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Mini review

# Recent developments in HIV protease inhibitor therapy

Akhteruzzaman Molla *, G. Richard Granneman, Eugene Sun, Dale J. Kempf

*Pharmaceutical Products Division, Abbott Laboratories, 47D, AP52-N, 200 Abbott Park Road, Abbott Park, IL 60064, USA*

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## 1. Introduction

Human immunodeficiency virus (HIV), the causative agent of AIDS, is a member of retrovirus family, *Retroviridae*. Upon encountering a T-lymphocyte, the virus binds to the CD4 receptor via the envelope gp120. We now know that secondary binding to a chemokine receptor on the cell surface is necessary for fusion. Following fusion, the contents of the virion enter into the cytoplasm, where the first of three virus-specific enzymatic processes occurs, that is reverse transcription (RT). The proviral DNA thus produced migrates to the nucleus, and in a second virus-specific step, is integrated into the host cell genome by the viral enzyme integrase. Upon cell activation, proviral DNA transcription is unregulated, and the RNA thus produced serves two purposes. First, it becomes the genetic material for new virus particles, and second, it serves as a template for translation into the various viral proteins. The structural and enzymatic proteins of HIV are encoded as a single *gag* or *gag-pol* polyprotein. The polyproteins are myristylated and aggregate at the cell surface, where they bind viral RNA and form the core of a nascent viral particle. As virions bud from the cell, the *gag* ($Pr55^{gag}$) and *gag-pol* ($Pr160^{gag\text{-}pol}$) polyproteins are cleaved by the third viral enzyme, HIV protease to generate *gag* matrix (p17), capsid (p24), and nucleocapsid (p15) proteins, the *pol* RT and integrase enzymes, and other viral proteins. The nucleocapsid protein is further processed into the smaller proteins p2, p7, p1 and p6. This process is essential for normal viral maturation and the generation of infective viral particles. Inhibition of this cleavage process by either specific inhibitors or by mutation of the active site aspartic acid residue leads to the accumulation of noninfectious, immature virus particles and impairs the spread of virus infection in cell culture (Kohl et al., 1988; Dubouck, 1992). Therefore, HIV protease is an attractive therapeutic target.

* Corresponding author. Tel. +1 847 9381094; fax: +1 847 9382756; e-mail: akhter.m.molla@abbott.com


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HIV-1 protease is a member of the aspartic protease group of enzymes typified by the human enzymes renin, pepsin and cathepsins D and E, and by several fungal proteases. Among aspartic proteases, HIV and other retroviral proteases are unique in that they exist in active form as an obligatory homodimer stabilized only by noncovalent interactions (Navia et al., 1989; Wlodawer et al., 1989). Both subunits are required to form the composite active site, with each subunit contributing one of two conserved triads (Asp-Thr-Gly) containing the catalytically active aspartate residue. These aspartates are located at the center of a hydrophobic cavern of sufficient length to encase six amino acids of the substrate in an extended conformation. To date, numerous peptidomimetic inhibitors of HIV protease with diverse structural features have been reported, many of which display high potency and selectivity in biochemical assays and in tissue culture. Moreover, unlike RT inhibitors, which only block HIV replication in acutely infected cells, protease inhibitors also inhibit production of infectious virus from chronically infected cells (Lambert et al., 1992). Currently licensed protease inhibitors include saquinavir (Ro 31-8959, Roche), ritonavir (ABT-538, Abbott), indinavir (MK-639, Merck), and more recently nelfinavir (AG 1343, Agouron). A number of other compounds such as amprenavir (141W94 or VX-478, Glaxo Wellcome), ABT-378 (Abbott), PNU-140690 (Pharmacia-Upjohn), DMP-450 (Triangle), and DMP-851 (DuPont Merck) are in clinical development.

HIV protease inhibitors have served as important tools to investigate important aspects of viral replication in HIV-infected individuals. Recent studies of HIV replication dynamics suggest variable, but finite half-lives for elimination of HIV from various cell populations if mutant virus outgrowth can be completely suppressed by protease inhibitors. The genotypic and phenotypic patterns that emerge in those patients on protease inhibitor therapy in whom suppression of HIV replication is incomplete are being characterized. A clear understanding of the mechanism of viral resistance can aid in the formulation of successful treatment strategies employing protease inhibitors. Furthermore, there is a need for the identification and development of agents that target resistant HIV. This review (1) describes the genotypic patterns observed in HIV in response to selective pressure by current protease inhibitors, in vitro and in vivo, (2) discusses the mechanisms by which mutations confer phenotypic resistance to the inhibitors, (3) describes the pharmacological and metabolic characteristics of protease inhibitors that impact the development of rational strategies for combination therapies, and (4) surveys new protease inhibitors currently in development.

## 2. In vitro anti-HIV activity

All of the current available HIV protease inhibitors show potent inhibition of HIV replication in vitro (Table 1). HIV protease inhibitors are hydrophobic and often highly bound to human serum proteins. Both $\alpha_1$-acid glycoprotein and albumin are involved in the serum binding of these agents, and the fraction unbound and available to partition into infected lymphocytes and macrophages is under 3% for all agents except indinavir. In the presence of 50% human serum the activity of ritonavir, saquinavir and nelfinavir shows a substantial drop in potency while indinavir, which has very low protein binding, only loses about one half of its potency. Amprenavir is moderately influenced by serum proteins (Table 1). These values represent realistic levels above which plasma concentrations must be maintained for a potent antiviral effect in vivo.

Table 1
Antiviral activity of protease inhibitors against wild-type HIV-1 (pNL4-3) in the presence or absence of human serum[a]

| Drug | $EC_{50}$ (nM) virus | |
|---|---|---|
| | 0% human serum | 50% human serum |
| Saquinavir | $21 \pm 7$ | $678 \pm 168$ |
| Ritonavir | $76 \pm 24$ | $1663 \pm 288$ |
| Indinavir | $53 \pm 8$ | $101 \pm 42$ |
| Nelfinavir | $37 \pm 16$ | $1338 \pm 276$ |
| Amprenavir | $101 \pm 20$ | $975 \pm 238$ |

[a]Determined at Abbott Laboratories, mean value of three experiments.

## 3. Resistance

### 3.1. In vitro resistance

In the light of the resistance problems encountered with nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs) as well as protease inhibitors in vivo, it is important to identify mutations in the protease gene during drug development. The common method employed to select mutants in vitro is by passage of virus in tissue culture in the presence of increasing concentrations of drugs. These experiments have resulted in a variety of mutant viruses with reduced sensitivity to a number of HIV protease inhibitors. In the following sections in vitro resistance of most advanced protease inhibitors are outlined.

#### 3.1.1. Saquinavir

In vitro studies have shown that reduced sensitivity to saquinavir arises slowly during extended selection in tissue culture. This reduction in susceptibility appears to be the result of two distinct mutations at position 48 (G48V) and position 90 (L90M) (Eberle et al., 1995). Both mutations together cause a greater change in antiviral susceptibility than either mutation alone (Jacobsen et al., 1995a,b). Selection of mutations at codon 54, 71 and 84 in vitro have also been reported (Eberle et al., 1995). The sequences of intermediate passaged viruses suggested a contribution from positions 12, 36, 57, and 63 in the early steps of resistance development. The selected virus containing both G48V and L90M displayed a 40-fold change in susceptibility to saquinavir. Growth kinetics of resistant virus were comparable to wild-type virus and the resistant genotype proved to be stable in the absence of inhibitor.

#### 3.1.2. Ritonavir

HIV-1 variants with diminished susceptibility to ritonavir were derived by passaging HIV in MT4 cells in the presence of escalating, subinhibitory concentrations of ritonavir (Markowitz et al., 1995a). Measurable resistance was not observed until passage 19, after which point the concentration of ritonavir was raised further from 0.8 to 2.0 $\mu$M at passage 22. The viral population selected at passage 19 exhibited a 5–6-fold decrease in sensitivity compared to the wild-type HIV. A further 2–4-fold decrease in sensitivity was observed with the passage 22 virus population. Two mutations (I84V and M46I) were predominantly observed from passage 19. In addition, one of 13 clones from passage 19 contained the alteration of V82F. This mutation persisted through passage 22, where it, along with M46I and I84V, were observed in all of the clones sequenced. Seven out of ten clones from passage 22 also contained an L63P or A71V substitution. Of all the cloned viruses with single mutations, only those containing V82F or I84V exhibited measurable resistance to ritonavir. The growth kinetics of A71V and V82F mutant viruses were slightly slower; however, the double mutant (V82F/I84V) and the triple mutant (L63P/V82F/I84V) displayed significantly impaired growth properties (Markowitz et al., 1995a).

#### 3.1.3. Nelfinavir

In vitro serial passage of HIV-1 in the presence of increasing concentrations of nelfinavir produced HIV variants with a 30-fold reduction in susceptibility to nelfinavir after 28 passages (Patick et al., 1996). Molecular analysis of the protease gene from this variant identified double mutations at residues 46 (M46I) and 84 (I84V/A). Other changes in the protease gene were also observed such as L63P, A71V. D30N was found after passage 22, but was not detected from passage 28 (Patick et al., 1996).

#### 3.1.4. Amprenavir

DNA sequence analyses of the protease gene after serial passage of HIV in the presence of increasing concentrations of amprenavir revealed, after seven passages, the initial mutations I84V and L10F (Partaledis et al., 1995). As the concentration was increased, the L10F, I84V mutant virus populations were replaced by variants with a mutation at I50V, suggesting a possible growth advantage over HIV-1 isolates with a mutation at position 84 at higher drug concentrations. When these substitutions were introduced as a single mutation into an infectious HIV clone, only the

I50V mutant showed reduced sensitivity (2–3-fold). However, an infectious HIV clone carrying three mutations at positions 46, 47, and 50 showed a 14–20-fold change in $EC_{50}$. The passaged viruses showed no cross-resistance to saquinavir but six-fold resistance to indinavir.

#### *3.1.5. Combination of two or more protease inhibitors*

Combination passage of amprenavir with indinavir or saquinavir resulted in a delay in selection of resistant variants compared to passage with the individual inhibitors (Tisdale et al., 1995). Dually resistant variants were isolated in combination passage with amprenavir and ritonavir (16E, 32I, 46I, 82I, and 84V) or nelfinavir (32I, 46I, 54M and 71V). Resistant mutants were also isolated after selection with the combination of indinavir and saquinavir (10F, 48V, 54S, 71V, 77I and 90M) (Tisdale et al., 1995, 1996). Selection using the combination of each of the possible pairs of the three protease inhibitors indinavir, saquinavir and ritonavir has recently produced virus populations with eight or more mutations in the protease gene (Smith and Swanstrom, 1996). Notably, there was a significant overlap in the pattern of mutations observed with each of the three pairs of inhibitors. The presence of greater number of mutations in the cultures selected with pairs of inhibitors suggests that the genetic barrier to resistance to two protease inhibitors simultaneously may be greater than a single protease inhibitor. However, data from in vitro studies do not completely predict the pattern of amino acid changes actually seen in patients receiving protease inhibitors.

### *3.2. In vivo resistance*

The replication of retroviruses is accompanied by a high error rate due to the infidelity of the retroviral RT and the lack of proof reading mechanism (Holland et al., 1992). Mansky and Temin (1995) recently assayed the replication fidelity in HIV and estimated a mutation rate of $3 \times 10^{-5}$ mutations per base pair per cycle. The availability of sensitive viral load assays, as well as a variety of highly potent drugs, has provided the means to quantitatively assess the dynamics of HIV infection in humans. Initial estimates of virus production, obtained by modeling changes in viral load response to anti-HIV drugs, suggested a very high rate of virus turnover with a minimum production of $10^8$–$10^9$ virions per day on average per infected individual (Ho et al., 1995; Wei et al., 1995). More recently, Perelson et al. (1996) raised that estimate to $10.3 \times 10^9$ virions per day per infected individual, based on viral load data obtained during a clinical trial with ritonavir. With this level of virus production, the number of HIV mutants that can potentially exist within the quasispecies is vast. Given the size of the HIV genome ($10^4$ nucleotides), the error rate of RT, and the kinetics of viral replication, every point mutation can theoretically occur $10^4$–$10^5$ times per day in an untreated, infected individual (Coffin, 1995). Thus, the degree of suppression of viral replication in vivo required to prevent the selection and outgrowth of mutant HIV in the face of drug pressure is very high.

