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## **Population Pharmacokinetics of HIV Therapy in the Swiss HIV Cohort: A First Data Exploration**

### **Study Report**

SHCS Pharmacology #472

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## 1. Introduction

Despite the success of highly potent antiretroviral therapy (HAART), there is still a significant percentage of patients not reaching an adequate suppression of plasma HIV RNA or experiencing drug toxicity. Treatment failure is clearly multifactorial, and has been mainly attributed to pharmacokinetic heterogeneity, genetic variability and poor adherence to therapy. The merge between the SHCS and the Therapeutic Drug Monitoring of Antiretroviral Drugs databases (TDM-ART) initiated early 2005 provided pharmacokinetic and clinical variables together with toxicological and efficacy endpoints, which offers a unique resource for research in the constant evolving field of HIV therapy. Two participating laboratories in Switzerland are providing routine level monitoring since 1999 for 14 antiretroviral drugs to date. Of note, both laboratories participate now to an International External Quality Assurance Program for the analysis of concentrations of antiretroviral drugs (KKG, *Stichting Kwaliteitsbewaking Klinische Geneesmiddelenanalyse en Toxicologie*, Association for Quality Assessment in TDM and clinical Toxicology, The Hague, The Netherlands).

By integrating all the components made available by the SHCS database, a cluster of large-scale population pharmacokinetic (PK), pharmacodynamic (PD) and pharmacogenetic (PG) analyses have been initiated. In order to perform such analyses, a thorough analysis of the SHCS data was done, with a specific focus on collecting information relevant to population PK, PD and PG analyses. Owing to the problems inherent to epidemiological data collected in unselected patients over a long period of time, this first data exploration analysis was primarily focused on the following objectives:

1. Identification and selection of the variables of interest from the SHCS database.
2. Data exploration and cleaning
3. Extraction and formatting of the data for population analyses using appropriate queries
4. Descriptive statistics of the data
5. First pharmacokinetic-pharmacogenetic analyses.

The present report presents the data covering the time frame of 1.1.1999 to 1.6.2008.

## 2. Procedure for the merging of data into the SHCS database

PK data emanating from the Division of Clinical Pharmacology and Toxicology of the Lausanne University Hospital (PCL) and from the Laboratory of Clinical Chemistry of the Zürich University Hospital are merged into the SHCS. A schematic representation of the merging procedure is presented in **Figure 1**.

**Figure 1:** Overview of the data merging procedure between the PCL and the ZH centre and the SHCS database.

**Lausanne Laboratory:** Information regarding drugs pharmacokinetics (drug, dosage, time of drug administration and concentrations) as well as co-medications (ART and other drugs) are provided by the TDM-ART database of the PCL. All other information (demographics, physiopathological, environmental and others) is emanating directly from the SHCS database. PK data extraction is undertaken once a week from the PCL database by the SHCS data centre which is integrated into the Lausanne University Hospital, using the tube identification number ("TUBE") as a match between the two databases. Once a month, a back check is performed using the patient identification number ("ID<sub>SHCS</sub>") in order to capture potentially missing data from a patient already present in the SHCS database. This data transfer concerns SHCS participants for any physician mandating the Lausanne laboratory.

**Zürich Laboratory:** PK data measured in the Department of Clinical Chemistry of the Zürich University Hospital is provided electronically by the centre of Zurich along with all the other laboratory results. The data are identified by the SHCS cohort number ("ID<sub>SHCS</sub>"). The laboratory data are imported into the SHCS database as soon as the corresponding clinical information is computerized. The data transfer from the Zurich laboratory is limited to patients under care at the University Hospital.

PK information and co-medications from both laboratories are finally stored in three tables: PHA-IDENTIF, PHA\_RESULT and PHA\_COMEDIC that can be linked to other SHCS tables, enabling the extraction of the needed items for pharmacokinetic analyses (see below).

### 3. Data selection from the SHCS database

The variables selected from the SHCS database that were considered relevant for the analyses can be grouped in five categories that hold the following data items:

- a. **Pharmacokinetic variables:** patient personal identification number, sample identification number, drug analyzed, date and time of blood sampling, date and time of last drug administration, dose in milligrams, number of drug administration per day, measured plasma concentration in  $\mu\text{g/L}$ .
- b. **Demographic variables:** year of birth, weight in kg measured within 6 months of drug analyzed, date of bodyweight measure, height in cm, gender, and ethnicity.
- c. **Comedications:** antiretroviral drugs within 2 weeks of drug analyzed and other medications within 2 weeks of drug analyzed.
- d. **Physiopathological variables:** CD4 cells per  $\mu\text{l}$  measured, date of last CD4 count measure, HIV-1 viral load, date of last viral load measure, bilirubine blood level measurement and date of test, serum creatinine level and date of test.
- e. **Environmental variables:** number of cigarettes per day.
- f. **Others:** Informed consent for genetic testing, DNA sample identification number.

