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Therapeutic drug monitoring of anti-HIV drugs

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1. Introduction

If we look back over the development of anti-HIV therapies, a key event is undoubtedly the introduction of protease inhibitors (PIs) in 1995. As a component of antiretroviral therapy, PIs have produced a dramatic decrease in mortality and morbidity in HIV infection [1] most clearly demonstrated by the reduction of opportunistic infections and hospital admissions. Today, a triple or quadruple drug combination regimen contains a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs) plus PIs or non-nucleoside reverse transcriptase inhibitors (NNRTIs),and this constitutes the standard of care for patients commencing therapy (see Table 1).

However, the reality of the present status is that combination antiretroviral therapy (ART) still lacks sufficient potency and durability. Large prospective studies (e.g. [2]) suggest that greater than 30% of HIV positive patients will fail to achieve adequate suppression of plasma HIV RNA (i.e. <50 copies/ml) for any of the current ART regimens. Even when this is achieved, viral rebound develops in a significant proportion of patients within 1 year of follow-up [3–5]. For example, in the Swiss cohort study [3], rebound HIV viraemia (from <400 copies/ml to detectable) was approximately 10% per year in ART-naive patients commencing therapy and 20% in ART-experienced patients switching therapy. Although 80% of ART-naive patients achieved viral load below 400 copies/ml at 6 months, this was only sustained in 66% at 30 months and treatment changes were necessary in approximately half of patients by 24 months. In the Frankfurt cohort [4],

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Table 1
Currently licensed antiretrovirals

Protease inhibitors (PIs)	Nucleoside reverse transcriptase inhibitors (NRTIs)	Nonnucleoside reverse transcriptase inhibitors (NNRTIs)
Amprenavir	Abacavir (ABC)	Delavirdine
Indinavir	Didanosine (ddI)	Efavirenz
Nelfinavir	Lamivudine (3TC)	Nevirapine
Ritonavir	Stavudine (d4T)	
Saquinavir (soft gel, hard gel)	Zalcitabine (ddC) Zidovudine (ZDV)	

Recommendation for initial treatment is:

- PI[a] + 2 NRTIs
- NNRTI + 2 NRTIs
- 3 NRTIs[b]

[a] PI might be a combination of two PIs.
[b] Not in official guidelines, but increasingly used.

over half of patients developed viral rebound within 12 months of achieving undetectable RNA. There are also a growing number of patients who fail treatment despite exposure to most anti-retroviral agents and consequently receive salvage therapy, which may include 'mega-HAART' regimens. Such regimens are associated with increased potential for toxicity and serious drug interactions. In this context, some of the most pressing clinical pharmacology questions are:

- Why do some patients fail treatment regardless of the regimen selected?
- How can existing therapies be improved or optimised?
- Will the new drugs in development show significant advantage?

Treatment failure is clearly multifactorial, and may include the development of antiviral resistance, poor adherence to therapy and pharmacokinetic reasons. This review will focus primarily on pharmacokinetic variability as an important consideration in treatment failure and current approaches to address the problem.

Pharmacokinetic variability is particularly important in relation to PIs—a group of peptidomimetic drugs with considerable inter- and intra-individual variability in plasma levels and marked potential for drug interactions leading to reduced or elevated PI plasma concentrations [6,7]. Reduced concentrations will potentially compromise efficacy while elevated concentrations will predispose to adverse events. When considering a possible role of therapeutic drug monitoring (TDM) in antiretroviral therapy, the primary focus is therefore on PIs. However, before proceeding to develop the arguments for TDM of PIs, it is important to highlight the main issues around plasma concentrations of the other two main classes of antiretrovirals.

For NRTIs, establishing a relationship between plasma concentration and antiviral effect has been difficult simply because it is the intracellular triphosphate anabolite that is the active moiety (Fig. 1). Plasma concentrations of parent nucleosides and intracellular

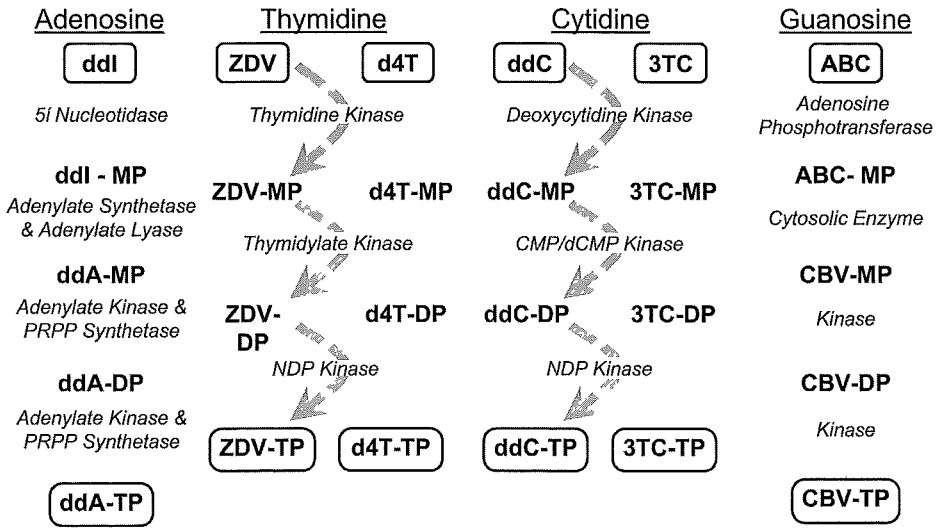

Fig. 1. Activation pathways of nucleoside analogues.

concentrations of triphosphates show only a weak correlation. Fig. 2 displays data from a study with lamivudine (3TC)—see also Refs. [8,9]. Therefore, meaningful data require cell separation (i.e. to generate peripheral blood mononuclear cells, PBMCs), a technique which is time consuming, followed by analysis of the active triphosphate, a procedure which is currently available only in a handful of laboratories. This effectively precludes

