

Pharmacokinetics, Pharmacogenetics, and HIV: The Aim to Optimize Antiretroviral Therapy

David J. Back, PhD
Professor of Pharmacology
Department of Pharmacology
and Therapeutics
University of Liverpool
Liverpool, United Kingdom

SUMMARY BY TIM HORN
EDITED BY CHARLES FLEXNER, MD

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THERE ARE HUNDREDS OF THOUSANDS OF HIV-INFECTED INDIVIDUALS around the world who are benefiting virologically, immunologically, and clinically from antiretroviral therapy. But without doubt, many of these individuals grapple with an array of short- and long-term side effects and virtually all of them are at risk of—or are currently dealing with the realities of—drug resistance and therapeutic failure. Fortunately, plans to remedy these situations are not simply based on the development of antiretrovirals with unique resistance patterns or adjunctive therapies to counteract side effects. Efforts are under way to better understand the pharmacology of antiretroviral therapy and how best to individualize treatment to yield the safest, most effective results.

According to Dr. David Back, the move toward this goal is evident in the recent move toward once-daily regimens to simplify dosing as well as boosted protease inhibitor (PI) regimens to both simplify and improve the effectiveness of treatment. Interest in therapeutic drug monitoring (TDM) has heightened considerably, with much of Europe already incorporating it into the HIV standard-of-care and with studies suggesting its usefulness sprouting up from all corners. Most recently, there has also been growing interest in the potential role for host genotyping, stemming from the fascinating study of pharmacogenetics.

I. Once-Daily Antiretrovirals

SINCE 2001, ALL OF THE ANTIRETROVIRALS APPROVED BY THE U.S. FOOD AND Drug Administration have been once-a-day, with the exception of enfuvirtide (Fuzeon). Tenofovir (Viread), emtricitabine (Emtriva), and atazanavir (Reyataz) all demonstrated favorable QD pharmacokinetics and were approved accordingly. Other once-daily drugs approved for use are older antiretrovirals, including lamivudine (Epivir), which now carries a dosing recommendation of 300 mg once a day; didanosine (Videx EC), a capsule formulation that only needs to be taken once a day and can be taken at the same time as other medications (although it still must be taken on an empty stomach and, thus, cannot be taken with other medications requiring administration with food); and stavudine (Zerit XR), a 100 mg capsule that can also be taken once a day, although it is not yet commercially available.

As explained by Dr. Back, there are actually three approaches to developing a once-daily drug. “First, there are some molecules that are naturally QD molecules and are studied and developed as such,” he said. “For other drugs, it’s possible to take the current BID formulation and increase the dose so that QD administration is possible. And for some drugs, it’s sometimes possible to change the formulation from a BID drug to a QD drug, extended-release formulations for example.”

The pharmacokinetic properties of stavudine, reformulated as an extended-release capsule and containing a higher daily dose of the drug (100 mg) than is typically used with the current twice-daily formulation

(40 mg BID), have been evaluated in healthy adult volunteers and HIV-infected adults. The slow release of stavudine from the extended-release capsule maintains measurable plasma concentrations for 24 hours after once-daily dosing. With once-daily dosing of the extended-release capsule, there is approximately 50% lower fluctuation of plasma concentration than observed with twice-daily dosing of the immediate-release formulation of stavudine.

In a crossover study in healthy volunteers, equivalent values for stavudine AUC (total daily exposure) were observed for the extended-release and immediate-release formulations. AUC increased proportionally with dose in the oral dose range of 37.5 to 100 mg (Kaul, 2002).

In parallel groups of HIV-infected patients, stavudine exposure was on average 23% lower following administration of 100 mg once daily of the extended-release formulation compared with 40 mg twice daily of the immediate-release formulation. The maximum plasma concentration (C_{max}) for the extended-release capsule is 43% of the value for the immediate-release capsule, and the time to reach C_{max} (T_{max}) is approximately three hours for the extended-release capsule compared with one hour for the immediate-release capsule. No significant accumulation of stavudine was observed after repeated administration of the extended-release capsule every 24 hours.

Dr. Back also discussed the pharmacokinetics of efavirenz, “a true once-a-day molecule.”

In HIV-infected patients, time-to-peak plasma concentrations are approximately three to five hours and steady-state plasma concentrations are reached within six to 10 days. The half-life of efavirenz is approximately 30 to 50 hours, although more recent data presented at the 11th Conference on Retroviruses and Opportunistic Infections, held in San Francisco in February, highlighted that in some patients the half-life could be greater than 100 hours (Taylor, 2004).