### 4. Data Exploration and Cleaning

#### 4.1 Historical merge of SHCS and TDM-ART databases:

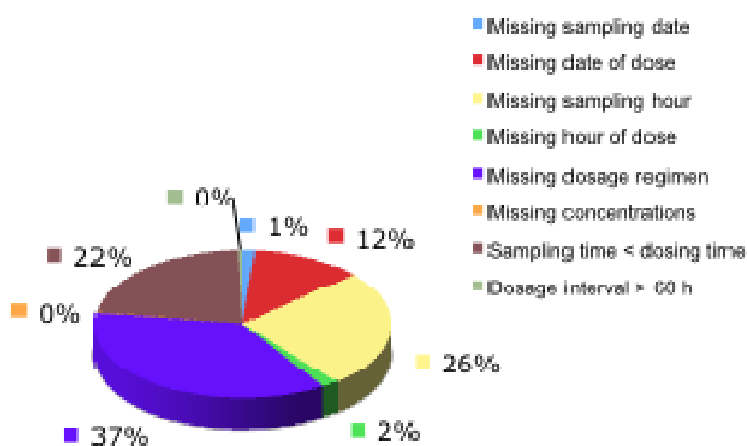
The merge of PK data from the TDM-ART database to the SHCS database was made retrospectively for the period 1999 to 2004 (within the frame of the previous project SHCS # 423, « *Capture of historical pharmacokinetic data in the SHCS*») and prospectively from 2005 onwards. An evaluation of the adequacy of the retrospective merge revealed that only a low percentage (between 9 % and 17 %) of the concentration data were missing in the SHCS database. On the opposite, a much more important number of data (51% and 72% for 2005 and 2006, respectively) were missing after the introduction of the *prospective* merging of data into the SHCS database. This discrepancy was explained by the wrong coding of a variable that was used for data extraction from the TDM-ART. After correction and retrieval of missing data from SHCS by systematically asking all the treating physicians to replace the patients name by the SHCS cohort number, the percentage of mismatch was reduced to about 20% (see below), which was considered acceptable for further investigations. These observations led to a change in the extraction procedure from the TDM-ART database, which is now routinely based on the tube identification number that limits extraction errors.

#### 4.2 Cleaning Therapeutic Drug Monitoring Database

A data cleaning procedure was conducted based on the selection of mandatory variables for population pharmacokinetic analyses. These variables are those stipulated under 3.a. Other variables (3.b to 3.e) were not considered compulsory at that stage.

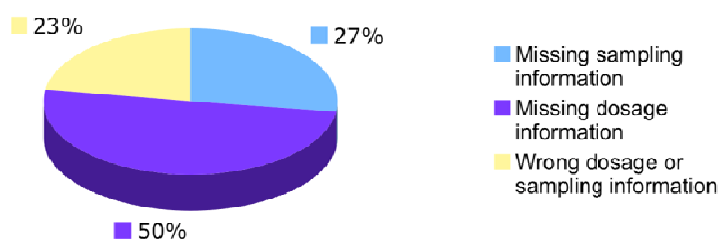
As on January 2008 and before cleaning, the data set consisted of 16'307 drug level measurements. The overall number of pharmacokinetic data available for population analyses was good with only 16 % of the data missing or inappropriately reported. Missing variables including date (day, month and year) and time (hour, minute) of blood sampling or drug intake, missing dosage regimen (dose and frequency of administration) and missing

concentrations were excluded. In certain cases, sampling time was identical to the time of dose intake or the time interval between last reported administered dose and sampling time was more than 60 hours. These data were considered inappropriate and also excluded. A representation of the repartition of the missing pharmacokinetic variables is presented in **Figure 2**.



**Figure 2:** Repartition of the missing pharmacokinetic variables.

The percentage of missing variables grouped by criteria regarding sampling information (date, time, concentrations), dosing information (date, time, dosage regimen), or inappropriate sampling and dosing information is summarized in **Figure 3**. Half of the missing information regarded doses or frequency of drug administration and time of drug intake.