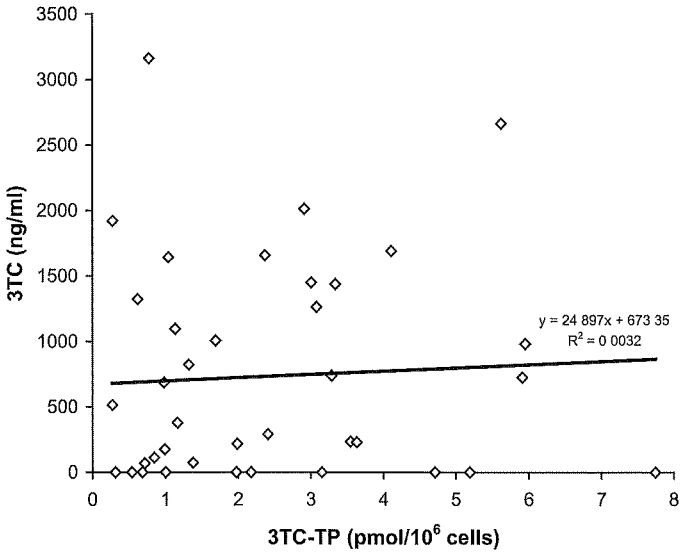

Fig. 2. The relationship between intracellular nucleoside triphosphate and plasma concentration of parent drug for lamivudine (3TC).

any realistic consideration of TDM for NRTIs in clinical practice until such time as a more rapid throughput analytical system is developed.

Data from pharmacokinetic studies of NNRTIs indicate that two of the drugs, efavirenz and nevirapine, have a prolonged half-life, normally achieve adequate steady-state plasma concentrations during a dosing interval, and the pharmacokinetics are less variable than PIs. Although we would not entirely rule out a role for TDM of NNRTIs in some situations (e.g. in relation to CNS side effects of efavirenz), at this point in time attention is focussed on PIs.

2. The arguments for TDM of PIs

2.1. Drug concentrations correlate with antiviral effect

This probably represents the strongest case for TDM. An association between plasma saquinavir levels and virological response was observed in patients participating in a dose ranging study of the original hard-gel formulation [10] and upon initiation of ART [11]. Gieschke et al. [12] investigated the relationship between systemic exposure to monotherapy saquinavir (soft-gel formulation administered at 400, 800, 1200 mg tds) and plasma HIV RNA and CD4 cell counts using empirical mathematical modelling, and indicated the area under the plasma concentration–time curve, which gives maximal viral suppression (Fig. 3). In the ADAM study, plasma saquinavir and nelfinavir concentrations were strongly associated with the initial rate of HIV clearance [11].

Dose ranging monotherapy studies of ritonavir and indinavir have also demonstrated a relationship between dose and clinical response with rapid emergence of antiviral resistance with the use of lower than recommended doses [13–16].

A phase I/II study of indinavir demonstrated a good relationship between indinavir exposure (AUC and C_{min} levels) and virological response [17]. The TRILEGE study [18]

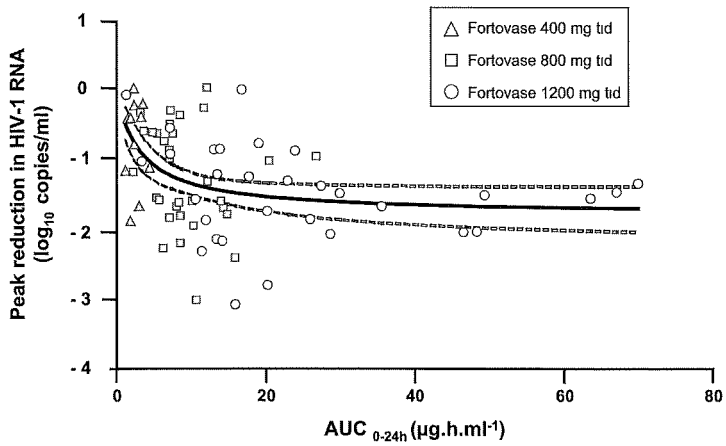

Fig. 3. Dose ranging study (NV 15107) to determine the optimal dose of saquinavir soft gel (Fortovase); from Gieschke et al. [12] with permission of Adis Press.

reported an association between low indinavir levels and treatment failure, although a similar US study (ACTG 343) failed to observe this association [19]. Several other US [20,21] and European [22,23] studies have reported an association between indinavir levels (using AUC, C_{max}, C_{min} or concentration ratios) and virological response in both adults and children. The evidence, therefore, is that maintaining therapeutic levels is potentially critical for PIs, not just in preventing drug resistance, but also cross-resistance to other PIs. Exposure of a patient to subtherapeutic levels of a PI may result in the stepwise accumulation of mutations in the HIV protease with subsequent resistance to both the prescribed drug and other class members. Recently results from the VIRADAPT study have been reported [24]. This is a randomised controlled trial of HIV genotyping to guide decision making versus standard of care (SOC). However, plasma samples were also analysed retrospectively for PI concentrations and patients were categorised as having optimal or suboptimal concentrations based on trough concentrations being above or below the IC_{50} of the drug. The results provide evidence of the benefit of both genotyping and having optimal PI concentrations. For example, at 6 months there was only a 0.23 log drop in viral load in patients receiving SOC with suboptimal drug concentrations, but a 1.3 log drop in patients having both genotyping and optimal drug concentrations.

