HIV-1 Protease Inhibitors

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Treatment of human immunodeficiency virus type 1 (HIV-1) infection with regimens that include protease inhibitors (PIs) has contributed to marked improvements in HIV-related disease progression and mortality. Five PIs are approved by the US Food and Drug Administration and have potent activity in vitro. PIs with 2 nucleoside analogue reverse transcriptase inhibitors have demonstrated prolonged suppression of HIV-1 replication in treated patients and improvements in disease progression and mortality. PIs combined with nonnucleoside reverse transcriptase inhibitors or other PIs produce marked antiretroviral effects. Although not all patients have prolonged responses to PIs, and salvage treatment has had mixed results for patients who have not responded to initial PI therapy or whose HIV RNA levels have relapsed during such therapy, newer PIs currently being developed hold promise. Most patients can successfully tolerate PI-including regimens; however, long-term side effects, such as body fat redistribution, insulin resistance, and increased serum lipids, are now being observed in some patients receiving PI-including therapy.

The treatment of HIV-1-infected patients has changed dramatically over the past 5 years. Multiple observations in a variety of settings have demonstrated a decrease in mortality due to HIV-related illnesses, and the incidence of HIV-1-related opportunistic infections has decreased [1]. Much of the improvement in HIV-1-related clinical outcomes has been attributed to the use of protease inhibitors (PIs) in combination antiretroviral therapy [2].

In HIV-1, as in all retroviruses, the production of infectious virus invariably requires an active viral protease. The protease is a viral-encoded enzyme that consists of a 99-amino-acid protein that forms a homodimer. In the replication cycle of HIV-1, a precursor protein or polyprotein is synthesized. This polypeptide is composed of structural proteins (Gag proteins) and viral-encoded enzymes, including the reverse transcriptase, integrase, and the protease itself (reviewed in [3]). Cleavage of the Gag polyprotein is required for the formation of infectious particles. Cells containing HIV-1 proviral DNA that does not produce a functional protease do produce viral particles, but these particles are immature and are noninfectious [4].

Knowledge of the protease substrate and protease inhibitor structures led to a “structure-informed” strategy of drug development. This strategy led to the design of an initial set of inhibitors that proved that inhibition of HIV-1 protease would result in inhibition of HIV replication [5]. This knowledge has ultimately led to successful drug discovery by a number of different research groups [6–8]. These inhibitors are highly potent in cell culture assays and highly specific for the HIV-1 protease.

Clinical Studies of PIs

The in vitro antiretroviral activity of HIV-1 PIs was quickly put to the test in clinical trials. In initial studies, the PIs were given as single agents in single- or multiple-dose studies, to define pharmacokinetic parameters of each of the agents and to establish initial in vivo antiretroviral activity. The clinical development of PIs coincided with the development of quantitative amplification assays to measure the amount or copy number of HIV RNA in blood plasma [9, 10]. Unlike previous techniques that had been used to measure HIV replication, such as p24 antigen assays or culture of virus from peripheral blood mononuclear cells or plasma, which were positive in a minority of infected patients, these newer techniques enabled the measurement of most patients’ HIV-1 RNA levels. These assays proved to be sensitive measures of both ongoing HIV replication by antiretroviral agents [11, 12]. The measurement of HIV RNA levels in blood plasma became an essential tool for assessing antiretroviral activity of new antiretroviral compounds and an indicator of the clinical benefits of a treatment regimen [13].

PIs that were developed initially had poor oral bioavailability and were administered through iv infusion. Improvements in the solubility of these agents enhanced oral bioavailability to allow for larger-scale clinical development. Five compounds—ritonavir, saquinavir, indinavir, nelfinavir, and amprenavir [6–8, 14]—have been approved by the US Food and Drug Administration (FDA) for the treatment of HIV-1 infection (table 1). Saquinavir is currently available in 2 formula-
occurs to predominantly 2 proteins, albumin and obstacle to clinical development. Plasma protein binding, which currently available PIs ranges from 4% for saquinavir-HGC to availability of saquinavir. The oral bioavailability of the cur-

The protein binding of PIs to plasma proteins offered another obstacle to clinical development. Plasma protein binding, which occurs to predominantly 2 proteins, albumin and α1-acid glycoprotein, ranges from 60% with indinavir to >98% with ritonavir, nelfinavir, amprenavir, and saquinavir [3, 15, 16]. The extent and avidity of protein binding influences the amount of free drug that is available for entry into cells and for clearance by metabolic pathways. One HIV PI, SC52151, which showed substantial antiretroviral activity in vitro, had limited antiretroviral activity when given orally to HIV-infected patients, at least in part because of tight protein binding [17].

Ritonavir. Initial studies of ritonavir (ABT-538; Norvir; Abbott Laboratories, Abbott Park, IL) demonstrated that the compound had antiretroviral activity over a range of doses when administered as a single agent [18, 19]. A clear dose-response relationship was observed, with the highest dose level (600 mg twice daily) associated with the most durable antiretroviral response. Doses of ritonavir higher than this proved intolerable [20], and the dosage of 600 mg twice daily was chosen for further development. Ritonavir has a relatively high (>98%) degree of protein binding but remains active in vitro in the presence of human plasma proteins [3].