**Figure 3:** repartition of the missing or wrong pharmacokinetic variables grouped by sampling or dosing criteria.

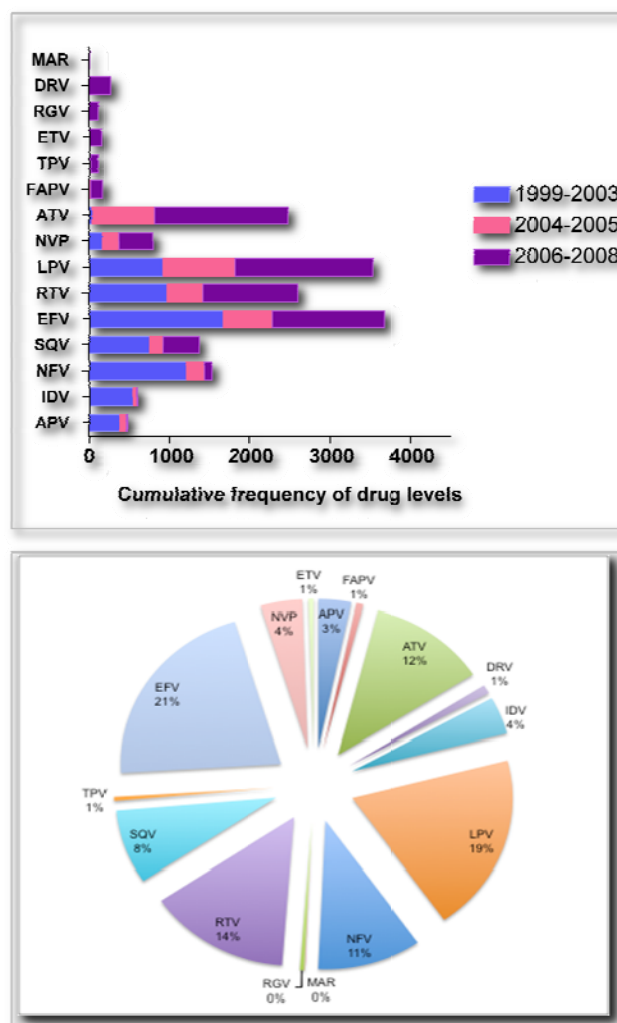
## 5. Data extraction and formatting

Several steps were performed in order to implement the appropriate query for data retrieval from the SHCS database into a format suitable for the population modeling software (NONMEM®) after an automated correction of inappropriate data. Except from extensive formatting issues that will not be detailed, we introduced a new definition table for the classification of non-ART co-medications (captured in the TDM-ART database as free text) into functional variables. In total, 443 co-medications showing influence (based published evidence) on ritonavir, lopinavir, efavirenz and atazanavir elimination as well as absorption for ATV were categorized as “inducers”, “inhibitors” or “having no effect” on the above 4

drugs analyzed as well as “decrease in absorption” for ATV in particular. The codification of the co-medications influencing other antiretroviral drugs needs to be performed.

## 6. Descriptive Statistics

As of May 2008, 13'997 antiretroviral plasma concentration data have been collected from the Lausanne (86%) and Zürich (14%) laboratories and are fully exploitable for data analyses. They involve 3'192 patients and 14 drugs, namely 11 protease inhibitors: amprenavir (APV), fos-amprenavir (FAPV), indinavir (IDV), nelfinavir (NFV), saquinavir (SQV), ritonavir (RTV), lopinavir (LPV), atazanavir (ATV), tipranavir (TPV), darunavir (DRV), 1 integrase inhibitor: raltegravir (RAL), 3 NNRTI: efavirenz (EFV), nevirapine (NVP) and etravirine (ETV) and 1 CCR5 antagonist: maraviroc (MRV). Representations of the frequency of plasma sample measurements per drug over the past 10 years are depicted in **Figure 4**.



**Figure 4:** Cumulated frequency of ART drugs blood level measurements (upper panel) and percentage of cumulative frequency (lowerpanel) from 1998 to 2008.