2.2. *Variability in plasma drug concentration*

PI concentrations in plasma show marked interpatient variability following standard dosing regimens. Based on studies with hard-gel saquinavir showing a greater than 20-fold variability in trough concentrations, we suggested that TDM of saquinavir could have a role in improving therapeutic outcome [25]. We have subsequently developed a TDM service for selected patients in the UK. Fig. 4 shows trough PI concentrations from this

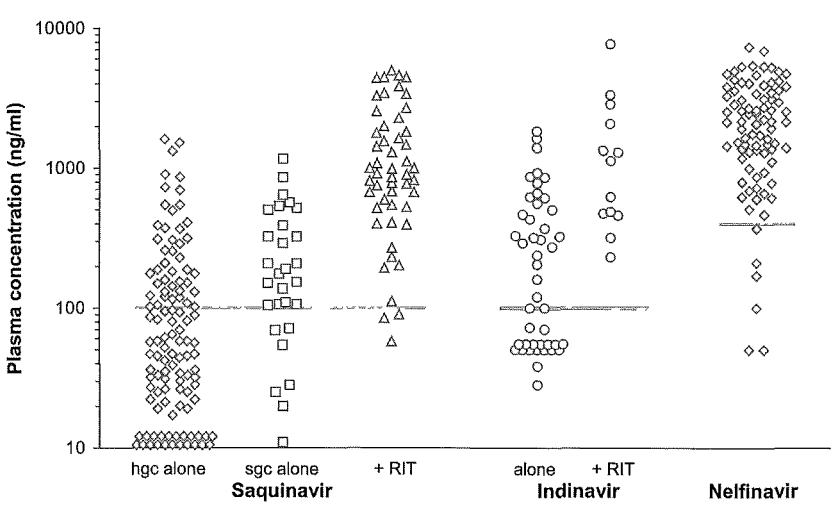

Fig. 4. Trough plasma concentrations of saquinavir, indinavir and nelfinavir after various dosing regimens alone and in the presence of ritonavir. Data from Liverpool HIV Pharmacology Group. Hgc = hard gel capsule; sgc = soft gel capsule. The horizontal line indicates the minimum effective concentrations used during 1999/2000.

study. There are a number of features. Clearly there is huge inter-patient variability for all the PIs. Note, for example, that a high proportion of patients receiving the original hard-gel formulation of saquinavir have trough concentrations (C_{trough}) below what we have suggested is a target minimum effective concentration (MEC). The latter is derived substantially from in vitro studies where the concentration of each PI to inhibit wild type HIV is determined (IC_{50} or IC_{95} values) in the presence of 50% human serum [26,27]. The latter is important since PIs (particularly saquinavir, nelfinavir, ritonavir, amprenavir) are extensively bound to plasma proteins. Using these protein-corrected inhibitory concentration values, we and others have estimated the in vivo MEC for each PI (Table 2). However, we are aware of the inherent difficulties of extrapolating in vitro cell culture data (with 50% not 100% human serum) to the patient situation, and therefore these values should be judged as best estimates.

Plasma concentrations following the introduction of soft-gel saquinavir are generally much higher, although some patients still present trough concentrations below the desired value. Indinavir, ritonavir and nelfinavir show similar variability. It should be stressed that the concentrations presented in Fig. 4 are from both daily clinical practice and some carefully monitored clinical trials. They represent a cross-section of patients, some of whom will have undergone TDM because of suspected virological failure, drug interaction or a change in therapy.

In relation to the marked inter-patient variability, although it is difficult to pin point a single factor, we know that there are clear effects of food (on absorption), drug interactions and hepatic dysfunction. Since PIs are substrates for enterocytic and hepatic CYP3A4 [6] and P-glycoprotein (P-gp) [28–32], drugs which inhibit CYP3A4 (e.g. other PIs, azole antifungals, macrolide antibiotics) or P-gp may result in marked elevation of PI concentrations. Conversely, the induction of CYP3A4 by drugs such as some NNRTIs, rifampicin and rifabutin may decrease PI concentrations. A patient failing a regimen will be given at least two new drugs and in salvage therapy increasingly complex combinations of drugs (as many as 4–8 agents), which may include PI–PI combinations plus an NNRTI. These regimens have considerable potential for complex drug interactions and toxicity. For a complete listing of the important drug interactions in HIV therapy, see http://www.hiv-druginteractions.org.

Table 2
Range of target minimum effective concentrations (MEC) based on protein binding corrected IC_{95} values for individual PIs

	MEC	
Protease inhibitors (PIs)	nM	ng/ml
Amprenavir	588–785	300[a]–400
Indinavir	97–160	60–100[a]
Nelfinavir	700–1228	400[a]–700
Ritonavir	2080–2910	1500–2100[a]
Saquinavir	150–300	100[a]–200

Note: Different laboratories have used slightly different cut-off values.
[a] Indicates values used in the Liverpool HIV Pharmacology Group, 1999–2000.

2.3. Drug concentrations may correlate with excessive toxicity

Reversible liver toxicity is seen with high-doses of PIs or dual PI combinations. High peak plasma concentrations (C_{max}) of indinavir are associated with urological complications [33]. Similarly, high C_{max} values of ritonavir are related to circumoral paraesthesia [34]. We have successfully changed ritonavir dosing regimens (e.g. to 300 mg, four times daily) to overcome this effect [34]. Data are emerging which suggest an association between saquinavir and indinavir levels and plasma triglycerides (e.g. [35]).

2.4. Altered clearance in hepatic dysfunction

Pre-existing liver impairment, particularly with co-existent chronic hepatitis B or C infection, is not uncommon in HIV infection. Wide variability in the pharmacokinetics of nelfinavir in a small group of patients has been demonstrated [36] and liver dysfunction is likely to affect the disposition of all PIs. In one study of over 1200 patients receiving PIs, most cases of serious liver toxicity (> grade 3) were seen in patients co-infected with Hepatitis C virus [37]. Plasma levels may assist in optimising doses, minimising hepatotoxocity and discriminating between drug toxicity and other causes of liver impairment.