In the first demonstration of the clinical benefit of PIs, a large-scale clinical (phase III) trial was undertaken in which ritonavir or placebo was added to the antiretroviral therapy for infected patients in a blinded fashion. Study patients had advanced HIV disease with CD4 cell counts <100 cells/μL. Once ritonavir was added to their treatment regimens, patients who were receiving ≥1 reverse transcriptase inhibitor were also receiving a combination of antiretrovirals; however, because of the sequential administration of the ritonavir, these patients were functionally receiving ritonavir monotherapy. Despite the fact that patients had very advanced HIV disease, the addition of ritonavir had a remarkable effect on both mortality and progression to AIDS-defining complications [21]. Over a median follow-up of 29 weeks, the addition of ritonavir resulted in a 47% reduction in occurrences of AIDS-defining events or death.

Indinavir. Initial clinical studies of indinavir sulfate (MK-639; Crixivan; Merck, West Point, PA) demonstrated that plasma concentrations that exceeded the 95% inhibitory concentration (IC95) for HIV-1 in cell culture were achievable [22]. The agent is not tightly bound by human plasma [8], and, in HIV-1–infected patients given 600 mg of indinavir 3 times a day, mean peak concentrations and mean trough concentrations of approximately 50 and 3 times the in vitro IC95, respectively, for clinical isolates were achieved [23]. Indinavir demonstrated a dose-response effect [24, 25] with the most potent antiretroviral activity at 2400 mg/d, as measured by changes in HIV-1 RNA levels [23, 26]. Daily doses >2400 mg/d did not appear to increase the antiretroviral effect [26].

Multiple larger studies included an indinavir monotherapy arm [27–30]. All showed similar findings for indinavir monotherapy, which was uniformly potent, with decreases in HIV-1 RNA ranging from 1.5 log10 copies/mL to 3.1 log10 copies/mL over 12–24 weeks, when a lower limit of quantification of 400–500 copies/mL was used. However, sustained responses (i.e., HIV-1 RNA level below quantifiable limits for 6 months) were seen in only ~40% of patients in each study. Although use of PI monotherapy is neither logical nor recommended [31], this degree of success with indinavir as a single agent speaks to the overall potency of this agent. Another potentially important characteristic of indinavir is its penetration into the CNS and CSF.

Saquinavir. Saquinavir (Invirase; saquinavir-HGC) in its original formulation has a bioavailability of ~4% when given

<table>
<thead>
<tr>
<th>Table 1. Currently available protease inhibitors.</th>
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<tbody>
<tr>
<td>Agent</td>
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<tr>
<td>Trade name</td>
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<tr>
<td>Dosing requirement(s)</td>
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<tr>
<td>Dosage</td>
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<td>Common side effects</td>
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<tr>
<td>Indinavir</td>
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<tr>
<td>Crixivan (Merck, West Point, PA)</td>
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<tr>
<td>Empty stomach, or with a very light meal</td>
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<tr>
<td>800 mg every 8 h</td>
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<td>Nephrolithiasis</td>
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<td>Nelfinavir</td>
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<tr>
<td>Viracept (Agouron, La Jolla, CA)</td>
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<tr>
<td>With meals</td>
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<tr>
<td>750 mg 3 times daily;</td>
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<tr>
<td>1250 mg twice daily</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Ritonavir</td>
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<tr>
<td>Norvir (Abbott Laboratories, Abbott Park, IL)</td>
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<tr>
<td>With or without food, though may be better tolerated with food</td>
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<tr>
<td>600 mg twice daily;</td>
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<tr>
<td>400 mg twice daily in combination with other protease inhibitor</td>
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<tr>
<td>Nausea, diarrhea, and perioral paresthesias</td>
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<td>Saquinavir-HGC</td>
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<td>Invirase (Roche Labs, Nutley, NJ)</td>
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<td>With high-fat meals</td>
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<td>600 mg 3 times daily</td>
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<td>Gastrointestinal</td>
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<td>Saquinavir-SGC</td>
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<tr>
<td>Fortovase (Roche Labs, Nutley, NJ)</td>
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<tr>
<td>With meals (high fat increases absorption)</td>
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<tr>
<td>1200 mg 3 times daily</td>
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<tr>
<td>Gastrointestinal</td>
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<td>Amprenavir</td>
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<tr>
<td>Agnerase (GlaxoWellcome, Research Triangle Park, NC; Vertex Pharmaceuticals, Cambridge, MA)</td>
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<td>With or without food</td>
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<tr>
<td>1200 mg twice daily</td>
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<td>Gastrointestinal, predominantly nausea</td>
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NOTE: HGC, hard-gel capsule; SGC, soft-gel capsule.
with a high-fat meal. Concentrations are decreased ~5-fold if saquinavir is given in the fasting state. The low bioavailability is, in part, due to extensive first-pass metabolism in the intestinal wall and the liver by the cytochrome p450 enzyme system. Saquinavir-HGC was studied as monotherapy in HIV-1-infected men who had minimal symptoms related to their disease, had CD4 cell counts <500 cells/μL, and had received no previous antiretroviral therapy [32]. In this trial, patients received 25, 75, 200, or 600 mg 3 times a day for 16 weeks. The highest dose, 600 mg 3 times a day, produced a peak median decrease in HIV-1 RNA of 80% (or ~0.7 log₁₀ copies/mL).