In parallel, the genetics project of the Swiss HIV Cohort Study includes genetic information on an increasing number of participants. In total, 6960 patients have provided consent for genetic testing, and a DNA repositorium already has purified DNA for 2783 patients. A total of 52 genes (212 selected SNPs) have been interrogated for the purpose of various genetic

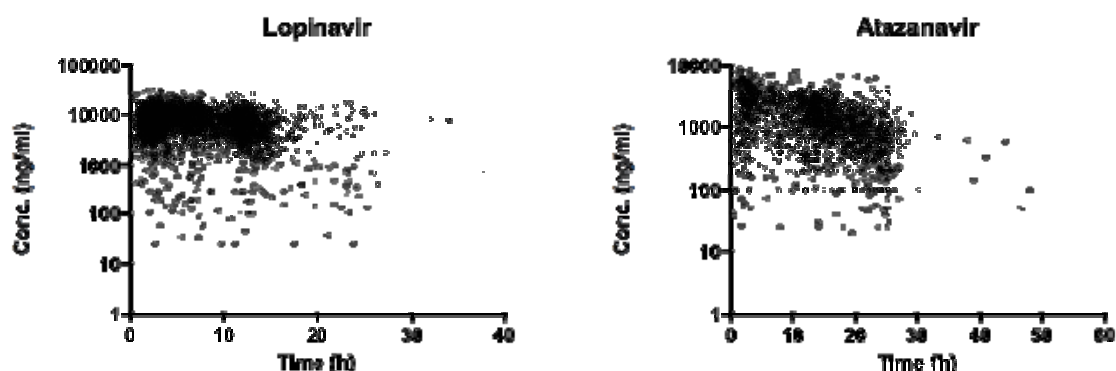
projects. The current database contains 103394 genetic analyses (mean 45 tests/patient). In addition, HLA data is available on 1569 patients and genome-wide genotype on 1095 patients. These numbers are currently expanding at a high rate.

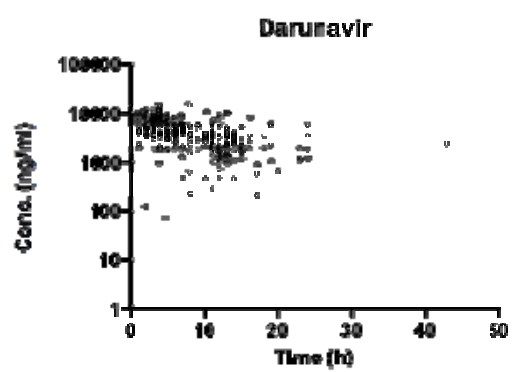
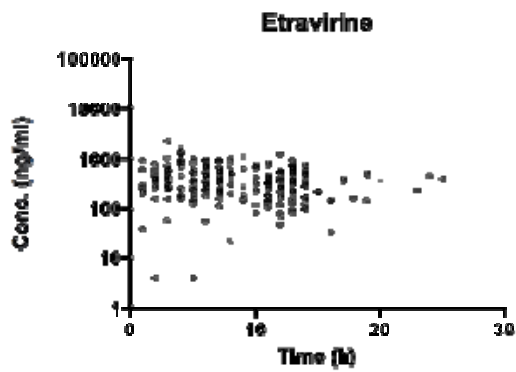
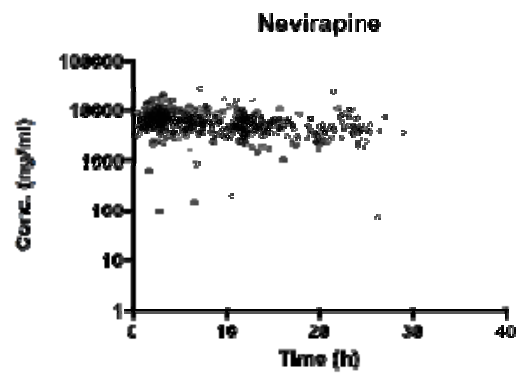
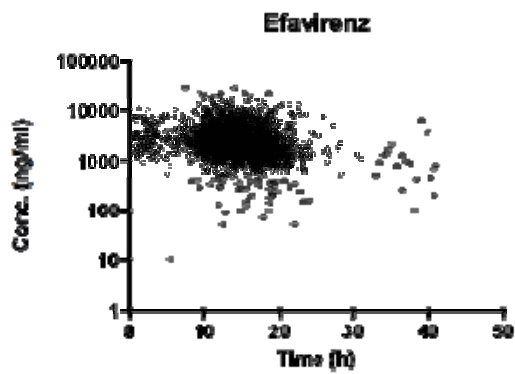
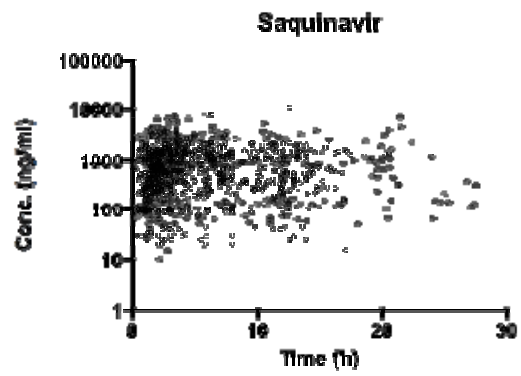
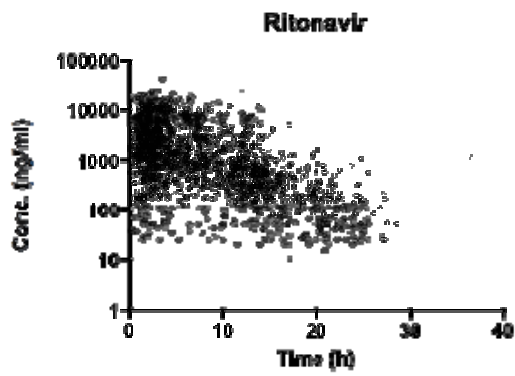
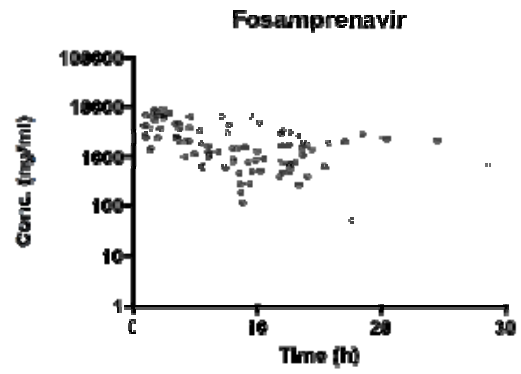
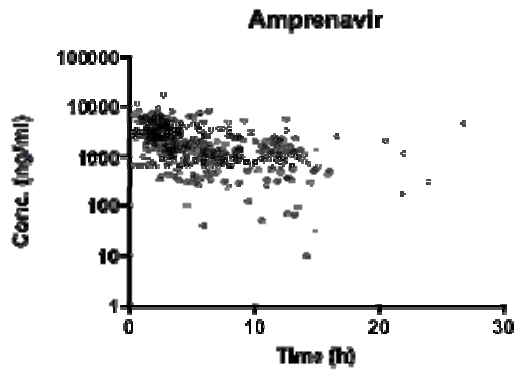
The system in place offers the possibility to retrieve updated data at anytime, including new patients and new drugs, which are now directly accessible for population pharmacokinetic analyses (i.e cleaned and formatted). The SHCS-connected pharmacokinetic database is being maintained and improved by the ongoing, prospective collection of current and recently marketed drugs (RAL, ETV, MRV). Collection of data for new drugs or families of drugs at the late stage of clinical development – rilpivirine, elvitegravir, vicriviroc – with clinical, pharmacokinetic and pharmacogenetic information will be insured. A summary table of the number of drug measurements is provided in **Table 1**.