2.5. To assess adherence to therapy

Lack of adherence to therapy due to the complexity of drug regimens is a major problem. HIV treatment appears to be very 'unforgiving' in this respect, since therapeutic failure is very closely associated with failure to adhere to prescribed therapy. For example, a study using pill bottles fitted with electronic caps demonstrated that successful HIV suppression in patients with >95% adherence was 81%, with 90–95% adherence this was 64%, with 80–90% adherence this was 50%, with 70–80% adherence this was 25%, and with <70% adherence only 6% patients achieved successful HIV suppression [38]. Although the plasma half-life of PIs is comparatively short (2–8 h), monitoring of plasma drug levels may be useful for selected patients in whom nonadherence is suspected [39]. In this situation, TDM may identify poor adherence although adequate plasma drug levels would not automatically imply good adherence.

3. Potential problems with TDM

One of the major problems with TDM, to which we have already alluded, is knowing the target concentration. Minimum target PI concentrations have largely been defined on the basis of monotherapy concentration–effect modelling or in vitro IC_{95} data for laboratory or clinical isolates of HIV, with allowance made for protein binding. But how are these values affected by other antiretroviral agents in a given combination? Are data derived from studies using PI monotherapy or complex four drug regimens including dual PIs, appropriate to the general clinic population of HIV-positive patients?

In using a single target trough concentration, we assume that all patients have viral isolates with the same susceptibility. In reality many patients with resistant isolates will require higher concentrations (see Fig. 5).

Then there is the lack of agreement concerning the best measure to use: AUC, C_{min} (C_{trough}), or concentrations ratios (i.e. concentration obtained at any time point related to population profile data). While AUCs represent a robust measure, there are logistical difficulties in instituting their use on a wide scale. There is currently a move to consider using C_{min}/IC_{50} values (IC_{50} being for the patient isolate) and this would certainly seem to be the way forward. In this connection the term inhibitory quotient (IQ) has been introduced, where $IQ = C_{min}/IC_{50}$ and there is clearly an advantage if the IQ for a PI is in excess of 1.

Clearly, the case for TDM for this class of drugs has yet to be fully established. Questions such as "Does TDM improve patient outcome?" and "Is it cost-effective?" urgently need to be addressed. Assessing the costs of PIs and of monitoring their levels is a health service issue of great immediacy. It is clear that randomized controlled trials to assess TDM are urgently required. If such studies are not performed there will be mounting demand for TDM to be instituted, in a situation analogous to viral resistance testing. Results of one TDM study, the ATHENA trial in the Netherlands, has recently been reported [40]. In 1999 the Pharmacology Committee of the US AIDS Clinical Trials Group (ACTG) in a position paper [41] issued guidelines stating that routine TDM was not recommended "except in the context of supervised clinical studies designed to assess the utility of TDM." However, this cautious approach must be viewed in the context of increasing numbers of patients failing therapy; the durability of ART is limited. While newer PIs, NNRTIs and NRTIs are being evaluated, there are no imminent plans for the introduction of a new major class of compound into Phase III studies. The urgent need to improve efficacy and preserve treatment options has therefore led to calls for the institution of routine TDM by some clinicians, leading experts and patient advocacy groups particularly in Europe. Despite the lack of definitive studies to evaluate the clinical

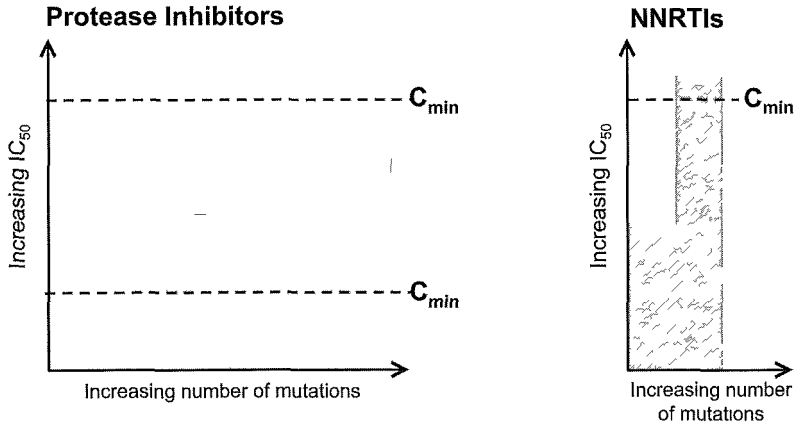

Fig. 5. Increasing mutations in the virus (stepwise) lead to an increase in IC_{50} and the need for higher plasma concentrations.

benefits of and indications for TDM, some national treatment guidelines (e.g. BHIVA [42]) have incorporated TDM as an option for the management of HIV infection.

4. Pharmacoenhancement

A critical examination of current PIs suggests that there is a need for better drugs that achieve higher plasma concentrations reliably without associated toxicity.

This urgent need to improve efficacy and preserve treatment options has led to the use of ritonavir as a pharmacoenhancer with the aim of obtaining plasma concentrations of unbound drug that are in excess of the IC_{95} of both WT and mutant virus [43–45]. The benefits include improving bioavailability through inhibition of first-pass loss (e.g. metabolism by CYP3A4 and efflux by the transporter P-gp) and reducing clearance (Fig. 6). Data from twice daily dosing are convincing {e.g. indinavir–ritonavir 800/100 or 400/400 mg [see Fig. 6], ABT378/r (lopinavir) 400/100 mg, saquinavir–ritonavir 400/400 mg, amprenavir–ritonavir 600/100 mg}, although it is probably too early to say if the data from once daily dosing are anything more than promising. Eventually we will move into the once daily single agent PI (without pharmacoenhancement) and there are a couple of drugs in the pipeline. A further consideration is whether the pharmacoenhancement of ritonavir will overcome any enzyme induction effect of co-administered NNRTI. Although this appears to hold for saquinavir/ritonavir + efavirenz, amprenavir/ritonavir + efavirenz, ABT378r + nevirapine, recent data indicate this is not so for ABT378r + efavirenz.

One issue with pharmacoenhancement is whether an increase in toxicity will be seen. Here TDM may have a role in dose reduction.

