



**HAL**  
open science

## **Influence of pharmacogenetics on indinavir disposition and short-term response in HIV patients initiating HAART**

Julie Bertrand, Jean-Marc Treluyer, Xavière Panhard, Agnes Tran, Solange Auleley, Elisabeth Rey, Dominique Salmon-Céron, Xavier Duval, France  
Mentré

► **To cite this version:**

Julie Bertrand, Jean-Marc Treluyer, Xavière Panhard, Agnes Tran, Solange Auleley, et al.. Influence of pharmacogenetics on indinavir disposition and short-term response in HIV patients initiating HAART. *European Journal of Clinical Pharmacology*, 2009, 65 (7), pp.667-678. 10.1007/s00228-009-0660-5 . hal-00534961

**HAL Id: hal-00534961**

**<https://hal.science/hal-00534961>**

Submitted on 11 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Influence of pharmacogenetics on indinavir disposition and short-term response in HIV patients initiating HAART

Julie Bertrand · Jean-Marc Treluyer ·  
Xavière Panhard · Agnes Tran · Solange Auleley ·  
Elisabeth Rey · Dominique Salmon-Céron ·  
Xavier Duval · France Mentré ·  
the COPHAR2-ANRS 111 Study Group

Received: 12 January 2009 / Accepted: 7 April 2009 / Published online: 14 May 2009  
© Springer-Verlag 2009

## Abstract

**Aims** To assess the relationship between genetic polymorphisms and indinavir pharmacokinetic variability and to study the link between concentrations and short-term response or metabolic safety.

**Methods** Forty protease inhibitor-naïve patients initiating highly active antiretroviral therapy (HAART) including indinavir/ritonavir and enrolled in the COPHAR 2–ANRS 111 trial were studied. At week 2, four blood samples were taken before and up to 6 h following drug intake. A population pharmacokinetic analysis was performed using the stochastic approximation expectation maximization (SAEM) algorithm implemented in MONOLIX software. The area under the concentration–time curve (AUC) and maximum ( $C_{\max}$ ) and trough concentrations ( $C_{\text{trough}}$ ) of indinavir were derived from the population model and tested for their correlation with short-term viral response and safety measurements,

while for ritonavir, these same three parameters were tested for their correlation with short-term biochemical safety. **Results** A one-compartment model with first-order absorption and elimination best described both indinavir and ritonavir concentrations. For indinavir, the estimated clearance and volume of distribution were 22.2 L/h and 97.3 L, respectively. The eight patients with the \*1B/\*1B genotype for the *CYP3A4* gene showed a 70% decrease in absorption compared to those with the \*1A/\*1B or \*1A/\*1A genotypes (0.5 vs. 2.1,  $P=0.04$ , likelihood ratio test by permutation). The indinavir AUC and  $C_{\text{trough}}$  were positively correlated with the decrease in human immunodeficiency virus RNA between week 0 and week 2 ( $r=0.4$ ,  $P=0.03$  and  $r=-0.4$ ,  $P=0.03$ , respectively). Patients with the \*1B/\*1B genotype also had a significantly lower indinavir  $C_{\max}$  (median 3.6, range 2.1–5.2 ng/mL) than those with the \*1A/\*1B or \*1A/\*1A genotypes (median 4.4, range 2.2–8.3 ng/mL) ( $P=0.04$ )

J. Bertrand (✉)  
UMR 738,  
INSERM, Université Paris Diderot,  
UFR de Médecine, 16, rue Henri Huchard,  
75018 Paris, France  
e-mail: julie.bertrand@inserm.fr

J.-M. Treluyer  
EA3620,  
Université Paris Descartes,  
Paris, France

J.-M. Treluyer  
Service de Pharmacologie Clinique,  
Hôpital Cochin-Saint-Vincent-de-Paul, AP-HP,  
Paris, France

X. Panhard · F. Mentré  
UMR 738, AP-HP,  
INSERM, Université Paris Diderot,  
Paris, France

A. Tran · E. Rey  
Service de Pharmacologie Clinique,  
Hôpital Cochin-Saint-Vincent-de-Paul, AP-HP,  
Paris, France

S. Auleley · X. Duval  
UMR 738, INSERM, Université Paris Diderot,  
Paris, France

D. Salmon-Céron  
Service de Médecine Interne,  
Hôpital Cochin-Saint-Vincent-de-Paul, AP-HP,  
Paris, France

X. Panhard · F. Mentré  
UF de Biostatistiques, Hôpital Bichat,  
Paris, France

X. Duval  
Centre d'Investigation Clinique, Hôpital Bichat, AP-HP,  
Paris, France

and a lower increase in triglycerides during the first 4 weeks of treatment (median 0.1, range  $-0.7$  to  $1.4$  vs. median 0.6, range  $-0.5$  to  $1.7$  mmol/L, respectively;  $P=0.02$ ). For ritonavir, the estimated clearance and volume of distribution were 8.3 L/h and 60.7 L, respectively, and concentrations were not found to be correlated to biochemical safety. Indinavir and ritonavir absorption rate constants were found to be correlated, as well as their apparent volumes of distribution and clearances, indicating correlated bioavailability of the two drugs.

**Conclusion** The *CYP3A4\*1B* polymorphism was found to influence the pharmacokinetics of indinavir and, to some extent, the biochemical safety of indinavir.

**Keywords** CYP3A4 · Efficacy · Nonlinear mixed effects modeling · Pharmacokinetics · Protease inhibitors · Safety

## Introduction

Indinavir has been one of the preferred protease inhibitor (PI) included in highly active antiretroviral therapy (HAART). Even if not recommended as initial therapy, indinavir is currently still used in patients who initiated their therapy with this PI and have kept a viral load below the limit of quantification with an acceptable safety profile. Compared to others PI, indinavir exhibits a high penetration into viral reservoirs, such as genital compartments and the central nervous system (CNS) [1], and it has been determined that the better distribution of indinavir leads to better outcomes in neurological complications related to human immunodeficiency virus (HIV) [2]. The pharmacokinetics (PK) of indinavir is characterised by high maximal concentrations, leading to potential toxicity, notably nephrolithiasis [3], and low minimum concentrations with respect to the 95% inhibitory concentration of the virus. These low residual concentrations result from an extended oxidative metabolism by the cytochrome P450 (CYP) 3A isoenzyme [4]. The co-administration of ritonavir, whose molecular structure leads to CYP3A inhibition, therefore enhances exposure to indinavir [5, 6]. Ritonavir is given at a lower dose as a booster than for therapeutic use, but it has been shown nevertheless to influence metabolic profiles, especially those associated with lipid disorders [7, 8].

The large inter-patient and intra-patient variability of indinavir pharmacokinetics is well referenced [9–11]. Genetic polymorphisms partly explain this variability, as far as the proteins involved in the metabolism and transport of PI are concerned. However, few studies have investigated the impact of ABCB1 polymorphisms, a gene coding for P-glycoprotein, and *CYP3A5* and *CYP3A4\*1B* polymorphisms on indinavir pharmacokinetics. Solas et al. [12]

reported that the ABCB1 C3435T genotype affects the absorption constant of indinavir, whereas Verstuyft et al. [13] found an absence of association. Anderson et al. [14] observed that *CYP3A5* expressors (*CYP3A5\*1* carriers) have a significantly faster oral clearance than non-expressors. To date, no relationship has been found between the *CYP3A4\*1B* polymorphism and alterations in *CYP3A* substrate metabolism, but clinical data have shown an association between the *CYP3A\*1B* polymorphism and disease risk/treatment toxicity [15].