A number of clinical trials evaluating complete regimens that only need to be taken once a day have been completed, with preliminary and final data reported in various forms. Generally speaking, QD regimens employing two or three classes of drugs—an NNRTI or a boosted PI plus NRTIs—have performed well, whereas QD regimens employing single-class regimens—most notably all-NRTI combinations—have performed poorly and are not recommended.

Boosted PI Regimens

AS WAS EXTENSIVELY REVIEWED IN THE MARCH 2004 ISSUE OF *THE PRN Notebook*, in an article focusing on boosted and double-boosted PI regimens, pharmacokinetic enhancement of PIs—most notably with low doses of ritonavir (Norvir)—has rendered many PIs easier to take and more effective, particularly for patients who have tried and failed protease inhibitor therapy in the past.

As summarized by Dr. Back, most of the boosted PI options are associated with twice-daily dosing. Once-daily boosted options include low-dose ritonavir with either atazanavir (Reyataz), amprenavir (Agenerase), or fosamprenavir (Lexiva). Once-daily options being studied in clinical trials include 1600 mg saquinavir (Invirase; Fortovase) plus 100 mg ritonavir and 800 mg lopinavir plus 200 mg ritonavir (coformulated as Kaletra).

There are a number of lingering questions regarding boosted PIs, particularly if, when, and how to provide additional boosting of PI concentrations using higher doses of ritonavir. Another important question focuses on ritonavir alternatives for clinicians hoping to boost PI levels. “In the literature, people have talked about fluconazole or itraconazole being a booster,” Dr. Back said. “There’s also cimetidine to consider. In my view, these are not appropriate boosters, but we do need to think about other boosting drugs, apart from ritonavir, to try to broaden the horizon.”

Pharmacokinetics of NRTIs As Once-Daily Options

ALL OF THE AVAILABLE NRTIS—WHICH INCLUDES NUCLEOSIDE AND nucleotide analogues—are “prodrugs” and must undergo anabolic phosphorylation by intracellular kinases to form triphosphates (or diphosphates in the case of tenofovir). The triphosphates/diphosphates are the active moiety. Numerous methods are used to elucidate the intracellular metabolic pathways of these agents and both intracellular and extracellular factors affect intracellular phosphorylation. “Intracellular triphosphates, and not the plasma levels of these drugs, are what’s clinically relevant,” Dr. Back explained.

Dr. Back drew upon one study, conducted at the University of Colorado Health Sciences Center, that set out to quantify the pharmacological characteristics of zidovudine (Retrovir) and lamivudine triphosphate in HIV-infected patients (Anderson, 2003). Peripheral blood mononuclear cells (PBMCs) were obtained at multiple planned intervals from antiretroviral-naïve adults participating in a study of zidovudine, lamivudine and indinavir (Crixivan). Triphosphate levels were determined by immunoassay and high-performance liquid chromatography/mass spectrometry.

Thirty-three patients were evaluated. The estimated intracellular half-life of zidovudine was seven hours. “Intracellular zidovudine goes through three to four half-lives in a 24 hour period, so that concentrations in the cell are likely to be very low or undetectable at the end of a 24-hour dosing interval,” Dr. Back added. “In other words, there is unlikely to be sufficient zidovudine triphosphate for once-daily zidovudine.” With respect to lamivudine, the intracellular half-life was 22 hours. “With lamivudine, the PK data support a once-a-day molecule.” Dr. Back pointed out that in the University of Colorado study intracellular lamivudine triphosphate levels are much higher than intracellular zidovudine triphosphate levels. Approximately an hour after zidovudine is taken, zidovudine triphosphate levels peak around 100 femtomoles (fmoles) per million PBMCs, whereas with lamivudine, the peak triphosphate concentration is in the ballpark of 10,000 fmoles per million PBMCs. “It remains uncertain as to why the lamivudine triphosphate levels inside the cell are so high,” he added. “It is also interesting to note the large spread of values in different individuals.”

