

The Relevance of Pharmacologic Monitoring In HIV Disease

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DETERMINING THE REASONS FOR FAILURE of highly active antiretroviral therapy (HAART) has long been a frustrating question for researchers and clinicians alike. Very often, the underlying causes of therapeutic failure in individual HIV-positive patients are established after the fact—when viral load has rebounded and a switch to a second-line regimen is inevitable. But times are changing and, with new laboratory markers, intervention before virologic failure occurs has become a possibility.

The emergence of drug-resistant virus is often described as the root cause of virologic failure in patients receiving HAART. Yet, when reviewing what is currently known about the various pathways that can lead to virologic rebounds in patients receiving HAART, the development of drug resistance is much more of a consequence of failure than a factor contributing to it.

As explained by Dr. Hoetelmans and a number of his peers, virologic failure—along with the emergence of drug-resistant virus—often begins with insufficient drug exposure in patients receiving HAART. And while it is true that poor adherence and other behavioral factors are frequently linked to substandard plasma concentrations, the actual pharmacokinetics of individual drugs can also be blamed.

It is important to note that plasma concentrations may also be associated with excessive toxicity. A number of studies have shown that high doses of protease inhibitors are associated with reversible liver damage, kidney stones (indinavir; Crixivan), and circumoral parasthesia (ritonavir; Norvir).

Enter therapeutic drug monitoring (TDM), a somewhat complicated laboratory test that allows researchers and clinicians to measure blood levels of antiretroviral drugs and, as a result, tinker with dosing in an individualized manner.

TDM is currently recommended by the British HIV Association and the French Department of Health in its HIV treatment guidelines released last year (see the special report on these guidelines in the December 1999 issue of *The PRN Notebook*) and is being offered by a number of laboratories in the Netherlands, France, and England. While TDM is not typically employed in the United States, most clinicians are familiar with its usefulness, particularly for patients being treated with narrow therapeutic index drugs (e.g., phenytoin (Dilantin), digoxin (Lanoxin), lithium bicarbonate, and theophylline).

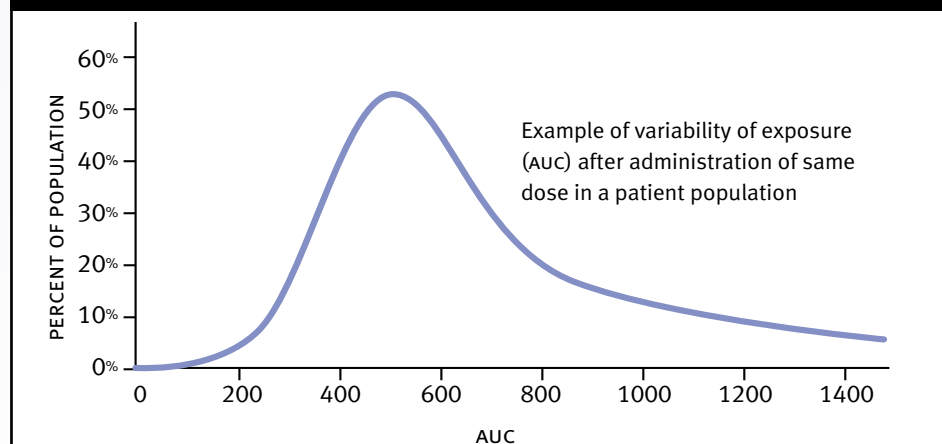
Whether or not TDM will enter the clinical care vernacular for HIV-infected patients is still not known. Answers to this central question will eventually come from the studies—both retrospective and prospective—currently under way and planned for the future.

Drug Exposure and Response

AS DISCUSSED ABOVE, THE SUCCESS OF ANY fixed dosing regimen is largely based on patients' responses measured using clinical and surrogate markers. Fixed dosing regimens are designed to generate plasma drug concentrations within a therapeutic range—the dose of each drug needed to achieve the necessary outcome while avoiding side effects. It is important to note, however, that fixed dosing regimens used in clinical practice are based on average responses reported in studies and are not necessarily adequate for those on the fringe of response ranges seen in clinical trials.

The factors determining drug disposition are all amenable to change in the HIV-infected patient. Physiologic, pathologic and pharmacologic factors can profoundly alter the disposition of a drug such that therapeutic failure or adverse reactions occur. Changes in drug metabolism and excretion induced by age, sex, stage of HIV disease, or drug interactions are among the more important factors that can cause drug concentrations to be higher or lower than expected.