Efficacy [16, 17] as well as adverse events [3, 18] have been related to indinavir plasma concentrations. Thus, therapeutic drug-monitoring appears to be a potent tool to achieve undetectable HIV-RNA and prevent toxicity for this drug. The COPHAR 2–ANRS 111 trial is a multi-centre, non-comparative pilot trial of early therapeutic drug-monitoring in HIV-positive patients naive for PI-containing HAART [19]. We focused on the PK sub-study from the group of patients receiving indinavir boosted with ritonavir. The aims of this paper were to estimate the population PK parameters and variability of indinavir and ritonavir in HIV patients, to evaluate the impact of genetic polymorphisms on indinavir PK and to study the link between indinavir concentrations and short-term efficacy and metabolic safety.

## Methods

### Study

The COPHAR 2–ANRS 111 study is a multi-centre non-comparative prospective pilot trial of early-dose adaptation in HIV-positive PI-naive patients starting a PI-containing HAART treatment. The trial started on July 2002 and was completed by the end of March 2005. The objective was to assess the benefit of pharmacological advice based on trough plasma concentrations of PI. The study involved three groups treated with indinavir, nelfinavir or lopinavir, respectively. In the study reported here we analysed the data obtained during the first month of treatment in the indinavir group. A similar analysis of data in the nelfinavir group was performed by Hirt et al. [19, 20; see these papers for details].

Patients were required to have a baseline plasma viral load value  $>1000$  copies/mL and to be PI treatment-naive. Patients were started on a HAART treatment containing 400, 600 or 800 mg of indinavir twice daily (b.i.d.) associated with ritonavir booster (100 mg b.i.d.) and two nucleoside analogues. The first dose was left to the treating physicians' discretion, and no dose adaptation was performed from week 0 (W0) to W4. A detailed PK study was performed at W2. Adherence was evaluated at W2 by means of a validated auto-questionnaire [21], and patients

were classified as adherent when they reported no shift in their treatment schedule during the last 4 days; in all other cases, they were classified as non-adherent.

Data on viral load and CD4 count were collected at baseline (D0) and at W2. Biochemical profiles of total cholesterol, high-density lipoprotein cholesterol, triglyceride and glycaemia as well as creatinine clearance and clinical events (diarrhoea grade of 2) were determined 4 weeks before treatment initiation (W–4) and at W4.

The study was performed in accordance with the Declaration of Helsinki and its amendments. All subjects provided written informed consent, and the protocol as well as the amendment for the pharmacogenetic study was approved by the Ethics Committee of the Bicêtre Hospital (France).

#### Indinavir and ritonavir concentration measurements

During a visit to the hospital at W2, the patients were sampled on arrival to measure trough concentrations. Patients were asked to record the time at which the dose was taken on the previous evening, given their medications, and then sampled again 1, 3 and 6 h after drug administration. Plasma concentrations were assumed to be at steady state with trough concentrations considered as following the drug intake using the delay reported by the patient the from previous dosing. Plasma concentrations were determined in the laboratories of the hospitals by a specific high-performance liquid chromatography protocol. The participant laboratories were cross-validated before starting the study. Results of the blind inter-laboratory quality control at three concentrations for indinavir and for ritonavir were within 15% of the target values for medium and high values and within 20% for low values. Lower limits of quantification (LOQ) were 0.02 mg/L for indinavir and 0.025 mg/L for ritonavir.

#### Genetic polymorphisms

All of the genotyping analyses were performed in the same laboratory. Total DNA was extracted from plasma samples using the QIAamp DNA Blood Mini kit (Qiagen, Courtaboeuf, France). ABCB1 polymorphisms in exons 21 (GG, GT, TT) and 26 (CC, CT, TT) were determined using previously published methods [22]. The genotyping of *CYP3A5* (\*1\*1, \*1\*3, \*3\*3, \*1\*6, \*6\*6) was performed by real-time PCR applying TaqMan MGB probe technology (Applied Biosystems, Foster City, CA). Genotyping for *CYP3A4* (\*1B\*1B, \*1B\*1A, \*1A\*1A) was determined by PCR, followed by direct sequencing. The PCR analysis was performed using a GenAmp PCR System 9700 (Applied Biosystems) according to a previously published method [23]. Amplified DNA was purified using the QiaQuick DNA

Purification System (Qiagen) and sequenced using BigDye Terminator chemistry and an ABI PRISM 3100 genetic analyser (Applied Biosystems). At least two positive controls were used for each genotyping analysis: one homozygous for the wild-type allele and one heterozygous (and, when available, one homozygous) for the mutated allele. These controls were DNA that had already been sequenced.

Allele frequencies ( $p$  for the wild allele and  $q=1-p$  for the mutant allele) were estimated by gene counting. Departure from Hardy–Weinberg proportions ( $p^2$ ,  $2pq$ ,  $q^2$ ) was tested by a  $\chi^2$  test with 1 *df* within each ethnic group [24]. We used two approaches to define patients belonging to an ethnic group: (1) classification of the patient according to town, birth area and nationality; (2) classification by means of genotype information using the Structure software [25]. This software is based on a Bayesian approach and computes the a posteriori probabilities of each individual of belonging to a given ethnic group. We assumed each locus to be at the Hardy–Weinberg equilibrium and patients to originate in one ethnic group (with its own characteristic set of allele frequencies).

#### Population PK analysis

We used a population approach to analyse the concentration–time data at W2 for indinavir and for ritonavir separately. Model fitting and estimation of the population model parameters were performed using the stochastic approximation expectation maximization algorithm (SAEM) for nonlinear mixed-effects models implemented in the MONOLIX software ver. 2.1 [26–28]. Both indinavir and ritonavir concentrations were fitted by a one-compartment model with first-order absorption and first-order elimination parameterised in the absorption rate constant ( $k_a$ ), oral clearance (Cl/F) and oral volume of distribution (V/F). Each model was assumed at steady state with trough concentrations considered as following the drug intake.

An exponential model was used for inter-individual variability where random effects were assumed to follow a normal distribution with zero mean and diagonal variance matrix. Additive, proportional and combined error models were tested, and model choice was based on the likelihood ratio test (LRT) and goodness-of-fit plots (observed vs. predicted population and individual concentrations; population and individual weighted residuals vs. predicted concentrations and vs. time). We performed a visual predictive check (VPC) with 1000 simulated data sets to evaluate the basic model [29].

Interaction between ritonavir and indinavir PK was evaluated with the individual parameters estimated from the basic model for each drug. All of the different correlations were tested with the Spearman non-parametric correlation test.

### Assessment of the effect of covariates

The effects of the following covariates were evaluated from the basic model: dose, concomitant use of the zidovudine lamivudine combination (AZT/3TC), co-infection by hepatitis C or B (VHC/VHB), adherence as previously defined, sex, ethnic group, the four studied genetic polymorphisms (ABCB1 exon 26, ABCB1 exon 21, *CYP3A5* and *CYP3A4*) and the CDC classification for HIV infection as categorical variables; age, body mass index (BMI), body weight, creatinine clearance, albumin and orosomucoid levels as continuous variables. The latter were centered to the median and log-transformed for model interpretation convenience.

Each of the four genetic polymorphisms was analysed by means of two binary categorisations: first, wild homozygotes versus heterozygotes or mutant homozygotes; second, heterozygotes or wild homozygotes versus mutant homozygotes. Categorisation in three classes was also tested: wild homozygotes versus heterozygotes versus mutant homozygotes. Missing continuous covariates were replaced with the median, and patients with missing discrete covariates were discarded for the corresponding analysis. The effects of covariates on the empirical Bayes estimates (EBE) of each individual PK parameter from the basic model were tested with the Wilcoxon non-parametric test for categorical variables and the Spearman non-parametric correlation test for continuous variables. The population covariate model was built with the covariates, which were found to have an effect in this first step with a  $P$  value  $< 0.1$ . When a genetic covariate was found to have an effect whatever the categorisation, the same categorisation as other genetic covariates also found to have an effect was chosen in model selection for consistency.