Higher doses of saquinavir-HGC have been tested. A dosage of 3600 mg/d resulted in a maximal mean decrease in plasma HIV RNA level of 1.06-log RNA copies/mL, and, at week 24, the plasma HIV RNA level remained 0.48-log RNA copies/mL lower than baseline. A higher dose of saquinavir (7200 mg/d) produced a mean maximal decrease in the plasma HIV RNA level of 1.34-log RNA copies/mL, and, at week 24, the plasma HIV RNA level remained 0.85-log RNA copies/mL lower than at baseline. Higher plasma drug concentrations in individual patients correlated with greater reductions in plasma HIV RNA levels over the 2 doses [33]. However, the higher dose (7200 mg/d) requires the ingestion of 36 capsules per day.

Saquinavir-SCG (Fortovase) is a more bioavailable formulation of saquinavir. This formulation has been tested only in combination with other agents, although the potency of saquinavir-SCG would be expected to be greater than that of the hard-gel formulation because of improved bioavailability. Saquinavir-SCG has a greater bioavailability than saquinavir-HGC, but the recommended dose is twice as high (1200 mg 3 times daily), which results in substantially higher plasma concentrations with the newer formulation.

Nelfinavir. Nelfinavir mesylate (Viracept; Agouron, La Jolla, CA) has an oral bioavailability >75% and high protein binding [3]. Nelfinavir has a half-life of ~4 h and, when given at a dosage of 750 or 1000 mg 3 times a day, trough concentrations remain above the IC₅₀ of nelfinavir for wild-type HIV in the presence of α₁-acid glycoprotein. There is a dose response with respect to effects of nelfinavir on HIV-1 RNA and CD4 cell counts at dosages from 500 mg twice daily to 1000 mg 3 times daily. Doses of 750 mg twice daily or less resulted in a decline in antiviral activity over 28 days, following a peak effect at 14 days [34]. Patients treated with 750 or 1000 mg 3 times daily had an antiviral effect ≥1.5 log₁₀ over 28 days, and 60% of patients had RNA levels fall below the detection limit of 500 copies/mL. However, in contrast to indinavir recipients, virtually all patients who continued nelfinavir monotherapy after 28 days had HIV RNA levels return to or surpass their pretherapy HIV-1 RNA levels [34].

Amprenavir. Amprenavir is the most recently approved PI in the United States. Amprenavir (GW-141, Vertex-478; GlaxoWellcome, Research Triangle Park, NC; Vertex Pharmaceuticals, Cambridge, MA) has high potency in vitro [35], a long half-life of 7–9.5 h, and an oral bioavailability that is unaffected by food [36]. The protein binding of this compound, when these proteins are present in physiological concentrations, has not been great [15, 16]. However, higher concentrations of α₁-acid glycoprotein may have a substantial effect on the activity of amprenavir [15].

Amprenavir has been administered as monotherapy in 2 clinical trials. In 1 trial, amprenavir therapy resulted in an ~2-log₁₀ decrease in HIV-1 RNA plasma levels over 28 days of therapy, with use of a limit of quantification of 400 copies/mL [37]. However, in a larger trial that compared amprenavir monotherapy with amprenavir/lamivudine/zidovudine combination therapy, the antiretroviral effect of amprenavir monotherapy diminished quickly in a substantial minority of patients. After a median follow-up of 88 days, the HIV RNA levels in more than one-third of patients returned to baseline or were >10-fold higher than the nadir values [14]. Only 26% of patients who reached 12 weeks of amprenavir monotherapy had HIV-1 RNA levels below the limit of quantification, at 500 copies/mL.

PIs in Combination Therapy

The use of combinations of nucleoside analogues and the use of PIs as monotherapy or added to a failing regimen have been shown to have substantial antiretroviral activity and clinical benefit [21, 38–41]. Each of the PIs currently available has been shown to have potent antiretroviral activity when given as a single agent and, as is the case with ritonavir, to have clinical benefit [21]. However, PI monotherapy would be expected to result in evolution of resistant virus and loss of antiretroviral effect in most patients. Currently, the goal of antiretroviral therapy is to suppress HIV-1 replication to the greatest extent possible, that is, below the detectable limits of the most sensitive assays available [31, 42]. Suppression of replication below the limits of detection is associated with more durable suppression of plasma HIV-1 RNA levels. The initiation of therapy with multiple agents is now the recommended standard for achieving this result [31, 43].