A median reduction in plasma viral load of more than two logs in HIV infected patients treated with protease inhibitors has been reported (Danner et al., 1995; Markowitz et al., 1995b; Stein et al., 1996; Gulick et al., 1997). A clear relation was observed between increasing drug doses and the duration of response. The plasma viral RNA in most patients initially falls below detection limit. However, due to a variety of factors, e.g. pharmacokinetic variability, inadequate dosing or poor adherence, the viral load in many patients may rebound during continued drug therapy, accompanied by the emergence of viral variants with reduced susceptibility to the inhibitor. Early interest in HIV protease as a therapeutic target was bolstered by the hope that minimal resistance would develop against protease inhibitors because of the enzyme's need to recognize and process at least nine distinct polyprotein sequences. In spite of this requirement, over 24 distinct mutations in the 99 amino acid protease sequence have been identified as emerging under selective pressure with protease inhibitors (Schinazi et al., 1996). The most complete studies of resistance emergence in vivo have documented the response to protease inhibitor

monotherapy, which produces a higher rate of treatment failure and viral rebound than do combination regimens. These data, along with those collected during combination therapy, allow a comparative assessment of the genotypic and phenotypic changes occuring over time as the virus progresses from a quasispecies that is susceptible to the concentrations of drug achieved in patients to variants that display partial or high-level resistance. This information is vital for devising optimal treatment regimens with current agents, as well as in developing novel therapies. In the following sections, the in vivo resistance patterns of currently available protease inhibitors are outlined. Because of more extensive clinical experience, documentation of the genotypic response to ritonavir and indinavir therapy is most complete. Characterization of in vivo resistance to saquinavir has been hampered by the low drug concentrations (and consequent modest levels of viral suppression and selective pressure) observed with that agent. Finally, the reported genotypic response to amprenavir in vivo is based on treatment for a short duration.

### *3.2.1. Saquinavir*

Analysis of protease sequences isolated at baseline and during saquinavir therapy showed that the predominant change occurring in patients on monotherapy to be the L90M mutation. The G48V mutation seen in vitro is uncommon and the double mutation such as L90M + G48V was even rarer. The L90M mutation had little effect on viral replication, while the double mutation appeared to reduce the replication rate and hence the viability of virus. Mutations at codons 48 and 90 in proviral PBMC DNA also developed concurrently with plasma viral RNA rebound (Schapiro et al., 1996b). Analysis of plasma sequences with high dose saquinavir suggested a possible supplemental role for mutations at codons 63 and 71, since both were seen with increasing frequency following therapy (Schapiro et al., 1996a). In addition, the mutations G48V and L10I tended to appear together.

### *3.2.2. Ritonavir*

Mutations selected during treatment with ritonavir appeared in a step-wise fashion (Fig. 1 and Molla et al., 1996), with almost uniform primary appearance of a change at position 82 (V82 to A, F or T). Following the initial mutation, the changes most commonly observed were at residues I54, A71, or M36. Subsequent mutations appeared at several other positions, but were limited to a small set of amino acid positions (Molla et al., 1996; Schmit et al., 1996). Fig. 1 demonstrates the response of four representative patients to ritonavir therapy. The first patient received 400 mg *bid* and experienced only a transient response followed by initial rebound in viral RNA coincidence with the emergence of a virus population carrying an alanine mutation at position 82. As the population expanded, additional mutations at position 54 and 71 were observed. Over time, this triple mutant gave rise to fourth and fifth mutations at 36 and 20. The second and third patients, who received 500 mg *bid*, had a similar pattern of mutations, beginning at position 82 and followed by 54 and 71, but the mutations appeared significantly later. The fourth patient, who received 600 mg *bid*, remained suppressed below the limit of detection of the first generation bDNA assay and did not develop mutations. Viral isolates with multiple mutations conferring high level resistant to ritonavir exhibited cross-resistance to indinavir and nelfinavir but remained sensitive to saquinavir and amprenavir in vitro (Molla et al., 1996)

### *3.2.3. Indinavir*

Resistance to indinavir has been shown to require an accumulation of three or more mutations. The mutations that were reported to occur with highest frequency were L10, M46, L63, A71, V82 and I84. The development of substitutions at 82 was observed in the majority of the patients who received indinavir and whose viral load rebounded on therapy (Condra et al., 1995). V82A/F appeared as early as 6 weeks after the start of the therapy and persisted throughout the course of the study (48 weeks) (Vasudevachari et al., 1996). Double mutants such as V82A/F, L90M were seen at lower frequency and appeared later

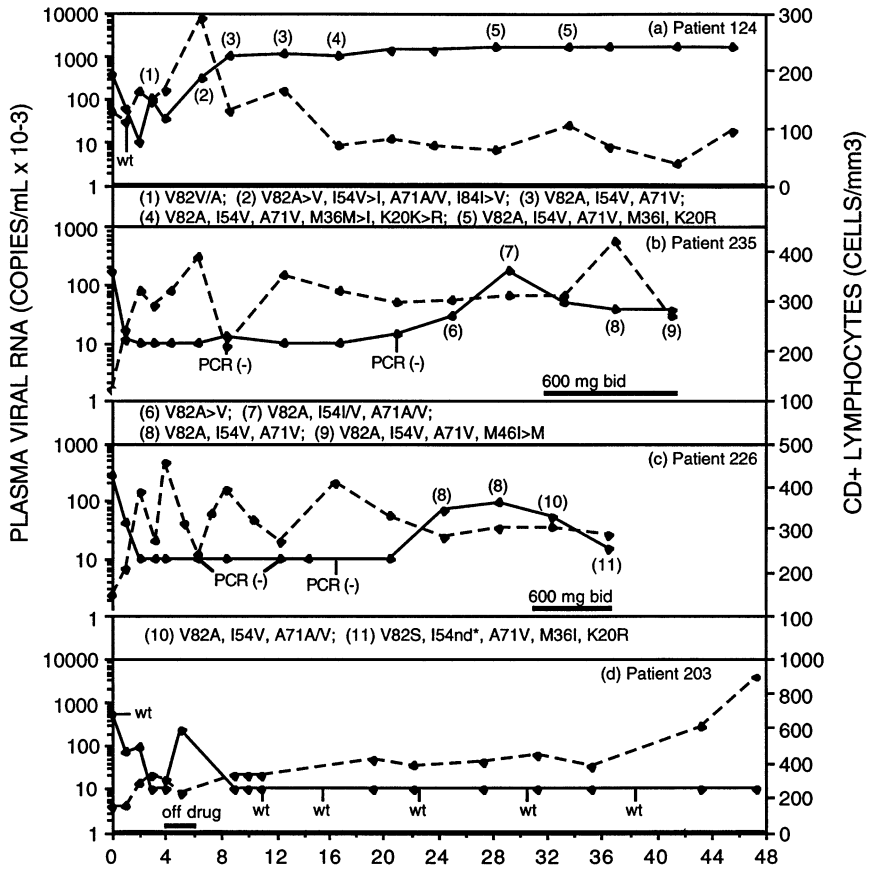


Fig. 1. Correlation of virological and immunological response to mutation patterns for individual patients. Solid lines: plasma RNA (measured using the branched DNA assay), dashed lines: CD4 levels. HIV protease sequences that varied from the pretreatment, baseline sequence at the consensus residues defined in Fig. 1 are listed: (a) patient 124 (400 mg *bid*), (b) patient 235 (500 mg *bid*), (c) patient 226 (500 mg *bid*), (d) patient 203 (600 mg *bid*). Wild-type sequences were defined as the absence of consensus resistance mutations that did not preexist in the baseline sequence. Samples labeled PCR negative refer to the sequencing procedure, not to the plasma RNA quantitation protocol. The dose of ritonavir in patients 235 and 226 was escalated to 600 mg *bid* where indicated. Reproduced from Molla et al. (1996) with the permission of Nature Medicine.

than the change at position 82. In addition to these changes, M46L/I, G48V and I54V mutations were also observed (Vasudevachari et al., 1996). Sequence analyses of the protease gene during indinavir therapy revealed that there was a sequential appearance of protease mutations at amino acids M46L/I, V82A/F, I54V, A71V/T, L89M/P and L90M (Zhang et al., 1997). M46 and/or V82 mutations arose first, followed by I54V or A71A/T, L89M/P and L90M. Amino acid substitutions at positions 10, 24 and 63 have also been observed in drug-resistant virus isolates. Molecular clones containing M46L/I and V82A mutations did not display significantly reduced susceptibility to indinavir. However, the addition of a third mutation, I54V to a background of M46L and V82A produced a significant increase in the $EC_{50}$ (8-fold) (Vasudevachari et al., 1996). Indinavir-resistant viral exhibited various patterns of cross-resistance to a diverse panel of HIV-1 protease inhibitors (Condra et al., 1995; Miller et al., 1997).

### *3.2.4. Nelfinavir*

DNA sequence analysis of the protease gene from nelfinavir treated patients indicated that

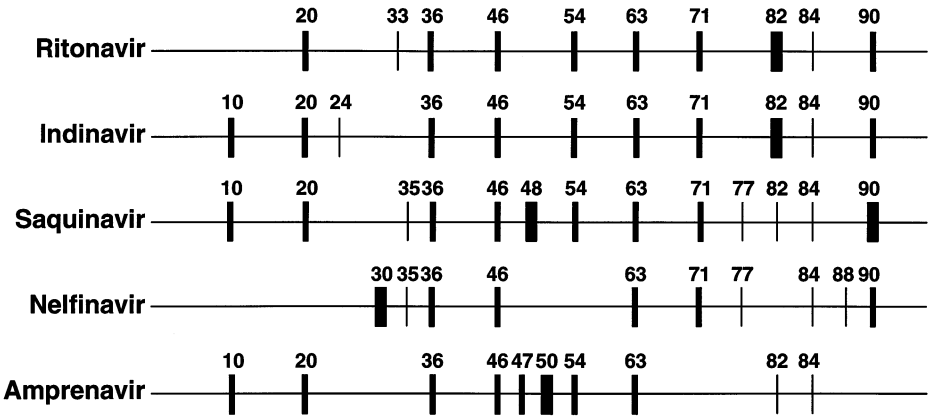


Fig. 2. In vivo resistance patterns of HIV protease inhibitors. Thick bars, primary mutations; medium bars, secondary mutations common to several protease inhibitors; thin bars, other secondary mutations or (as is the case for the mutation at position 84) other active site mutations not clearly identified as a primary mutation for a given inhibitor.

D30N is the primary mutation in the protease gene. The appearance of D30N was associated with the emergence of other changes including E35D, M36I, M46I, A71T/V, V77I, N88D/S and L90M. In phenotypic assays, HIV clinical isolates with significant resistance to nelfinavir were susceptible to indinavir, saquinavir, ritonavir and amprenavir (Patick et al., 1997). In the other studies, the majority of isolates with nelfinavir resistance demonstrated cross-resistance with other protease inhibitors (Hertogs et al., 1998).

*3.2.5. Amprenavir*

Viral rebound during amprenavir monotherapy produced two distinct patterns of mutations. In the first, the I50V mutation, which was observed in vitro, was found in conjunction with secondary mutations at several sites, including positions 36, 46, 47, 54, 63, 84 and the p1/p6 *gag* substrate cleavage site. The I50V mutation was not present in the second pattern, instead, changes at positions 10, 20, 54, 82 and 84, commonly selected with indinavir or ritonavir were observed (De Pasquale et al., 1998).