Figure 1. Variability of Exposure to Saquinavir and Other PIs



Source: Richard Hoetelmans, PhD. Adapted and published with permission.

There is a great deal of inter- and intra-patient variability in plasma concentrations of antiretroviral drugs (see figure 1). According to a paper published in the *British Journal of Clinical Pharmacology*, protease inhibitor plasma concentrations may vary more than tenfold between patients (Barry, 1998). “We initially saw this in early trials involving both the old and newest formulations of saquinavir,” explained Dr. Hoetelmans. “We’ve definitely seen variability with all the PIs.”

Both PIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs) are metabolized by way of the cytochrome P450 isoenzyme system. Not only can the activity of this pathway vary from person to person, but it can also make for complex drug interactions, particularly among the antiretroviral agents and AIDS treatments used today. There is also protein binding to consider. What’s more, all three classes of antiretroviral drugs may be affected by the cellular influx and efflux system mediated by P-glycoproteins and other transport pumps, which has been implicated in multiple-drug resistance (see “The Evolution of Drug Resistance and Altered Viral Fitness” in the December 1999 issue of *The PRN Notebook*).

Dr. Hoetelmans explained that current knowledge regarding the relationship between plasma concentrations and the effectiveness of antiretroviral drugs is in a state of flux. For example, there is no clear connection between plasma levels and intracellular levels of the nucleoside reverse transcriptase inhibitors (NRTIs). “The intracellular triphosphate concentrations of NRTIs are difficult to measure and, because of the variability we see in the triphosphate anabolite concentrations, simply measuring plasma levels isn’t going to tell us much.”

As for the PIs, Dr. Hoetelmans pointed out that there is a connection between suboptimal concentrations and virologic failure, but the jury is still out with respect to the population of patients that may be best served using TDM. There are also data indicating an association between NNRTI concentrations and HIV-RNA rebounds, but this, Dr. Hoetelmans said, “has not been fully explored.”

A handful of studies have explored the connection between suboptimal plasma concentrations and virologic response in both treatment-naïve and -experienced patients. “We’re seeing a difference be-

tween these two groups of patients,” explained Dr. Hoetelmans. “It is necessary for us to consider these differences in current and future studies of TDM.”

Protease Inhibitor-Experienced Patients

IN ACTG 359, 277 PATIENTS EXPERIENCING VIROLOGIC failure while receiving an initial HAART regimen were randomly assigned to one of two dual PI regimens plus either delavirdine (Rescriptor), adefovir (Preveon), or both. Interestingly, the ACTG team found that patients allotted to either of the delavirdine groups (either with or without adefovir) fared better than those who received adefovir without delavirdine.

The executive summary prepared by the ACTG 359 team reasoned that the inferior virologic effect in the adefovir arms may have been because patients had extensive NRTI experience or discontinued 3TC upon study entry. The ACTG 359 also suggested that the lack of additivity or synergy in the delavirdine plus adefovir combination arms may well have been caused by an adverse pharmacokinetic interaction between delavirdine and adefovir. In an intensive pharmacokinetic sub-study of ACTG 359 (ACTG 884), the investigators demonstrated that delavirdine levels were halved when coadministered with adefovir. In addition, saquinavir levels were reduced by about half in the delavirdine plus adefovir combination arms, possibly as a direct result of the decreased delavirdine levels.

The VIRADAPT study, conducted in France, the Netherlands, and Israel, aimed to determine whether genotypic-resistance testing results would improve virologic responses to second-line or subsequent treatment regimens. In the study, 108 patients who were failing a HAART regimen and had HIV-RNA levels >10,000 copies/mL at the time of entry were randomized to a control group (group A) or

a group that received genotypic testing results before switching therapies (group B).

After three months, the study volunteers in group B had viral loads that were significantly lower than those in group A (-1.04 vs. -0.46 logs, respectively). After six months of therapy, patients in group B were almost twice as likely to have undetectable viral loads (32% vs. 14%, respectively).