PIs in combination with nucleoside reverse transcriptase inhibitors (NRTIs). The use of a potent PI in combination with 2 nucleoside agents has become one of the recommended standard therapy for previously treatment-naïve HIV-infected patients [31, 43]. A substantial body of information exists on the effects of PI combination regimens on HIV viral load and on clinical improvement and survival [2]. Each of the currently approved PIs has been tested in combination with nucleoside analogues in ≥1 clinical trials.

One of the first studies to demonstrate the profound effects of a PI plus 2 NRTIs on HIV-1 RNA levels compared indinavir/ lamivudine/zidovudine with indinavir alone and with zidovudine/lamivudine [29, 44, 45]. In this study, the triple-drug combination resulted in a decrease in HIV RNA levels to below the limit of quantification of 500 copies/mL in >80% of patients.
at 6 months of treatment. This level of effectiveness has persisted in the patients initially treated with triple-drug therapy for >148 weeks [44, 45]. This study used an intent-to-treat analysis, in which all patients were included unless they left the study for nontreatment-related reasons, in which case they were then excluded from the analysis (2 of 32 patients over 148 weeks).

Two-thirds of patients who received indinavir/lamivudine/zidovudine from the outset had HIV RNA levels <50 copies/mL after 148 weeks. Indinavir, lamivudine, and zidovudine have also been shown to have potent effects on HIV-1 RNA levels and CD4 cell counts in patients with very advanced HIV disease whose CD4 cell counts were <50 cells/µL [30]. Similar potent results were seen with indinavir combined with didanosine and zidovudine [28]. Indinavir has been combined with lamivudine and stavudine [46] and with didanosine and stavudine [47] in clinical trials. In each of these studies, the triple-drug combination was compared with indinavir/lamivudine/zidovudine. The effects on HIV RNA plasma levels of the combination of indinavir with lamivudine/stavudine and with didanosine/stavudine were almost identical to those of indinavir/lamivudine/zidovudine. The proportions of patients whose HIV RNA levels decreased to <500 copies/mL at 48 weeks in an intent-to-treat analysis, in which any missing data are considered a treatment failure, were also very similar with each of the 3 indinavir-including triple-drug combinations.

The triple combination of indinavir with 2 nucleoside analogues has also been shown to improve survival and to delay HIV-1 disease progression [48]. In the AIDS Clinical Trials Group (ACTG) Study 320, patients with CD4 cell counts <200 cells/µL who had received zidovudine and other nucleosides but not lamivudine or PIs were randomized to receive either zidovudine plus lamivudine or the triple-drug combination of zidovudine, lamivudine, and indinavir. The addition of lamivudine to zidovudine-including regimens had been shown to improve survival rates and to delay disease progression in a recently completed trial [41]. Despite this fact, the triple-drug regimen of zidovudine/lamivudine/indinavir resulted in a marked improvement in survival rates and a significant delay in HIV-1 disease progression, compared with zidovudine/lamivudine. The relative risks of death and disease progression were both improved by >50% [48].

When the virologic data from this trial were examined, the antiretroviral effectiveness of the triple combination was less than that seen in previous studies [49]. Demeter et al. [49] demonstrated that only about half of the patients in this trial treated with indinavir/lamivudine and zidovudine had HIV RNA levels decrease and remain below detectable limits after 48 weeks. In the subgroup of patients who had CD4 cell count <50 cells/µL at the time of enrollment, the proportion of patients with HIV RNA levels below quantifiable limits (500 copies/mL) at 48 weeks was <40%. For approximately half of those patients, HIV-1 could still be cultured from their peripheral blood mononuclear cells by use of a simple coculture assay. These results suggest that triple-drug therapy with a PI and 2 nucleoside analogues may not produce the desired antiviral result for a majority of patients with low CD4 cell counts.

The use of nelfinavir in combination with zidovudine and lamivudine in treatment-naive patients also resulted in suppression of HIV RNA to <500 copies/mL in 75% of the patients over 6 months [50]; this activity also seemed to persist over time. Two dosages of nelfinavir were used in this study. Nelfinavir at a dosage of 750 mg 3 times daily had the more profound and durable effect on HIV-1 RNA levels in plasma, compared with the effect of 500 mg 3 times daily. Nelfinavir administered at a dose of 1250 mg twice daily has been compared with a 3-times-daily dose of 750 mg, each combined with lamivudine and stavudine, and was shown to have similar antiretroviral activity [51]. Twice-daily nelfinavir is now commonly used.

Saquinavir-SGC appears to be more potent that saquinavir-HGC [52] and to have potency and antiretroviral efficacy similar to those of indinavir when either drug is combined with zidovudine and lamivudine [53]. Saquinavir-SGC given as 1600 mg twice a day has now been compared with the 1200-mg 3-times-daily dose, both combined with 2 nucleoside analogues [54]. These 2 dosing strategies appear similarly effective; in ~60% of patients, an HIV-1 RNA level <400 copies/mL was achieved in an intent-to-treat analysis. However, the final analysis of this study, which will occur when all patients reach week 48, is not complete.