A forward selection of these covariates for the population model was performed using the LRT with a significance threshold at  $P < 0.05$ . From this ascending method, a backward elimination procedure was performed. In order to correct the inflation of the LRT type I error on small sample size [30], the backward selection was realized using permutation [31]. More specifically, 1000 data sets are generated by permuting the rows of the covariates matrix from the original data set. For each covariate, one likelihood ratio statistic,  $LRT^{obs}$ , is estimated from the original data and one likelihood ratio statistic,  $LRT^{perm}$ , is estimated from each of the 1000 data sets. Thus, we obtain  $j = 1, \dots, 1000 LRT^{perm_j}$ . The permutation  $P$  value is the proportion :  $card(LRT^{perm_j} > LRT^{obs})/1000$ .

### Short-term efficacy and safety and link with concentrations

As there was no change of dose before W4, we studied the link between concentration at W2 and efficacy or safety during the first 2 or 4 weeks of treatment. For short-term

efficacy, the difference of log viral load between the day of treatment initiation and W2 ( $\Delta \log VL$ ) was studied. The significance of the viral load decrease was tested by a Wilcoxon non-parametric paired test.

Individual area under the concentration–time curve (AUC), maximal plasma concentration ( $C_{max}$ ) and trough concentrations ( $C_{trough}$ ) of indinavir at steady-state were derived for each patient using the EBE of the individual parameters from the basic model and their corresponding dose of indinavir. The relationship between indinavir dose, indinavir AUC,  $C_{max}$ ,  $C_{trough}$  and  $\Delta \log VL$  was evaluated using the Spearman correlation test. A Wilcoxon non-parametric test was performed to compare the  $\Delta \log VL$  between patients with or without a  $C_{trough}$  below the lower limit of the therapeutic range used in the COPHAR 2–ANRS 111 trial: 150 ng/mL.

Safety was analysed by determining the difference between 4 weeks before and 4 weeks after treatment initiation in terms of total cholesterol ( $\Delta TC$ ), high-density lipoprotein cholesterol ( $\Delta HDL$ ), triglyceride ( $\Delta trig$ ) and glycaemia ( $\Delta gly$ ) and also by the appearance of diarrhoea (grade 2) between treatment initiation and W4. To the best of our knowledge, no precocious biological markers exist for nephrolithiasis; however, creatinine clearance has been found to relate to the occurrence of severe adverse events (including nephrolithiasis) in a multivariate analysis [3]. Thus, we also analysed the difference in creatinine clearance ( $\Delta CrCl$ ), computed with the Cockcroft–Gault formula using body weight and serum creatinine 4 weeks before and 4 weeks after treatment initiation. The significance of these differences was tested using a Wilcoxon non-parametric paired test.

We performed Spearman correlation tests between indinavir dose, indinavir AUC,  $C_{max}$ ,  $C_{trough}$  and  $\Delta TC$ ,  $\Delta HDL$ ,  $\Delta trig$ ,  $\Delta gly$  and  $\Delta CrCl$ . We used Wilcoxon non-parametric tests to compare these differences between patients with or without an indinavir  $C_{trough}$  over the upper limit defined in the therapeutic index (550 ng/mL). We studied the link between the appearance of grade 2 diarrhoea (yes/no) between treatment initiation and W4 and indinavir dose, indinavir AUC,  $C_{max}$  and  $C_{trough}$  using a Wilcoxon non-parametric test, and we studied the association with or without an indinavir  $C_{trough} > 550$  ng/mL using a Fisher exact test.

We assessed the relation between the genetic polymorphisms remaining in the final population model and indinavir dose, indinavir AUC,  $C_{max}$  and  $C_{trough}$  and the relation between these genetic polymorphisms and the short-term efficacy and safety outcomes using Wilcoxon non-parametric tests.

We also derived AUC,  $C_{max}$  and  $C_{trough}$  for ritonavir and performed Spearman correlation tests with  $\Delta TC$ ,  $\Delta HDL$ ,  $\Delta trig$ ,  $\Delta gly$  and  $\Delta CrCl$  as well as Wilcoxon non-parametric tests on the appearance of grade 2 diarrhoea.

**Results**

**Patients**

Forty-two patients were included in this treatment group of the COPHAR 2 ANRS–111 trial. However, one patient withdrew from the study, and one switched to another PI during the first week of treatment. We therefore obtained PK data from 40 patients (27 men, 13 women) with a median age of 36.5 years (range 20.0–59.0 years). Table 1 summarizes the main characteristics of the patient cohort.

Both of the approaches used to allocate the ethnic group provided corroborating results. Using the civic information we allocated 20 patients to the African group and 20 to the Caucasian group. Because information for all genotypes was missing for all genotypes, the Structure software allocated 19 patients to the Caucasian group and 20 to the African group. In the resulting two ethnic groups, Hardy–Weinberg proportions were respected for all polymorphisms under study, as shown in Table 2.

**Indinavir pharmacokinetics**

Two samples were missing, the trough and the 6 h concentrations, for two patients, and only the trough concentration was available for a second patient. Among the 155 samples, two indinavir plasma concentrations in one patient were below the LOQ (at 1 h and at trough), and these were

discarded from further analysis. Figure 1a shows the plot of indinavir plasma concentrations at W2 versus time, revealing a high inter-individual variability.

The best error model was a proportional error model. The population estimates are displayed in Table 3. All of the relative standard errors (RSE) were below 25% with the exception of  $k_a$  and  $\omega_{V/F}$  (around 30 and 60%, respectively). The inter-individual variance of  $k_a$  in this study was rather important (above 100%). The simulated median and the 90th interval are given in Fig. 2a together with all of the observed concentrations of indinavir. This graph provides good evidence of the adequacy of the model.

From that basic model, we first tested the effects of the covariates on the individual parameter estimates. Effects of age ( $P=0.03$ ) and the ABCB1 exon 26 polymorphism ( $P=0.09$ ) on  $Cl/F$  and of the Centers for Disease Control (CDC) classification ( $P=0.09$ ) and the *CYP3A4*\*1B polymorphism ( $P=0.09$ ) on  $k_a$  were found. Both ABCB1 exon 26 and the *CYP3A4*\*1B polymorphism variables were dichotomised in mutant homozygotes versus other genotypes. Following a forward selection based on LRT, the population model had *CYP3A4* effect on  $k_a$  ( $P=0.02$ ) and an age effect on  $Cl/F$  ( $P=0.03$ ). The age effect on clearance was withdrawn from the model after the backward selection based on the permutation test. In the final model, the absorption rate constant was decreased by 70% ( $P=0.04$ , LRT by permutation) in patients with the \*1B\*1B genotype for the *CYP3A4* allele:

$$k_a = 2.1 \times e^{-1.3 \times CYP3A4} \text{ with } \begin{cases} CYP3A4 = 0 \text{ for patients } CYP3A4 * 1A * 1A \text{ or } CYP3A4 * 1A * 1B \\ CYP3A4 = 1 \text{ for patients } CYP3A4 * 1B * 1B \end{cases}$$

**Table 1** Characteristics of the patient cohort ( $n = 40$ )

Characteristics of the patients	Median (range)
Age (years)	36.5 (20.0–59.0)
BMI (kg/m <sup>2</sup> )	22.6 (17.5–35.8)
Weight (kg)	68.0 (45.0–103.0)
Creatinine clearance (mmol/L)	95.4 (57.4–245.1)
Albumin (g/L)	38.4 (25.5–47.4)
Orosomucoid (g/L)	1.0 (0.5–2.9)
	Number of patients (%)
Dose (400/600/800 mg)	26 (65)/8 (20)/6 (15)
Coadministration of AZT/3TC (y/n)	33 (83)/ 7 (17)
Coinfection VHB/VHC (yes/no) <sup>a</sup>	7 (18)/32 (82)
Good adherence (yes/no)	15 (38)/25 (62)
Sex (male/female)	27 (68)/13 (32)
Ethnic group (African/Caucasian)	20 (50)/20 (50)
CDC classification for HIV infection (A or B/C)	30 (75)/10 (25)