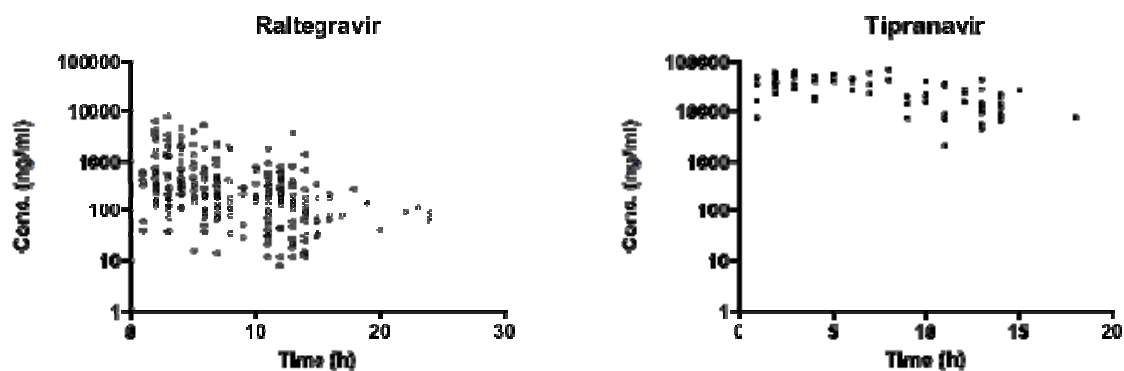
**Table 1:** Summary of the numbers of drug level measurements from the Lausanne and Zürich laboratories from 1999 to 2008.