Overall, 70% of the patients in VIRADAPT did not achieve an undetectable HIV-RNA level upon switching. To determine whether suboptimal plasma concentrations of the drugs used might be contributing to this sobering finding, the investigators performed a pharmacokinetic analysis of protease inhibitor plasma levels (Durant, 2000). The pharmacologic substudy included 81/108 patients. Serial PI plasma trough levels were performed in both arms throughout the study and were measured using high-performance liquid chromatography (HPLC). Samples were collected before the morning dose, and the analysis was performed using batched frozen samples.

Virologic response was analyzed by whether subjects achieved optimal PI concentrations with no more than one PI level less than twice the IC95, or suboptimal concentrations with two or more levels less than twice the IC95. Of the 81 patients studied, 32.1% had suboptimal PI concentrations; a breakdown of the substudy data are provided in tables 1 and 2.

Table 1. VIRADAPT HIV-RNA Changes by PI Concentration (log₁₀)

	3 months	6 months	9 months	12 months
SOC PI*	-0.31	-0.45	-0.49	-0.36
OC PI**	-0.99	-1.21	-1.12	-1.20

*SOC = suboptimal concentration; **OC = optimal concentration

Table 2. VIRADAPT Factors Predictive of Virologic Response

	or	95% c.i.	p-value
PI Concentration (>IC95 x 2)	2.37	2.0 – 7.0	0.017
Genotypic therapy	2.24	1.22 – 19.56	0.025

Source: Adaptation of data reported in *AIDS* (Durani, 2000).

Protease Inhibitor-Naive Patients

DR. HOETELMANS WAS CAREFUL TO POINT OUT that ACTG 359 and VIRADAPT involved patients who had failed their first-line PI-based regimen and that at least for these patients, TDM looks promising. For anti-retroviral-naive patients, or more specifically patients currently receiving their first PI-based regimen, Dr. Hoetelmans warned that TDM might not yield a significant connection between plasma concentrations and outcomes. Such was the case in the ADAM and CHEESE studies, both of which were reported in *AIDS*.

In the CHEESE study, Drs. Stuart Cohen, Hoetelmans, and their colleagues compared the efficacy and tolerability of the second saquinavir formulation (Fortovase) and indinavir (Crixivan), both in combination with AZT and 3TC, in antiretroviral-naive patients (Cohen, 1999). In the ADAM study, a randomized trial looking at the feasibility of induction-maintenance therapy, a team of researchers that included Dr. Hoetelmans at the University of Amsterdam conducted a substudy looking at toxicity and drug exposure in patients receiving a quadruple-drug regimen containing nelfinavir and saquinavir (Reijers, 2000).

In both studies, Dr. Hoetelmans and his colleagues were unable to find a significant relationship between plasma concentrations of the PIs used and the percentage of viral undetectability at 48 weeks. This finding differs from other studies, including those described above, which did report an association between plasma concentrations and viral undetectability, even in PI-naive patients.

Dr. Hoetelmans commented that, “simply because we didn’t find an association in patients being treated with PIs for the first time, does not necessarily mean that an association does not exist. Perhaps the follow-up was too short for this particular group of patients. We also think that the minimum drug exposure needed in antiretroviral-naive patients is much lower than the minimum drug exposure required to treat antiretroviral-experienced patients.”

As for the NNRTIs, Dr. Hoetelmans said that blood plasma concentrations are significantly associated with viral undetectability for treatment-naive patients. Drawing upon data from the INCAS trial—a randomized study comparing combinations of nevirapine, ddI, and AZT in antiretroviral-naive patients—Dr. Hoetelmans indicated that low plasma concentration of

nevirapine were significantly associated with viral rebounds among the 151 patients enrolled. “But we don’t have much evidence of an association in treatment-experienced patients,” he said. “In this way, we have an opposite effect of what we see in patients receiving protease inhibitors.”

TDM: Which Measurement Is Meaningful?

DR. HOETELMANS ADDRESSED THE issue as to which measurement of drug levels is the most meaningful. He said that the trough concentration (C_{min}) is typically the most meaningful and is easier to measure than the area-under-the-curve (AUC) concentration. “Trough levels haven’t been validated as the most important PK parameter when it comes to determining efficacy. But we know it’s just as important as the AUC. We know that the trough level is extremely important when looking at antibiotic resistance and this certainly applies to HIV.”