Data are also available from CNA10905, which evaluated the pharmacokinetics of once-daily abacavir (Ziagen), which is converted to carbavir triphosphate (Pillero, 2003). In this study, 20 HIV-positive patients underwent pharmacokinetic sampling over a 24-hour period to determine the concentrations of abacavir in plasma and intracellular carbavir triphosphate in PBMCs. On the pharmacokinetic sampling day, patients took their usual regimen in the morning, but withheld their evening dose

TABLE 1. Tenofovir Interactions

	Impact (%) of Coadministration on Exposure (AUC)	
	Coadministered Drug	Tenofovir
Didanosine (Videx; Videx EC)	↑ 44% to 60%*	↔*
Lamivudine (Epivir)	↔*	↔*
Emtricitabine (Emtriva)	↔	↔
Stavudine (Zerit; Zerit XR)	↔	↔
Abacavir (Ziagen)	↔*	↔*
Indinavir (Crixivan)	↔	↔
Lopinavir/ritonavir (Kaletra)	↓15%	↑34%
Atazanavir (Reyataz)	↓26%	↑25%
Atazanavir/ritonavir	↓25%	ND
Efavirenz	↔	↔
Methadone	↔	↔
Oral Contraceptives	↔	↔

* plasma levels

Source: David Back, PhD; Gilead Sciences

of abacavir in order to better characterize the pharmacokinetics of carbavir triphosphate. The geometric mean abacavir terminal half-life in plasma was 2.59 hours; the geometric mean CBV-TP terminal half-life in PBMCs was 20.64 hours. “Again,” Dr. Back commented, “we seem to be looking at a molecule that can be taken once a day instead of twice a day, based on intracellular triphosphate concentrations.”

Unfortunately, once-daily regimens employing antiretrovirals that are supported by once-daily pharmacokinetic data have not always yielded positive results in clinical trials. One example is GlaxoSmithKline’s ESS300009 trial, in which 345 patients were randomized to receive either tenofovir or efavirenz, combined with a fixed-dose combination tablet containing 600 mg abacavir and 300 mg lamivudine, all to be taken once a day (Gallant, 2003). Patients were naïve to antiretroviral therapy prior to starting the study and had an average baseline viral load of 4.63 log₁₀ copies/mL and a baseline CD4+ cell count of 260 cells/mm³. Because of a glaringly high number of premature treatment failures being documented in the triple-NRTI group, the study investigators conducted an unplanned analysis involving the first 194 patients to complete eight weeks of follow-up. Approximately 49% of patients in the triple-NRTI group met the definition of virologic failure, compared to only 5.4% of patients in the efavirenz-based arm. Among patients who had viral loads that were high enough to test for drug resistance, 64% of the triple-NRTI failures had both the K65R and M184V mutation and 36% had only the M184V mutation. Based on these preliminary results, the study was immediately halted and closed to enrollment.

The reason for the glaring differences in response to therapy is still being debated. One possibility was the low genetic barrier for selection of resistance for the regimen. Tenofovir, abacavir, and lamivudine can all select for the K65R mutation and both abacavir and lamivudine can select for the M184V mutation, which can potentially yield rapid and high-level resistance to a regimen consisting of these three drugs. “However,” Dr. Back commented, “we also know that efavirenz has a low genetic barrier for selection of resistance, with development of the K103N mutation. And we didn’t see the rapid emergence of this mutation in this study.”

Another possible explanation may be a pharmacological mecha-

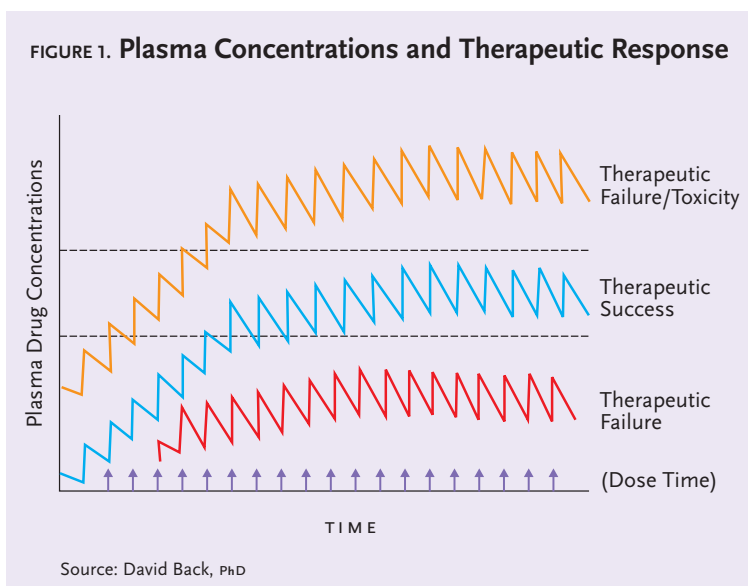
nism. One possibility is an interaction at the phosphorylation level, perhaps resulting in decreased carbovir triphosphate and/or tenofovir diphosphate with the coadministration of these NRTIs. However, two new studies reported at the 5th International Workshop on Clinical Pharmacology of HIV Therapy, held in early April in Rome, indicate that such an interaction does not occur (Ray, 2004; Hawkins, 2004). "Another possibility may be an effect of tenofovir on influx/efflux transport, or vice versa, across cell membranes," Dr. Back said. "However, to date there are no data supporting an interaction between abacavir and tenofovir in relation to transport."

