Phenotypic assays measure the susceptibility of HIV-1 to antiretroviral drugs, and are used to guide treatment regimen choices. Analyses of baseline predictors of virological failure support the use of a 0.4-fold cutpoint for defining increased susceptibility (hypersusceptibility) to efavirenz in the PhenoSense HIV assay. The enhancement of the virological activity of efavirenz, associated with resistance to reverse transcriptase inhibitors, provides an opportunity to consider mutational and drug interactions in optimizing treatment.

High-throughput phenotypic assays, which measure the inhibition of reverse transcriptase and protease activity by increasing concentrations of drug, provide accurate and reproducible measures of drug susceptibility to HIV-1. With one of the commercial phenotypic assays, the ViroLogic PhenoSense assay (ViroLogic, Inc., South San Francisco, CA, USA), increased susceptibility or hypersusceptibility to each class of antiretroviral drugs has been observed. Increased susceptibility to non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs is more common in nucleoside reverse transcriptase inhibitor (NRTI)-experienced patients and is most closely associated with resistance to zidovudine. Site-directed mutagenesis studies have shown that combinations of the classic thymidine analog mutations, such as M41L, D67N, T215Y, and K219R, or other NRTI-associated mutations, such as M184V and T69 insertions, are associated with NNRTI hypersusceptibility [1]. In the ACTG 364 study cohort of highly zidovudine-experienced subjects, nearly 40% demonstrated hypersusceptibility associated with D67N, L210W and T215Y/F [2,3]. In three clinical trials, CCTG 575 [4], ACTG 364 [3,5] and ACTG 398 [6] and one observational study [7], hypersusceptibility to NNRTI drugs has been associated with improved virological outcome, and with immunological outcome in one study [4]. The original definition of NNRTI hypersusceptibility, a less than 0.4-fold change in susceptibility relative to a wild-type reference virus, was based on the assay variability and the variability of drug susceptibility among wild-type clinical isolates [1]. With a view to refining the definition to a more clinically relevant threshold for efavirenz hypersusceptibility, we examined multiple potential cutpoints in the ACTG 364 trial database and related these to the virological outcome of treatment over 144 weeks.

Two different approaches were used to evaluate thresholds for efavirenz hypersusceptibility. In the first approach, a separate co-variate for baseline efavirenz hypersusceptibility was examined in models that included other predictors of treatment outcome. Cox proportional hazards models evaluated the relationship between baseline efavirenz hypersusceptibility and the risk of on-treatment virological failure, defined as confirmed HIV-1 RNA 2000 copies/ml or greater at week 16 or later [3].

Table 1 displays the hazard ratios for efavirenz hypersusceptibility, using various efavirenz hypersusceptibility thresholds among efavirenz-treated subjects, adjusted for baseline log_{10} HIV-1 RNA and phenotypic susceptibility score.

<table>
<thead>
<tr>
<th>Cutpoint</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
<th>No. subjects less than cutpoint (out of n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.54</td>
<td>(0.1, 2.6)</td>
<td>0.44</td>
<td>4</td>
</tr>
<tr>
<td>0.3</td>
<td>0.41</td>
<td>(0.2, 1.1)</td>
<td>0.08</td>
<td>21</td>
</tr>
<tr>
<td>0.4</td>
<td>0.43</td>
<td>(0.2, 1.0)</td>
<td>0.04</td>
<td>32</td>
</tr>
<tr>
<td>0.5</td>
<td>0.73</td>
<td>(0.4, 1.5)</td>
<td>0.38</td>
<td>46</td>
</tr>
<tr>
<td>0.6</td>
<td>1.10</td>
<td>(0.5, 2.3)</td>
<td>0.80</td>
<td>56</td>
</tr>
<tr>
<td>0.7</td>
<td>1.28</td>
<td>(0.6, 2.8)</td>
<td>0.52</td>
<td>59</td>
</tr>
<tr>
<td>0.8</td>
<td>0.92</td>
<td>(0.4, 2.0)</td>
<td>0.84</td>
<td>65</td>
</tr>
<tr>
<td>0.9</td>
<td>1.50</td>
<td>(0.5, 4.3)</td>
<td>0.45</td>
<td>71</td>
</tr>
</tbody>
</table>