BMI, Body mass index; AZT/3TC, zidovudine lamivudine combination; VHC/VHB, coinfection by hepatitis C or B; CDC Centers for Disease Control; HIV, human immunodeficiency virus

<sup>a</sup>Data on one patient are missing

**Table 2** Distribution of the genetic polymorphisms within each ethnic group and Hardy–Weinberg P values

Genetic polymorphisms	Number of patients (%)	H-W P-value
African		
ABCB1 exon 26 (CC/CT/TT)	11 (55)/9 (45)/ 0 (0)	0.43
ABCB1 exon 21 (GG/GT/TT)	19 (95)/1 (5)/ 0 (0)	0.99
<i>CYP3A5</i> (4*1/3*1/_2*1)	0 (0)/8 (40)/12 (60)	0.53
<i>CYP3A4*1B</i> (*1A*1A/*1A*1B/*1B*1B)	9 (45)/8 (40)/3 (15)	0.86
Caucasian		
ABCB1 exon 26 (CC/CT/TT)	2 (12)/12 (70)/3 (18)	0.22
ABCB1 exon 21 (GG/GT/TT)	4 (21)/11 (58)/4 (21)	0.79
<i>CYP3A5</i> (4*1/3*1/_2*1)	18 (100)/0 (0)/0 (0)	1
<i>CYP3A4*1B</i> (*1A*1A/*1A*1B/*1B*1B)	0 (0)/3 (16)/16 (84)	0.93

H-W P value, Hardy–Weinberg P value according to the H–W proportions test

Data on all genotypes were missing for one patient; data on the ABCB1 exon 26 and *CYP3A4* genotypes were both missing for a second patient; data on the genotype for ABCB1 exon 26 were missing for a third patient

The population parameters of this final model and their RSE are given in Table 3 for the 38 patients with data available genotyping for *CYP3A4\*1B* polymorphism. The inter-individual variability for  $k_a$  decreased by 27% from the basic model with the incorporation of the covariate, and residual variability was 44.7%.

#### Ritonavir pharmacokinetics

For one patient, only data on the indinavir concentrations were available and there was no data on ritonavir concentration; consequently, we only analysed ritonavir data for 39 patients. The same five samples for indinavir mentioned in the preceding section were also missing. Among the 151 samples, two ritonavir plasma concentrations at 1 h and at trough in one patient and one concentration at 12 h in another

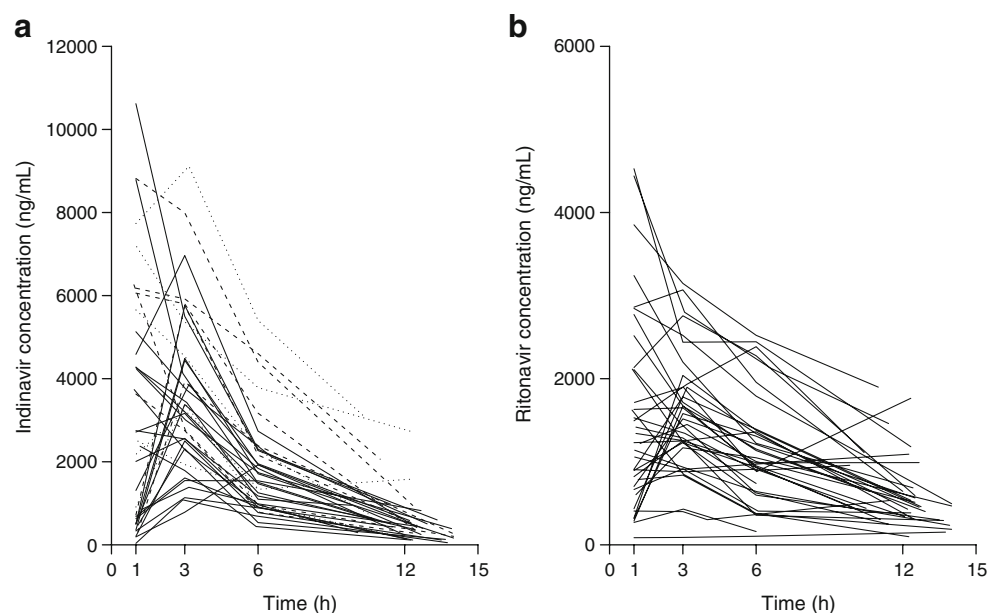
patient were below the LOQ and were discarded. Observed plasma concentrations are given in Fig. 1b, and it should be noted that some patients showed high plasma concentrations (above 2000 ng/mL) for a dose of 100 mg b.i.d.

A proportional error model was selected. The population estimates are displayed in Table 4. All of the RSE were below 25% with the exception of  $k_a$ ; this was partly attributable to the sparse design and to the  $\omega_{Cl/F}$ , as observed for the indinavir data. The VPC obtained with the basic model parameters estimates is given in Fig. 2b, together with the concentrations observed.

The results of the basic model evaluation were very satisfactory.

Effects of orosomuroid ( $P=0.03$ ), albumin levels ( $P=0.04$ ) and *CYP3A5* polymorphism (patients with two wild alleles at most vs. other genotypes,  $P=0.04$ ) on Cl/F were

**Fig. 1** Observed plasma indinavir concentration (a) and plasma ritonavir concentration (b) versus time in samples collected 2 weeks after treatment initiation, in 40 human immunodeficiency virus (HIV) naive-patients receiving indinavir plus 100 mg of ritonavir twice daily (b.i.d). In the indinavir plot, the *solid lines* correspond to an indinavir dose of 400 mg b.i.d., the *dashed lines* to 600 mg b.i.d. and the *dotted lines* to 800 mg b.i.d. Sampling times following drug administration were measured by the nurse. Concentrations were assumed at steady state, trough concentrations are those of samples taken following drug intake at sampling times deduced from the patient record



**Table 3** Population pharmacokinetic parameters of indinavir for the basic and the final model: estimates and relative standard error

Parameters	Basic model (n=40)		Covariate model (n=38)	
	Estimates	RSE (%)	Estimates	RSE (%)
$k_a$ (h <sup>-1</sup> )	1.3	33.7	2.1	44.1
$\beta_{k_a}^{CYP3A4}$	-	-	-1.3	42.0
Cl/F (L/h)	21.9	6.9	22.2	6.9
V/F (L)	93.9	8.2	97.3	9.3
$\omega k_a$ (%)	118.0	22.9	98.2	28.7
$\omega$ Cl/F (%)	34.4	15.0	34.9	15.0
$\omega$ V/F (%)	19.3	66.8	21.6	57.8
$\sigma$ (%)	44.5	8.9	44.7	8.6

$k_a$ , absorption rate constant; Cl/F, oral clearance; V/F, oral volume of distribution; RSE, relative standard error

found on the individual parameters by the non-parametric tests, as were effects of HIV disease status ( $P=0.05$ ) on  $k_a$  and creatinine clearance ( $P=0.1$ ) on V/F. In the final model, an increase of 0.5 g/L in orosomucoid from the median (1 g/L) was associated with a clearance decrease of 28% ( $P=0.03$ , LRT by permutation):

$$Cl/F = 8.3 \times Orosomucoid^{-0.8}$$

The population parameters of this model and their RSE are given in Table 4.