Amprenavir has also been studied in combination with nucleoside analogues. Amprenavir in combination with lamivudine and zidovudine was compared with amprenavir monotherapy [14]. The triple-therapy arm was found to be superior to monotherapy. At 24 weeks, 63% of patients who were still receiving therapy and could be evaluated (an as-treated analysis) were found to have <500 copies/mL. These results are similar to those of a second comparative trial, in which amprenavir/lamivudine/zidovudine was compared with lamivudine and zidovudine [55]. The triple-drug regimen was superior to the dual-nucleoside regimen.

In an intent-to-treat analysis of the proportion of patients with HIV RNA levels <400 copies/mL, in which any missing value is considered to be >400 copies/mL, 41% of patients treated with amprenavir/lamivudine/zidovudine were observed to have <400 copies/mL [55]. Amprenavir has also been combined with a single nucleoside, abacavir. This combination has been shown to have potent activity in patients with early stage HIV disease (>400 CD4 cells/mm3), with 40% having HIV RNA levels <5 copies/mL at 48 weeks in an intent-to-treat analysis [56].

≥2 PIs in combination. Each of the currently available PIs has limitations that decrease the usefulness of these agents or, at best, make long-term adherence to them challenging. Some of these limitations include low bioavailability, short half-lives,
toxicity, food requirements for administration, and potentially limited potency. In addition, single PIs with 2 NRTIs may not have adequate potency in all circumstances, as outlined above. Some of the limitations of PI potency may be due to inadequate exposure of HIV to the inhibitor.

The best example of this concept is the PI saquinavir, with a bioavailability of 4%–20%, depending on the drug formulation. Optimal exposure to saquinavir—indeed, probably to any of the currently available PIs—is limited by bioavailability, protein binding, drug half-life, formulation (i.e., pill number, size, and dose frequency), and/or toxicity. The use of 2 PIs in combination has been proposed as a way to overcome some of these limitations. Combining PIs may improve bioavailability and reduce dosing frequency through pharmacokinetic interactions. Even if there are no pharmacokinetic interactions, the use of 2 PIs may increase overall exposure to PI concentrations, if full doses of the 2 PIs are administered and if they do not have substantial overlapping toxicity. However, if there is no substantial pharmacokinetic interaction between the 2 agents, the number of doses of medications and the number of pills consumed may be quite high. The cost of such regimens might also be prohibitively high.

Multiple-PI combinations are under active study in clinical research trials. Ritonavir-including dual-PI combinations appear to have the most favorable pharmacokinetic interactions. The combination of ritonavir and saquinavir has been the most extensively studied. Ritonavir dramatically enhances the pharmacokinetic profile of saquinavir by inhibiting the P450-mediated metabolism of saquinavir in the gut and liver. In addition, 400 mg of ritonavir coadministered with saquinavir increases saquinavir levels by 20- to 50-fold [57].

The combination of ritonavir and saquinavir has been studied in a randomized trial that involved PI-naive patients [58]. Several different doses of ritonavir and saquinavir were used. At 48 weeks, 60% of all patients had HIV RNA levels <200 copies/mL in an intent-to-treat analysis. Approximately one-quarter of these patients had nucleoside analogues added to their regimens after 12 weeks of administration of ritonavir/ saquinavir alone. Ritonavir (400 mg) and saquinavir (400 mg), both administered twice daily, were the most tolerable doses.

Liver function abnormalities occurred more commonly in patients who had underlying hepatitis B or C. Diarrhea, asthenia, perioral paresthesias, and nausea were the most common adverse events at these doses. The potential for limited penetration of these 2 agents into other tissue compartments, such as the male genital tract and the CNS, has been increased as a concern with regard to this treatment combination [59, 60].

Ritonavir has also been studied in combination with nelfinavir and indinavir. When 400 mg of ritonavir twice daily was given in combination with nelfinavir at doses of either 500 or 750 mg twice daily, the area-under-the-curve (AUC) concentration for nelfinavir was similar to that seen with the approved dose of nelfinavir (750 mg 3 times daily). With the higher doses of nelfinavir, the AUC concentration of M8 (the active metabolite of nelfinavir) are raised even further [61]. The antiretroviral effect of this combination was substantial in the small number of patients studied [62]. Moderate to severe diarrhea was a common side effect, seen in almost half of the 20 patients treated.

Indinavir, although potent, has practical limitations. The recommended dosing is 800 mg every 8 h on an empty or near-empty stomach. However, when 400 mg of indinavir is given with 400 mg of ritonavir, both twice daily, AUC measurements are almost identical, and higher trough concentrations and lower peak concentrations occur than with indinavir alone, given at 800 mg every 8 h. These results are seen even when the ritonavir/indinavir combination is administered with a meal [63].

