High-throughput genetic screening and pharmacokinetic population modeling in drug development

Tessier A[1,2], Bertrand J[3], Fouliard S[2], Comets E[1], Chenel M[2]

1. Univ Paris Diderot, Sorbonne Paris Cité, UMR 738, F-75018 Paris, France and INSERM, UMR 738, F-75018 Paris, France
2. Clinical Pharmacokinetic Department, Institut de Recherches Internationales Servier, Suresnes, France
3. University College London, Genetics Institute, London, UK

CONTEXT

- Drug S is currently in phase I in SERVIER laboratories.
- In order to follow the EMA recommendations **[1]**, a large number of genetic variants have been genotyped, with two consequences:
  - this number is superior to the number of subjects,
  - some of these variants are correlated due to linkage disequilibrium.

  We expect the genetic part of Drug S variability to be shared among several polymorphisms with low to intermediate effect sizes **[2]**.

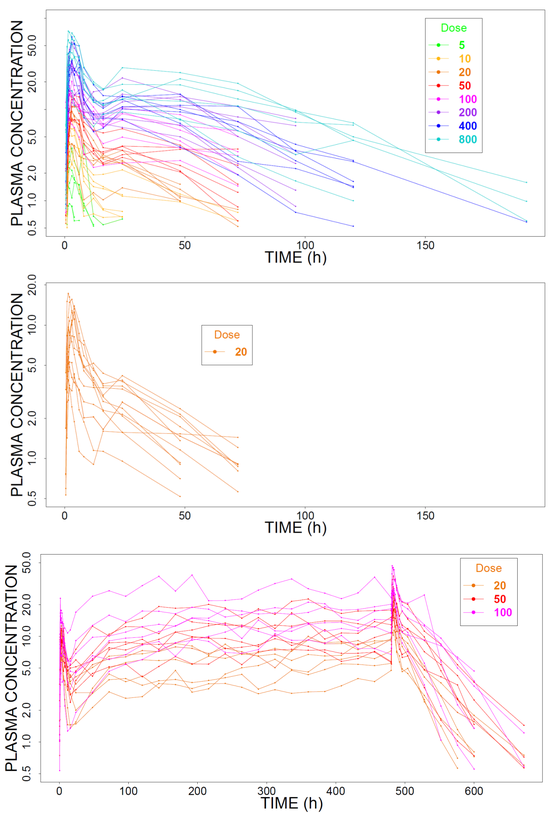
OBJECTIVES

- To develop a population PK model.
- To build the covariate model using a stepwise model selection algorithm adapted to genetic variant features.

METHODS

Pharmacokinetic data

- **Study 1:**
  - oral single dose
  - 8 doses
  - 48 subjects
- **Study 2:**
  - oral single dose
  - 1 dose
  - 12 subjects
- **Study 3:**
  - oral repeated doses
  - 3 doses
  - 18 subjects



Individual Concentrations Profiles - Concentrations versus time profiles in log scale for each subject

Population Pharmacokinetic Analysis

- FOCE-I algorithm in **NONMEM 7.2 [3]**.
- Exponential model for the random effects.
- Combined error model.
- Model evaluation using **individual fits** and **normalized prediction distribution error** (NPDE) **[4]**.

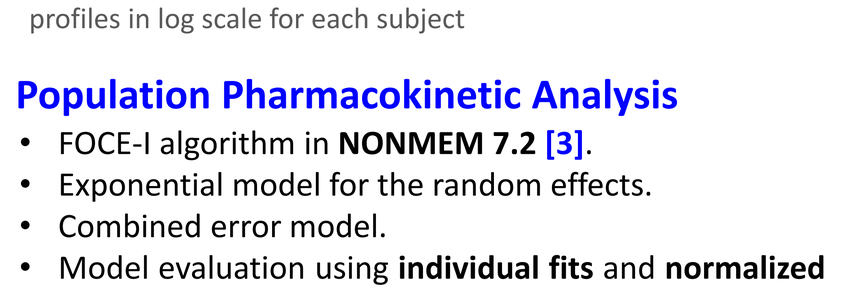
Drug S Proposed Model

- **PK profile showed a rebound at approximately 24h:**
  - described assuming an **enterohepatic circulation** (EHC◊).
- **Non-linearity in the PK with dose:**
  - modeled through a bioavailability-dose Imax model (with fixed baseline).