#### Link between indinavir and ritonavir PK parameters

Four positive correlations between individual parameters of ritonavir and indinavir were found to be significant. There was a relationship between the indinavir and ritonavir absorption rate constant ( $r=0.4$ ,  $P=0.005$ ). Indinavir clearance was strongly correlated to ritonavir clearance ( $r=0.6$ ,  $P < 0.0001$ ) and to a smaller degree to ritonavir volume of distribution ( $r=0.4$ ,  $P < 0.01$ ), while indinavir volume of distribution was

highly correlated to ritonavir volume of distribution ( $r=0.5$ ,  $P < 0.002$ ).

#### Concentrations link with short-term efficacy and safety

There was a significant decrease in viral load in the first 2 weeks of treatment, and a significant increase in total cholesterol, glycaemia and triglycerides in the first 4 weeks of treatment, as shown in Table 5.

The decrease in log viral load was significantly associated with higher indinavir AUC ( $r = -0.4$ ,  $P = 0.03$ ) and  $C_{trough}$  ( $r = -0.4$ ,  $P = 0.03$ ), as shown in Fig. 3. No significant difference in viral load decrease was found between the five patients with a  $C_{trough}$  below the lower limit of the therapeutic range and the 35 patients with a  $C_{trough}$  above this value.

Further, no significant relationship was found between indinavir nor ritonavir concentrations and safety measurements or grade 2 diarrhoea. No nephrolithiasis has been reported in the COPHAR 2–ANRS 111 trial, which has prevented us from analysing the link between concentrations and this adverse event associated with indinavir.

The genetic covariate kept in the final population PK model was the *CYP3A4*\*1B polymorphism categorised in two classes: \*1B\*1B versus other genotypes. Both  $C_{max}$  and increase in triglycerides were found to be significantly associated with the *CYP3A4*\*1B polymorphism, although the correlation was not significant. The  $C_{max}$  was significantly lower in patients homozygous for the \*1B allele (median 3.6, range 2.2–5.2 ng/mL) than in the other groups (mean 4.4, range 2.2–8.3 ng/mL) ( $P=0.04$ ), and the increase in triglycerides was also significantly smaller (mean 0.1, range -0.7 to 1.4 vs. mean 0.6, range -0.5 to 1.7 mmol/L, respectively;  $P=0.02$ ), as illustrated by Fig. 4. In terms of the efficacy, no significant association was found between the *CYP3A4*\*1B\*1B genotype and the  $C_{trough}$  or the log viral decrease.

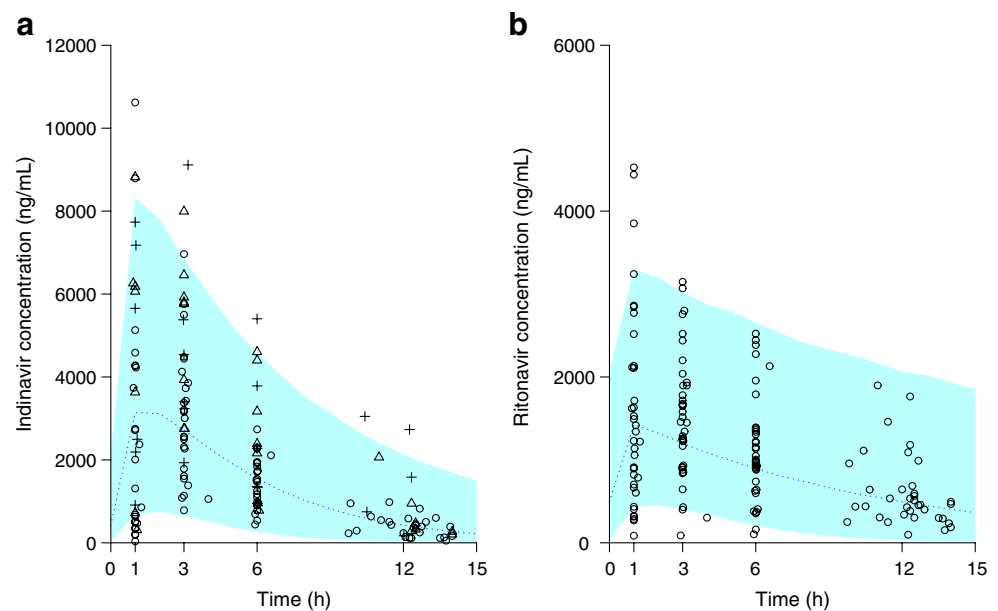
The various doses of indinavir were not found to be associated with the *CYP3A4*\*1B polymorphism, short-term

**Table 4** Population pharmacokinetic parameters of ritonavir for the basic and the final model: estimates and RSE

Parameters	Basic model (n=39)		Covariate model (n=39)	
	Estimates	RSE (%)	Estimates	RSE (%)
$k_a$ (h <sup>-1</sup> )	2.4	98.7	2.2	93.5
Cl/F (L/h)	8.7	9.4	8.3	9.0
$\beta_{Cl}^{Orosomucoid}$	-	-	-0.8	46.5
V/F (L)	61.6	8.6	60.7	8.7
$\omega k_a$ (%)	357.7	21.9	346.8	21.5
$\omega$ Cl/F (%)	55.9	12.2	52.4	12.4
$\omega$ V/F (%)	22.8	53.9	23.3	51.5
$\sigma$ (%)	30.4	8.4	30.3	8.4



**Fig. 2** Visual predictive check of the basic population pharmacokinetics (PK) model: comparison between the median (line) and the 90th interval (shaded area) predicted for 1000 simulated data sets and the observed concentrations of indinavir (a) and of ritonavir (b). Indinavir plot: open circles indinavir dose of 400 mg, open triangles indinavir dose of 600 mg, crosses indinavir dose of 800 mg



efficacy or safety, which negated its potential confounding effect.

## Discussion

The PK of indinavir was analysed using a one-compartment model with first-order absorption and elimination at steady-state. The estimated clearance and volume of distribution were 22.2 L/h and 97.3 L, respectively, both of which are in the range of those obtained in previous studies [9, 11, 32]. In this study, ABCB1 exons 26 and 21 and the *CYP3A5*\*3 and \*6 polymorphisms were not found to significantly influence the PK of indinavir: the absorption rate was  $0.6 \text{ h}^{-1}$  for *CYP3A4*\*1B\*1B patients and  $2.1 \text{ h}^{-1}$  for *CYP3A4*\*1A\*1A or *CYP3A4*\*1A\*1B patients. The *CYP3A* enzymes are distributed in both hepatocytes and enterocytes [33] and

their inhibition by ritonavir is well-documented [34–36]. In vivo, the genotype–phenotype correlation for *CYP3A4*\*1B remains a subject of debate [37–40]; however, *CYP3A4*\*1B has been related to increased transcription [41] in vitro. We hypothesised that in *CYP3A4*\*1B\*1B patients, the ritonavir inhibition potency is lowered, leading to a higher first pass effect of indinavir, although this does not impact on its clearance. The potential confounding effect of the ethnic group was discarded, as this covariate was not significantly related to indinavir individual parameters in the sample. However, this finding is more relevant clinically in an African population given the extremely low frequency of the *CYP3A4*\*1B\*1B genotype among Caucasians. The primary objective of the COPHAR2 study was not to assess the influence of genetic polymorphisms on indinavir PK, and the use of modelling has helped to circumvent the limited sample size of 40 patients in the study. In addition, most of