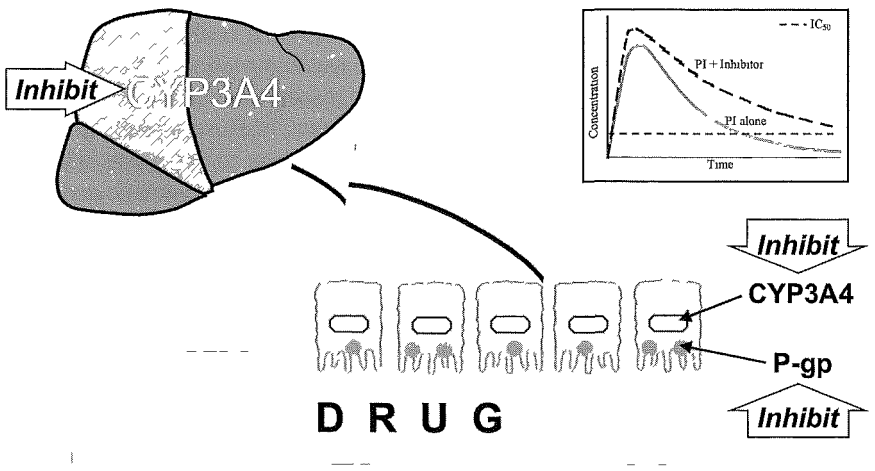

Fig. 6. Inhibition of first pass metabolism and hepatic clearance increases plasma PI concentrations.

5. Conclusion

In conclusion, there are clear pointers to a role for TDM in ART, and in particular, if TDM is shown to be beneficial in controlled clinical trials, it will assist clinicians in optimising the management of HIV-positive patients and by delaying the onset of virological failure, preserve future treatment options. We also need to remember that expert interpretation of a concentration is absolutely critical to understanding what the results actually mean. This is not therapeutic drug *measurement* but therapeutic drug *monitoring*.

Appendix A. Discussion 13

T. Blaschke: We're all really still having trouble deciding about the role of therapeutic drug monitoring. As you pointed out, there's an enormous number of unpredictable drug interactions that we have to worry about, both inhibitors as well as inducers, and of course the inducers are a great concern in terms of therapeutic failure. What has always dampened my enthusiasm for therapeutic drug monitoring, are some of the things that you and other speakers have mentioned. Primarily, it is the difficulty of knowing what to do with values for one or two samples that we get from a patient, because we know that there is a tremendous amount of between-patient variability as well as a substantial amount of within-patient variability. On top of that, most of these protease inhibitors are very extensively protein-bound, and many of these patients are reasonably ill. This can have a significant effect on their plasma protein concentrations. Some of the protease inhibitors are bound to α_1-acid glycoprotein, which can be affected by the disease or by the other infections that these patients also have. I'm struck by the real quandary about knowing whether we can improve outcome and show cost benefit using therapeutic drug monitoring. We certainly need to do the right studies to evaluate the benefit, because I'm afraid that if we don't, what's going to happen is that physicians and patients will use it in a willy-nilly fashion. Unlike the cases you presented to us, and it is always possible to pick out the best examples, in many situations TDM doesn't appear to have value for the individual patient. One possible result is that we could discard something that ultimately, if used properly, could provide significant benefit to patients, because of the complexity of the regimens that patients with HIV have. I'm really am trying to encourage what you're doing; that is, to really look carefully and prospectively, in a very pragmatic and practical fashion, and determine whether or not therapeutic drug monitoring does have cost-effective value. One of the possibilities I've been thinking about is whether or not we should be even more rigorous and intensive about the kinds of sampling that we do in order to really define individual pharmacokinetics. In some ways we are short-changing the patient and ourselves by trying to use minimal numbers of samples rather than obtaining, for example, a more complete pharmacokinetic profile on an individual to really understand reasonably accurately, that individual's pharmacokinetics. And I think there are some approaches that ought to be considered and some ideas that could be used to try to enhance the value of serum concentration measurements to really try to understand an individual's within- and between-patient variability in plasma concentrations.

D. Back: I absolutely agree with your caution. We've been trying to persuade our Medical Research Council now for 18 months to fund the trial OPIUM and at the moment I think we're going to be linking with a therapeutic drug monitoring study in Canada, in order to increase the power of the study. They have got a 3:1 randomisation, whereas we have a 1:1 randomisation, so we are going to data share. Hopefully that trial will get started within the next few weeks.

M. Ingelman-Sundberg: What struck me a bit was that you can increase the concentration of one of the drugs by a factor of 10. When do the patients envision side effects? I mean, what is the clean window?

D. Back: Side effects are a major issue in antiretroviral therapy. For example, with indinavir one of the problems is nephrolithiasis; a number of patients who have boosted with ritonavir, on an 800/100 dose, have got flank pain and we've monitored the levels which are often much higher than they potentially need be. We have recommended dose reduction from 800/100 to 600/100 or even 400/100. In addition to short-term toxicity, there is long-term toxicity. Dr. Pirmohamed has got a particular interest in lipodystrophy which emerges as a chronic adverse event in many patients on antiretrovirals. This may or may not have a direct relationship with high plasma concentrations. I've got major concerns about how high with concentrations you can actually go. If you're maintaining concentrations 20, 30 or 40 times higher than maybe you need for a wild-type virus, that may not be too high for a mutant virus, but the trade-off is long-term toxicity (all the drugs have got their own toxicities). With ritonavir, for example, one of the simple things is oral parasthesia, this seems to correlate with the maximum concentration. You can reduce the maximum concentration and you can actually limit the oral paraesthesia that patients have.

W. Evans: When you do the measurements of the intracellular concentrations of these agents, what cells are you looking at? Is there any attempt to look at the subset of cells that are targeted by the virus? And my second question is: does viral load in any way affect the turnover rate of these drugs in the lymphocytes?