Dose < 20: $F_0 = 0.3$

Dose ≥ 20:

$$F = \left(1 - \frac{(DOSE - 20) \times Imax}{DOSE - 20 + D_{50}}\right) \times F_0$$



Where,

- F : bioavailability (expressed in terms of parameters Imax and D_{50})
- D_1 : zero order absorption
- Tlag : lag-time on absorption
- V_c : central compartment volume
- V_p : peripheral compartment volume
- Q : intercompartmental clearance between V_c et V_p
- V_{GB}◊ : gallbladder compartment volume
- Q_{GB}◊ : intercompartmental clearance between V_c et V_{GB}
- T_{start}◊ : gallbladder emptying time
- T_{dur}◊ : gallbladder emptying duration

◊*EHC parameters*

Pharmacogenetic data

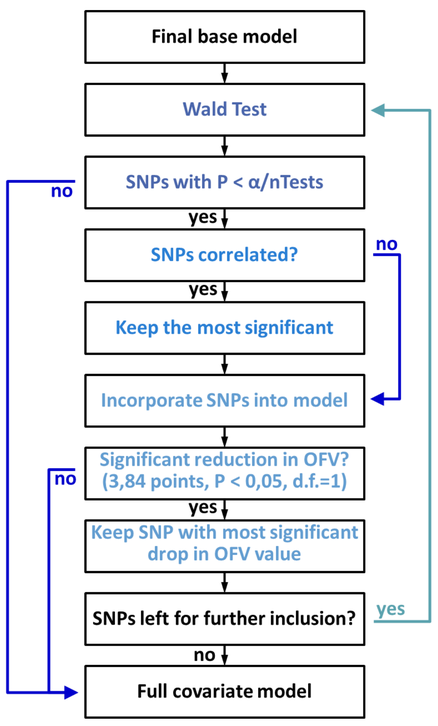
- **176 Single Nucleotide Polymorphisms (SNPs):**
  - genotyped using specific SERVIER DNA microarrays,
  - 39 genes chosen for their known implication in drugs pharmacokinetics.

Population Pharmacogenetic Analysis

We applied a **stepwise method** inspired by Lehr et al **[5]**:

- **Univariate regression** on **Empirical Bayes Estimates**:
  - a **Wald test** was applied with **a Bonferroni correction,**
  - using 3 different genetic models (additive, dominant and recessive),
  - in **PLINK 1.07 [6].**
- Accounting for **linkage disequilibrium**:
  - **strong correlation** ($r^2 > 0.8$) among selected SNPs
  - only the most significant is kept.
- **Forward inclusion** in the model:
  - for each SNPs issuing the screening step,
  - using a Likelihood Ratio Test.

This two steps (i. screening and ii. model inclusion) are performed until no more SNPs enters the model.



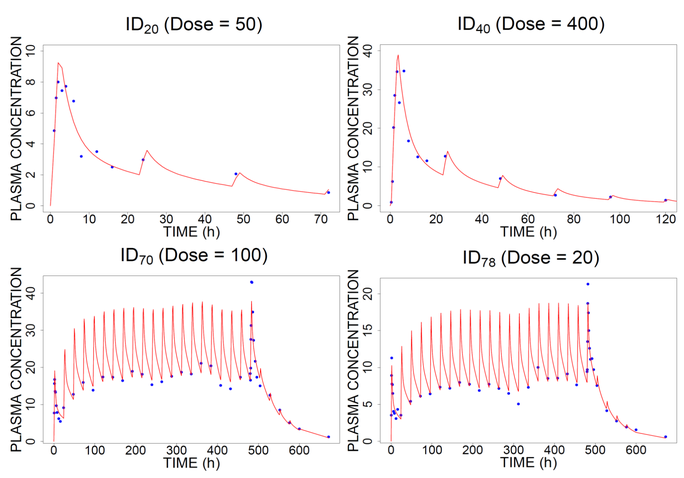
RESULTS

Pharmacokinetic Results

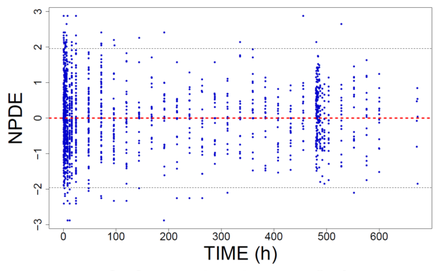
	Population Prediction (RSE%)	InterIndividual Variability in % (RSE%)	IntraIndividual Variability in % (RSE%)
F	Imax = 0.919 (0.55) D_{50} = 53 (26)	46.90 (26.4)	13.20 (29)
D_1	2 (7)	30.80 (58.2)	28.60 (41.5)
Tlag	0.387 (6.7)	38.70 (23.5)	
V_c	1640 (6.2)		
Q	242 (18.5)	89.10 (33.2)	
V_p	1960 (8.6)		
CL	89.3 (8)	26.80 (31.7)	18.90 (14.2)
T_{start}	23.9 (fixed)		
T_{dur}	0.5 (fixed)		
V_{GB}	6.19 (fixed)		
Q_{GB}	39.4 (fixed)	65 (31.9)	
σ_{inter}	0.241 (9.5)		
σ_{slope}	15.80% (2.8)		

Parameter Estimates (Relative Standard Error) - All parameters are estimated with good precision

- A first model was developped on single dose studies.
- The addition of data from repeated doses study makes the EHC parameters unidentifiable since new administrations mask the rebound:
  - T_{start}, T_{dur}, Q_{GB} and V_{GB} were set to the values estimate with single dose data.