CI, Confidence interval.
susceptibility to drugs in the treatment regimen. The phenotypic susceptibility score (PSS) was used as a summary measure of the susceptibility of baseline virus to the assigned treatment regimen. The PSS of baseline viruses was determined for each study subject by summing the levels of drug susceptibility to each drug in the treatment regimen [3]. In the PSS calculation, each drug in the regimen could receive a maximum score of 1 for complete sensitivity, and a minimum score of 0 for complete resistance, with a continuous range between 0 and 1 for “partly sensitive” viruses. Although many hypersusceptibility cutoffs were independently associated with treatment failure hazard ratios of less than 1, the best estimate for the cutoff was 0.4-fold (based on maximizing the partial likelihood of the Cox model). Whereas an efavirenz hypersusceptibility cutoff of 0.3-fold was associated with an even lower risk of treatment failure (Table 1), the P value observed at this cutoff was greater than 0.05 as a result of the smaller patient numbers. The a priori choice of 0.4 as a cutoff, as used in three clinical trials [3,4,6], is thus supported by these additional analyses.

The second approach to evaluate the efavirenz hypersusceptibility threshold utilized models that incorporated efavirenz hypersusceptibility into the baseline PSS. In these models, the PSS could be increased by a maximum of 0.5 units for efavirenz hypersusceptibility, such that an efavirenz score between 1.0 and 1.5 was given to viruses that showed hypersusceptibility to efavirenz. For example, when evaluating the efavirenz hypersusceptibility definition of less than 0.4-fold, the PSS contribution for efavirenz was calculated as

\[
1 + 0.5 \times \left(0.4 - \text{efavirenz fold-change}\right)/0.4
\]

for efavirenz drug susceptibility values less than 0.4-fold, 1 for efavirenz drug susceptibility values between 0.4- and 2.5-fold, and between 0 and 1 for susceptibility values above 2.5-fold [3]. In multivariate Cox models using this approach, the threshold of 0.4-fold was again shown to be the maximum likelihood estimate, and yielded the smallest hazard ratio (hazard ratio 0.45, \( P = 0.003 \), for composite PSS incorporating efavirenz hypersusceptibility).

In summary, this re-analysis of ACTG 364 outcome data supports the use of a 0.4-fold cutoff for defining efavirenz hypersusceptibility in the PhenoSense HIV drug susceptibility assay.

The increase in virological efficacy of efavirenz in three clinical trials (ACTG 398, ACTG 364 and CCTG 575) among NRTI-experienced subjects with efavirenz hypersusceptibility, compared with those without evidence for efavirenz hypersusceptibility (all with a cutoff of 0.4) provides new challenges and opportunities. A challenge is how to use these observations in the sequencing of antiretroviral treatments to sustain a long-term virological response. Treatment regimens that contain efavirenz in combination with other active drugs may have enhanced potency in salvage therapy for hypersusceptible viruses. Among these patients, it may be advantageous to continue NRTI treatment to sustain the resistance mutations associated with efavirenz hypersusceptibility. The enhancement of the virological activity of efavirenz, associated with resistance to NRTI, provides an opportunity to consider mutational and drug interactions in optimizing treatment.

\[\text{ References} \]


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Increased bleeding in HIV-positive haemophiliac patients treated with lopinavir–ritonavir

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In this retrospective study, performed on 16 HIV-infected patients with congenital coagulation disorders who were part of a French clinical cohort, the incidence rate of bleeding with a lopinavir–ritonavir-containing regimen was almost four times the rate with other protease inhibitor-containing regimens. Lopinavir–ritonavir also appeared to be associated with exceptionally severe haemorrhagic events. If these results are confirmed, they suggest that when other therapeutic options are available, we should reconsider lopinavir–ritonavir use in haemophiliac patients.