D. Back: This was total PBLs, although it would be very nice to subfractionate into all the different categories. To actually do a full PK profile we need about 10 million cells, at each time point so we're taking 200 ml of blood from the patient over 24 h in order to do these PK studies. Certainly not every patient wants to take part in this sort of study. But we'd like to be able to fractionate down into all the different subsets, CD4, CD8, CD56s. I'm not sure about the viral load issue.

W. Evans: You would think it might, in fact, if these drugs are substrates for RT, or they're being incorporated into viral RNA, DNA. That's the basis of selectivity, when the viral load's high, you might think that the turnover rate of the drug would be higher as well.

D. Back: One of the issues in relation to intracellular concentrations is transporters. We are interested in P-gp and MRP and in particular the role of viral infection on the regulation of the transporters, the effect that different drugs have on inducing the transporter, etc. What we see, intracellularly, for different drugs is actually affected by the transporters, and what we'd like to be able to do is to correlate intra-cellular concentration with transporter expression and function.

M. Pirmohamed: If you look at the history of treatment of HIV, it's becoming more and more complex; we have TDM, you also mentioned genotyping for viral resistant

strains, and in the future you can imagine doing a genotype for cellular resistance, and maybe pharmacogenetics of transporters, and so on. And so the layers of complexity are getting higher and higher. Do you think clinicians, first of all, have the knowledge to cope with all that complexity? And secondly, it's already pretty expensive to treat HIV, by adding these layers of complexity, the cost is going to increase and increase, and are the health care systems going to be able to afford it, or is it going to price itself out of existence?

D. Back: That's a very important issue, and rather than just genotyping, I think it's more likely to be phenotyping, where you have the phenotype of the virus, and so you relate drug concentration to the phenotype in an individual patient. I think we need to go along the road of saying, we'll look at the concentration and the phenotype and we'll take a decision from there. But the cost of phenotyping at the moment is about $300 or $400 per sample, and therapeutic drug monitoring is relatively cheap compared to that. At the end of the day, I suppose it's the trade-off and the balance between patients who are going to respond and keep responding, and keep responding versus the cost. That's an argument I guess we could go on for a long time, discussing the cost issues.

M. Reidenberg: I'm curious as to why after the ritonavir interaction was worked out, ritonavir was chosen to be the enzyme inhibitor in these mixtures, among all the possible, well-worked-up enzyme inhibitors that could have been used. And secondly, where adherence has been shown to be a real problem with treatment, has anybody attempted to look at directly observed therapy as a intervention, as we've done with tuberculosis?

D. Back: As a sort of anecdotal story, 5 or 6 years ago, there was great interest in grapefruit juice increasing the bioavailability of protease inhibitiors, and I remember, we were doing PK studies in Dublin, Ireland. At one stage patients were clearing out every single supermarket of all the concentrated grapefruit juice, because they knew they could push up their saquinavir levels by grapefruit juice. I think the ritonavir issue is very interesting, because you've got subtherapeutic concentrations of ritonavir, and there is still that question mark: what is happening by having subtherapeutic concentrations? And there's a lot of debate about that. In terms of the adherence, maybe Terry knows the details about a study done in prisoners with directly observed therapy.

T. Blaschke: It wasn't a prison study per se, but some of the subjects in a clinical trial were in prison and had directly observed therapy, and they had essentially 100% responses, whereas the nonincarcerated patients had a much higher failure rate. There is a study now being set up and proposed within the AIDS Clinical Trials Group for directly observed therapy in HIV, but it has already been shown that directly observed therapy appears to be quite effective.

N. Holford: The value of therapeutic drug monitoring—at least one of the putative areas—is that it actually predicts what dose that individual patient needs. It assumes that if you make a measurement today, then you can predict what the concentration will be tomorrow by changing the dose. In the examples you gave us of patients in which does changes were made, concentrations went up, but they weren't proportional to the dose. That suggests it was not predictable, and I think your examples are just examples of random variability which by chance happened to have an increase in the concentration when you measured it. I suspect that that means that there's a lot of within-subject variability, which means that therapeutic drug monitoring based upon assuming that you're

going to have a predictable change in concentration, doesn't work for this class of agents. Unless you can show me that the within-subject variability is relatively small, therapeutic drug monitoring is not going to be beneficial.

D. Back: I certainly take the point about the examples. I tried to give some relatively common examples. The within-subject variability needs to be worked out better. I guess Pieter could probably add something in terms of data on file in Roche for saquinavir and nelfinavir where the within-subject variability information must be there.

P. Joubert: It is very high. It makes a demonstration of bio-equivalence even with the same formulation, very difficult.

L. Sheiner: I wanted to make a remark apropos the question: What is the pattern of concentration versus time that is crucial for efficacy? I think we are all aware of the exquisite sensitivity of RNA response to what ordinarily would be regarded as modest variation in compliance. It leads one to speculate that it's the trough level, or the time above some critical level that counts, and that on the average current dosage yields concentrations quite close to this limit, so that small variations in intake or PK can cause inefficacy. With the kind of data that you're getting, the combination of concentration versus time data and viral load response data, one could ask the question: What function of concentration versus time seems to be most critical? But to do so would require collating the data and then using one or another modelling assumption to relate them—Mats Carlsson, for example, has a nice semi-empirical model that allows one to answer that question. It seems to me that if such data analyses are worth-while, it raises the larger issue of data-sharing and getting everybody working on the same problems, because the people who have the data are not necessarily as adept at modelling them as modellers who would like such data, but don't have access to them. I think that's a relatively little-explored benefit of TDM - to generate a database that can be used for multiple questions in addition to the single one of how to treat an individual patient.

D. Back: I agree; one of the great frustrations is that we have a database probably of 1500 patients, and it's just sitting there. I think that data sharing is absolutely critical, in order to answer some of these issues.