### *3.3. Primary and secondary mutations in HIV protease*

Fig. 2 shows a list of the mutations selected in patients by protease inhibitors in vivo. The general classification of mutations at individual residues within HIV protease as either primary mutations or secondary mutations (also called ancillary, accessory or compensatory) is useful for understanding the mechanism of resistance development and the possible responses of patients with resistant HIV to a subsequent regimen containing a second protease inhibitor. Ritonavir and indinavir both select the position 82 mutation most frequently and, at least with ritonavir, as the initial mutation. Saquinavir causes initial mutation at residues 90 or 48, and nelfinavir has been reported to select the 30 as the first mutation. Residues 82, 30, 50, 48 and 90, can be classified as primary mutations for their respective inhibitor because they tend to appear first and, with the exception of the 90 mutation, they lie within the active site. Because of their direct contact with the inhibitor, primary mutations generally contribute to phenotypic resistance by directly reducing the binding affinity of the drug to HIV protease (Gulnik et al., 1995). Moreover, because of the different structures of each inhibitor, primary mutations are, with the exception of the 82 mutation for ritonavir and indinavir, distinct for a given inhibitor. In contrast, secondary mutations, such as those at residues 10, 20, 71, 63, 36, 46, and 54 are common to other protease inhibitor and occur outside of the active site toward the surface of the enzyme and are usually found in

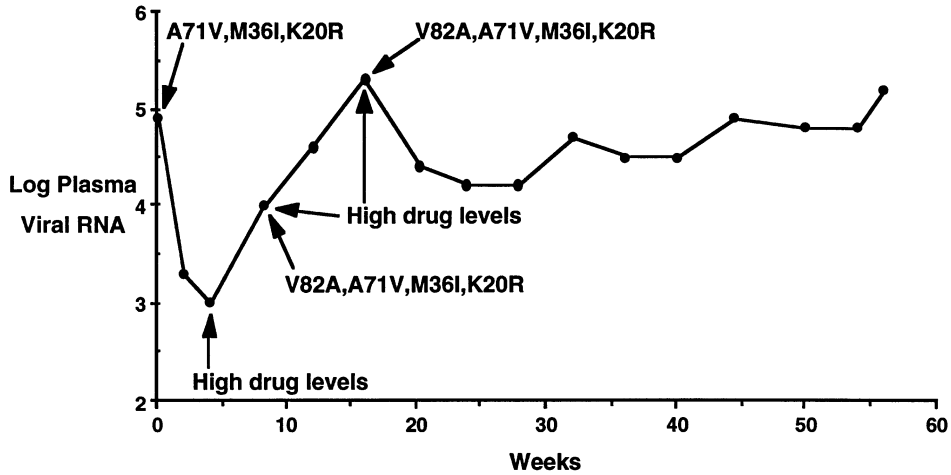


Fig. 3. Plasma HIV RNA curve for a patient who received 600 mg *bid* of ritonavir combined with 200 mg *tid* of AZT.

vivo after the initial appearance of a primary mutation. Both a primary mutation and several secondary mutations are generally required for high level phenotypic resistance in vitro; however, in the absence of a primary mutation, viruses with multiple secondary mutations have been reported to be sensitive to the inhibitors (Condra et al., 1995; Markowitz et al., 1995a; Molla et al., 1996). Consequently, many secondary mutations appear to contribute to phenotypic resistance by increasing the fitness of the initial (primary) mutant virus rather than (or in addition to) by decreasing drug binding. Several studies have shown that molecular clones containing a single active site mutation grow more slowly in vitro than viruses containing the primary mutation plus one or more secondary, non-active site mutations (Ho et al., 1994). Furthermore, the presence of two secondary (L63P, M46I) mutations increased the catalytic efficiency of either wild-type HIV protease or the protease containing two active site (V82A, I84V) mutations (Schock et al., 1996). This more general mechanism may operate regardless of the identity of the primary mutation and is consistent with the observation that, in contrast to primary mutations, many secondary mutations appear in response to all protease inhibitors as a class.

The generic nature of secondary mutations may have implications for the ease by which mutant virus selected by one protease inhibitor may develop cross-resistance to a subsequent protease inhibitor in vivo. Virus selected by one inhibitor, containing the primary mutation for that inhibitor plus several secondary mutations, is often phenotypically sensitive to other inhibitors that select a different primary mutation (Molla et al., 1996; Patick et al., 1996; Jacobsen et al., 1995a,b; Schapiro et al., 1996a,b). However, the number of additional mutations required to confer cross-resistance to a second protease inhibitor might be expected to vary considerably. At one extreme, a different primary mutation and several new secondary mutations might be required for resistance to the second drug regimen and accompanying virological failure. At the other extreme, virus selected by one protease inhibitor need to add only the primary mutation specific for the second inhibitor to become highly cross-resistant, even if phenotype at the time change of therapy was sensitive. The possible consequences of selection of multiple, common secondary mutations following failure of any protease inhibitor-based regimen are illustrated in Fig. 3 for a patient, who instituted therapy with ritonavir plus AZT. At baseline, the viral population within this patient contained three secondary mutations associated with ritonavir resistance (residues 71, 36 and 20). The population was phenotypically sensitive to ritonavir, as judged by the initial two-log decline in viral RNA. However, in spite of high drug

levels of ritonavir, within weeks the viral load began to increase and ultimately rebounded to baseline with only the addition of a mutation at position 82, the primary mutation for ritonavir. In this extreme example, the response to protease inhibitor therapy was similar to that observed with monotherapy with nevirapine, the activity of which is completely overcome with a single mutation in RT (Richman et al., 1994). The selection of secondary mutations during and after virological rebound on therapy with any protease inhibitor regimen is therefore likely to compromise the durability and, in some cases, even the initial response to a subsequent regimen containing another protease inhibitor. Preliminary clinical studies using protease inhibitor regimens as subsequent therapy in patients who have experienced rebound following initial therapy with any of the currently licensed inhibitors support this prediction (Deeks et al., 1997; Kaufmann et al., 1997; Sampson et al., 1997). Thus, protease inhibitors exert their greatest efficacy in patients who are naive to the protease inhibitor class. Two important therapeutic strategies arise from these considerations. First, the most powerful combination regimens should be used at the initiation of antiretroviral therapy in order to block viral replication and thus delay or possible completely prevent resistance development in the greatest number of patients. Second, a delay in a change of regimen following virological rebound on protease inhibitor therapy is likely to allow for the selection of a greater number of secondary mutations, thus increasing the risk that salvage therapy will be ineffective.

### *3.4. Kinetics of in vivo resistance emergence*

The emergence of resistant HIV in vivo is generally temporally associated with the rebound in plasma RNA toward baseline, pretreatment levels during continued therapy. This association reflects the mechanistic requirement for both viral replication, which produces mutations in the HIV genome, and drug selection, which inhibits the majority of the HIV quasispecies and allows outgrowth of minor species with greater fitness in the presence of drug. The mutant virus that emerges will be the most fit under the particular circumstances, which may include, among others, the plasma and tissue concentrations of drug, the immune status of the patient, and the preexisting viral heterogeneity prior to treatment. The rate of emergence of resistance for a given drug or combination of drugs will be a function of both the genetic barrier (i.e. the number of new mutations required to overcome the inhibition by the drug) and the pharmacokinetic barrier (the degree of suppression of viral replication by particular plasma concentrations of inhibitors) exhibited by the drug regimen. In general, the genetic barrier to protease inhibitor resistance is relatively high, requiring several mutations. In contrast, a single mutation may produce high-level resistance to NNRTIs. The relationship of viral replication (and thus resistance development) and pharmacokinetic profile of protease inhibitors is a consequence of the mechanism of action (competitive inhibition) and mode of transport into cells (passive) of these agents. Thus, in contrast to NRTIs, which accumulate in cells as their respective active triphosphates, protease inhibitors may diffuse out of infected cells if plasma concentrations decline to low levels.

The rate at which mutations in HIV protease accumulated during monotherapy with ritonavir inversely correlated with the measured trough concentrations in the plasma of patients (Fig. 4). Thus, the HIV protease sequence in those patients with higher sustained drug concentrations exhibited a lower in vivo selection rate (defined as the number of new resistance-associated mutations per time on therapy) than in those with lower drug levels, regardless of dose (Molla et al., 1996). More powerful combination regimens produce a more prolonged plasma RNA response prior to rebound; however, little has been documented regarding the rate at which mutations accumulate between the nadir and the point at which viral RNA reaches baseline. In a related study (Kempf et al., 1998), the duration of suppression of viral RNA in patients on protease inhibitor therapy prior to viral rebound with resistance was shown to depend primarily on the absolute HIV RNA value at the nadir, rather than on the baseline RNA value of the initial decline in RNA. Both of

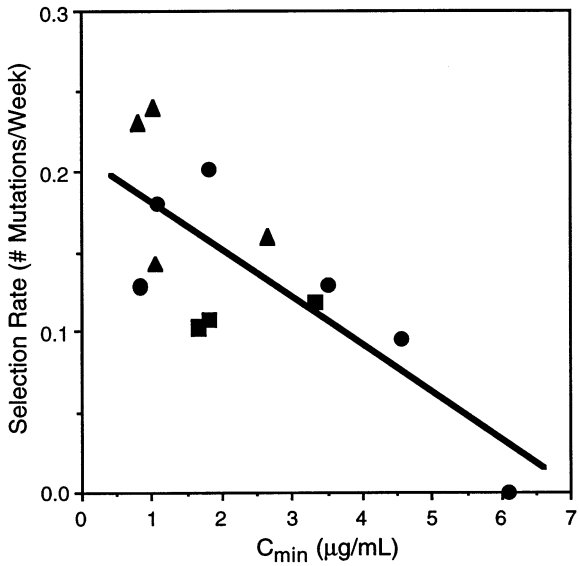


Fig. 4. Relationship of in vivo selection rate and total ritonavir exposure. Each dot represents one patient. Triangles: 400 mg *bid*; squares: 500 mg *bid*; circles: 600 mg *bid*. Reproduced from Molla et al. (1996) with the permission of Nature Medicine.

these studies support the hypothesis that resistance arises because viral replication is incompletely suppressed in the plasma or tissues during a periods of time between doses when drug concentrations are the lowest (Fig. 5, panel 1). Because of the inherent high error rate of RT, low level replication allows the outgrowth of mutants with slightly increased fitness in the presence of drug. As more mutations accumulate, viral fitness continues to increase either through decreasing the binding of the drug and/or by increasing the kinetics of proteolytic processing by HIV protease (panel 2). Ultimately, a sufficient number of mutations are present to overcome the suppression of the drug (panel 3), with viral RNA often returning to pretherapy levels. Therefore, therapeutic strategies which produce higher, more sustained plasma and tissue concentrations between doses would be expected to delay or prevent the emergence of resistance in a greater proportion of patients. The use of therapeutic drug level monitoring to guide dose adjustments of indinavir and produce more consistent trough levels is being investigated (Acosta et al., 1997). Moreover, the use of positive drug–drug interactions between ritonavir and other protease inhibitors to enhance plasma levels has provided the basis for powerful, dual protease inhibitor therapy (see below).

### 3.5. Protease cleavage site mutations

HIV protease recognizes and cleaves nine sites in *gag* and *gag-pol* precursor polyprotein. The

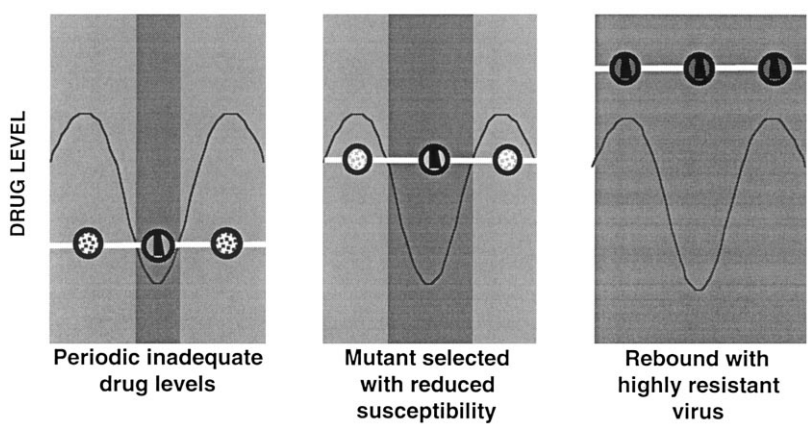


Fig. 5. Model for resistance development to protease inhibitors. Black line, plasma levels of drug; light grey, period of complete suppression of replication; dark grey, period of production of infectious virus; white line, drug concentration above which low level viral replication occurs; circles, representation of electron micrographs of mature, infectious particles following proteolytic processing by HIV protease (circles with dense, dark cores) or immature, noninfectious viral particles with incompletely processed structural proteins (opaque circles).

proper processing of each of these sites is required by either wild-type or mutant HIV protease to produce mature, infectious viral progeny. Studies using peptides corresponding to the natural cleavage sites have shown that the p2/p7 and transframe protein/protease (TF/PR) cleavage sites are processed most rapidly, while the p7/p1 and p1/p6 are most slowly cleaved (Darke et al., 1988; Tozser et al., 1991). If cleavage of the latter sites is rate-limiting for virion maturation, changes in substrate sequence at those sites might provide a mechanism for upregulating the fitness of variants containing mutations in HIV protease that otherwise might be lethal to viral replication. Recently, Doyon et al. (1996) identified changes in the HIV-1 *gag* p7/p1 and p1/p6 proteolytic cleavage sites during in vitro selection with the pipecolinic acid based inhibitors BILA 1906 BS and BILA 2185 BS. These second locus cleavage site mutations were shown not to contribute directly to the drug resistance but, rather, improve polyprotein processing and outgrowth of protease mutants. Thus, removal of cleavage site mutations from corresponding resistant clones led to a decrease or even an absence of viral growth. Similar mutations were observed during serial passage of HIV in the presence of increasing concentrations of the new protease inhibitor ABT-378 (Carrillo et al., 1997). Recently, a change in the *gag* p7/p1 cleavage site was observed in vivo, following rebound in plasma HIV RNA levels in patients on indinavir therapy (Zhang et al., 1997). A second mutation at the *gag* p1/p6 cleavage site was also observed in one patient. Gag cleavage site mutation P1/P6 (Leucine to phenylalanine) together with protease mutations was also observed during amprenavir therapy (De Pasquale et al., 1998). Studies with infectious clones containing a similar series of mutations indicated that a mutation in both the protease gene and substrate cleavage site led to the most replication-competent viruses. These studies indicate that, in addition to compensatory mutations in HIV protease that may increase the proteolytic efficiency of mutant protease, mutations in substrates can provide a second mechanism to increase the overall fitness of mutant HIV in the presence of a protease inhibitor.