Some of the most important drug-drug interactions involving tenofovir are reviewed in Table 1.

II. Therapeutic Drug Monitoring

THE BASIS OF THERAPEUTIC DRUG MONITORING (TDM) IS SIMPLY TO BE ABLE to individualize doses of antiretroviral medications in order to keep plasma concentrations of the drugs within the window of therapeutic success, meaning concentrations that are high enough to suppress either drug-sensitive or drug-resistant HIV but low enough to prevent or minimize toxicities (see Figure 1). "The therapeutic window will vary from person to person," Dr. Back said. "A number of viral and host factors can have varying effects on the minimum and maximum concentrations that define an individual's therapeutic window."

There are a number of possible scenarios in which TDM might be useful in terms of individualizing antiretroviral therapy. First, it may help in tailoring doses to address extreme differences in weight. Someone with a very low body weight may require lower doses of antiretroviral medications to avoid dose-dependent side effects, whereas someone with a very high body weight may require higher doses of antiretroviral medications to ensure high enough concentrations to suppress HIV replication. Second, race, gender, and age are associated with varying plasma concentrations of antiretroviral drugs. It is important to note, however, that this may apply only to some drugs; body weight, race, gender, and age may not have an impact on plasma concentrations of all antiretrovirals. Third, TDM may help identify clinically significant drug-drug or drug-food interactions that may result in reduced efficacy or increased dose-related toxicities. Fourth, it may help guide dosing of an-



tiretrovirals in the setting of specific diseases or coinfections associated with the impairment of gastrointestinal, hepatic, or renal function that can alter drug absorption, distribution, metabolism, or elimination. Fifth, TDM may be useful in monitoring HIV-positive pregnant women undergoing treatment, who may be at risk for virologic failure or at an increased risk of toxicities as a result of varying pharmacokinetics not typically seen in non-pregnant HIV-positive patients.

TDM: Determining the Need for a Dosage Increase

ONE SCENARIO IN WHICH TDM MAY BE USEFUL IN DETERMINING IF A dose of a particular antiretroviral needs to be increased to achieve maximal effectiveness is that of drug interactions, particularly with drug regimens that involve combining two protease inhibitors with ritonavir as a pharmacokinetic booster. As was explained by Dr. Back, and explored in detail in the March 2004 issue of the *Notebook*, ritonavir may inhibit the metabolism of one protease inhibitor, while at the same time the first protease inhibitor may induce the metabolism of the other protease inhibitor prescribed. In this way, some drug-drug interactions can be very unpredictable.

There have been a number of studies demonstrating that lopinavir and amprenavir appear to be problematic antiretroviral partners. In studies reviewed by Dr. Back, the combination of amprenavir and lopinavir/ritonavir resulted in reduced plasma concentrations of lopinavir relative to the usual lopinavir/ritonavir pharmacokinetics and reduced plasma concentrations of amprenavir relative to the usual amprenavir/ritonavir pharmacokinetics (although amprenavir concentrations were elevated relative to unboosted amprenavir 1200 mg BID). Data presented at the 11th Conference on Retroviruses and Opportunistic Infections (CROI), held in San Francisco in February, also indicate that fosamprenavir (Lexiva) and lopinavir concentrations are reduced when coadministered, both with ritonavir (Wire, 2004). "These data show us that an increase in the dose of both drugs may be needed to optimize therapy in patients taking these particular combinations," Dr. Back said. "And this would be an indication for therapeutic drug monitoring."

Another scenario discussed by Dr. Back is the need to ensure adequate drug concentrations in antiretroviral-experienced patients. According to a letter published in *AIDS*, authored by Dr. Marta Boffito of St. Stephen's Chelsea and Westminster Hospital in London and her colleagues, intraindividual variability in lopinavir plasma concentrations strongly supports TDM (Boffito, 2003). The letter detailed the experience of 35 HIV-positive antiretroviral-experienced patients being treated with a lopinavir/ritonavir-based regimen. Twenty-two of the patients were responders, defined as a viral load below 50 copies/mL, and 13 were nonresponders, defined as detectable or increasing viral load while on therapy. Nonresponders were more likely to have lower lopinavir trough levels than the responders. "According to this study," Dr. Back explained, "a lopinavir trough above 5.7 µg/mL was an independent predictor of response. So here we have another example of using TDM strategically to maximize responses to treatment."