Soon after the introduction of the first protease inhibitor (PI) drugs, there were several reports of increased bleeding complications in haemophiliac patients after starting treatment with these drugs [1,2]. Ritonavir was shown to be associated with the highest incidence of bleeding, followed by indinavir. Until now, the most recently introduced PI drugs, including nelfinavir, amprenavir and lopinavir–ritonavir, were considered to be less frequently associated with an increased bleeding tendency than other PI. Therefore, their use as part of highly active antiretroviral therapy was suggested in patients with congenital coagulation disorders [3].

In the Tourcoing Clinical Cohort in northern France, we conducted a study to estimate the risk and severity of bleeding associated with regimens containing lopinavir–ritonavir in patients with congenital coagulation disorders. The study was prompted by the occurrence of fatal intracranial bleeding in a 22-year-old patient with severe haemophilia who was on a lopinavir–ritonavir regimen.

Details of the Tourcoing Clinical Cohort have been published elsewhere [4]. In brief, all patients referred to the Tourcoing AIDS Reference Centre in northern France who had HIV-1 infection confirmed by Western blot and were over 13 years of age were enrolled in this clinical cohort. An initial medical evaluation was performed for every new patient. Standardized questionnaires were used to collect extensive demographic, clinical, laboratory, and pharmaceutical information. Data included age, sex, probable transmission route, year of diagnosis, date of AIDS-defining events before inclusion and at inclusion, CD4 lymphocyte count and viral load, during the period since 1995. Follow-up data were collected at each outpatient visit, hospital admission or at least every 6 months by technicians who abstracted the information collected by clinicians onto standardized computer records. These data included the occurrence of new clinical events and especially bleeding disorders.

In patients with congenital coagulation disorders who were followed between April 1996 and September 2002, we identified the episodes of bleeding associated with antiretroviral regimens containing lopinavir–ritonavir and for comparison all bleeding episodes caused by other PI antiretroviral regimens. A patient was considered to have a bleeding disorder if he was experiencing bleeds in sites not normally associated with haemophilic bleeding. Purpura was also considered to be a bleeding disorder; however, a sensitivity analysis was performed in which cases of purpura were excluded from the incidence analysis. We estimated the incidence rate of bleeding for lopinavir–ritonavir and other PI regimens. The incidence rate was defined as the number of bleeding disorders divided by the total number of person-years at risk of bleeding. Patients were considered at risk from the date they started the regimen until they stopped it, or until the day of their last visit if they were still on that regimen. Incidence rates 95% confidence interval (CI) were estimated using the Poisson distribution.

Of 1641 HIV-infected patients enrolled during the study period, 16 had congenital coagulation disorders. Fifteen of these had severe congenital haemophilia and one von Willebrand’s disease. The median age at the first PI prescription was 26 years (range 16–41), and 94% of the patients were men. The median CD4 lymphocyte count and HIV viral load was 196 cells/μl (range 1–822), and 4.5 log10 copies/ml (range 1.6–6.1), respectively. The median duration of follow-up on a PI regimen was 2.5 years (range 0.1–6.2). The incidence rate for the lopinavir–ritonavir regimen (3.7 per 100 person-months; 95% CI 0.70–10.84) was above the upper bound of the 95% CI of the incidence of bleeding for other PI (i.e. ritonavir, saquinavir, indinavir, nelfinavir, saquinavir–ritonavir; 0.96; 95% CI 0.35–2.09; P < 0.05). When purpura was not considered as a bleeding complication, the incidence rate of bleeding for the lopinavir–ritonavir regimen was still three times higher than for other PI regimens (P < 0.05).

Six patients had received a lopinavir–ritonavir-containing regimen. Three of them (50.0%) developed at least one bleeding complication (Table 1). At the time of this complication, the three patients had been on a lopinavir–ritonavir regimen for a median period of 4 months (range 0.3–7). All of the bleeding complications required inpatient admissions and one resulted in the patient’s death.