## 4. Pharmacokinetics and metabolism

All of the HIV protease inhibitors are extensively metabolized by cytochrome *P*-450 (CYP) enzymes which are present predominantly in liver, but also in other tissues, notably gastrointestinal enterocytes. The principal metabolism for all protease inhibitors is mediated by CYP3A, the major CYP isoform in human liver. In addition to CYP3A, there is evidence that some of the protease inhibitors (nelfinavir and ritonavir) are partially metabolized by CYP2D6, which usually accommodates basic, lipophilic substrates. Direct *O*- and *N*-glucuronidation has been confirmed as a minor route in the clearance of nelfinavir and indinavir, respectively. Most protease inhibitors appear to have distributional phases after oral administration; thus, comparison of terminal-phase half-lives may be misleading because the inferred intradose fluctuation is smaller than the observed $C_{max}/C_{min}$ ratio, which ranges up to 50:1 for indinavir. The terminal-phase half-lives of the agents range from 1.8 h for saquinavir to 7.8 h for ritonavir, with *tid* dosage intervals recommended for all agents except ritonavir which is administered twice daily. Except for saquinavir, these regimens maintain trough concentrations above the $EC_{50}$ against wild-type HIV in tissue culture. In the following sections, the pharmacokinetics, metabolism and interactions of the protease inhibitors are outlined. Key pharmacokinetic and metabolic information is provided in Table 2.

### 4.1. Saquinavir

Saquinavir has the highest apparent clearance of all marketed protease inhibitors primarily due to extensive first-pass gastrointestinal and hepatic clearance, primarily by CYP3A4 isoform of cytochrome *P*-450. For single 600 mg doses, the absolute bioavailability averaged 4%, apparently as a result of first pass metabolism and possibly incomplete absorption. The clearance appears to decrease with multiple oral dosing. In a study evaluating 3600 and 7200 mg/day regimens, AUCs of 2.23 and 9.74 $\mu$g h/ml, respectively were obtained (Schapiro et al., 1996a). Saquinavir is highly sensitive to metabolic inhibition by riton-

Table 2
Comparison of HIV protease inhibitor pharmacokinetics

| Standard regimen | Saquinavir[a] FORTOVASE 1200 mg q8h | Ritonavir NORVIR 600 mg q12h | Indinavir CRIXIVAN 800 mg q8h | Nelfinavir VIRACEPT 750 mg q8h | Amprenavir 141W94; VX-478 1200 mg q12h |
|---|---|---|---|---|---|
| $C_{min}$ ($\mu$g/ml)[b] | 0.2 | 3.7 | 0.15 | ~2 | 0.35 |
| $C_{max}$ ($\mu$g/ml)[b] | 2.5 | 11.2 | 7.7 | 3.5 | 9.5 |
| CL/$F$ (l/h)[c] | 166 | 9 | 43 | 37 | 42 |
| Plasma unbound fraction (%) | 2 | 1–2 | 40 | <2 | ~10 |
| Unchanged renally | <3 | 3.5 | 9–12 | 1–2 | <2 |
| AUC, effect of food (%) | 570 high fat | 14, capsule | −77 | 100–200 | minor |
| AUC, with Rifampin (%) | −80 HG | −35 | −92 | −82 | −81 |
| AUC, with Ketoconazole (%) | 200 HG | 18 | 62 | 35 | 32 |
| CL/F, increasing dose | Decreases | Decreases | Decreases | Increases | NA |
| Major Metabolism[d] | CYP3A4 | CYP3A4 | CYP3A4 | CYP3A4 (~50%) | CYP3A4 |
| Minor Metabolism[d] | None | CYP2D6 | GT[e] | GT[e], CYP2C, CYP2D6 | NA |
| Active Metabolites | No | No | No | Yes | NA |
| In vivo Inhibition[d] | CYP3A | CYP3A ≫ CYP2D6 | CYP3A | CYP3A | CYP3A |
| Effect on Saquinavir AUC | NA | Increase 20X SG | Increase 6X SG | Increase 5–13X HG | Ongoing |
| In vivo Induction[d] | No | CYP3A, CYP1A2, GT[e] | No | GT[e] | No |

[a] Refer to current soft-get formulation (SG), unless otherwise specified for original hard gel formulation (HG);
[b] $C_{min}$ and $C_{max}$ are reported average steady-state minima and maxima;
[c] CL/$F$ is the reported or imputed (CL/$F$ = dose/AUC) steady-state apparent oral clearance;
[d] CYP refers to human cytochrome $P$-450 isoforms;
[e] GT is glucuronosyl transferase; NA, results not available.

avir (Kempf et al., 1997). As expected, no interactions were found between saquinavir and ddC and AZT. Both inductive and inhibitory effects on saquinavir metabolism have been reported. The NNRTI nevirapine, which demonstrates autoinduction, appears to slightly reduce (27%) saquinavir concentrations. A new soft gel capsule formulation of saquinavir (Fortovase™) with improved oral bioavailability has recently been licensed. As a 1200 mg *tid* regimen, it produces a mean AUC of 7.25 $\mu$g h/ml over an 8 h dosing interval (Buss, 1998).

### 4.2. Ritonavir

Ritonavir has the lowest apparent clearance, approximately 9 l/h at steady state, of the currently marketed protease inhibitors. The pharmacokinetics of ritonavir are being investigated in children, and preliminary results indicate that in children greater than 2 years old doses of 250–400 mg/m$^2$ are tolerated and confer antiviral activity (Mueller et al., 1997). It has been demonstrated that the human metabolism of ritonavir is principally mediated by CYP3A4, with

a minor contribution from CYP2D6 (Kumar et al., 1996). Other CYP isoforms and glucuronsoyl transferase do not appear to be important in the human metabolism. Ritonavir coadministration is associated with decreases in the AUC of AZT ($-25\%$) and has little effect on the AUC of ddI ($-13\%$). However, ritonavir potently inhibits CYP3A4 and, to a lessor extent, CYP2D6. Consequently, ritonavir increases the AUC of several other CYP3A substrates, including clarithromycin (1.8-fold) and rifabutin plus its active metabolite (6-fold), as well as other HIV protease inhibitors (see below).

### 4.3. Indinavir

Indinavir is rapidly absorbed in the fasted state with a time to peak plasma concentration ($T_{max}$) of 0.8 h. The clearance of indinavir after multiple dosing with a 800 mg q8h regimen is around 43 l/h, producing an AUC0-8 of 18.8 $\mu$g h/ml (30.7 $\mu$M h). Indinavir has a relatively short functional half-life, with the above regimen having mean $C_{max}$ and 8-h (trough) values of $7.7 \pm 2.5$ and $0.15 \pm 0.10$ $\mu$g/ml, respectively (Winchell et al., 1997). Indinavir is extensively metabolized by CYP3A (Chiba et al., 1996). Seven metabolites have been identified, one glucuronide conjugate and six oxidative metabolites after incubation in human liver microsomes. Experiments with recombinant *P*-450 isoforms showed that CYP3A4 efficiently produced most of the metabolites, and that CYP2D6 could produce three of the metabolites as well as a *cis*-diol—a metabolite that is not observed in vivo. In vitro studies indicated that indinavir inhibited CYP3A4-dependent testosterone 6 $\beta$-hydroxylase activity, with a $K_i$ of ~0.5 $\mu$M (McCrea et al., 1997). Plasma concentrations of indinavir decrease when it is administered with food ($-77\%$), or co-dosed with rifampin ($-92\%$), DMP-266 ($-35\%$), nevirapine ($-28\%$) or rifabutin ($-34\%$) (Fiske et al., 1997; Murphy et al., 1997; Winchell et al., 1997). Smaller decreases ($\leq 26\%$) are reported for fluconazole and grapefruit juice. Increases in indinavir AUCs have been demonstrated with other protease inhibitors (see below) and ketoconazole (McCrea et al., 1996).

### 4.4. Nelfinavir

In 12 adult subjects receiving nelfinavir 500 mg q8h for 16 doses, the apparent clearance was 27.4 l/h, $V/F$ was 124 l and the terminal phase $t_{1/2}$ averaged 3.1 h (Kerr et al., 1997). In another Phase I study, mean CL/$F$ was 37.4 l/h, mean $V/F$ was 150 l and mean $t_{1/2}$ was 2.8 h after a 750 mg q8h regimen (Yuen et al., 1997). The pharmacokinetics of nelfinavir have been assessed in neonates ($3.3 \pm 0.5$ kg) dosed with 2 mg/kg oral doses $57 \pm 10$ h after birth. Consistent with developing metabolism, the clearance of nelfinavir was low (22–40 ml/kg per h) and the mean terminal-phase half-life was prolonged 36.8 h. The maximum concentrations after dosing ranged from 0.64 to 1.61 $\mu$g ml, declining slowly to a median of 0.22 $\mu$g/ml at one week. Nelfinavir is principally metabolized by CYP3A4. In vitro experiments with various substrates indicate that nelfinavir is capable of CYP3A inhibition ($K_i = 4.8$ $\mu$M), with little effects on the other isoforms. Nelfinavir is reported to have no effect on the NRTIs, 3TC and ddI.

### 4.5. Amprenavir

Inhibition studies with human liver microsomes showed that amprenavir inhibited CYP3A4 and 2C19. The primary phase I metabolic pathway for amprenavir utilizes CYP3A4. Amprenavir is not an inducer of the CYP1A, 2B, or 3A isozymes in animals. A side by side study with other protease inhibitors in human liver microsome showed the following rank order of CYP inhibition: ritonavir $\gg$ indinavir $\approx$ nelfinavir $\approx$ amprenavir $>$ saquinavir (Woolley et al., 1997). The $C_{max}$ and AUC of amprenavir after single dose administration was 5.5 mg/ml and 15.5 h mg/ml, respectively. Coadministration with ketoconazole increased the AUC of amprenavir by 32% and that of ketoconazole AUC by 44% (Polk et al., 1997). The Cmax, AUC, and Cmin for amprenavir were reduced by 5, 14, and 10, respectively by rifabutin and 67, 81, and 91%, respectively by rifampin (Polk et al., 1998). Mean AUCs, $C_{max}$, and $C_{min}$ decreased by 36, 39 and 43% respec-

tively, in the presence 600 mg DMP-266 (Piscitelli et al., 1998).