Individual predictions versus Time - observation (●) and model predictions (—) for 4 subjects

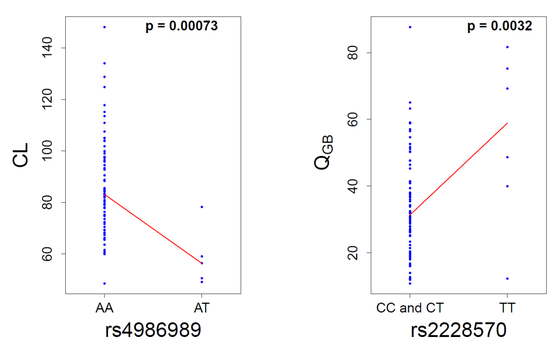


Internal Evaluation - scatterplot of NPDE versus time

- **Good description** of drug S concentration.
- Selected model **describes the observed rebound**.

Pharmacogenetic Results

SNPs Impact on Individual Parameters



Gene Effect - plot of the effect of the SNP rs4986989 on CL according to an additive model (AA: common homozygotes ; AT: heterozygote ; rare homozygotes TT have not been observed), and the SNP rs2228570 on Q_{GB} according to a recessive model (CC and CT: respectively common homozygotes and heterozygotes ; TT: rare homozygotes)

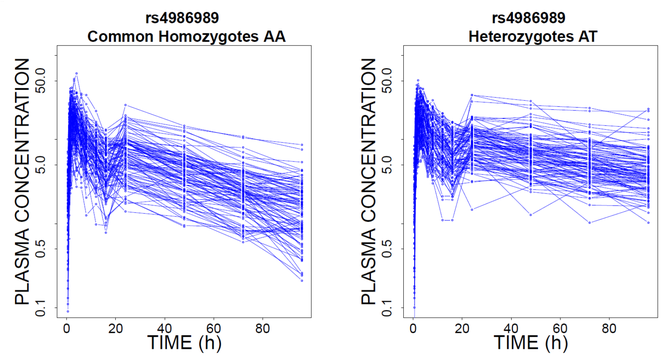
- The **screening step on EBEs** allows to detect the impact of two SNPs:
  - a marker of **metabolic enzyme NAT-1** on CL,
  - a marker of **nuclear receptor VDR** on Q_{GB}.
- Both markers are not correlated:
  - positioned on chromosome 8 and 12 respectively for NAT and VDR.

Inclusion of SNP in the Model

- The covariates model: $\theta_i = \theta \times e^{SNP.\beta} \times e^{\eta_i}$
  - SNP = {0, 1 or 2}: the genotype,
  - β: impact coefficient.

- **rs2228570 as covariate on QGB:**
  - No decrease in the objective function value (OFV).
- **rs4986989 is included as covariate on CL:**
  - Significant decrease of OFV (ΔOFV=-26),
  - **explains 19% of CL variability.**

Only SNP rs4986989 remains in the final covariate model.



Simulated PK Profiles - Simulation of 100 rs4986989 common homozygotes and 100 rs4986989 heterozygotes from the covariate model

DISCUSSION

- CYP3A4, 2D6 and 1A2 metabolize Drug S (*in vitro*):
  - None of the corresponding markers were detected by the Wald test in the univariate step.
- The involvement of NAT1 and VDR in the Drug S PK must be confirmed by *in vitro* studies.

The **stepwise method:**

- reduces the number of genetic variants to include in the model,
- takes into account the correlation between these variants,
- is limited by the small number of subjects:
  - lack of power,
  - absence of the less frequent mutations.

CONCLUSION

The combination of NonLinear Mixed Effects Model and Genetic statistic methods allows:

- to describe the complex pharmacokinetics of drug S (nonlinearity, EHC),
- to explore the effect of many SNPs on separate phases of the ADME process and thereby accurately predict its effect on the drug PK.

[1] EMA. *Draft* 2010
[2] Manolio et al. *Nature* 2009
[3] Sheiner LB et al. *Computers and Biomedical Research* 1972
[4] Brendel K et al. *Pharmaceutical Research* 2006
[5] Lehr T et al. *Pharmacogenetics and Genomics* 2010
[6] Purcell S et al. *American Journal of Human Genetics* 2007