Two patients with bleeding complications maintained...
platelet counts above $100 \times 10^9/l$. The remaining patient’s count was $88 \times 10^9/l$, but this was no lower than the values found before starting lopinavir–ritonavir. The results of the prothrombin time before and after taking lopinavir–ritonavir revealed no differences explaining a greater bleeding tendency.

One of the three patients had presented with an AIDS-defining event before the bleeding complication occurred. At the time of bleeding all of these patients had viral loads below 1000 copies/mm³; two had CD4 cell counts above 100 cells/µl, whereas before taking lopinavir–ritonavir none of the patients had an undetectable viral load and all had CD4 cell counts below 100 cells/µl.

When lopinavir is at a dose of 400 mg combined with ritonavir 100 mg twice daily, the mean trough plasma lopinavir concentrations are at least 75 times as high as the protein binding connected concentrations needed to inhibit the replication of wild-type HIV by 50% [5]. The two drugs were originally combined to exploit this pharmacokinetic advantage, in order to minimize the risk of treatment failure and maximize the durability of a response to treatment that was partly confirmed by clinical trials. Although the mechanism of increased bleeding associated with PI remains unexplained [3], one may postulate that higher trough plasma concentrations of lopinavir might explain the higher risk of bleeding with this drug, but this requires further exploration.

The results of this retrospective study on a small sample size highlight the greater risk of bleeding with lopinavir–ritonavir regimens than with other PI in haemophilic patients. Moreover, lopinavir–ritonavir regimens appear to be associated with exceptionally severe haemorrhagic events. On the basis of these results, which should be further investigated in other centres, it seems advisable to reconsider prescribing lopinavir–ritonavir for haemophilic patients when other therapeutic options are available. When no other options are available, the benefits of lopinavir–ritonavir regimens may outweigh the risk of bleeding and these drugs should be used. However, patients should be warned of the potentially severe risk of bleeding, and should be closely monitored. The prophylactic administration of factor VIII concentrate may be beneficial.

Y. Yazdanpanah and N. Viget performed the data analysis and prepared the manuscript. A. Cheret and H. Guerroumi contributed to data collection and data analysis. Y. Gerard and F. Ajana contributed to data collection and the preparation of the report. J. Caron assisted with data interpretation and preparation of the report. Y. Mouton coordinated work.

### Acknowledgements

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Sex differences in nevirapine disposition in HIV-infected patients

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To evaluate the role of sex in nevirapine pharmacokinetics, we analysed the pharmacokinetic profile of nevirapine in 11 male and 11 female HIV-positive patients chronically treated with nevirapine 400 mg once a day. Statistically significant inter-group differences in the plasma concentration and fluctuations between that and the concentration just before the next dose, at steady-state, were observed. Variations in lean body mass and composition affecting nevirapine distribution might account for inter-sex discrepancies in nevirapine plasma levels.

Sex differences reported in the plasma levels for some antiretroviral drugs may be involved in the higher incidence of adverse reactions observed in women compared with men [1,2]. In particular, an overall higher frequency of nevirapine-induced rash has been compared with men [1,2]. In particular, an overall incidence of adverse reactions observed in women of the quality control samples were always less than 10% for all evaluated concentrations.

The maximum nevirapine plasma concentration (C max), corresponding time (T max), concentration just before the next dose (C trough), and the difference between C max and C min (ΔC) during the dosing interval τ were determined by analysing the individual plasma concentration–time profiles for both groups of patients.

The area under the plasma concentration–time curve from time 0 to 24 h (AUC0–24) was calculated using the trapezoidal method. Oral clearance (CL/F, with F as the fraction of the absorbed dose, assumed to be equal to 1) was calculated as dose/AUC.