## 5. Clinical effects of protease inhibitors

Treatment regimens containing a protease inhibitor produce a pronounced effect on surrogate markers in HIV-infected patients. In preliminary studies, therapy with ritonavir or indinavir reduced plasma HIV RNA by 90 to $>99\%$ and substantially elevated CD4 levels (Danner et al., 1995; Markowitz et al., 1995b; Stein et al., 1996). The rapid rate of decline in viral load following initiation of treatment provided unprecedented insight into the dynamics of HIV particle production and CD4 cell destruction at steady state (Ho et al., 1995; Wei et al., 1995). Monotherapy with these agents, however, was insufficient to prevent the emergence of resistant HIV in most patients. Therefore, treatment regimens using a protease inhibitor in combination with inhibitors of RT have been assessed in hopes of more completely and durably suppressing replication in vivo. The rationale for such combinations was based both on in vitro virological studies showing an additive or synergistic relationship between the two classes of agents. Initial investigation of triple therapy using indinavir (Gulick et al., 1997) or ritonavir (Danner, 1996; Mathez et al., 1997) with two nucleoside analogs produced a decline in plasma HIV RNA to unquantifiable (400–500 copies/ml) levels in a high percentage of patients. The median durability of response to triple therapy was much longer than to therapy with either a single protease inhibitor or a combination of two nucleosides (Gulick et al., 1997). Furthermore, the response was greater in patients who had received no prior antiretroviral therapy of any class. More recent studies have shown that triple therapy with nelfinavir (Markowitz et al., 1996) or an enhanced formulation of saquinavir (Slater, 1998) along with two nucleosides produces a similar effect. Importantly, the reduction in viral load in lymph nodes in patients on triple therapy with ritonavir was found to be similar to that observed in plasma (Cavert et al., 1997). Finally, the combination of indinavir with the NNRTI DMP 266 was also shown to produce potent, durable anti-HIV activity with no decrease in tolerability (Mayers et al., 1997).

The pronounced effect of protease inhibitor therapy on surrogate markers has been shown to produce an improved clinical outcome. In a phase III study, a group of patients with a median CD4 of 20 cells/$\mu$l added either ritonavir or placebo to their current regimen. Compared to placebo, ritonavir therapy reduced the incidence of death and disease progression about 47% (Cameron et al., 1998a). More recently, triple therapy with indinavir, AZT and 3TC was shown to be clinically superior to AZT/3TC combination therapy (Hammer et al., 1997). At least partial restoration of immune function on highly suppressive combination therapy with a protease inhibitor has been demonstrated (Autran et al., 1998; Connick et al., 1998), although the degree of possible immune reconstitution has not been established. Finally, although continuous therapy can suppress viral load below the level of detection in excess of two years, careful studies have documented the persistence of reservors of infectious HIV, suggesting that eradication of HIV is unlikely without additional approaches designed to selectively deplete infected resting memory T-cells (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997).

## 6. Dual protease inhibitor therapy

In addition to the powerful combinations described above, the unique antiviral and pharmacological properties of protease inhibitors have prompted the clinical investigation of dual protease inhibitor therapy, with and without additional agents. Although significant cross-resistance between some protease inhibitors has been documented, several theoretical considerations support the use of dual protease inhibitor combinations. First, protease inhibitors are highly potent drugs when used individually and use of two such potent agents simultaneously is likely to produce significantly greater suppression of viral replication. Second, the initial mutations selected by most protease inhibitors are distinct, and the selection of high-level resistance to two protease

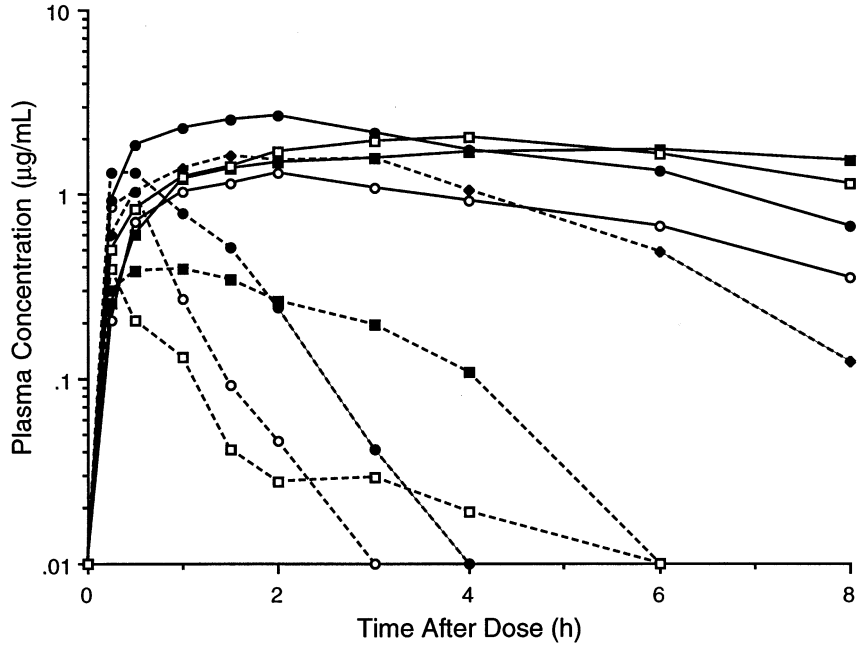


Fig. 6. Plasma concentrations of HIV protease inhibitors following a 10 mg/kg dose in rats, either as single agents or in combination with 10 mg/kg of ritonavir. Dashed lines, inhibitors dosed as single agents; solid lines, inhibitors coadministered with ritonavir. Open squares, saquinavir; open circles, indinavir; filled squares, nelfinavir; filled circles, amprenavir. Reproduced from Kempf et al. (1997) with the permission of American Society for Microbiology.

inhibitors simultaneously may require a greater number of mutations than that required to overcome a single inhibitor, thus raising the 'genetic barrier' to drug resistance. Third, most of the current protease inhibitors undergo relatively rapid clearance mediated predominantly by CYP3A (see above), and thus are characterized by trough levels only modestly in excess of concentrations required to inhibit HIV in vitro. Several protease inhibitors, in particular ritonavir, reversibly inhibit CYP3A (Kumar et al., 1996). The pharmacokinetics of protease inhibitor combinations have been studied thoroughly with ritonavir. In rats coadministration of ritonavir increased the plasma exposure of saquinavir, indinavir, nelfinavir or amprenavir from eight to 36-fold (Kempf et al., 1997 and Fig. 6). Therefore, combinations of protease inhibitors with a significant pharmacokinetic interaction can raise the 'pharmacokinetic barrier' to drug resistance. Simultaneously, they can potentially result in regimens with lower and less frequent dosing, enhancing patient adherence and thus reducing the likelihood of viral breakthrough.

The degree of inhibition of protease inhibitor metabolism by ritonavir was similar in both rat and human liver microsomes, and in human studies, ritonavir was shown to substantially increase the AUC of saquinavir ($>20$-fold), indinavir (three–five-fold) and nelfinavir (2.5-fold). Multiple dosing of ritonavir and saquinavir, each at 400 mg twice daily, provided a mean saquinavir AUC of 31 $\mu$g h/ml, which is at least twenty times that obtained for saquinavir alone at 600 mg *tid* (hard gel formulation), and exceeds that reported after 1200 mg q8h administration (soft gel formulation). Saquinavir concentrations with ritonavir cotherapy are comparable using both the hard gel and new soft gel formultion of saquinavir. Of equal importance, the trough levels of saquinavir in combination with ritonavir were well in excess of the in vitro $EC_{50}$ value. The trough levels of indinavir are also significantly affected such that indinavir dosed twice daily with ritonavir (400 mg

each) produces trough levels that are even higher than those observed with standard (800 mg *tid*) indinavir doses (Hsu et al., 1997). The interaction of ritonavir with nelfinavir also allows *bid* dosing regimen with reduced nelfinavir doses of 500–750 mg *bid* (Gallant et al., 1998). Pharmacokinetic interactions between protease inhibitors other than ritonavir are generally more modest, corresponding with less potent CYP3A inhibition. Indinavir increases the AUC of nelfinavir by less than 2-fold (83%) and that of saquinavir by 7.2-fold (indinavir 800 mg q8h + 800 mg saquinavir soft gel capsule) (McCrea et al., 1997; Kerr et al., 1997). In turn, nelfinavir increases the AUC of indinavir by 51%. The single dose AUC of saquinavir, administered as the soft gelatin capsule, was increased 5-fold when coadministered with nelfinavir 750 mg *tid* (Kravcik et al., 1997). In a different study, nelfinavir have been shown to increased total and peak plasma exposures of saquinavir hard gel capsule by 12.5- and 10.1-fold, respectively (Gallicano et al., 1998). Nelfinavir is reported to have no effect on ritonavir. Coadministration of amprenavir with indinavir resulted in increase in the AUC of amprenavir of 64% with no change in indinavir AUC (Sadler et al., 1997).

The pronounced pharmacokinetic interaction of ritonavir with saquinavir prompted a clinical study of 141 HIV-infected patients with CD4 cell counts of 100–500 per ml. Patients were randomly assigned a ritonavir-saquinavir regimen (400–400 mg *bid*, 600–400 mg *bid*, 400–400 mg *tid*, or 600–600 mg *bid*, respectively). After 12 weeks, 19% (27/141) of the patients had their treatment regimen intensified with two RT inhibitors for failure to achieve or maintain viral load $\leq 200$ copies/ml. Thus, a majority of the patients (81%) received only ritonavir-saquinavir (Farthing et al., 1997). Viral load in approximately 89% (89/100) of patients who remained on study and were treated for 60 weeks was $\leq 200$ copies/ml, and the median CD4 change from baseline was $+174$ cells mm$^3$. After 56 weeks, CSF HIV RNA was $\leq 400$ copies/ml in 14/15 patients with unquantifiable plasma HIV RNA (Cameron et al., 1998b). The combination of these two drugs has been safe and well tolerated. The dose combination at 400 mg *bid*/400 mg *bid* (ritonavir/saquinavir) had the best tolerability, with only one patient out of 35 discontinuing treatment for adverse events.

Subsequently, studies of many other dual protease inhibitor regimens, usually in combination with other antiretrovirals, have recently been initiated. Based on the ability to sustain higher plasma levels than with single protease inhibitor therapy, many of these regimens hold promise for a more complete and durable suppression of infection. Two doses of ritonavir-nelfinavir regimen (400–500 mg *bid* and 400–750 mg *bid*) are under investigation in protease inhibitor-naive patients. At week 12, intensification with RT inhibitors was allowed. After 16 weeks, 6/10 and 9/10 patients, respectively, in the two groups experienced a decline in viral load to $\leq 400$ copies/ml (Gallant et al., 1998). A pilot combination study of saquinavir soft gel capsule and nelfinavir demonstrated a 5-fold increase in saquinavir levels and prompted a clinical study of 54 patients who received saquinavir 800 mg *tid* and nelfinavir 750 mg *tid*. By week 32, the plasma RNA of 69% of 40 patients remaining on the original regimen was $\leq 400$ copies/ml (Opravil, 1998).

## 7. New protease inhibitors

### *7.1. ABT-378*

ABT-378 is a novel HIV protease inhibitor developed by Abbott Laboratories with approximately ten-fold greater in vitro potency than ritonavir. The $K_i$ of ABT-378 for wild-type and for V82A and V82F mutant HIV protease is 1–3 pM (Sham et al., 1997). The $EC_{50}$ of ABT-378 in the presence of 50% human serum is approximately 100 nM (Molla et al., 1997). In primary PBMC cultures, patient HIV isolates were inhibited with an average $EC_{50}$ of 6.5 nM. Against a battery of HIV isolates from patients who had experienced a loss of plasma viral suppression during ritonavir therapy, ABT-378 demonstrated consistently superior activity compared with ritonavir (Korneyeva et al., 1997). ABT-378 displayed an attenuation of activity against some strains, how-

ever, the degree of resistance of these isolates to ABT-378 was substantially less than to ritonavir. Furthermore, ABT-378 inhibited a variety of mutant clones, many of which are highly resistant to ritonavir, at similar or lower concentrations than those required for inhibition of wild-type virus by ritonavir (Molla et al., 1997). A predominant I84V mutation emerged after passage 6 when wild-type HIV molecular clone was passaged in the presence of increasing concentrations of drug (Carrillo et al., 1997). Accompanying the I84V mutation, two secondary mutations (M46I, L10I) were observed after passage 6. Two protease cleavage site mutations (p1/p6 junction and p7/p1 junction) also appeared after seven and 16 passages, respectively. Molecular clone containing four or five mutations in the protease gene, which were viable only in the context of both cleavage site mutations, displayed a 243-fold decrease in susceptibility to ABT-378. Pharmacokinetic studies in animals demonstrated modest bioavailability of ABT-378 when dosed alone. Coadministration of ABT-378 with ritonavir, however, substantially improved the pharmacokinetic profile of ABT-378 to provide plasma concentrations that remained 50-fold in excess of the in vitro $EC_{50}$ at 8–12 h following dosing (Marsh et al., 1997). These pharmacokinetic properties are attributable to the rapid CYP3A-mediated metabolism of ABT-378 and its inhibition by ritonavir. In phase I studies, the pharmacokinetics of ABT-378 (200–600 mg *bid*) coadministered with low (50 and 100 mg *bid*) doses of ritonavir were evaluated in healthy volunteers. Each regimen was well tolerated and produced trough concentrations of ABT-378 of 3.3–8.3 $\mu$g/ml, $>$ 50-fold in excess of the in vitro $EC_{50}$ for wild-type HIV-1 in the presence of human serum (Lal et al., 1998).