X. Carné: My question was raised already by Marcus: Do you have any experience about using inhibitors of CYP450 other than ritonavir, drugs that are not in fact anti-virals?

D. Back: Apart from grapefruit juice and ketoconazole, I'm not actually aware of other studies that have been formally done with non-PI-type inhibitors.

T. Blaschke: It was mainly for historical reasons that ritonavir was chosen, but there is also a pretty good reason above and beyond that. Ritonavir is probably one of the most potent inhibitors of CYP3A, and it also has an unusual characteristic, in that the effect persists well beyond its half-life. It appears to be a pseudo-irreversible inhibitor of the enzyme, so very low doses can be quite effective and last for relatively long periods of time, whereas other inhibitors might have to be given more often or in larger doses. Again, some of it's use is historical just because people started using it, but on the other hand, it does, I think, have some biochemical pharmacology rationale.

P. Joubert: What are the potential problems or worries you mentioned about subtherapeutic doses of anti-retroviral drugs?

D. Back: There have been concerns, obviously, that if you've got a subtherapeutic concentration you are going to allow viral escape, and you are going to actually have a

mutant virus. The overlapping pattern with indinavir is that you get the same mutations as with ritonavir. So the concern is not that you are going to actually get a different mutation pattern. But I think there are still unanswered questions about that. It goes against our normal principles of trying to make sure you have adequate drug: if you've got a subtherapeutic level there what are you actually going to do? But the general concensus of virologists is that you do not produce mutations.

W. Evans: On that point, do these drugs both inhibit the enzyme in the same mechanism and in the same site? Are they additive or synergistic? You've got a low concentration of ritonavir, but you've got a much higher concentration of another protease inhibitor? Although it is also an inhibitor, you're not going to leave the enzyme uninhibited, just because ritonavir in low concentrations, you've still got another protease inhibitor. If they're inhibiting the enzyme at the same site, for example. I'm not sure how, it would set the stage for selecting a ritonavir-resistant strain, since you've still got that target enzyme inhibited, the virus can't escape that.

D. Back: But you've got different mutation patterns with different protease inhibitors. It depends on the protease.

W. Evans: That suggests they are inhibiting slightly different mechanisms or site of the enzyme, binding to different sites to inhibit.

References

[1] F.J. Pallella, K.M. Delaney, A.C. Moorman, et al., Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection, N. Engl. J. Med. 338 (1998) 853–860.
[2] The AVANTI Steering Committee, Analysis of HIV-1 clinical trials: statistical magic? Lancet 353 (1999) 2061–2064.
[3] B. Ledergerber, M. Egger, M. Opravil, et al., Clinical progression and virological failure on highly active anti-retroviral therapy in HIV-1 patients: a prospective cohort study, Lancet 353 (1999) 863–868.
[4] S. Staszewski, V. Miller, C. Sabin, et al., Virological response to protease inhibitor therapy in an HIV clinic cohort, AIDS 13 (1999) 367–373.
[5] F.W.N.M. Wit, R. van Leeuwen, G.J. Weverling, et al., Outcome and predictors of failure of highly active anti-retroviral therapy: one-year follow-up of a cohort of human immunodeficiency type-1-infected persons, J. Infect. Dis. 179 (1999) 790–798.
[6] M.G. Barry, F. Mulcahy, C. Merry, S. Gibbons, D.J. Back, Pharmacokinetics and potential interactions amongst anti-retroviral agents used to treat patients with HIV infection, Clin. Pharmacokinet. 36 (1999) 289–304.
[7] Http://www.hiv-druginteractions.org.
[8] M.G. Barry, S.H. Khoo, G.J. Veal, et al., The effect of zidovudine dose on the formation of intracellular phosphorylated metabolites, AIDS 10 (1996) 1361–1367.
[9] K.H.P. Moore, J.E. Barrett, S. Shaw, et al., The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1, AIDS 13 (1999) 2239–2250.
[10] J.M. Schapiro, M.A. Winters, F. Stewart, et al., The effect of high dose saquinavir on viral load and CD4+ T-cell counts in HIV-infected patients, Ann. Intern. Med. 124 (1996) 1039.
[11] R.M.W. Hoetelmans, M.H.E. Reijers, G.J. Weverling, et al., The effect of plasma drug concentrations on HIV-1 clearance rate during quadruple drug therapy, AIDS 12 (1998) F111–F115.
[12] R. Gieshke, B. Fotteler, N. Buss, J-L. Steiner, Relationship between exposure to saquinavir monotherapy and antiviral response in HIV-positive patients, Clin. Pharmacokinet. 37 (1999) 75–86.
[13] J. Chodakewitz, P. Deutsch, R. Leavitt, et al., Relationship between indinavir pharmacokinetics and antiviral activity in phase I/II trials, 12th World AIDS Conference, Geneva, 1998, abstract 42266.

