Extensive Population Pharmacokinetic-Pharmacogenetic Study of nevirapine in HIV-Infected Cambodian Patients

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CONTEXT

- **Importance of nevirapine**
  - ✓ backbone of HAART in resource limited countries
  - ✓ No pharmacokinetic studies in the Cambodian population
- **Pharmacokinetics of nevirapine**
  - ✓ metabolized by CYP2B6 and CYP3A which are regulated by the Pregnane X receptor (PXR)
  - ✓ weak substrate of the P-glycoprotein (P-gP) efflux transporter
- **Pharmacogenetics of nevirapine**
  - ✓ effect of five polymorphisms investigated on nevirapine apparent clearance in the Cambodian population investigated using nonlinear mixed effect models (1)
    - ➲ *CYP2B6 516G/T* associated with steady-state NVP clearance (Cl/F) in HIV-infected Cambodians
  - ✓ Sequenom assay previously designed for the pharmacogenetics of efavirenz and nevirapine (2)

ANRS 12154 - PECAN- study design

- **Patients**
  - ✓ 170 HIV-1 infected from the ESTHER cohort
  - ✓ Hospital Calmette, Phnom Penh Cambodia
- **Treatment**
  - ✓ nevirapine (200mg bid)
  - ✓ nucleoside reverse transcriptase inhibitors
    - ➲ Stavudine + Lamivudine or Zidovudine + Lamivudine
  - ✓ WHO prequalified generic fixed dose combinations from India

OBJECTIVE

- To more thoroughly investigate *CYP2B6* and other genes using the **Sequenom assay** design and **nonlinear mixed effect modeling**
- To inform on genetic variation of these polymorphisms in the Cambodian population

METHODS

- **PK sampling**
  - ✓ before morning dose intake
  - ✓ 18 months (M18) and 36 months (M36) after antiretroviral drug regimen onset
  - ✓ additional samples at 1, 2, 4 and 8h post intake in 10 patients
- **Population pharmacokinetic model**
  - ✓ built on the 170 patients of the PECAN study
  - ✓ one compartment model parameterized in first order absorption (ka), apparent clearance (Cl/F) and apparent volume of distribution (V/F)
  - ✓ exponential model for the between and within subject random effects on Cl/F only
  - ✓ constant error model for the residuals
  - ✓ estimation using the SAEM algorithm implemented in Monolix 2.4
- **Phenotype**
  - ✓ derivation of the Empirical Bayes Estimates of the individual Cl/F at each occasion
  - ✓ computation of the average individual Cl/F across the occasions

- **Genotyping**
  - ✓ 129 patients agreed for an extended genotyping
  - ✓ Sequenom platform
    - ➲ chromosome 3: 49 SNPS on NR1I2 (PXR)
    - ➲ chromosome 7: 63 on ABCB1 (P-gp), 1 on CYP3A5 and 36 on CYP3A4
    - ➲ chromosome 19: 1 on CYP2A6 and 47 on CYP2B6
- **Haplotypes definition**
  - ✓ blocks defined with the D' confidence intervals method in Haploview (3)
  - ✓ phasing inferred using the standard E-M algorithm in Plink (4)
- **Analyses**
  - ✓ regression on rare allele dosage
    - ➲ additive genetic model assumption
  - ✓ Wald test on effect coefficients
  - ✓ adjustment for the *CYP2B6 516G/T* status
- **Multiple tests correction using the False Discovery Rate** (5)

RESULTS

Population Pharmacokinetics

Residual concentrations — Complete pharmacokinetics (N=10)

- 3.4% and 5.6% of the patients with nevirapine residual concentrations ≤3,000 ng/ml at M18 and M36

Parameters (unit)	Estimates	95%CI
ka (/h)	1.64	$(0.35 - 7.75)$
V/F (L)	213	$(120 - 377)$
Cl/F (L/h)	2.67	$(2.51 - 2.84)$
$\omega_{Cl/F}$ (%)	28	$(24 - 32)$
$\gamma_{Cl/F}$ (%)	17	$(15 - 19)$
σ (ng/mL)	519	$(408 - 630)$

- Estimates in accordance with the literature
- Large CI around the volume due to the design
- Low between (ω) and within (γ) subject variances on nevirapine elimination clearance

Genetic Analyses

- 196 single nucleotide polymorphisms (SNPs) across 3 chromosomes
  - ✓ 126 polymorphic SNPs
- **Chromosome 3**
  - ✓ 4 haplotype blocks
- **Chromosome 7**
  - ✓ 9 haplotype blocks
- **Chromosome 19**
  - ✓ 3 haplotype blocks

Linkage Disequilibrium (LD) plot of all polymorphic SNP genotyped on chromosome 19. The top bar marks the location of each SNP in genomic order and each square represent the correlation (r²) between two SNPs, darker shade indicates stronger correlation, e.g the dark square in the top right corner indicates a r²=0.94 between SNP 44 and 48. The 3 haplotype blocks defined using the D' confidence intervals method are represented by 3 black triangles starting from SNP 4 to 6, 6 to 43 and 44 to 48 respectively.

Each haplotypes in the haplotype blocks with its population frequency and connections from one block to the next. In the crossing areas, the multiallelic D' is shown, which is a value of the level of recombination between the two blocks. Above the haplotypes are marker numbers along with a tick beneath haplotype tag SNPs.

Pharmacogenetic Analyses

- Median (range) average individual Cl/F : **2.6 (1-7.8) L/h**
- Univariate associations with Cl/F (P=0.03 to 4.6×10^{-4})
  - ✓ 17 SNPs in CYP2B6
  - ✓ 1 in CYP3A4
  - ✓ 6 haplotypes in CYP2B6
- No other SNP independently associated to Cl/F once conditioning on *CYP2B6 516G>T* status (as shown on the figure below)

Genetic polymorphisms are designated by their reference SNP (rs) number, all are on the CYP2B6 gene except for rs2687116 on the CYP3A4.
extens.=extensive metabolizers; intermed.=intermediate metabolizers; slow=slow metabolizers for the *CYP2B6 516G>T* polymorphism

DISCUSSION

- Quantification of low between and within subject variability of NVP Cl/F in this HIV-infected Cambodian population using nonlinear mixed effect models
  - ✓ flexible design to ensure a satisfactory number of patients of each genotype for most SNPs under study to meet the requirements of the medical authorities
  - ✓ low precision on volume of distribution and absorption constant rate parameters
- Strong association between *CYP2B6 516G/T* and steady-state NVP Cl/F
  - ✓ 36% reduction predicted by *CYP2B6* 516TT homozygosity
- Effect of an **extended *CYP2B6* haplotype block** encompassing promoter regions and multiple exons
- No individual SNPs beyond this haplotype block independently predict NVP Cl/F.

REFERENCES

1. M. Chou, et al., "Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients.," Antimicrobial Agents and Chemotherapy, vol. 54, 2010, pp. 4432-4439.
2. D.W. Haas, et al., "Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in North americans.," Journal of Infectious Diseases, vol. 199,2009, pp. 872-880.
3. J.C. Barrett, et al., "Haploview: analysis and visualization of LD and haplotype maps.," Bioinformatics, vol. 21, 2005, pp. 263-265.
4. S. Purcell, et al., "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses," The American Journal of Human Genetics, vol. 81, 2007, pp. 559-575.
5. Y. Benjamini, D. Yekutieli, " The control of the false discovery rate in multiple testing under dependency," Annals of Statistics, vol. 29, 2001, pp.1165-1188