### 7.2. PNU-140690

PNU-140690 is a member of a new class of nonpeptide HIV protease inhibitor developed by Pharmacia Upjohn. PNU-140690 displayed a $K_i$ of 0.01 nM and an $EC_{50}$ of about 75 nM against laboratory strains as well as clinical isolates. PNU-140690 retains good anti-HIV activity in vitro in the presence of human serum proteins (Poppe et al., 1997). PNU-140690 retained activity against HIV-1 variants resistant to ritonavir (Chong and Pagano, 1997). After a 10 mg/kg oral dose in rats, the absolute bioavailability was 30% and levels of PNU-140690 in blood exceeded 1 $\mu$M for 8–12 h (Thaisrivongs et al., 1996). Repeated oral administration of PNU-140690 to rats increased the clearance due to induction of cytochrome *P*-450 3A and/or glucuronidation enzymes. In phase I studies, steady-state trough concentrations of PNU-140690 averaged $>1$ $\mu$M, the predicted $EC_{90}$, for dose regimens of 900 mg *tid* and higher. Absorption was significantly increased with high-fat meal, producing a ca. 2- and 1.5-fold increase in AUC and $C_{max}$, respectively, relative to fasting conditions (Borin et al., 1998).

### 7.3. DMP-450

DMP-450 is symmetric member of the cyclic urea class of nonpeptide protease inhibitors discovered at DuPont Merck is being developed by Triangle Pharmaceuticals. The in vitro $K_i$ of DMP-450 using a peptide substrate was 0.28 nM. The average value of the $EC_{50}$ against different HIV strains was 10–20 nM. This compound is moderately bound to human serum, and the $EC_{90}$ for wild-type virus was 4–8 fold higher in the presence of $\alpha$1 acid glycoprotein and human serum albumin. A 45 to 60-fold decrease in potency observed when mutant HIV-1 was selected in vitro in the presence of increasing concentration of DMP-450. This was associated with the presence of two-five mutations, including the I84V in the protease gene. Sequencing of viruses with intermediate levels of sensitivity to DMP-450 inhibition suggested that the mutation at position 46 was an early event, followed by the mutation at position 84 and subsequently by mutations at positions 82, 90 and 45 (Hodge et al., 1996). Molecular clones containing the V82A and V82F mutations showed 2.5 and 5.3-fold resistance to DMP-450, respectively. The I84V mutant virus showed about 10-fold resistance. Highly ritonavir and indinavir resistance virus displayed significant cross-resistance to DMP-450 (Jadhav et al., 1997). The highest plasma concentration achieved after

dosing ($C_{max}$) was 11.19 $\mu$M in dogs, and 1.53 $\mu$M in chimpanzees. Bioavailability ranged from 24% in chimpanzees to 79% in the dogs (Hodge et al., 1996). Single doses of 10 mg/kg DMP-450 result in plasma levels in humans in excess of that required to inhibit wild-type and several mutant viral strains (Hodge et al., 1996).

### *7.4. DMP-851*

A potent member of the asymmetric cyclic urea series, DMP-851 discovered at DuPont Merck displayed a $K_i$ of 21 pM against HIV protease. The protein-adjusted $EC_{50}$ values against wild-type HIV-1 and the I84V mutant virus were 0.8 and 3.8 $\mu$M, respectively. The degree of loss of potency against multiply mutated viruses resistant to ritonavir, indinavir, saquinavir and amprenavir with DMP-851 was less than with DMP-450. Addition of 45 mg/ml human serum albumin and 1 mg/ml $\alpha$1-acid glycoprotein in the culture medium caused a 16.7-fold loss of anti-HIV activity. A single 10 mg/kg oral dose in dogs produced 8 h trough levels (oral bioavailability 63%) of DMP-851 which equaled or exceeded the protein-adjusted $EC_{90}$ for wild-type and single amino acid mutants of HIV-1 (Bacheler et al., 1997).

## 8. Conclusion

The management of HIV infection has become more complex as the options for treatment have increased. Among available anti-HIV drugs, protease inhibitors produce the greatest suppression of viral replication. Although long-term experience with these agents is lacking, the degree of suppression of viral replication obtained with regimens that include one or two protease inhibitors coupled with one or two RT inhibitors can delay or prevent the emergence of resistance. However, adherence to the regimen is critical to ensure a durable virological response. In spite of their potency, currently available protease inhibitors are limited by gastrointestinal side effects, drug–drug interactions, moderate bioavailability, and short plasma half-life. Most require frequent (three times daily) dosing and/or food restriction. A new generation of protease inhibitors with higher potency, superior pharmacokinetics, fewer drug interactions, excellent tolerability and convenient dosing schedule is needed. Current compounds under development should display many of the above properties.

## References

Acosta, E.P., Henry, K., Weller, D., Page, L.M., Bacon, L., Rhame, F., Gilson, I., Rosenstein, H., Schacker, T., Fletcher, C.V., 1997. Indinavir pharmacokinetics and relationship between exposure and antiviral effect. 37th ICAAC, Abstract A-15, Toronto, Canada.

Autran, B., Carcelain, G., Li, T.S., Blanc, C., Mathez, D., Tubiana, R., Katalaba, C., Debre, P., Leibowitch, J., 1998. Positive effects of combined antiretroviral therapy on CD + T cell homeostasis and function in advanced HIV disease. Science 277, 112–116.

Bacheler, L.T., Rodgers, J.D., Lam, P.Y.S., Wright, M., Garber, S., Reid, C., Erickson-Viitanen, S., 1997. New cyclic urea-based protease inhibitors with improved potency, oral bioavailability and resistance profiles. Abstract-2, Sixth International Workshop on HIV drug resistance, treatment strategies and eradication, St. Petersburg, Florida.

Borin, M.T., Wang, Y., Schneck, D.W., Li, H., Brewer, J.E., Daenzer, C.L., 1998. Multi-dose safety, tolerance, and pharmacokinetics of the protease inhibitor PNU-140690 in healthy volunteers. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 648, IL.

Buss, N. on be half of the fortovase study group, 1998. Saquinavir soft gel capsule (Fortovase™): pharmacokinetics and drug interactions. 5th conference on retroviruses and opportunistic infections. Abstract 354, IL.

Cameron, D.W., Heath-Chiozzi, M., Danner, S., Cohen, C., Kravcik, S., Maurath, C., Sun, E., Henry, D., Potthoff, A., Leonard, J.M. and the advanced HIV disease ritonavir study group, 1998. Prolongation of life and prevention of AIDS complications in a randomized placebo-controlled clinical trial of ritonavir in patients with advanced HIV disease. Lancet 351, 543–549.

Cameron, D.W., Japour, A., Mellore, J., Farthing, C., Cohen, C., Markowitz, M., Poretz, D., Follansbee, S., Ho, D., Mahon, D.M., Berg, J., Nieves, J., Xu, Y., Rode, R., Salgo, M., Leonard, J., Sun, E., 1998b. Antiretroviral safety and durability of ritonavir-saquinavir in protease inhibitor naive patients in year two of follow-up. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 388, IL.

Carrillo, A., Sham, H., Norbeck, D., Kempf, D., Kohlbrenner, W., Plattner, J.J., Leonard, J., Molla, A., 1997. Mutation in the proteolytic sites is required for the growth of mutant HIV selected by ABT-378, a new HIV protease inhibitor. Abstract I 116. 37th ICAAC, Toronto, Canada.

Cavert, W., Notermans, D.W., Staskus, K., Wietgrefe, S.W., Zupancic, M., Gebhard, K., Henry, K., Zhang, Z.-Q., Mills, R., McDade, H., Goudsmit, J., Danner, S.A., Haase, A.T., 1997. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science 276, 960–964.

Chiba, M., Hensleigh, M., Nishime, J.A., Balani, S.K., Lin, J.H., 1996. Role of cytochrome *P*-450 3A4 in human metabolism of MK-639, a potent human immunodeficiency virus protease inhibitor. Drug. Metab. Disp. 24, 307–314.

Chong, K.-T., Pagano, P.J., 1997. In vitro combination of PNU-140690, a human immunodeficiency virus type 1 protease inhibitor, with ritonavir against ritonavir-sensitive and -resistant clinical isolates. Antimicrob. Agents Chemother. 41, 2367–2373.

Chun, T.W., Stuyver, L., Mizell, S.B., Ehler, L.A., Mican, J.A., Baseler, M., Lloyd, A.L., Nowak, M.A., Fauci, A.S., 1997. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acd. Sci. U.S.A. 94, 13193–13197.

Coffin, J.M., 1995. HIV population dynamics in vivo: implication for genetic variation, pathogenesis, and therapy. Science 267, 483–489.

Condra, J.H., Schleif, W.A., Blahy, O.M., Gabryelski, L.J., Graham, D.J., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., Titus, D., Yang, T., Deutsch, P.J., Holder, D.J., Emini, E.A., 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature (London) 374, 569–571.

Connick, E., Lederman, M., Kotzin, B., Wade, M., Spritzler, J., Kuritzkes, D., St. Clair, M., Kessler, H., Roe, J., Fox, L., Martinez, A., Heath-Chiozzi, M., Rousseau, F., Landay, E., ACTG 315 protocol team, 1998. Immunologic effects of 48 weeks of AZT/3TC/ritonavir. Abstract LB14. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Danner, S.A., 1996. Immediate versus delayed triple therapy with ritonavir, AZT and 3TC. Program and abstracts of the International Congress on Drug Therapy in HIV Infection. Birmingham, UK.

Danner, S.A., Carr, A., Leonard, J.M., Lehman, L.M., Gudiol, F., Gonzales, J., Raventos, A., Rubio, R., Bouza, E., Pintado, V., Aguado, A.G., Garcia deLamos, J., Delgado, R., Borleffs, J.C.C., Hsu, A., Valdes, J., Boucher, C.A.B., Cooper, D.A., 1995. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1protease. New Engl. J. Med. 333, 1528–1533.

Darke, P.L., Nutt, R.F., Brady, S.F., Garsky, V.M., Ciccarone, T.M., Leu, C.-T., Lumma, P.K., Freidinger, R.M., Veber, D.F., Sigal, I.S., 1988. HIV-1 protease specificityide cleavage is sufficient for processing of gag and pol polyprotein. Biochem. Biophys. Res. Commun. 156, 297–303.

Deeks, S., Grant, R., Horton, C., Simmonds, N., Follansbee, S., Eastman, S., 1997. Virologic effect of ritonavir (RTV) plus saquinavir (SQV) in subjects who have failed indinavir (IDV). 37th ICAAC, Abstract I-205, Toronto, Canada.

De Pasquale, M.P., Murphy, R., Gulick, R., Smeaton, L., Sommadossi, J.-P., Degruttola, V., Calindo, A., Kuritzkes, D., Sutton, L., Savara, A., D'Aquila, R., 1998. Mutations selected in HIV plasma RNA during 141W94 therapy. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 406a, IL.

Doyon, L., Croteau, G., Thibeault, D., Poulin, F., Pilote, L., Lamarre, D., 1996. Second locus involved in human immunodeficiency virus type 1 resistance to protease inhibitors. J. Virol. 70, 3763–3769.

Dubouck, C., 1992. The HIV protease as a therapeutic target for AIDS. AIDS Res. Hum. Retroviruses 8, 153–160.

Eberle, J., Bechowsky, B., Rose, D., Hauser, U., Von Der Helm, K., Gurtler, L., Nitschko, H., 1995. Resistance of HIV type 1 to proteinase inhibitor Ro31-8959. AIDS Res. Hum. Retroviruses 11, 671–675.