TDM: Determining the Need for a Dosage Decrease

THERE ARE ALSO SCENARIOS IN WHICH TDM MAY BE USED TO DETERMINE if it's suitable to reduce the dose of a particular drug. In one French study reported at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), pharmacokinetic characteristics of efavirenz were studied in 30 HIV-infected patients, 13 of whom were female and 12 of whom were coinfecting with HCV (Jeantils, 2003).

While the therapeutic range of efavirenz was defined by the investigators to be between 1100 and 3000 ng/mL, 40% of the patients in this study had efavirenz levels that were above the upper limit of the therapeutic range. In these patients, an efavirenz dose reduction to 400 mg once-daily, was initiated, and all patients have maintained undetectable viral loads for the six months of follow-up reported thus far. “I’m not entirely convinced of this narrow therapeutic range,” Dr. Back commented, “but I do think these are interesting data to keep an eye on.”

Also of interest are recent data evaluating low-dose indinavir (400 mg BID) combined with low-dose ritonavir (100 mg BID). In one study reported by a French team, patients with HIV-RNA levels below 200 copies/mL while taking a standard indinavir-based regimen (800 mg TID) were switched to 400 mg indinavir/100 mg ritonavir and maintained on their previous NRTI selection (Ghosn, 2003). Twenty patients were enrolled and, at the time of study entry and while still receiving standard-dose indinavir, the median indinavir C_{min} was 194 ng/ml and median IDV C_{max} was 8449 ng/ml. Upon switching to 400 mg indinavir and 100 mg ritonavir, the median indinavir C_{min} increased to 536 ng/ml at week two and 475 ng/ml at week four, while the C_{max} decreased to 2983 ng/ml at week two and 2997 ng/ml at week four. The median indinavir half-life, using the boosted low-dose regimen, was 4.4 hours. Encouragingly, after 48 weeks of follow-up, all patients maintained HIV-RNA levels below 200 copies/mL and tolerability was reported to be excellent.

To provide a closer look at the efficacy of this low-dose PI combination, Dr. Back reviewed more recent data, from the same French team, involving an open-label clinical trial of 400 mg indinavir and 100 mg ritonavir, combined with two NRTIs, in 40 antiretroviral-naïve patients (Duvivier, 2003). At baseline, the median viral load was 5.36 \log_{10} copies/mL and the median CD4+ count was 84 cells/mm³. In the intent-to-treat analysis, after 48 weeks of treatment, 65% patients had HIV-RNA levels below 200 copies/mL and 50% had HIV-RNA levels below 50 copies/mL. In the as-treated analysis, 96% had viral loads below 200 copies/mL and 74% had viral loads below 50 copies/mL. The median CD4+ count increase among the study participants was 167 cells/mm³. Ten patients discontinued therapy before week 48: eight because of adverse events, one because of personal choice, and one because of a change in therapy. Three patients were lost to follow-up. Only one virologic failure occurred among those completing the 48-week treatment course and was associated with poor adherence.

Another scenario discussed by Dr. Back was the potential utility of TDM as a tool for optimizing dosing in HIV-positive individuals with hepatitis C virus (HCV) coinfection. As has been extensively reviewed in previous issues of the *Notebook*, HIV/HCV-coinfection is a common problem and it is well known that hepatic disease can affect the ability of the liver to metabolize various drugs, including antiretrovirals. The problem is, there has been a paucity of sound scientific study involving antiretroviral therapy in the setting of HIV/HCV coinfection, particularly biopsy data categorizing liver damage.

Altered antiretroviral pharmacokinetics have been documented in HIV/HCV-coinfected patients. In coinfecting patients with moderate or severe hepatic dysfunction and/or cirrhosis, nelfinavir plasma concentrations are increased, along with decreased formation of the M8 metabolite. “No dose adjustment is likely needed with nelfinavir,” Dr. Back commented, “although TDM might be useful.” Indinavir plasma concentrations are also increased in HIV/HCV-coinfecting patients with hepatic dysfunction. “Dose modification, perhaps 200 mg of indinavir and 100 mg of ritonavir, may be possible,” he said. “However, this hasn’t been studied.” As for lopinavir/ritonavir, exposure levels are increased in coin-

fecting patients, with changes in protein binding noted as well. With the NNRTI efavirenz, data are still limited, and with nevirapine, HIV/HCV-coinfection does not appear to be generally associated with increased plasma concentrations of the drug. However, this is not to say that increased plasma levels of nevirapine, for whatever reason, are not associated with an increased risk of hepatotoxicity in both HIV/HCV-coinfecting and HIV-monoinfecting patients.