The antiretroviral effect of this combination has only been studied in a small number of patients, but 18 of 18 treatment-naïve patients receiving ritonavir/indinavir plus lamivudine and stavudine had HIV RNA levels <400 copies/mL after 12 weeks of therapy [64]. The duration of study has so far precluded a detailed examination of adverse events. Indinavir has now been studied with ritonavir in several different dose combinations [65, 66]. Each combination increased indinavir trough levels 15- to 35-fold. Combinations of indinavir (800 mg) with either 100 mg or 200 mg of ritonavir and indinavir (400 mg) with ritonavir (400 mg) are all undergoing additional study.

Indinavir has also been studied with nelfinavir in a small phase I/II pharmacokinetic trial. Indinavir (1000 mg) and nelfinavir (750 or 1000 mg) given every 12 h resulted in steady-state indinavir levels similar to those with every-8-h dosing, but the trough concentrations of nelfinavir were low, and diarrhea was a common side effect [67]. The combination of indinavir (1200 mg) with nelfinavir (1250 mg), both given twice daily, yields the most favorable trough levels and appears to have potent antiretroviral activity [68].

Nelfinavir combined with saquinavir has been studied in small pharmacokinetic trials and in a larger antiretroviral-efficacy trial. Single-dose studies suggested that nelfinavir would increase saquinavir levels by ~5-fold [69], although the effect in different patients was quite variable. However, the effects of nelfinavir on saquinavir concentrations over the long term may be less marked. In a larger study, patients randomized to receive saquinavir-SGC (800 mg 3 times daily) and nelfinavir (750 mg 3 times daily) had a relatively modest antiretroviral response [70]. The proportion of patients with HIV-1 RNA levels <50 copies/mL over time was similar or less than the proportion seen with either a PI alone or a PI with 2 nucleosides.

This dual-PI combination given with 2 NRTIs had the most potent antiretroviral effect in this study. Twice-daily dosing with the nelfinavir/saquinavir combination may be possible, but the pill number is high and a large majority of patients have some diarrhea. Nelfinavir (1250 mg) with saquinavir-SGC (1200 mg)
twice daily, combined with 1 nucleoside analogue, has activity similar to saquinavir-SGC with 2 nucleoside analogues [54].

Ampranavir has been studied in combination with indinavir, nelfinavir, or saquinavir, to obtain pharmacokinetic and preliminary antiretroviral activity data [71]. Each compound was administered 3 times daily. Indinavir and nelfinavir were given at their approved dosages, and saquinavir-SGC was administered at a dosage of 800 mg 3 times daily, as was ampranavir. Preliminary results show potent activity for each of the combinations, although patient numbers were small [71]. No marked pharmacokinetic interactions were seen. Only 1 patient has withdrawn from this study, because of an adverse event that occurred during the first 24 weeks of study.

The pharmacokinetic interaction of ampranavir with ritonavir has recently been studied, and 200 mg of ritonavir was found to increase ampranavir trough levels significantly.

Overall, combinations of 2 PIs appear to increase potency over single-PI therapy, although head-to-head comparisons of 2-PI regimens with single-PI regimens have yet to show dramatic differences [70, 72]. However, many of these combinations require large numbers of pills each day, and the side-effect profile and cost of some regimens may become significant considerations. Ritonavir-based dual-PI combinations seem to have the most favorable pharmacokinetics, allowing for decreased pill numbers and cost without reducing potency.

**PIs in combination with nonnucleoside reverse transcriptase inhibitors (NNRTIs).** PIs have been combined with NNRTIs, both with and without coadministration of NRTIs. To date, most studies of PI/NNRTI/NRTI combinations have been as salvage regimens for NRTI or NRTI/PI treatment failures (see below). When given without NRTIs, the combination of indinavir and efavirenz resulted in HIV RNA levels <400 copies/mL in a substantial proportion (80%) of patients who continued receiving therapy (as-treated analysis) in 2 relatively large clinical trials [73, 74]. Nelfinavir has also been combined with efavirenz, producing similar results after 16 weeks [75]. The use of combination PI/NNRTI regimens with or without NRTIs as initial therapy may limit subsequent treatment options for patients who do not respond completely to therapy or who relapse during therapy.

### Treatment of Patients Whose Initial PI Therapy Fails

PIs are clearly an important component of effective salvage therapy for patients who have not responded to nucleoside analogue therapy. Indinavir in combination with lamivudine and zidovudine has been shown to be highly effective for patients who have had extensive zidovudine treatment [29, 44] and for patients who have had experience with multiple nucleoside analogues, as long as they are lamivudine-naive [48]. The combination of PI with NNRTIs and nucleosides has also been used successfully as salvage therapy for patients who have had extensive NRTI treatment [76, 77].