Farthing, C., Japour, A., Cohen, C., Cameron, W., Follansbee, S., Markowitz, M., Mellors, J., Poretz, D., Ho, D., Mahon, D.M., Berg, J., Nieves, J., Xu, Y., Rode, R., Salgo, M., Leonard, J., Sun, E., 1997. Cerebrospinal fluid and plasma HIV RNA suppression with ritonavir-saquinavir in protease inhibitor naive patients. Abstract LB-3. 37th ICAAC, Toronto, Canada.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D., Siliciano, R.F., 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278, 1295–1300.

Fiske, W.D., Mayers, D., Wagner, K., Riddler, S., Drusano, G., Stein, D., Bach, M., Havlir, D., Kahn, J. 1997. Pharmacokinetics of DNP 266 and indinavir multiple oral doses in HIV-1 infected individuals. Abstract 568. 4th Conference on Retroviruses and Opportunistic Infections, Washington, DC.

Gallant, J.E., Heath-Chiozzi, M., Raines, C., Anderson, R., Katz, T.K., Fields, C., Jackson, J.B., Flexner C., 1998. Safety and efficacy of nelfinavir-ritonavir combination therapy. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 394a, IL.

Gallicano, K., Sahai, J., Kravcik, S., Seguin, I., Bristow, N., Cameron, D.W., 1998. Nelfinavir increases plasma exposure of saquinavir in hard get capsule in HIV+ Patients. Abstract 353. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Gulick, R., Mellors, J., Havlir, D., Eron, J., Gonzalez, C., McMahon, D., Richman, D., Valentine, F., Jonas, L., Meibohm, A., Emini, E., Chodakewitz, J., 1997. Treatment with indinavir, zidovudine, and lamivudine in adaults with human immunodeficience virus infection and prior antiretroviral therapy. New Engl. J. Med. 337, 734–739.

Gulnik, S.V., Suvorov, L.I., Liu, B., Yu, B., Anderson, B., Mitsuya, H., Erickson, J.W., 1995. Kinetic characterization and cross-resistance patterns of HIV-1 protease mutants selected under drug pressure. Biochemistry 34, 9282–9287.

Hammer, S.M., Squires, K.E., Hughes, M.D., Grimes, J.M., Demeter, L.M., Currier, J.S., Eron, J.J., Feinberg, J.E.,

Balfour, H.H., Deyton, L.R., Chodakewitz, J.A., Fischl, M.A., 1997. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. New Engl. J. Med. 337, 725–733.

Hertogs, K., Mellors, J.W., Schel, P., Van Cauwenberghe, A., Larder, B., Kemp, S., Miller, V., Stasewski, S., Conant, M., Pauwels, R., 1998. Patterns of cross-resistance among protease inhibitors in 483 clinical HIV isolates. Abstract 395. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Ho, D.D., Toyoshima, T., Kempf, D.J., Norbeck, D., Chen, C.-M., Wideburg, N.E., Burt, S.K., Erickson, J.W., Singh, M.K., 1994. Characterization of HIV-variants with increased resistance to a C2-symmetric protease inhibitor. J. Virol. 68, 2016–2020.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infections. Nature (London) 373, 123–126.

Hodge, C.N., Aldrich, P.E., Bacheler, L.T., Chang, C.-H., Eyermann, C.J., Garber, S., Grubb, M., Jackkson, D.A., Jadhab, P.K., Korant, B., Lam, P.Y.S., Maurin, M.B., Meek, J.L., Otto, M.J., Rayner, M.M., Reid, C., Sharpe, T.R., Shum, L., Winslow, D.L., erickson-Vitanen, S., 1996. Improved cyclic urea inhibiyors of the HIV-1 protease: synthesis, potency, resistance profile, human pharmacokinetics and X-ray crystal structure of DMP 450. Chem. Biol. 3, 301–314.

Holland, J.J., Torre De La, J.C., Steinhauer, D.A., 1992. RNA virus populations as quasispecies. Curr. Top. Microbiol. Immunol. 176, 1–20.

Hsu, A., Granneman, G.R., Japour, A., cao, G., Locke, C., Carothers, L., Dennis, S., El-Shourbagy, T., Leonard, J., 1997. Evaluation of potential ritonavir and indinavir combination BID regimen. Abstract A-57. 37th ICAAC, Toronto, Canada.

Jacobsen, H., Haenggi, M., Ott, M., Duncan, I.B., Vella, S., Mous, J., 1995a. In vivo resistance to HIV protease inhibitor saquinavir-an update. AIDS Res. Hum. Retrovirues 11, S169.

Jacobsen, H., Yasargil, K., Winslow, D.L., Craig, J.C., Krohn, A., Duncan, I.B., Mous, J., 1995b. Characterization of human immunodeficiency virus type 1 mutants with decreased sensitivity to proteae inhibitor Ro 31-8959. Virology 206, 527–534.

Jadhav, P.K., Francis, P.A., Woerner, F.J., Chang, C.-H., Garber, S.S., Anton, E.D., Bacheler, L.T., 1997. Cyclic urea amides: HIV-1 protease inhibitors with low nanomolar potency against both wild type and protease inhibitor resistant mutants of HIV. J. Med. Chem. 40, 181–191.

Kaufmann, G., Duncombe, C., Beveridge, A., Chuah, J., Carr, A., Cunningham, P., Cooper, D.A., 1997. Efficacy of a four drug combination therapy including two protease inhibitors in patients with advanced HIV-1 illness. Abstract I-200, 37th ICAAC, Toronto, Canada.

Kempf, D.J., Marsh, K.C., Kumar, G., Rodrigues, A.D., Denissen, J.F., McDonald, E., Kukulka, M.J., Hsu, A., Granneman, G.R., Baroldi, P.A., Sun, E., Pizzuti, D., Plattner, J.J., Norbeck, D.W., Leonard, J.M., 1997. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. Antimicrob. Agents Chemother. 41, 654–660.

Kempf, D.J., Rode, R.A., Xu, Y., Sun, E., Heath-Chiozzi, M.E., Valdes, J., Japour, A.J., Danner, S., Boucher, C., Molla, A., Leonard, J.M., 1998. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. AIDS 12, F9–F14.

Kerr, B., Lee, C., Yuen, G., Anderson, R., Daniels, R., Grettenberger, H., Liang, B.-H., Quart, B., Sandoval, T., Shetty, B., Wu, E., Zhang, K., 1997. Overview of in vitro and in vivo drug interaction studies of nelfinavir mesylate, a new HIV-1 protease inhibitor. Abstract 373. 4th Conference on Retrovirus and Opportunistic Infections, Washington, DC.

Kohl, N.E., Emini, E.A., Schleif, W.A., Davis, L.J., Heimbach, J.C., Dixon, R.A.F., Scolnick, E.M., Sigal, I.S., 1988. Active human immunodefiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA 85, 4686–4690.

Korneyeva, M., Chernyvskiy, T., Norbeck, D., Sham, H., Kempf, D., Kohlbrenner, W., Plattner, J.J., Leonardf, J., Molla, A., 1997. Virological Evaluation of Ritonavir-Resistant Virus to the HIV Protease Inhibitor ABT-378. Abstract-212. 4th Conference on Retrovirus and opportunistic Infections, Washington DC.

Kumar, G.N., Rodrigues, A.D., Buko, A., Denissen, J.F., 1996. Cytochrome *P*-450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. J. Pharmacol. Exp. Ther. 277, 423–431.

Kravcik, S., Sahai, J., Kerr, B., Anderson, R., Buss, N., Seguin, I., Bristow, N., Farnsworth, A., Salgo, M., 1997. Nelfinavir mesylate increases saquinavir-soft gel capsule exposure in HIV + patients. Abstract-371. 4th Conference on Retrovirus and Opportunistic Infections, Washington, DC.

Lal, R., Hsu, A., Grannemen, G.R., El-Shourbagy, T., Johnson, M., Law, W., Manning, L., Japour, A., Sun, E., 1998. Multi dose safety, tolerability and pharmacokinetics of ABT-378 in combination with ritonavir. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 647, IL.

Lambert, D.M., Petteway, S.R., McDanal, C.E., Hart, T.K., Leary, J.J., Dreyer, G.B., Meek, T.D., Bergelski, P.J., Bolognesi, D.P., Metcalf, B.W., Methews, T.J., 1992. Antimicrob. Agents Chemother. 36, 982–988.

Mansky, L.M., Temin, H.M., 1995. Lower in vivo mutation rate of human immunodefiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. J. Virol. 60, 5087–5094.

Markowitz, M., Cao, Y., Hurley, A., O'Donovan, R., Peterkin, J., Anderson, B., Smiley, L., Keller, A., Johnson,

P., Johnson, D., Ho, D.D., 1996. Triple therapy with AZT, 3TC in combimnation with nelfinavir mesylate in 12 antiretroviral naive subjects chronically infected with HIV. Abstract LB-B6031. Program and abstracts of the XI International Conferences on AIDS. Vancouver, BC, Canada.

Markowitz, M., Mo, H., Kempf, D.J., Norbeck, D.W., Bhat, T.N., Erickson, J.W., Ho, D.D., 1995a. Selection and analysis of human immunodeficiency virus type a variants with increased resistance to ABT-538, a novel protease inhibitor. J. Virol. 69, 701–706.

Markowitz, M., Saag, M., Powderly, W.G., Hurley, A.M., Hsu, A., Valdes, J.M., Henry, D., Sattler, M., Marca, A.L., Leonard, J.M., Ho, D.D., 1995b. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. New Engl. J. Med. 333, 1534–1539.

Marsh, K., McDonald, E., Sham, H., Kempf, D., Norbeck, D., 1997. Enhancement of ABT-378 pharmacokinetics when administered in combination with ritonavir. Abstract 210. 4th Conference on Retrovirus and opportunistic Infections, Washington DC.

Mathez, D., Bagnarelli, P., Gorin, I., Katalama, C., Pialoux, G., Saimot, G., Tubiana, P., De Truchis, P., Chauvin, J.-P., Mills, R., Rode, R., Clementi, M., Leibowitch, J., 1997. Reductions in viral load and increases in T-lymphocytes in treatment naive patients with advanced HIV infection, treated with ritonavir, zidovudine, and zalcitabine triple therapy. Antiviral Ther. 2 (3), 175–183.

Mayers, D., Riddler, S., Bach, M., Stein, D., Havlir, M.D., Kahn, J., Ruiz, N., Labriola, D.F., DMP 266 clinical development team, 1997. Durable clinical anti-HIV activity and tolerability for DMP 266 in combination with indinavir at 24 weeks. Abstract I-175. 37th ICAAC, Toronto, Canada.

McCrea, J., Woolf, E., Sterrett, A., Matthews, C., Deutsch, P., Yeh, K.C., Waldman, S., Bjornsson, T., 1996. Effects of ketoconazole and other *P*-450 inhibitors on the pharmacokinetics of indinavir. Abstract PPDM 8370. American Association of Pharmacological Scientists, p. 485.

McCrea, J., Buss, N., Stone, J., Carides, A., Dru, J., Stewart, F., Brown, A., Yeh, K., Salgo, M., Waldman, S., Deutsch, P., 1997. Indinavir-saquinavir single dose pharmacokinetic study. Abstract 608. 4th Conference on Retrovirus and Opportunistic Infections, Washington, DC.

Miller, V., Hertogs, K., Bethune, M.-P., Pauwels, R., Ivens, T., Azijn, H., Schlecht, C., Nolde, B., Sturmer, M., Morgenstern, B., Kortenbusch, M., Staszewski, S., 1997. Incidence of HIV-1 resistance and cross-resistance to protease inhibitors after indinavir failure: impact on subsequent ritonavir/saquinavir combination therapy. Abstract 81, Sixth International Workshop on HIV drug resistance, treatment strategies and eradication, St. Petersburg, FL.

Molla, A., Korneyeva, M., Gao, Q., Vasavanonda, S., Schipper, P.J., Mo, H.-M., Markowitz, M., Chernyavskiy, T., Niu, P., Lyons, N., Hsu, A., Granneman, G.R., Ho, D.D., Boucher, C.A.B., Leonard, J.M., Norbeck, D.W., Kempf, D.J., 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2, 760–766.