In a case-control study conducted at Hospital Carlos III in Madrid, 70 patients taking nevirapine-based regimens were classified into two groups, one including patients who developed any grade of hepatotoxicity, and a control group that included subjects without transaminase elevations (González de Requena, 2002). Patients were also stratified according to the presence of HCV infection. The peak in transaminase levels among the 33 subjects in the first group was reached after approximately six months of beginning treatment. Transaminase levels were mildly to moderately elevated in 70% of patients, twenty-three of whom were HCV-positive. The median nevirapine plasma concentrations—measured using high-performance liquid chromatography—in subjects who developed transaminase elevations was significantly higher than in controls (6.25 μ g/mL vs. 5.2 μ g/mL, respectively). When HCV infection was also included in the analysis, both higher nevirapine plasma levels and HCV seropositivity were found to be independent factors predicting elevated transaminases. Moreover, in patients with both chronic HCV and nevirapine plasma levels above 6 μ g/mL, the risk of elevated transaminases was approximately 92%. It is important to note, however, that this study did not perform biopsies to determine if peak elevations in transaminases were associated with bona fide liver damage.

“There’s growing evidence that TDM is useful in terms of knowing when to reduce antiretroviral drug dosages, particularly in the setting of hepatitis coinfection and when using antiretrovirals known to cause dose-dependent hepatotoxicity,” Dr. Back commented.

Where are we with TDM?

GIVEN THAT RESEARCH INTO THE CLINICAL UTILITY OF TDM IS STILL quite new, compounded by the fact that TDM is not widely available through commercial laboratories, the United States Department of Health and Human Services’ *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* do yet not recommend TDM for routine use in the management of HIV-infected patients. However, the *Guidelines* are in agreement with treatment guidelines published by Australian and European agencies that there are a number of possible scenarios in which TDM might be useful to clinicians, which includes many of the scenarios reviewed above.

As explained by Dr. Back, TDM truly is an evolving aspect of HIV management, with a number of lingering questions. First, he said, more research is needed to clearly define who should be tested using TDM, when the optimal time for testing is, and how best to translate the results of TDM into optimized therapy. Second, with current TDM technologies unable to measure intracellular concentrations of NRTI triphosphates and diphosphates, is it simply “okay” to ignore these drug concentrations? Third, there is very much a need to define target concentrations for all of the antiretrovirals, particularly for people who are antiretroviral experienced. Fourth, more research needs to be done to better understand the influence of various covariables—including race, gender, age, weight, etc.—on the pharmacokinetics of antiretrovirals. Finally, it is vital that clinical researchers, the pharmaceutical industry, and consensus panelists work together to standardize dose-adjustment procedures.

For clinicians in the United States who are interested in ongoing TDM

TABLE 2. Therapeutic Ranges for Antiretroviral Drugs

Drug	Minimum trough levels (mg/L)	Maximum peak levels (mg/L)
Saquinavir (Invirase; Fortovase)	0.10	ND
Ritonavir (Norvir) ^a	2.1	ND
Indinavir (Crixivan)	0.10	10
Nelfinavir (Viracept)	0.8	ND
Amprenavir	0.4/1.2 ^c	ND
Lopinavir/ritonavir	1.0/4.0 ^c	ND
Nevirapine (Viramune) ^b	3.4	ND
Efavirenz (Sustiva) ^b	1.0	4.0 ^d

(a) RTV given as single PI

(b) due to a long elimination half-life, the variability in NVP and EFV levels during a dose interval is minimal; samples may be taken without regard to time between intake and sampling; values for NNRTIs represent minimum effective concentrations (MEC) based on clinical outcome data.

(c) Minimum trough levels for treatment experienced patients

(d) Maximum trough level

Source: HIVpharmacology.com

research—and wish to find out more about commercial laboratories offering TDM—an excellent source of information is <http://www.hivpharmacology.com>. Some therapeutic ranges for antiretroviral drugs have been recommended by the Editorial Board of HIVpharmacology.com (see Table 2), of which Dr. Back is currently a member.