For patients who have persistent or recurrent evidence of HIV replication during therapy with NNRTIs in combination with nucleoside analogues, the use of a combination including a PI also seems to be effective as salvage therapy, although studies have had only short follow-up periods [78, 79]. A good response to PI-based therapy after therapy directed only at the reverse transcriptase enzyme is theoretically plausible.

Successful treatment options are needed for patients whose HIV-1 RNA levels have not fallen below detection limits or have rebounded to above these limits during initial therapy with PI-including regimens. The use of indinavir or ritonavir after prolonged saquinavir therapy had only modest antiretroviral effect [80, 81]. The use of saquinavir in an incompletely suppressive regimen appears to predispose to indinavir resistance, even if resistance mutations cannot be documented when therapy is changed. The failure of single-PI therapy after failure of therapy with another PI is not surprising, given the substantial degree of cross-resistance between PIs [82, 83]. Cross-resistance seems greatest if the virus has a high level of resistance to 1 inhibitor.

Dual-PI therapy with ≥1 agent to which virus from the patient would be expected to be susceptible is commonly used as salvage therapy after PI failure. The combination of ritonavir and saquinavir has been used with variable success to treat patients whose single-PI-including regimens have failed [84–86]. Following nelfinavir-including regimens, treatment with ritonavir/saquinavir in combination with lamivudine and stavudine resulted in HIV RNA levels <400 copies/mL in 65% of patients after 6 months of therapy, in an intent-to-treat analysis [87].

Initial resistance to nelfinavir may result predominantly from a single-point mutation that conveys limited cross-resistance to other PIs [7, 34, 88, 89]. Ritonavir plus saquinavir-based regimens appear to be less successful after initial failure of an indinavir- or ritonavir-including regimen, in part because of the occurrence of resistance at the time of the switch [84, 86]. However, changing from the initial therapy early after the detection of HIV-1 RNA in plasma (i.e., early virologic failure) may enhance the response to subsequent dual-PI regimens.

In a retrospective study, an early switch from an indinavir-including regimen to a ritonavir/saquinavir–based regimen resulted in 56% of patients having sustained HIV RNA levels <400 copies/mL [90]. Earlier switching from an indinavir-including regimen to a salvage regimen may limit the degree of cross-resistance between the initial PI regimen and the salvage regimen [82, 91]. Adding an NRTI to a dual PI salvage regimen may be another way to improve response rates to PI salvage regimens in those patients who are naive to NNRTI therapy [92, 93].

### PIs in Development

PIs that are approved by the FDA have demonstrated potent antiretroviral activity and/or clinical benefits. However, they
have limitations collectively and individually, involving (for some or all of the currently available PIs) bioavailability, large pill numbers, dosing frequency, dosing schedule with meals, and toxicity. Potential cross-resistance among available PIs is an important issue, and the success of salvage treatment of patients whose treatment with a PI has failed is by no means guaranteed. Development of additional PIs that address some or all of these issues is essential.

**ABT-378.** ABT-378 is a peptidomimetic PI that is 10 times more active than ritonavir in vitro [94] and has in vitro activity against HIV variants that have decreased susceptibility to ritonavir [95, 96]. This agent is extensively metabolized by the P450 3A4 system, and its catabolism is substantially inhibited by ritonavir. When ABT-378 is administered with ritonavir to rats, the AUC is increased 13-fold and the half-life is increased substantially [97]. In HIV-1-seronegative volunteers, ABT-378 given twice daily with as little as 50 mg or 100 mg of ritonavir resulted in trough drug concentrations 20- to 80-fold greater than the IC50 of ABT-378 when tested against wild-type HIV-1 in vitro in the presence of plasma proteins [98]. ABT-378 is >98% protein-bound at protein concentrations in the physiological range [98].

This inhibitor combined with either 100 mg or 200 mg of ritonavir (ABT-378/r) has been studied with lamivudine and stavudine in 100 antiretroviral-naive patients. In an intent-to-treat analysis, 79% of patients had HIV RNA levels <50 copies/mL after 36 weeks of therapy. No patient discontinued therapy because of an adverse event related to the drug [99]. ABT-378, because of its activity against ritonavir-resistant isolates [96], is also being tested as a component of salvage therapy for cases in which virologic failure occurs during treatment with a PI. Patients who had HIV-1 RNA levels >1000 copies/mL after at least 3 months of treatment with a single-PI-including regimen were given the combination of ABT-378/r with nevirapine and a least 1 new nucleoside agent [99]. In an intent-to-treat analysis, 67% of these patients had an HIV RNA level <400 copies/mL at 36 weeks of therapy. Phase III studies of ABT-378/r that involve treatment-naive and PI-experienced patients are ongoing.

**Tipranavir.** Tipranavir (PNU-140690) is the first nonpeptidic HIV PI to reach clinical development. This agent has been given to HIV-1-seronegative patients at doses ranging from 300 mg to 2000 mg as a single dose and 3 times a day. Doses of 900 mg 3 times daily or greater produced trough concentrations >1 μmol, the IC50 for wild-type HIV-1 to PNU-140690 in vitro [100]. This agent has good activity in vitro, is synergistic with NRTIs and NNRTIs, and appears to be active in vitro against HIV-1 variants that have decreased susceptibility to the currently approved PIs [101]. Tipranavir also shows additive to synergistic activity with ritonavir in vitro, even against ritonavir-resistant isolates [102].