The maximum concentration (C_{max}) provides much less information that would be associated with drug efficacy or viral undetectability. The reasoning for the C_{min} being important is that if the minimum or lowest drug concentration falls below a crucial level, then HIV replication could occur.

Before assessing plasma concentrations, it is necessary to take into consideration the IC_{50} for each drug. Using phenotypic drug-resistance testing, the IC_{50} is needed to determine the degree of drug resistance—or lack thereof—for each patient’s dominant HIV strain. The end result is a ratio— C_{min}/IC_{50} —which, according to Dr. Hoetelmans, will provide the most useful information.

The required ratios for the C_{min}/IC_{50} varies between drug classes. For example, the optimal C_{min}/IC_{50} for the NNRTI drug class may well be greater than 500. For the PIs, Dr. Hoetelmans said, the required ratio may be smaller than 1. He also stressed that C_{min}/IC_{50} ratios most likely cannot be used to compare the potency of difference classes of drugs.

Dr. Hoetelmans argues that threshold values for C_{min}/IC_{50} ratios should be established for each drug and stressed the

Figure 2: Intracellular Kinetics of PIs

	Durability of this Response Following Removal of Drug from Culture	
	Half-Life of Antiviral Activity (h) HeLa Cells	PBMCS
SQV	>24	8
NFV	>24	8
RTV	12	3
APV	5	1
IDV	5	1

Source: Adaptation of data reported in *Antimicrobial Agents and Chemotherapy* (Nascimbeni, 1999).

importance of considering protein binding, penetration of various systemic compartments, intracellular accumulation, active metabolites, and synergy and antagonism between the antiretroviral drugs. “These are all important factors that can affect the levels of unbound and free drug in plasma and in cells,” he said.

Also discussed by Dr. Hoetelmans were data pertaining to the respective half-lives of five of the six currently available PIs (all except lopinavir). The data were originally published by researchers at the Laboratoire de Recherche Antivirale in Paris (Nascimbeni, 1999).

As shown in Figure 2, the *in vitro* half-life within PBMCS of either saquinavir or nelfinavir was over twice that of three other PI drugs. Specifically, the half-lives within PBMCS for saquinavir was eight hours, nelfinavir eight hours, ritonavir three hours, amprenavir one hour, and indinavir one hour. The Parisian researchers added a 5 μ m concentration of each drug to HIV-infected PBMCS. Within six hours, all HIV growth was blocked. Dr. Hoetelmans commented that, while the situation within the human body might be different due to many cofactors, “this study was important because it revealed differences in the concentration of each PI that would remain in cells where HIV replication occurs.”

It is important to note, however, that this study did not explore the possibility of non-specific binding of these drugs to cellular proteins that may have been responsible for the seemingly prolonged intracellular half-life. Because intracellular drug levels do not equate free drug capa-

The DONUT Study: Combining Nevirapine and Efavirenz

BYOND THE UTILITY OF THERAPEUTIC drug monitoring, the study of pharmacokinetics remains one of the most important aspects of an antiretroviral drug's development, marketability, and use in clinical practice. This is especially true in this day and age, in which novel combinations of drugs high in antiretroviral potency and with limited side effects are coveted prizes, especially for patients with high baseline viral loads or prior treatment experience.

One particular combination that has not been explored fully in clinical trials involves efavirenz (Sustiva) and nevirapine (Viramune), two potent non-nucleoside reverse transcriptase inhibitors. Yet, since both drugs can induce and/or inhibit CYP450 isoenzymes, a pharmacokinetic interaction cannot be ruled out. In order to evaluate the potential interactions between these two compounds, Dr. A.I. Veldkamp, Dr. Hoetelmans, and their colleagues in Amsterdam and London conducted the DONUT study and reported their results recently at the 7th Conference on Retroviruses and Opportunistic Infections and at the September PRN meeting.

As explained by Dr. Hoetelmans, HIV-infected individuals who had already been receiving efavirenz (600 mg qd) for at least two weeks were included. The pharmacokinetics of efavirenz were determined during 24 hours at baseline. Subsequently, nevirapine was added to the regimen: the first two weeks at 200 mg qd, followed by 400 mg qd. The pharmacokinetics of efavirenz and nevirapine were assessed on day 29.