III. Pharmacogenetics

A FRONT-PAGE HEADLINE IN THE DECEMBER 8, 2003 EDITION OF *THE Independent*, one of the United Kingdom's leading daily newspapers, couldn't have been more shocking: "Glaxo chief: Our drugs do not work on most patients." The article, relying on statements made by Dr. Allen Roses, Worldwide Vice-President of Genetics at GlaxoSmithKline, went on to explain that "most drugs work in fewer than one in two patients, mainly because the recipients carry genes that interfere with the medicine. Although his comments were not specific to antiretrovirals, his comments illustrate the potential of pharmacogenetics to profile a population of patients with HIV infection and predetermine, based on their genetic makeup, those who are likely to benefit from standard doses of therapy and those who will likely need lower or higher doses to limit toxicities and maximize effectiveness" (see Figure 2).

MDR-1 Polymorphisms

THERE ARE A LARGE NUMBER OF GENETIC VARIANTS IN THE ENZYMES OF phase I and phase II drug metabolism, in drug transporters, and drug targets, all of which might account for differences in drug response. Polymorphisms in the cytochrome P450 enzyme system have been investigated most extensively. More recently, cellular drug transports—particularly p-glycoprotein, the ATP binding cassette efflux transporter encoded by the multidrug-resistance transporter-1 (MDR-1) gene—have been recognized as crucial determinants in pharmacokinetics. "There have

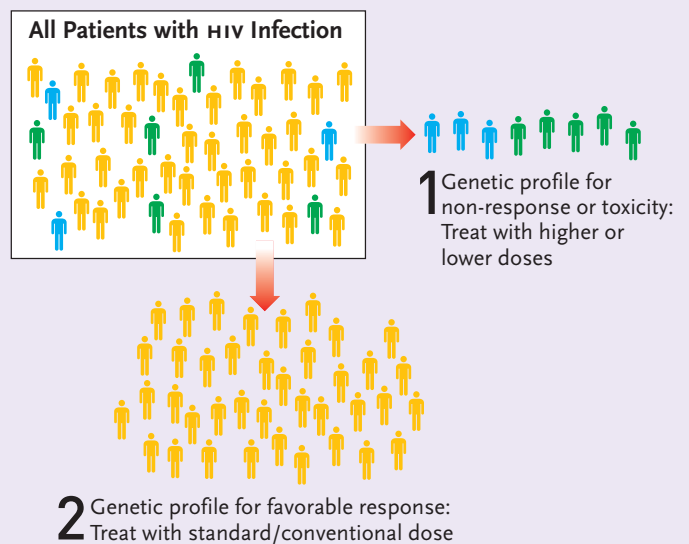
been a lot of studies looking at the different polymorphisms, or single nucleotide polymorphisms, in MDR-1," Dr. Back said. "Studies have focused on MDR-1 exons 1, 12, 21, and 26, with the most data thus far involving the polymorphism at exon 26."

In one Swiss study reviewed by Dr. Back, plasma concentrations of efavirenz and nelfinavir, levels of P-glycoprotein expression, CD4+ cell counts, and viral load were evaluated in 123 HIV-positive patients (Fellay, 2002). Median drug concentrations in patients with the MDR-1 T3435T, C3435T, and C3435C genotypes were at the 30th, 50th, and 75th percentiles respectively. Patients with the T3435T genotype, six months after starting treatment, experienced greater CD4+ cell count increases (257 cells/mm³) than patients with the C3435T (165 cells/mm³) and C3435C (121 cells/mm³) genotype, and the best recovery of naive CD4+ cells. However, these data have not been corroborated. In one study reported at the 10th Conference on Retroviruses and Opportunistic Infections, held in Boston in 2003, a study involving 73 children found that patients with the MDR-1 C3435C genotype were more likely to have lower plasma nelfinavir concentrations with no advantage in terms of CD4+ cell gains (Singh, 2003). And in another study involving 142 patients, MDR-1 variants were not associated with significant differences in CD4+ cell increases while taking either a nelfinavir- or efavirenz-based regimen (Peterson, 2003).

In another study, conducted by a team with the B.C. Center for Excellence in HIV/AIDS in Vancouver, the influence of single nucleotide polymorphisms in the MDR-1 gene on virological and immunological responses among 461 HIV-infected patients was assessed (Brumme, 2003). Among the patients with MDR-1 variants, no statistically significant differences in time to virological and immunological failure were documented. However, there was a trend to earlier virological failure in the MDR-1 C3435C genotype group, suggesting that polymorphisms in MDR-1 may be associated with premature treatment failure.