**Table 5** Median and range of the studied short-term efficacy and safety measurements and of the change from baseline

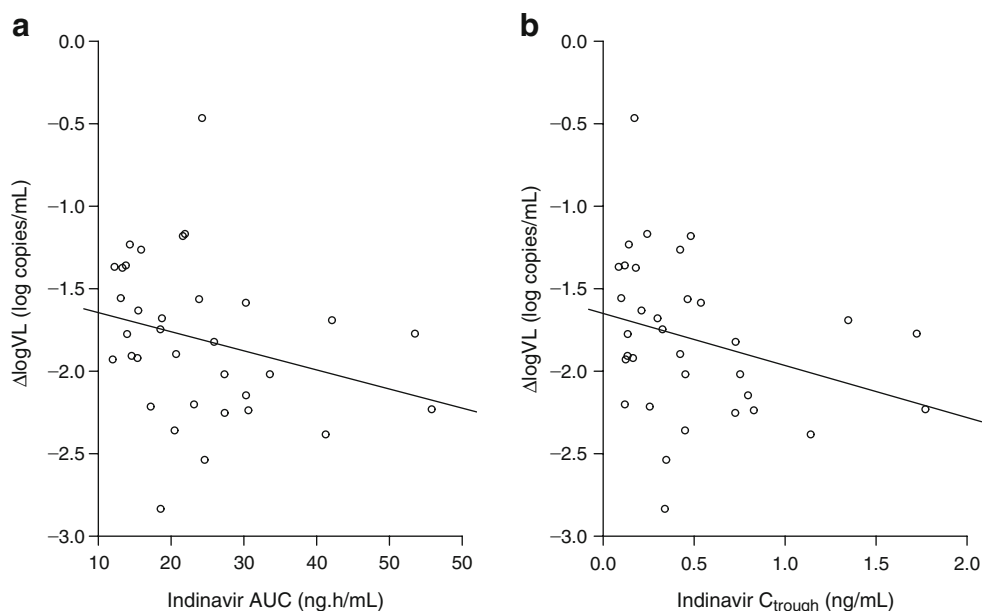
Short-term efficacy and safety measurements	Baseline <sup>a</sup>	W2 or W4 <sup>b</sup>	Difference from baseline	P value
<b>Efficacy</b>				
Log viral load (log copies/mL)	4.9 (3.4–6.3)	2.9 (1.8–4.1)	−1.8 (−2.8 to −0.5)	<0.001
<b>Safety</b>				
Total cholesterol (mmol/L)	4.3 (1.9–7.4)	5.0 (2.9–7.5)	0.8 (0.8–4.7)	<0.001
HDL cholesterol (mmol/L)	1.1 (0.5–1.8)	1.1 (0.4–2.1)	0.1 (−0.7–1.0)	0.09
Glycaemia (mmol/L)	4.7 (3.4–6.0)	4.9 (2.8–7.1)	0.2 (−1.0 to 2.7)	0.013
Triglycerides (mmol/L)	1.0 (0.4–3.0)	1.4 (0.6–4.0)	0.4 (−0.7 to 1.7)	<0.001
Creatinine clearance (mL/min)	98.4 (62.0–195.7)	97.4 (62.8–252.0)	−1.0 (−38.0 to 56.4)	0.5

HDL, High-density lipoprotein

<sup>a</sup> Baseline = Day 0 for log viral load and week (W) 4 for safety

<sup>b</sup> Week 2 for log viral load and week 4 for safety

**Fig. 3** Differences in log viral load ( $\Delta \log VL$ ) observed between treatment initiation and week 2 versus area under the concentration–time curve (a) and trough plasma concentration of indinavir (b) predicted by the model

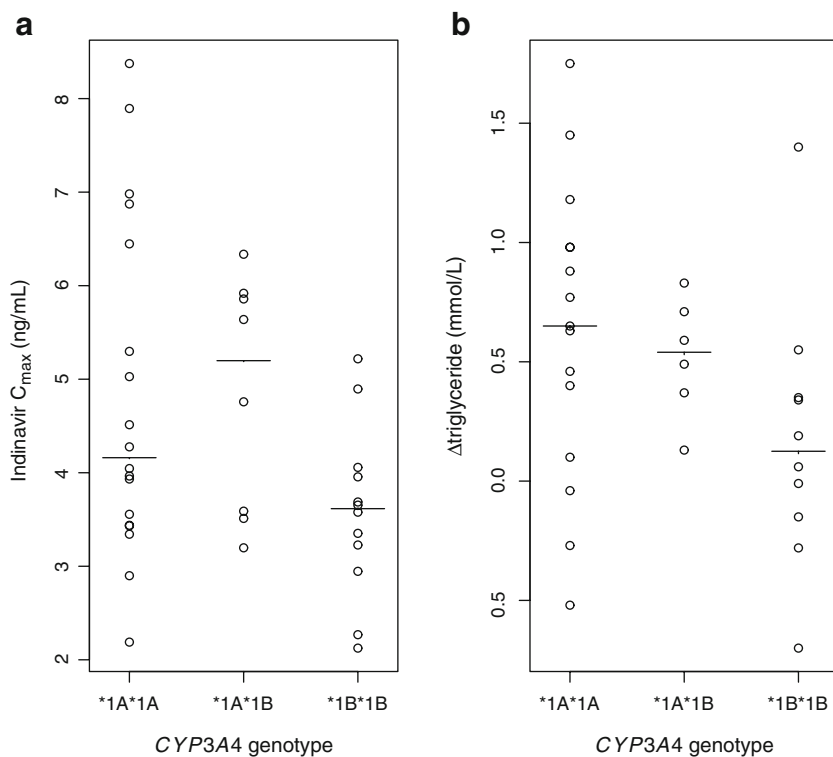


the tests in this study were performed as an exploratory step, and final inclusion in the model was based on permutation to cope with departure from the asymptotic assumption [30]. No evidence for a gender effect was found, as has been reported in a number of other studies on indinavir PK [9–11], but there were only 13 women in the present study. Dose has been found not to influence the PK of indinavir, and the use of ritonavir as a booster has been found to hide the dose non-linearity of indinavir [42]. We did not assess the impact of diet, as these data were not available, but patients were

recommended to ingest the pills with food containing a sufficient amount of fats.

We also performed a population PK analysis of ritonavir concentrations. Ritonavir profiles were adequately described by a one-compartment model with first-order absorption and elimination processes, with estimates of the parameters being in good agreement with those of previous studies [6, 43, 44]. The estimated inter-individual variance for the absorption constant was singularly large. We found a negative relationship between ritonavir clearance and orosomucoid level in

**Fig. 4** Peak indinavir concentrations predicted by the model (a) and differences in triglycerides ( $\Delta \text{triglyceride}$ ) 4 weeks before and after treatment initiation (b) versus *CYP3A4* genotype. The solid line represents the median in each group



plasma. The affinity of ritonavir for orosomucoid protein as well as its impact on PI intracellular concentrations and efficacy has been described in both in vitro and in vivo studies [45–47]. In patients with high orosomucoid plasma levels, the decrease in the unbound fraction of ritonavir led to a lower clearance.

In the analysis of both PI, the few concentrations (1.3 and 2% for indinavir and ritonavir, respectively) below the LOQ were discarded. Using this approach, SAEM acquires a less important bias than it would with LOQ/2 [48]. There is no proper method in MONOLIX 2.1 to handle LOQ.

In the analysis of the link between indinavir and ritonavir concentrations, we chose not to include ritonavir as a covariate in the indinavir model, as performed in previous studies [10, 11]. Indeed, such parameterisation assumes a unidirectional influence of ritonavir on indinavir, which is not true. Ritonavir concentrations, when ritonavir is given with lopinavir, are lower than when ritonavir is given with indinavir [44]. We have instead emphasised the different levels of interaction between indinavir and ritonavir PK, especially at the absorption step, with the strong correlation between their absorption constant, but also in terms of bioavailability, as the oral clearances and volumes of distribution were highly correlated.