Year	Number of drug level measurements	
	Lausanne	Zürich
1998	9	--
1999	1041	--
2000	2340	156
2001	1358	402
2002	902	513
2003	793	624
2004	1285	805
2005	1486	481
2006	1532	399
2007	1517	435
2008	1484	415

The concentration-time profile of the most currently used drugs (ATV, LPV, RTV, APV, FAPV, NVP, EFV) and of the newer molecules (RGV, DRV, ETV, TPV updated as of 28.1.2010) are shown in **Figure 5**.

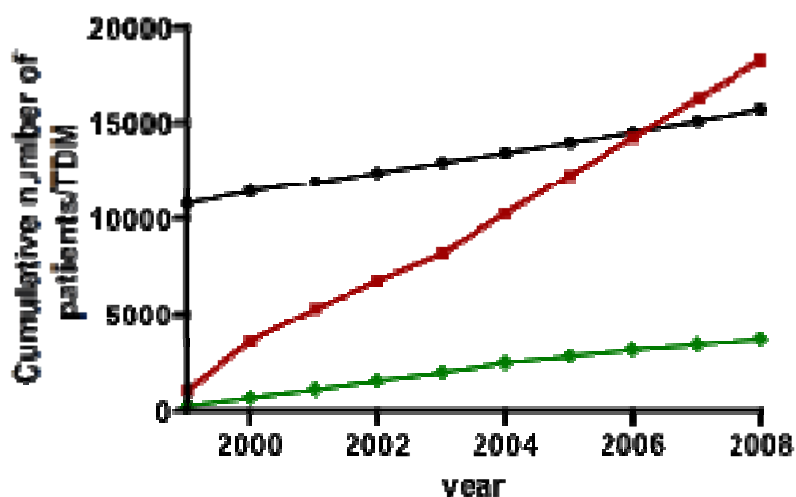






**Figure 5:** Concentration time profiles of the antiretroviral drugs available from the SHCS database.

As depicted in **Figure 6**, the number of patients enrolled in the SHCS is growing over the years. About 250 new patients have been included each year over the past four years in the PK database and we can therefore project a 30% increase in the number of patients and corresponding drug levels by 2013.



**Figure 6:** Cumulative number of SHCS registrations (black line), cumulated number of participants with at least 1 drug level measurement (TDM)(green line) and cumulated number of TDM measurements (red line) from 1999 to 2008 (data emanating from the SHCS database).

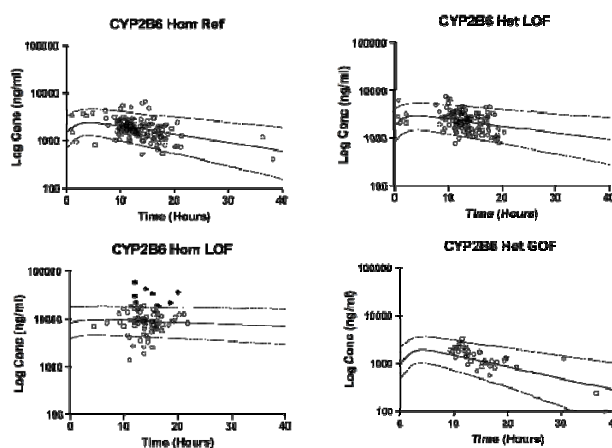
## 7. Antiretroviral population pharmacokinetic-pharmacogenetic modelling

The complexity of real data, which confounds multiple pharmacokinetic, host-related, viral, environmental and genetic variables to a phenotype, requires the implementation of complex and sophisticated models. Population-based approaches represent a highly suitable way to capture the contribution of multiple influences on plasma concentrations and to quantify both between and within-patients variances, which constitute two of the main rationales for studies on the identification of genetic determinants and the adequate characterization of pharmacokinetic or dynamic phenotypes [1-3]. The basic concept of population modeling is to include patient pharmacokinetic data obtained from observational studies into non-linear mixed effects regression models to analyze data pooled over all the sampled individuals. For such purposes, several well established software programs, in particular the NONMEM® (Non-linear Mixed Effect Modeling) system, have been developed [4].