Inter-group differences between the pharmacokinetic

Table 1. Mean (± SD) nevirapine pharmacokinetic parameters.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Women</th>
<th>Men</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C trough (µg/ml)</td>
<td>3.7 ± 1.7</td>
<td>3.6 ± 1.8</td>
<td>0.93 (ns)</td>
</tr>
<tr>
<td>C max (µg/ml)</td>
<td>7.3 ± 4.0</td>
<td>5.1 ± 2.1</td>
<td>0.042</td>
</tr>
<tr>
<td>T max (h)</td>
<td>2.6 ± 1.1</td>
<td>2.8 ± 1.1</td>
<td>0.7 (ns)</td>
</tr>
<tr>
<td>ΔC (C max – C min) (µg/ml)</td>
<td>3.7 ± 3.6</td>
<td>1.5 ± 1.2</td>
<td>0.020</td>
</tr>
<tr>
<td>AUC0–24 (µg·h/ml)</td>
<td>117.34 ± 42.49</td>
<td>98.24 ± 43.02</td>
<td>0.3 (ns)</td>
</tr>
<tr>
<td>CL/F (l/h)</td>
<td>3.9 ± 1.7</td>
<td>5.0 ± 2.4</td>
<td>0.24 (ns)</td>
</tr>
<tr>
<td>CL/F (l/h/kg)</td>
<td>0.076 ± 0.036</td>
<td>0.076 ± 0.040</td>
<td>0.88 (ns)</td>
</tr>
</tbody>
</table>

AUC0–24, Area under the plasma concentration–time curve from time 0 to 24 h; CL/F, oral clearance; C max, maximum plasma concentration; C trough, plasma concentration just before the next dose; T max, corresponding time to C max.
parameters of nevirapine were evaluated using the analysis of variance. A $P$ value of less than 0.05 was considered statistically significant.

The two groups of patients were comparable for age, CD4 cell count and plasma HIV-RNA levels. In men compared with women the total body weight (TBW; 70.4 versus 54.3 kg, respectively) and lean body mass (LBM; 64.4 ± 6.2 versus 42.7 ± 9.6, respectively) were significantly higher ($P < 0.005$). No adverse events occurred during the study. The mean pharmacokinetic parameters of nevirapine at steady-state in the two groups of patients, at the dosage regimen of 400 mg a day, are shown in Table 1.

The only slight increase (20%, $P = 0.29$) in AUC mean values observed in women is partly based on TBW differences between women and men. Correcting for the TBW differences, CL/F mean values overlapped in the two groups of patients, suggesting no differences in the TBW differences, CL/F mean values overlapped in the two groups of patients, at the dosage regimen of 400 mg a day, are shown in Table 1.

We found no inter-group differences for $C_{\text{trough}}$, but a statistically significant difference was seen for $C_{\text{max}}$ and $\Delta C$, which were higher in women than in men. While normalizing data to the same dose/kg, the inter-group difference was no longer statistically significant, pooled data showed a correlation between $\Delta C$ and LBM ($\Delta C$, $\mu g/ml = 18.3e^{-0.045} \times \text{LBM, kg}$, $r = 0.61$). This suggests that differences in nevirapine distribution, attributable to variations in LBM and composition, might account for the inter-sex discrepancies in nevirapine plasma levels.

Elevated drug concentrations may result in a higher risk of side-effects, as reported for the central nervous system in patients receiving efavirenz [5] or ritonavir [6]; thus, different pharmacokinetic values might be responsible for the inter-sex differences in side-effects. It has been demonstrated that women may be at a higher risk than men of cutaneous hypersensitivity reactions from nevirapine [7]. Rash pathogenesis remains uncertain as no relationship between rash and nevirapine plasma levels was found in one study [3], whereas a correlation was suggested in two other studies [8]. Administering the same drug dosage to women and men at the same frequency, without considering LBM differences, might predispose women to develop dose-related adverse reactions to nevirapine. From a pharmacokinetic viewpoint, twice daily dosing might be preferable in patients with very low LBM, to ensure lower peak and higher trough nevirapine plasma levels.

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