Yet another study discussed by Dr. Back was conducted by the AIDS Clinical Trials Group (Haas, 2003). This study explored relationships between MDR-1 polymorphisms and the first phase of viral decay among 31 HIV-infected individuals initiating antiretroviral therapy for the first

FIGURE 2. Potential of Pharmacogenomics



Source: Evans WE, Johnson JA. **Pharmacogenetics: The Inherited Basis for Interindividual Differences in Drug Responses.** *Annu Rev Genomics Hum Genet.* 2:9-39, 2001.

time. Seven (23%) patients harbored the MDR-1 C3435C genotype, 14 (45%) had the C3435T mutation, and 10 (32%) had the T3435T mutation. Additionally, eight (26%) patients had the G2677G genotype, 18 (58%) harbored the G2677T mutation, and five (16%) had the T2677T genotype. However, no significant relationships between allelic variants in either exon 26 or 21 and phase 1 or phase 2 viral decay were documented, nor were there any associations between these polymorphisms and antiretroviral drug concentrations or CD4+ cell responses to therapy over time. “So with these data,” Dr. Back commented, “we’re left with a really mixed message regarding the importance of these genetic changes.”


Dr. Back argued that these studies were limited in the sense that they looked only at the effect of single nucleotide polymorphisms in the MDR-1 gene, not various haplotypes (the overall structure of the gene combinations of polymorphisms). “We’ve got to look across the whole gene in order to be able to marry the data,” he explained. “Fortunately, studies are beginning to do that.”

Haplotype Structure of the MDR-1 Gene

TO EVALUATE THE VARYING EFFECTS OF MDR-1 HAPLOTYPES, BASED ON THE presence of polymorphisms, Dr. Back participated in a retrospective analysis of data from the MaxCmin1 clinical trial (Owen, 2004). The MaxCmin1 trial was a phase IV randomized, open-label, multicenter trial to evaluate the safety and efficacy of indinavir/ritonavir (800/100 mg twice daily) versus saquinavir/ritonavir (1000/100 mg twice daily). Comparable antiretroviral efficacy was observed between both groups, although a greater number of treatment-limiting adverse events were observed in the indinavir/ritonavir group. In order to determine whether MDR-1 haplotypes contributed to treatment endpoints and variability in pharmacokinetics, Dr. Back and his English and Danish colleagues genotyped patients for the common single-nucleotide polymorphisms C3435T and G2677T.

Of the 306 patients who initiated treatment, 229 patients were genotyped for either C3435T or G2677T or both. Additional blood samples were obtained for analysis of drug concentrations. Logistic regression models were constructed in order to determine whether either genotype or haplotypes were predictors of a viral load greater than 50 or 400 copies/mL or the time to a CD4+ cell increase of greater than 100 cells/mm³ from baseline. Similar analyses were carried out to determine whether clinical progression or time to an adverse event were dependent on these single-nucleotide polymorphisms. Finally, regression models were constructed in order to determine whether predicted trough concentrations of saquinavir, indinavir, or ritonavir were dependent on MDR-1 genetics. As reviewed by Dr. Back, no significant differences were observed in any of the regression models for changes in the CD4+ cell count or viral load relative to C3435T, G2677T, or haplotype in pooled analyses. No statistically significant differences were observed in drug levels at week four or week 48 for MDR-1 haplotypes or individual genotypes.

“With this study,” Dr. Back explained in his concluding remarks, “our genetic analysis of the MDR-1 gene failed to correlate with predicted trough concentrations of saquinavir, indinavir, and ritonavir. However, I don’t think that this study rules out the possibility of genetic factors being associated with antiretroviral treatment outcomes. Plus, we’ve seen data indicating that genetic changes in cytochrome P450, particularly CYP3A4 and CYP3A5, can have significant effects on the pharmacokinetics of immune-suppressive drugs, such as cyclosporine and tacrolimus. We need to begin applying this same research to anti-HIV drugs. Down the line, I really do believe that we may be able to use a patient’s genotype to help individualize drug treatment. It probably does constitute a

methodological advance and may give us a feasible approach to safer and more efficient drug therapies. But we need to remember the variables. We need to remember that this is only one factor that may influence drug handling. There are multiple environmental factors that can impinge upon pharmacokinetics, making genetic polymorphisms only one of the issues we really do need to face.” 

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