In order to properly model such an interaction between PI, a joint population analysis of concentrations of indinavir and ritonavir should be considered with correlated absorption constants and bioavailabilities.

We observed significant changes in viral load after 2 weeks of treatment, and we confirmed the association between high indinavir trough and mean concentrations and a greater decrease of viral load, which has already been described in PI-naïve patients [49–51]. We did not find any relationship between *CYP3A4\*1B* polymorphism and viral load decrease. We also observed a significant increase, after 4 weeks of treatment, of total cholesterol, glycaemia and triglycerides, as already reported [52], which was, however, not significantly related to indinavir concentrations at week 2. Ritonavir was found at singularly high levels in our study and is known to affect metabolic profiles, yet we found no evidence of an association between ritonavir levels and safety measurements. In patients homozygous for the *CYP3A4\*1B* allele, the ritonavir-decreased inhibition on indinavir metabolism led to significantly lower indinavir  $C_{max}$  and appeared to impact at a metabolic level through a significantly lower increase in triglycerides in these patients.

## Conclusion

We have developed and validated models for indinavir and ritonavir PK with reduced sampling in indinavir HAART patients. Both the average and trough concentrations were

found to be predictors of the viral load decline. Only the *CYP3A4\*1B* allele was found to influence indinavir absorption and biochemical safety, but no evidence was found of an impact of the five genetic polymorphisms studied on indinavir efficacy.

**Acknowledgements** Steering committee of the COPHAR2 ANRS-111 trial: principal investigators: D. Salmon- Céron, X. Duval, statistics: F. Mentré; other members: S. Auleley, M. Biour, M.J. Commo, B. Diquet, C. Goujard, C. Katlama, C. Lascoux, M. Legrand, A. Métro, G. Peytavin, E. Rey, A.M. Taburet, J.M. Tréluyer.

Safety committee: S. Auleley, M. Biour, A. Métro, C. Lascoux, D. Salmon-Céron. Pharmacological monitoring committee: X. Duval, E. Rey, J.M. Tréluyer. Independent committee: C. Rouzioux, C. Piketti, P. Flandre, M. Zenut, P. Marquet.

Clinical centers: Dr. Bentata, Dr. Mansouri, Mme Touam, Pr. Sereni, Dr. Lascoux, Dr. Pintado, Dr. Goujard, Mme Mole, Dr. Sellier, Dr. Bendenoun, Dr. Rami, Mme Parrinello, Dr. Jeantils, Mme Tassi, Pr. Vittecoq, Dr. Teicher, Mme Mallet, Pr. Dupont, Dr. Lahoulou, Soeur Azar, Pr. Rosebaum, Dr. Slama, Dr. Naï-Ighil Baakili, Dr. Courtial-Destembert, Pr. Vildé, Pr. Leport, Dr. Duval, Dr. Al Kaied, Pr. Salmon, Dr. Spiridon, Dr. Lesprit, Mme Chesnel, Pr. Katlama, Dr. Schneider, Mme Schoen, Pr. Molina, Dr. Ponscarne, Dr. Colin de verdière, Pr. Morlat, Dr. Bonarek, Dr. Joly, Dr. Ralaimazava, Mme Meridda, Mme Le Gac, P. Raffi, Dr. Allavena, Mme Hüe, Mme Sicot, Dr. Perré, Dr. Leautez, Dr. Aubry, Mme Suaud, Pr. Dellamonica, Dr. Rahelinirina, Pr. Michelet, Dr. Bouvier, Pr. Bazin, Dr. Goubin, Pr. May, Dr. Boyer, Pr. Rouveix, Dr. Dupont, Mme Berthé.

Pharmacological centers: Dr. Rey, Pr. Tréluyer, Dr. Abbara, Dr. Audoul, Dr. Tran, Dr. Sauvageon, Dr. Poirier, Dr. Taburet, Dr. Vincent, Dr. Aymard, Dr. Peytavin, Dr. Lamotte, Dr. Dailly, Dr. Garraffo, Dr. Lavrut, Dr. Mollimard, Dr. Titier, Dr. Tribut, Dr. Hulin, Dr. Huet, Dr. Delhotal, Dr. Hoizey.

Virological centers: Pr. Nicolas-Chanoine, Dr. Sousan, Pr. Dény, Dr. Baazia, Dr. Alloui, Pr. Brun-Vézinet, Dr. Chams, Pr. Fleury, Dr. Pellegrin, Dr. Garrigue, Pr. Fremut, Dr. Vabret, Pr. Lebon, Dr. Krivine, Pr. Calvez, Dr. Gourlain, Dr. Amellal, Pr. Bouvier-Alias, Pr. Norman, Dr. Idri, Pr. Chambreuil, Dr. Poirier, Pr. Mazon, Pr. Le Faou, Dr. Vénard, Pr. Billaudel, Dr. Ferre, Pr. Rouzioux, Dr. Burgard, Pr. Lefevre, Dr. Cottalorda, Pr. Dussaix, Dr. Bensidhoum, Pr. Colimon, Dr. Ruffault, Dr. Maillard, Pr. Morinet, Dr. Palmer, Pr. Nicolas, Dr. Zalta.

Monitoring: S. Auleley, E. Marcault, F. Mentré. Statistics: E. Bougen, F. Mentré, X. Panhard.

The authors thank the study participants and the Agence de Recherche Nationale sur le SIDA (ANRS, Essai 111) for financial support.

We also acknowledge Dr. Emmanuelle Comets, Pr. Marc Lavielle and Dr. Emmanuelle Génin for their valuable advices during the analysis.

J. Bertrand was supported by a grant from Servier Research Group, France.

## References

1. Solas C, Lafeuillade A, Halfon P et al (2003) Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 47:238–243
2. Letendre S, Marquie-Beck J, Capparelli E et al (2008) Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch Neurol* 65:65–70