The following pharmacokinetic parameters were deter-

mined: area under the plasma concentration versus time curve (AUC), maximum (C_{max}), and minimum (C_{min}) plasma concentration. The pharmacokinetics of nevirapine were compared with historical controls. Differences in efavirenz pharmacokinetics with and without nevirapine were analyzed using the Wilcoxon matched pairs signed ranks test.

As of September 2000, when Dr. Hoetelmans presented these data, a total of 19 male patients had been included in the preliminary analysis. Only 14 of these patients were evaluable, as two patients had undergone pharmacokinetics sampling only once and an additional three patients were not at steady-state levels (they ingested medication only during the pharmacokinetics sampling).

The pharmacokinetics data for each drug are reported in Tables A and B. It was determined that the pharmacokinetics of nevirapine were not affected by coadministration of efavirenz. The median decrease in the AUC and trough concentrations of efavirenz, when coadministered with nevirapine, were 22% and 36%, respectively.


Dr. Hoetelmans pointed out that no rashes or serious adverse events were observed and that HIV-RNA levels remained stable during the study period. With respect to the correct dose of efavirenz to be used if combined with nevirapine, Dr. Hoetelmans and his team indicated that an increase to 800 mg qd will suffice to achieve the necessary plasma concentrations. 

Figure A. Pharmacokinetics of NVP

	NVP+EFV	NVP alone
AUC ($h \cdot mg/L$)	112.1 (93.6–166.2)	101.8 (92.6–145.3)
C_{max} (mg/L)	6.19 (5.12–8.79)	6.69 (5.95–8.64)
C_{min} (mg/L)	3.66 (2.86–5.51)	2.88 (2.33–4.09)
T_{max} (mg/L)	2.00 (1.50–3.40)	1.50 (1.00–2.40)
$t_{1/2}$ (mg/L)	27.0 (16.4–35.2)	21.5 (15.0–32.8)

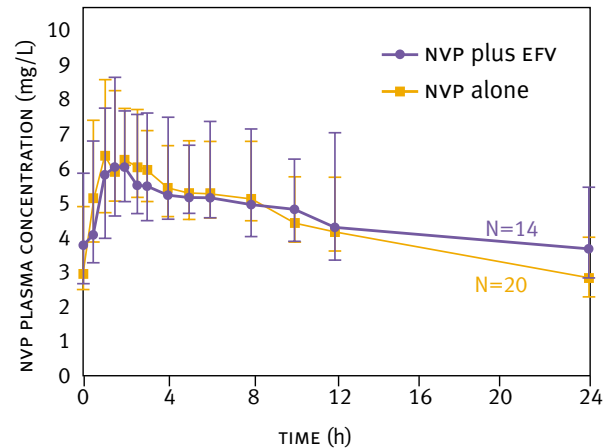
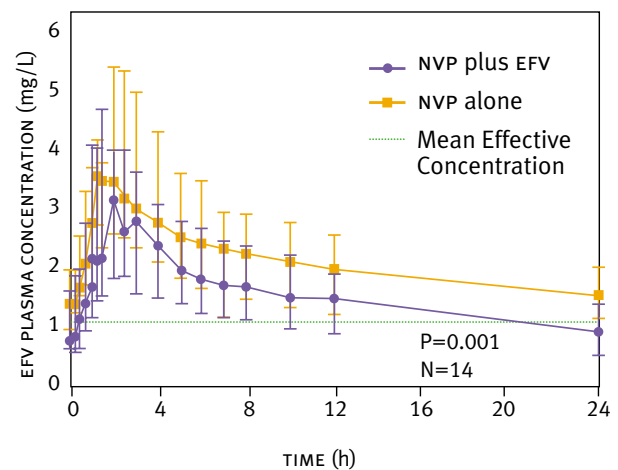


Figure B. Pharmacokinetics of EFV

	EFV+NVP	EFV alone
AUC ($h \cdot mg/L$)	54.8 (33.3–66.6)	38.8 (23.9–54.3)
C_{max} (mg/L)	3.63 (2.61–5.37)	3.36 (1.93–4.24)
C_{min} (mg/L)	1.55 (0.93–2.04)	0.96 (0.51–1.40)
T_{max} (h)	2.00 (1.50–3.00)	2.20 (1.50–2.50)
$t_{1/2}$ (h)	35.8 (18.1–50.6)	18.9 (14.8–34.0)



Source: Richard Hoetelmans, PhD. Adapted and published with permission.