A population-based approach is clearly superior to more classical and simple descriptive statistics with linear approximation in problematic design such as non-linear dependencies, sparse plasma sampling, censored or imbalanced data and offers physiological-based parameters that are more closely associated with pharmacokinetic processes that underlie drug exposure (metabolism and elimination pathways) and patient factors suspected of influencing drug exposure (e.g. disease state, sex, race, special populations) than the commonly used parameters (maximal or trough drug concentrations). Optimized large-scale studies aiming at defining the complex interactions between various elements involved in HIV therapy are strongly dependant on three fundamental resources: robust phenotypic data, efficient genotyping strategies and powerful population analysis methods. Problematic issues in observational data analyses lie in that may be confounded by numerous factors including non-standardized blood levels measurements, compliance issues, drug-drug, drug-host interaction or other environmental and demographic influences. Such problems can be best circumvented using population pharmacokinetic techniques, which offer individual estimates of pharmacokinetic parameters and variability around them and accounts for contributing individual/joint variants to drug pharmacokinetics. Noteworthy, the clinical usefulness of such an approach has been acknowledged in medicine, allowing for the possibility for Bayesian feed back adjustment of dosage regimens, which represents the best currently available therapeutic drug monitoring strategy [5].

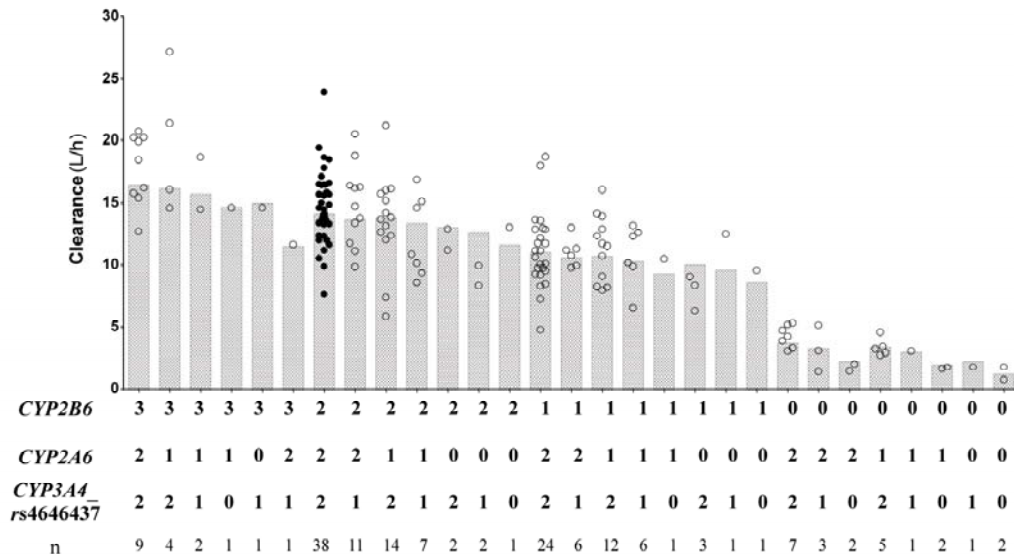
## 8. Pharmacokinetic-pharmacogenetic model explorations

The first exploration of pharmacokinetic-pharmacogenetic relationships was assessed for EFV using the population approach [6]. This study aimed at characterizing the joint impact of genetic polymorphisms in the main (CYP2B6) and accessory metabolic pathways (CYP2A6, CYP3A4/A5) involved in EFV elimination. We could demonstrate that while functional alleles of CYP2B6 accounted for the majority of EFV interindividual variability (**Figure 7**), genetic variations in EFV accessory metabolic pathways influenced EFV disposition as well (**Figure 8**). We also investigated and proposed an innovative model to describe the relationship between phenotypes and functional allelic variants. This study provided new insights in the understanding of the mechanisms of genetic influences.



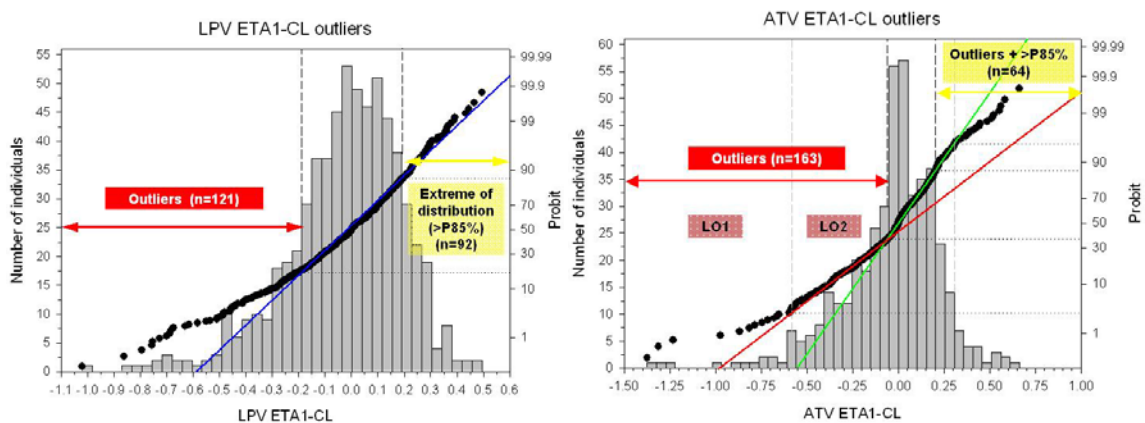
**Figure 7:** EFV plasma concentrations (n=393) in 169 HIV-1 individuals (open circles) according to CYP2B6 polymorphism with population predictions of the corresponding genotype represented by black lines and 90 % prediction interval (grey dotted lines). Left low panel: full dark circles represent concentrations in individuals Hom LOF for CYP2B6, CYP2A6 and CYP3A4\_rs46464337 while full dark diamonds represent concentrations in individuals Hom LOF for CYP2B6 and Het LOF for CYP3A4\_rs4646437.