Preliminary data from patients who had never received a PI showed single-agent tipranavir activity, with decreases in HIV-1 plasma RNA ranging from 1.0 to 1.5 log10 over an 11-day period, as revealed by an HIV-1 RNA assay with a lower limit of quantification of 400 copies/mL. A dose response was seen, with the highest dose (1500 mg 3 times daily) yielding the greatest activity. No serious adverse events were seen in this early phase I/II study. An analysis after 12 weeks revealed no distinct mutational pattern suggestive of resistance development.

This agent appears to require dosing 3 times a day, and, with the current formulation, the 1500-mg dose requires 10 capsules 3 times daily. Therefore, if tipranavir is active against protease-resistant HIV-1, this agent may be reserved for PI-experienced patients.

Additional PIs are in development. Two promising compounds are BMS-232632 and AG-1776. BMS-232632 is an azapeptide PI with potent in vitro activity, even in the presence of human serum proteins. This drug retains activity in vitro against HIV-1 variants resistant to other PIs [103]. The pharmacokinetics of this compound may also allow for once-daily dosing [104]. AG-1776 is also highly active in vitro. Clinical isolates of HIV-1 from patients whose treatment with regimens that include indinavir, nelfinavir, ritonavir, or saquinavir have failed to retain susceptibility to AG-1776 [105]. Recombinant viral strains with multiple mutations in the protease gene that are highly resistant to currently available PIs were also inhibited in vitro by AG-1776 [105]. Initial pharmacokinetic studies of this compound are under way.

### Adverse Effects

Each of the approved PIs has a relatively distinct side-effect profile, which may limit therapy (table 1). Ritonavir causes nausea, vomiting, diarrhea, and perioral paresthesias. Indinavir increases serum bilirubin levels predominantly by increasing the indirect fraction. Indinavir administration also leads to nephrolithiasis or urinary tract sludging in 5%-15% of treated patients. Saquinavir is associated with gastrointestinal complaints, and nelfinavir causes diarrhea in a substantial proportion of patients, although the diarrhea is commonly self-limited. Amprenavir is also associated with nausea and perioral paresthesias and can cause rash. Frequently, each of these side effects can be managed with supportive therapy or occasionally by the exchange of 1 PI for another.

Recently, however, more pervasive side effects that may be class-specific have been observed. PI therapy is associated with elevated triglyceride levels and, occasionally, increased cholesterol levels. Diabetes has developed in patients treated with PIs, and although this event is uncommon, insulin resistance appears to occur more frequently [106]. Finally, redistribution of fatty tissue from subcutaneous to visceral locations, termed lipodystrophy or fat redistribution syndrome, has been reported to occur in patients receiving PIs [106, 107]. Although this phenomenon occurred before the introduction of PI into antiretroviral therapy, its frequency appears to be greater with these agents.

A specific interaction of PIs with human proteins involved
in lipid metabolism has been hypothesized as a mechanism for this dysregulation [108]. However, clear definition(s) for this syndrome or syndromes are only now being developed, and the contribution of long-term nucleoside analogue therapy to changes in body fat is under investigation [109].

Conclusions
PI therapy for HIV-1–infected patients has radically changed the expectations for antiretroviral therapy. Clinical improvement is now the rule, and the goal of antiretroviral therapy is to attain HIV RNA levels in plasma that are below measurable limits. Currently, 5 PIs are widely available in North America and Europe. These agents are commonly coupled with 2 nucleoside analogues, although alternative combinations are frequently being used. The combination of 2 PIs also frequently results in potenter therapy and, because of pharmacokinetic interactions, may enable the dosing frequency to be reduced and tolerability to increase.

Unfortunately, the plasticity of the HIV genome, coupled with the high failure rate of HIV reverse transcriptase and the potential for high levels of replication, has led to the emergence of HIV-1 variants resistant to PIs. The consequences of resistance to PIs (and other antiretrovirals) are not yet fully explored, but loss of treatment effects and progression of clinical disease have been noted.

Potent antiretroviral regimens are needed for use after inadequate viral suppression or relapsing viral replication during use of PI-including combinations. New PIs in development may have better activity against variants resistant to PIs. The consequences of resistance to PIs (and other antiretrovirals) are not yet fully explored, but loss of treatment effects and progression of clinical disease have been noted.

Finally, the newly recognized side effects of PI therapy, such as hypertriglyceridemia, insulin resistance, and lipodystrophy, must be better characterized. Whether these side effects are common to the class or occur more commonly with 1 PI versus another is not yet clearly known. Despite these caveats, PIs remain one of the backbones of potent antiretroviral therapy.

References


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