3. Collin F, Chêne G, Retout S, Peytavin G, Salmon D, Bouvet E, Raffi F, Garraffo R, Mentré F, Duval X, ANRS CO8 APROCO-Copilote Study Group (2007) Indinavir trough concentration as a determinant of early nephrolithiasis in HIV-1-infected adults. *Ther Drug Monit* 29:164–170
4. Flexner C (1998) HIV-protease inhibitors. *N Engl J Med* 338:1281–1292
5. Von Moltke LL, Greenblatt DJ, Grassi JM et al (1998) Protease inhibitors as inhibitors of human cytochromes P450: high risk associated with ritonavir. *J Clin Pharmacol* 38:106–111
6. Hsu A, Granneman GR, Bertz RJ (1998) Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet* 35:275–291
7. Soriano V, Garcia-Gasco P, Vispo E, Ruiz-Sancho A, Blanco F, Martyn-Carbonero L, Rodryguez-Novoa S, Morello J, de Mendoza C, Rivas P, Barreiro P, Gonzalez-Lahoz J (2008) Efficacy and safety of replacing lopinavir with atazanavir in HIV-infected patients with undetectable plasma viraemia: final results of the SLOAT trial. *J Antimicrob Chemother* 61:200–205
8. Collet-Teixeira S, De Lorenzo F, Waters L, Fletcher C, Back D, Mandalia S, Pozniak A, Yilmaz S, McGregor JL, Gazzard B, Boffito M (2009) Impact of different low-dose ritonavir regimens on lipids, CD36, and adipophilin expression. *Clin Pharmacol Ther* 85(4):375–378
9. Csajka C, Marzolini C, Fattinger K et al (2004) Population pharmacokinetics of indinavir in patients infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 48:3226–32
10. Brendel K, Legrand M, Taburet A et al (2005) Population pharmacokinetic analysis of indinavir in hiv-infected patient treated with a stable antiretroviral therapy. *Fundam Clin Pharmacol* 19:373–383
11. Kappelhoff BS, Huitema ADR, Sankatsing SUC et al (2005) Population pharmacokinetics of indinavir alone and in combination with ritonavir in HIV-1-infected patients. *Br J Clin Pharmacol* 60:276–286
12. Solas C, Simon N, Drogoul MP et al (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
13. Verstuyft C, Marcellin F, Morand-Joubert L et al (2005) Absence of association between MDR1 genetic polymorphisms, indinavir pharmacokinetics and response to highly active antiretroviral therapy. *AIDS* 19:2127–2131
14. Anderson PL, Lamba J, Aquilante CL et al (2006) Pharmacogenetic characteristics of indinavir, zidovudine, and lamivudine therapy in HIV-infected adults: a pilot study. *J Acquir Immune Defic Syndr* 42:441–449
15. Lamba JK, Lin YS, Schuetz EG et al (2002) Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliver Rev* 54:1271–1294
16. Lichterfeld M, Nischalke HD, Bergmann F et al (2002) Long-term efficacy and safety of ritonavir/indinavir at 400/400 mg twice a day in combination with two nucleoside reverse transcriptase inhibitors as first line antiretroviral therapy. *HIV Med* 3:37–43
17. Duval X, Mentre F, Lamotte C et al (2005) Indinavir plasma concentration and adherence score are codeterminant of early virologic response in HIV-infected patients of the APROCO cohort. *Ther Drug Monit* 27:63–70
18. Dieleman JP, Gyssens IC, van der Ende ME et al (1999) Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. *AIDS* 13:473–478
19. Duval X, Mentré F, Rey E et al (2009) Benefit of therapeutic drug monitoring of protease inhibitors in HIV-infected patients depends on PI used in HAART regimen—ANRS 111 trial. *Fundam Clin Pharmacol* (in press)
20. Hirt D, Mentré F, Tran A et al (2008) Effect of CYP2C19 polymorphism on nelfinavir to M8 biotransformation in HIV patients. *Br J Clin Pharmacol* 65:548–557
21. Carrieri P, Cailleton V, Le Moing V et al (2001) The dynamic of adherence to highly active antiretroviral therapy: results from the french national APROCO Cohort. *J Acq Immun Def Syndr* 28:232–239
22. Cascorbi I, Gerloff T, Johne A et al (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 69:169–174
23. Dally H, Edler L, Jager B et al (2003) The CYP3A4\*1B allele increases risk for small cell lung cancer: effect of gender and smoking dose. *Pharmacogenetics* 13:607–618
24. Crow J (1999) Hardy, Weinberg and language impediments. *Genetics* 152:821–825
25. Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
26. Kuhn E, Lavielle M (2005) Maximum likelihood estimation in nonlinear mixed effects models. *Comput Stat Data Anal* 49:1020–1038
27. Lavielle M (2008) MONOLIX (MOdèles NON Linéaires à effets mixtes). MONOLIX group, Orsay, France. Available at: <http://software.monolix.org/index.php>
28. Lavielle M, Mentré F (2007) Estimation of population pharmacokinetic parameters of saquinavir in HIV patients with the MONOLIX software. *J Pharmacokinet Pharmacodyn* 34:229–249
29. Gelman A, Carlin JB, Stern HS et al (1995) Bayesian data analysis. Chapman & Hall, London
30. Bertrand J, Comets E, Mentré F (2008) Comparison of model-based tests and selection strategies to detect genetic polymorphisms influencing pharmacokinetic parameters. *J Biopharm Stat* 18:1084–1102
31. Manly B (1998) Randomization, bootstrap and Monte Carlo methods in biology. Chapman & Hall, London
32. Goujard C, Legrand M, Panhard X et al (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
33. Fuhr U, Jetter A, Kirchheiner J (2007) Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the “cocktail” approach. *Clin Pharmacol Ther* 81:270–283
34. Ikezoe T, Hisatake Y, Takeuchi T et al (2004) HIV-1 protease inhibitor, ritonavir: a potent inhibitor of CYP3A4, enhanced the anticancer effects of docetaxel in androgen-independent prostate cancer cells in vitro and in vivo. *Cancer Res* 64:7426–7431
35. Zhou S, Yung Chan S, Cher Goh B et al (2005) Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. *Clin Pharmacokinet* 44:279–304
36. Rittweger M, Arastéh K (2007) Clinical pharmacokinetics of darunavir. *Clin Pharmacokinet* 46:739–756
37. Spurdle A, Goodwin B, Hodgson E et al (2002) The CYP3A4\*1B polymorphism has no functional significance and is not associated with risk of breast or ovarian cancer. *Pharmacogenetics* 12:355–366
38. He P, Court MH, Greenblatt DJ et al (2005) Genotype-phenotype associations of cytochrome P450 3A4 and 3A5 polymorphism with midazolam clearance in vivo. *Clin Pharmacol Ther* 77:373–387
39. Hesselink DA, van Schaik RH, van der Heiden IP et al (2003) Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 74:245–254

40. Tran A, Jullien V, Alexandre J et al (2006) Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. *Clin Pharmacol Ther* 79:570–580
41. Amirimani B, Ning B, Deitz AC et al (2003) Increased transcriptional activity of the CYP3A4\*1B promoter variant. *Environ Mol Mutagen* 42:299–305
42. Yeh K, Stone J, Carides A et al (1999) Simultaneous investigation of indinavir nonlinear pharmacokinetics and bioavailability in healthy volunteers using stable isotope labeling technique: study design and model-independent data analysis. *J Pharm Sci* 88:568–573
43. Lu JF, Blaschke TF, Flexner C et al. (2002) Model-based analysis of the pharmacokinetic interactions between ritonavir, nelfinavir and saquinavir after simultaneous and staggered oral administration. *Drug Metab Dispos* 30:1455–1461
44. Kappelhoff BS, Huitema ADR, Crommentuyn KML et al (2005) Development and validation of a population pharmacokinetic model for ritonavir used as a booster or as an antiviral agent in HIV-1-infected patients. *Br J Clin Pharmacol* 59:174–182
45. Schön A, del Mar Ingaramo M, Freire E (2003) The binding of HIV-1 protease inhibitors to human serum proteins. *Biophys Chem* 105:221–230
46. Jones K, Hoggard PG, Khoo S et al (2001) Effect of alpha1-acid glycoprotein on the intracellular accumulation of the HIV protease inhibitors saquinavir, ritonavir and indinavir in vitro. *Br J Clin Pharmacol* 51:99–102
47. Zhang X, Schooley R, Gerber J (1999) The effect of increasing alpha1-acid glycoprotein concentration on the antiviral efficacy of human immunodeficiency virus protease inhibitors. *J Infect Dis* 180:1833–1837
48. Samson A, Lavielle M, Mentré F (2006) Extension of the SAEM algorithm to left-censored data in nonlinear mixed-effects model: Application to HIV dynamics model. *Comput Stat Data Anal* 51:1562–1574
49. Fletcher CV (1999) Pharmacologic considerations for therapeutic success with antiretroviral agents. *Ann Pharmacother* 33:989–995
50. Burger DM, Hugen PWH, Aarnoutse RE et al (2001) A retrospective, cohort-based survey of patients using twice-daily indinavir+ritonavir combinations: pharmacokinetics, safety and efficacy. *J Acquir Immune Defic Syndr* 26:218–224
51. Rayner CR, Galbraith KJ, Marriott JL et al (2002) A critical evaluation of the therapeutic range of indinavir. *Ann Pharmacother* 36:1230–1237
52. Behrens G, Dejam A, Schmidt H et al (1999) Impaired glucose tolerance, beta cell function, and lipid metabolism: HIV patients under treatment with protease inhibitors. *AIDS* 13:F63–F70