Such an original approach serves as a proof-of-concept for further investigations of multiple genotypes-phenotypes interactions involving other anti-HIV drugs.



**Figure 8:** EFV individual predicted Bayesian clearances (open circles) and average predicted clearance (bars) for each CYP2B6, CYP2A6 & CYP3A4\_rs4646437 allelic combinations 0: Hom LOF/DOF, 1 Het LOF/DOF, 2 Hom Ref, 3 Het GOF; n = number of individuals carrying the allelic combination, Hom= homozygote, Het = heterozygote, LOF/DOF = loss/decrease of function, GOF = gain of function.

As part of a pharmacogenomic study targeted at evaluating the contribution of genes and SNPs involved in the whole absorption-distribution-metabolism-elimination (WADME) of LPV and ATV (see below), we conducted a population pharmacokinetic analysis of LPV and ATV in 638 and 446 HIV-infected patients, respectively (SHCS #531. *An ART Pharmacogenomics study of two pharmacokinetic and one pharmacodynamic (toxicity) phenotype*). The description of LPV and ATV pharmacokinetic profile and variability served as a robust phenotypic determination to identify extreme plasma concentration levels and to select outlier patients for genetic investigations (**Figure 9**).



**Figure 9:** Frequency histogram of LPV (left panel) and ATV (right panel) ETA-CL (unexplained variability on CL) values and the corresponding probits in the study population. Individuals with lower (high concentrations) or higher ETA-CL values (low concentrations) than predicted by normal distribution are clearly noticeable.

The phenotype distribution suggested the existence of a case population with low estimated clearance. This method served for patients' selection and inclusion in the WADME genetic project according to a case-control approach. The WADME study has generated a ranked list of new relevant candidate SNPs that have been integrating into population pharmacokinetic/pharmacogenetics (PK/PGx) models [7-8]. This paradigm will be further exploited and extended to the further phenotype-genotype association studies.

These examples are representative of gene interactions that seem to influence the pharmacokinetics of antiretroviral drugs. This conceptual framework thus reveals its fertility to generate new hypotheses, which are the basis of further investigation planned in SHCS-wide prospective sets of patients, or in well-controlled gene-drug interactions trials.

## 9. Perspectives and Conclusion

The tremendous source of available and oncoming data regarding HIV drugs and metabolites, plasma and intracellular concentrations as well as pharmacogenetics and clinical outcomes calls for the completion of many projects that will offer a better understanding of the overall aspects involved in HIV therapy. In that respect, as part of our large scale project supported by the SNSF (Grant No FN 32430-124943 in 2009 entitled "Population Pharmacokinetics, Pharmacogenetics and Metabolic Profiling of Antiretroviral Therapy"), we are aiming to ensure state-of-the-art population pharmacokinetic modeling of most of the currently used and future anti-HIV drugs, in the perspective of providing a full characterization of drug exposure, including metabolites kinetics, to provide pharmacokinetic phenotypes for the selection of candidates for phenotypes-genotypes analyses and association studies, to explore drugs or metabolites concentrations-effect/toxicity relationships and to further build up a reference base for the rational exploitation of pharmacokinetic determination results as a medically important tool for the monitoring and optimization of antiretroviral therapy.

In conclusion, the support of SHCS for this first data exploration provided a very valuable help to construct the base necessary for appropriate data collection and retrieval and model explorations. Our results obtained with EFV and LPV strongly suggests that promising results can be expected by integrating pharmacokinetic, pharmacogenetic and clinical components into population analyses.

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