1 **Figure legends**

2

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

6

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

11

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

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

2

3

	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 ($\mu\text{mol/mL}$)	7.0 (5.0 – 32.0)	129	7.0 (5.0 – 37.0)	160
Creatinine ($\mu\text{mol/L}$)	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 \leq 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

1
2 Table II. Parameter estimates and their 95% confidence intervals for the basic model
3 (N=169) and the final model with covariates (N=152)
4
5

Parameter (unit)	Basic model		Final model		P-value*
	Estimates	95%CI	Estimates	95%CI	
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)	$9.9 \cdot 10^{-4}$
β_{CLCR}			0.23	(0.06 – 0.40)	$6.9 \cdot 10^{-3}$
ω_{CLF} (%)	28	(24 – 32)	24	(0.20 – 0.28)	
γ_{CLF} (%)	17	(15 – 19)	17	(0.14 – 0.20)	
σ (ng/mL)	519	(408 – 630)	580	(454 – 716)	

6
7 * Permutation test of covariate effect
8
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10