[14] M. Markowitz, M. Saag, W.G. Powderly, et al., A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection, N. Engl. J. Med. 333 (1995) 1534–1539.
[15] A.P. Lea, D. Faulds, Ritonavir, Drugs 52 (1996) 541–546.
[16] E.A. Emini, Resistance to anti-human immunodeficiency virus therapeutic agents, Adv. Exp. Med. Biol. 390 (1995) 187–195.
[17] D.S. Stein, D.G. Fish, J.A. Bilello, et al., A 24-week open-label Phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir), AIDS 10 (1996) 485–492.
[18] D. Descamps, P. Flandre, V. Calvez, et al., Mechanisms of virological failure in previously untreated HIV infected patients from a trial of induction-maintenance therapies, JAMA 283 (2000) 205–211.
[19] D.V. Havlir, N.S. Hellmann, C.J. Petropoulos, et al., Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir containing regimens, JAMA 283 (2000) 229–234.
[20] R.L. Murphy, J.P. Sommadossi, M. Lamson, et al., Antiviral effect and pharmacokinetic interaction between nevirapine and indinavir in persons infected with human immunodeficiency virus type 1, J. Infect. Dis. 179 (1999) 1116–1123.
[21] E.P. Acosta, K. Henry, L. Baken, et al., Indinavir concentrations and antiviral effect, Pharmacotheraphy 19 (1999) 708–712.
[22] D.M. Burger, R.M.W. Hoetlemans, P.W.H. Hugen, et al., Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1 infected patients on indinavir-containing triple therapy, Antiviral Ther. 3 (1998) 215–220.
[23] D.M. Burger, A.M. van Rossum, P.W. Hugen, et al., Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1 infected children, Antimicrob. Agents Chemother. 45 (2001) 701–705.
[24] J. Durant, P. Clevenberg, R. Garaffo, et al., Importance of protease inhibitor plasma levels in HIV infected patients treated with genotypic-guided therapy: pharmacological data from the VIRADAPT study, AIDS 14 (2000) 1333–1339.
[25] M.G. Barry, C. Merry, J. Lloyd, et al., Variability in trough plasma saquinavir concentrations in HIV patients—a case for therapeutic drug monitoring, Br. J. Clin. Pharmacol. 45 (1998) 501–502.
[26] A. Molla, S. Vasavanonde, G. Kumar, et al., Human serum attenuates the activity of protease inhibitors towards wild type and mutant human immunodeficiency virus, Virology 250 (1998) 255–262.
[27] J.H. Condra, C.J. Petropoulos, R. Ziermann, et al., Drug resistance and predicted virologic responses to human immunodeficiency virus type 1 protease inhibitor therapy, J. Infect. Dis. 182 (2000) 758–765.
[28] L. Profit, V.A. Eagling, D.J. Back, Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors, AIDS 13 (1999) 1623–1627.
[29] C.G.L. Lee, M.M. Gottesman, C.O. Cardarelli, et al., HIV-1 protease inhibitors are substrates for the MDR-1 multidrug transporter, Biochemistry 37 (1998) 3594–3601.
[30] R.B. Kim, M.F. Fromm, C. Wandel, et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest. 101 (1998) 289–294.
[31] M.T. Huisman, J.W. Smit, A.H. Schinkel, Significance of P-glycoprotein for the pharmacology and clinical use of HIV protease inhibitors, AIDS 14 (2000) 237–242.
[32] H. Gutmann, G. Fricker, J. Drewe, M. Toeroek, D.S. Miller, Interactions of HIV protease inhibitors with ATP-dependent drug export proteins, Mol. Pharmacol. 56 (1999) 383–389.
[33] J.P. Dieleman, I.C. Gyssens, M. van der Ende, et al., Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients, AIDS 13 (1999) 473–478.
[34] C. Merry, M.G. Barry, S. Gibbons, F. Mulcahy, D.J. Back, Improved tolerability of ritonavir derived from pharmacokinetic principles, Br. J. Clin. Pharmacol. 42 (1996) 787.
[35] D.R. Churchill, A.S. Pym, A.G. Babiker, D.J. Back, J.N. Weber, Rate of onset of hyperlipidaemia following treatment with ritonavir/saquinavir and correlation with drug levels, AIDS 12 (Suppl. 4) (1998) S9.
[36] Y. Khaliq, K. Gallicano, I. Seguin, et al., Therapeutic drug monitoring of nelfinavir in HIV patients with liver disease, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, 1999, PO 369. Available at http://www.retroconference.org/past_conferences.htm.
[37] F. Raffi, M. Saves, V. Le Moing, et al., Serious adverse effects (SAE) in a prospective cohort of HIV-infected adults started on protease inhibitor therapy (ANRS EP11 Study), 6th Conference on Retroviruses

and Opportunistic Infections, Chicago, 1999, PO 676. Available at http://www.retroconference.org/past_conferences.htm.

[38] D. Paterson, S. Swindells, J. Mohr, et al., How much adherence is enough? A prospective study of adherence to protease inhibitor therapy using MEMSCaps, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, 1999, PO 92. Available at http://www.retroconference.org/past_conferences.htm.

[39] D.M. Burger, P.W.H. Hugen, P.P. Koopmans, Monitoring of plasma drug levels to assess non-adherence to drug therapy, AIDS 12 (Suppl. 4) (1998) S61.

[40] D.M. Burger, P.W.H. Hugen, J. Droste, et al., Therapeutic drug monitoring (TDM) of indinavir (IDV) and of nelfinavir (NFV) in treatment-naive patients improves therapeutic outcome after 1 year: results from ATHENA. 2nd International Workshop on Clinical Pharmacology of HIV Therapy, Nordwijk, April 2001, AB 6.2. Available at http://194.134.33.80/tdc/documents/Abstract_6.2_Burger.pdf.

[41] AIDS Clinical Trials Group (ACTG) Pharmacology Committee, Position paper on therapeutic drug monitoring for anti-retroviral drugs: using drug concentrations to guide individual therapy, 9th Annual Clinical Care Options for HIV Symposium, California, June 3–6, 1999.

[42] British HIV Association, http://www.aidsmap.co.uk.

[43] R.P.G. Van Heeswijk, A.I. Veldkamp, R.M.W. Hoetelmans, et al., The steady-state plasma concentrations of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1 infected individuals, AIDS 13 (1999) F95–F99.

[44] M.S. Saag, M. Kilby, E. Ehrensing, et al., Modulation of saquinavir steady state pharmacokinetics with "baby" doses of ritonavir in healthy volunteers, 7th European Conference on Clinical Aspects and Treatment of HIV Infection, Lisbon, October 1999, abstract 829. Available at http://www.euro-aids99.com.

[45] N. Shulman, A. Zolopa, D. Havlir, et al., Ritonavir intensification in indinavir recipients with detectable HIV RNA levels, 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, February 2000, abstract 534. Available at http://www.retroconference.org/past_conferences.htm.