Molla, A., Vasavanonda, S., Denissen, J., Kumar, G., Grabowski, B., Sham, H., Norbeck, D., Kempf, D., Kohlbrenner, W., Plattner, J.J., Leonard, J., 1997. Effect of Human Serum Proteins on the Antiretroviral Activity of Ritonavir and ABT-378, Potent Inhibitors of HIV Protease. Abstract 213. 4th Conference on Retrovirus and opportunistic Infections, Washington DC.

Mueller, B.U., Zuckerman, J., Nelson, R.P., Sleasman, J., Saulis, R., Higham, C., Brouwers, P., Janelevich, S., Jarosinski, P., Sei, S., Serchuck, L.K., Wood, L.V., Zeichner, S., Heath-Chiozzi, M., Pizzuti, D., Pizzo, P.A., 1997. Update on the pediatric phase I/II study of the protease inhibitor ritonavir (ABT-538). Abstract 722. 4th Conference on Retrovirus and Opportunistic Infections, Washington, DC.

Murphy, R., Gagnier, P., Lamson, M., Dusek, A., Ju, W., Hsu, A., 1997. Effect of nevirapine on pharmacokinetics of indiavir, and ritonavir in HIV-1 patients. 4th conferences on retroviruses and opportunistic infections. Abstract 374, Washington, DC.

Navia, M.A., Fitzgerald, P.M.D., McKeever, B.M., Leu, C.T., Heimbach, J.C., Herber, W.K., Sigal, I.K.S., Darke, P.L., Springer, J.P., 1989. Three dimensional structure of the aspartyl protease from human immunodefiency virus HIV-1. Nature 337, 515–621.

Opravil, M., on behalf of the SPICE study team, 1998. Study of protease inhibitor combination in Europe (SPICE): saquinavir soft gel capsule and nelfinavir in HIV infected individuals. 5th Conference on Retroviruses and Opportunistic Infections. Abstract 394b, IL.

Partaledis, J.A., Yamaguchi, K., Tisdale, M., Blair, E.E., Falcione, C., Maschera, B., Myers, R.E., Pazhanisamy, S., Futer, O., Cullinan, A.B., Stuver, C.M., Byrn, R.A., Livingston, D.J., 1995. In vitro selection and characterization of human immunodeficiency virus type 1 (HIV-1) isolates with reduced sensitivity to hydroxyethylamino sulfonamide inhibitors of HIV-1 aspartyl protease. Antimicrob. Agents Chemother. 69, 5228–5235.

Patick, A.K., Mo, H., Markowitz, M., Appelt, K., Wu, B., Musick, L., Kalish, V., Kaldor, S., Reich, S., Ho, D., Webber, S., 1996. Antiviral and resistance studies of AG1343, an orally bioavailable inhibitor of human immunodeficiency virus protease. Antimicro. Agents Chemother. 40, 292–297.

Patick, A.K., Kuritzkes, D., Johnson, V.A., Shugarts, D., Bakhtiari, M., Potts, K.E., Farnsworth, A., Anderson, R., Koel, J.L., Hazelwood, J.D., Nail, C.D., Duran, M., Markowitz, M., Ho, D., Richman, D., 1997. Genotypic and phenotypic analyses of HIV-1 variants isolated from patients treated with nelfinavir and other protease inhibitors. Abstract 18, Sixth International Workshop on HIV drug resistance, treatment strategies and eradication, St. Petersburg, FL.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion

clearance rate, infected cell life-span, and viral generation time. Science 271, 1582–1586.

Piscitelli, S., Vogel, S., Sadler, B., Fiske, W., Metcalf, J., Masur, H., Falloon, J., 1998. Effect of efavirenz (DMP266) on the pharmacokinetics of 141W94 in HIV-infected patients. Abstract 346. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Polk, R.E., Israel, D.S., Pastor, A., Sadler, B.M., Chittick, G.E., Symonds, W.T., Crouch, M., Gouldin, W., Lou, Y., Rawls, C., Bye, A., 1997. Pharmacokinetic interaction between ketoconazol and the HIV protease inhibitor 141W94 after single dose administration to normal volunteers. Abstract A-61. 37th ICAAC, Toronto, Canada.

Polk, R.E., Israel, D.S., Pastor, A., Sadler, B.M., Chittick, G.E., Symonds, W.T., Brophy, D., Kristoff, D., Lou, Y., Bye, A., 1998. Pharmacokjinetic interaction between 141W94 and rifabutin and rifampin after multiple dose administration. Abstract 340. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Poppe, S.M., Slade, D.E., Chong, K.T., Hinshaw, R.R., Pagano, P.J., Markowitz, M., Ho, D.D., Mo, H., Gorman III, R.R., Dueweke, T.J., Thaisrivongs, S., Tarpley, W.G., 1997. Antiviral activity of the dihydropyrone PNU-140690, a new nonpeptidic human immunodeficiency virus protease inhibitor. Antimicro. Agents Chemother. 41, 1058–1063.

Richman, D.D., Havlir, D., Corbeil, J., Looney, D., Ignacio, C., Spector, S.A., Sullivan, J., Cheeseman, S., Barringer, K., Pauletti, D., Shih, C.-K., Myers, M., Griffin, J.J., 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68, 1660–1666.

Sadler, B.B., Eron, J., Wakeford, J., Pagano, G., Rawls, C., Mccrea, J., Mazina, K., Deutsch, P.J., 1997. Pharmacokinetics of 141W94 and indinavir after single-dose administration in HIV positive volunteers. Abstract A-56, 37th ICAAC, Toronto, Canada.

Sampson, M., Torres, R.A., Stein, A.J., Cochrane, J.M., Taylor, B., Mcintyre, K., Shay, W., Barr, M.R., Fischer, L., 1997. Ritonavir-saquinavir combination treatment in protease inhibitor experienced patients with advanced HIV disease. 37th ICAAC, Abstract I-104, Toronto, Canada.

Schinazi, R.F., Larder, B.A., Mellors, J.W., 1996. Mutations in retroviral genes associated with drug resistance. Antiviral News 4, 95–107.

Schapiro, J.M., Winters, M.A., Stewart, F., Efron, B., Norris, J., Kozal, M.J., Merigan, T.C., 1996a. The effect of high-dose saquinavir on viral load and CD4+ T-cell counts in HIV-infected patients. Ann. Int. Med. 124, 1039–1050.

Schapiro, J.M., Winters, M.A., Vierra, M., Jacobson, H., Mous, J., Merigan, T.C., 1996b. Resistance mutations in patients receiving saquinavir: simultaneous appearance in lymph nodes, peripheral blood mononuclears (PBM) and plasma. Abstract of the Fifth International workshop, Whistler, Canada, p. 28.

Schock, H.B., Garsky, V.M., Kuo, L.C., 1996. Mutational anatomy of an HIV-1 protease variant conferring cross-resistance to protease inhibitors in clinical trials—compensatory modulations of binding and activity. J. Biol. Chem. 271, 31957–31963.

Schmit, J.-C., Ruiz, L., Clotet, B., Raventos, A., Tor, J., Leonard, J., Desmyter, J., De Clercq, E., Vandamme, A.-M., 1996. Resistance-related mutations in the HIV-1 protease gene of patients treated for 1 year with protease inhibitor ritonavir (ABT-538). AIDS 10, 995–999.

Sham, H., Kempf, D., Molla, A., Marsh, K., Betebenner, D., Chen, X., Rosenbrook, W., Wideburg, N., Chen, C., Kati, W., Kumar, G., Korneyeva, M., Vasavanonda, S., McDonald, E., Saldivar, A., Chernyavskiy, T., Carrillo, A., Lyons, N., Park, C., Stewart, K., Plattner, J.J., Norbeck, D., 1997. Design, synthesis and biological properties of ABT-378, ahighly potent HIV protease inhibitor. Abstract 1, 4th Conference on Retrovirus and opportunistic Infections, Washington DC.

Slater, L., on behalf of the NV15355 study group, 1998. Activity of new formulation of saquinavir in combination with two nucleosides in treatment naive patients. Abstract 368. 5th Conference on Retroviruses and Opportunistic Infections, IL.

Smith, T., Swanstrom, R., 1996. Selection for high level resistance to HIV-1 protease inhibitors used in pairs. Abstract of the Fifth International Workshop on HIV Drug Resistance, Whistler, Canada, p. 20.

Stein, D.S., Fish, D.G., Bilello, J.A., Preston, S.L., Martineau, G.L., Drusano, G.L., 1996. A 24-week open-label phaseI/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 10, 485–492.

Thaisrivongs, S., Skulnick, H.I., Turner, S.R., Strohbach, J.W., Tommasi, R.A., Johnson, P.D., Aristoff, P.A., Judge, T.M., Gammill, R.B., Morris, J.K., Romines, K.R., Chrusciel, R.A., Hinshaw, R.R., Chong, K.T., Tarpley, W.G., Poppe, S.M., Slade, D.E., Lynn, J.C., Horng, M.-M., Tomichj, P.K., Seest, E.P., Dolak, L.A., Howe, W.J., Howard, G.M., Schwende, F.J., Toth, L.N., Padbury, G.E., Wilson, G.J., Shiou, L., Zipp, G.L., Wilson, K.F., Rush, B.D., Ruwart, M.J., Koeplinger, K.A., Zhao, Z., Cole, S., Zaya, R.M., Kakuk, T.J., Janakiraman, M.N., Watenpaugh, K.D., 1996. Structure based design of HIV protease inhibitor s: sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrones as non peptidic inhibitors. J. Med. Chem. 39, 4349–4353.

Tisdale, M., Meyers, R.E., Blair, E., Kohli, A., Kemp, S.D., Larder, B.A., 1995. In vitro studies support combination therapy with HIV protease inhibitors. Fourth International Workshop on HIV drug Resistance, Sardinia, Italy, p. 61.

Tisdale, M., Myers, R., Najera, I., Kohli, A., Kemp S., Larder, B.A., 1996. Analysis of resistance interactions with 141W94 (VX-478) and other HIV protease inhibitors. Abstract of the Fifth International Workshop on HIV Drug Resistance, Whistler, Canada, p. 27.

Tozser, J., Blahah, I., Copeland, T.D., Wondrak, E.M., Oroszlan, S., 1991. Comparison of the HIV-1 and HIV-2 proteinases using oligopeptide substrates representing cleavage sites in gag and gag-pol polyprotein. FEBS Lett. 281, 77–80.

Vasudevachari, M.B., Zhang, Y.-M., Imachi, H., Imachi, T., Falloon, J., Salzman, N.P., 1996. Emergence of protease inhibitor resistance mutations in human immunodeficiency virus type-1 isolates from patients and rapid screening procedure for their detection. Antimicro. Agents Chemother. 40, 2535–2541.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., Saag, M.S., Shaw, G.M., 1995. Viral dynamics in human immunodefiency virus type 1 infection. Nature (London) 373, 117–122.

Winchell, G.A., McCrea, J.B., Carides, A., Kusma, S.E., Chiou, R., Deutsch, P., Yeh, K.C. Waldman, S., Bjornsson, T.D., 1997. Pharmacokinetic interaction between indinavir and rifabutin. Abstract PI-66. American Association Clinical Pharmacolgical Therapists, p. 153

Wlodawer, A., Miller, M., Jaskolski, M., Sathyanarayana, B.K., Baldwin, E., Weber, I.T., Selk, L.M., Clawson, L., Schneider, J., Kent, S.B.H., 1989. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-a protease. Science 245, 616–621.

Wong, J.K., Hezareh, M., Gunthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., Richman, D.D., 1997. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278, 1291–1295.

Woolley, J., Studenberg, S., Boehlert, C., Bowers, G., Sinhababu, A., Adams, P., 1997. Cytochrome *P*-450 isozyme induction, inhibition and metabolism studies with HIV protease inhibitor, 141W94. Abstract A60. 37th ICAAC, Toronto, Canada.

Yuen, G.J., Anderson, R., Sandoval, T., Wu, E., Shetty, B.V., Kerr, B.M., 1997. The pharmacokinetics of nelfinavir administered alone and with rifampin in healthy volunteers (Abstract PI-40). Clin. Pharmacol. Ther. 61, 147.

Zhang, Y., Imamichi, H., Imamichi, T., Lane, C.H., Falloon, J., Vasudevachari, M.B., Salzman, N.P., 1997. Drug resistance dirung indinavir therapy is caused by mutations in the protease gene and in its gag substrate cleavage sites. J. Virol. 71, 67662–67667.