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Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients

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Boosted protease inhibitor regimens combine ritonavir with a second, 'boosted' protease inhibitor to enhance patient exposure to the latter agent, thereby preventing or overcoming resistance and allowing less frequent dosing, potentially improving adherence. The advantages offered by ritonavir boosting are primarily attributable to the drug's pharmacokinetic properties. Ritonavir's inhibition of the cytochrome P-450 CYP3A4 enzyme reduces the metabolism of concomitantly administered protease inhibitors and changes their pharmacokinetic parameters, including area under the curve (AUC), maximum concentration (C_{max}), minimum concentration (C_{min}) and half-life ($t_{1/2}$). As a result, the bioavailability of the boosted protease inhibitor is increased and improved penetration into HIV reservoirs may be achieved. Boosted protease inhibitor regimens that utilize a low dose of ritonavir (100–200 mg) appear to offer the best balance of efficacy and tolerability. At this dose, ritonavir boosts the bioavailability of the second protease inhibitor without contributing significantly to the side effect profile of the regimen. In clinical trials, regimens boosted with low dose ritonavir have demonstrated high levels of viral suppression in both antiretroviral naïve patients and patients who previously failed antiretroviral therapy, including protease inhibitor therapy. Side effects observed have generally been similar to those associated with the boosted protease inhibitor. Based upon their enhanced drug exposure and demonstrated efficacy, the boosted ritonavir regimens should be among the first options considered for use in clinical practice.

Keywords: pharmacokinetics, pharmacodynamics, clinical experience, dosing, tolerability

Introduction

Single protease inhibitor (PI)-based regimens have dramatically impacted HIV-related morbidity and mortality, contributing to a reduction in AIDS-related deaths of 47% in the United States following their introduction into clinical practice.[1] However, the limited bioavailability and lack of adherence because of the frequency of dosing and tolerability issues may lead to the development of resistant virus and virological failure among patients treated with single PI regimens. A population-based study at an urban clinic reported that 37% of PI-naïve patients had vRNA < 500 copies/mL after 1 year of treatment with single PI-based regimens (despite reports of higher rates of suppression in clinical trials).[2]

HIV treatment that includes a ritonavir-boosted PI regimen offers advantages over traditional single PI-based regimens. Ritonavir-boosted regimens combine low-dose ritonavir with a second PI, as well as two or more nucleoside reverse transcriptase inhibitors (NRTIs), to achieve higher sustained levels of the second PI. These regimens are utilized to prevent or overcome resistance and allow less frequent dosing, potentially improving adherence. Advantages offered by these regimens are attributable to the pharmacokinetic properties of ritonavir, and its effect on the second, 'boosted' PI. Concomitant administration with ritonavir increases the bioavailability and cellular penetration of the second PI despite reduced doses and less frequent administration.[3,4] The dose of ritonavir administered in boosted PI regimens is generally considered subtherapeutic (100–200 mg).[5] Plasma peak and trough levels of the second PI in boosted PI regimens generally exceed levels achieved when the agent is given alone because the second PI is cleared from the body more slowly. The antiretroviral activity of the second PI is, consequently, enhanced.

Regimens that combine ritonavir with saquinavir, indinavir, lopinavir or amprenavir have demonstrated efficacy in clinical studies of newly infected, treatment-naïve patients as well as patients who have failed treatment with one or more antiretroviral regimens.[4–7] In addition, genotype and phenotype assays can now identify patients, whether treatment naïve or previously exposed to antiretroviral therapy, infected with HIV resistant to single PI-based regimens. Thus, when PIs are prescribed, whether as first line or salvage therapy, they are commonly prescribed as a boosted regimen.

Rationale for boosted PI regimens: pharmacokinetics and pharmacodynamics

Inhibition of cytochrome P-450 (CYP-450) metabolic pathways by ritonavir forms the basis for its enhancement of concomitantly administered PIs. In particular, ritonavir is a potent inhibitor of the

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CYP3A4 isozyme, the primary enzyme involved in the metabolism of most PIs, and to a lesser extent CYP2C19, which is important for nelfinavir's metabolism and the metabolism of its active metabolite, M8.[3,6] CYP3A4 is present in the intestinal tract and liver where it plays a key role in PI first-pass metabolism. Ritonavir can also inhibit CYP3A4 in areas of the body outside the intestinal tract and liver. Ritonavir's inhibition of CYP3A4 reduces the metabolism of concomitantly administered PIs and changes their pharmacokinetic parameters, including area under the curve (AUC), maximum concentration (C_{max}), minimum concentration (C_{min}) and half-life ($t_{1/2}$) of the second PI.

The pharmacokinetics of the PIs vary, as does the effect ritonavir has on their pharmacokinetics. Saquinavir is removed by first-pass metabolism in the intestine, which limits its bioavailability (Table 1).[6] Ritonavir improves saquinavir's effectiveness by inhibiting first-pass intestinal metabolism and increasing AUC, C_{min} and C_{max} (Table 2).[5,6,8] Indinavir has relatively good bioavailability, but has a comparatively short $t_{1/2}$ (Table 1). Ritonavir improves indinavir's effectiveness primarily by inhibiting hepatic metabolism and decreasing systemic clearance.[5,6] This leads to larger increases in C_{min} than AUC, while having less effect on C_{max} (Table 2).[3–6] Trough indinavir levels are maintained above the concentration necessary to inhibit 95% of viral growth seen in the absence of drug.[9] The effect of ritonavir on nelfinavir pharmacokinetics is smaller than other PIs (Table 2), as nelfinavir is metabolized by several CYP-450 enzymes and has relatively good bioavailability.[4,10] Larger increases are observed in the AUC, C_{min} and C_{max} of nelfinavir's M8 metabolite, but the increases are generally no larger than one-fold (Table 2). Lopinavir is only available in combination with ritonavir and, like saquinavir, benefits from ritonavir's inhibition of first-pass intestinal metabolism. Ritonavir's effect on amprenavir appears to be similar to its effect on indinavir, with inhibition of hepatic metabolism leading to larger increases in C_{min} than AUC (Table 2).[3–5,11] Atazanavir was approved for use in June 2003, and is also associated with a larger percentage increase in C_{min} than AUC with ritonavir boosting (Table 2). The PI, tipranavir, is under investigation and not yet approved for use.

Another possible component of ritonavir boosting involves cellular transport via the P-glycoprotein and multidrug resistance-associated protein (MRP1 and MRP2) efflux channels in the membrane of epithelial cells in the intestinal tract, as well as cells lining the liver and kidneys.[12–14] These channels are believed to be involved in the active transport of PIs out of cells and high levels of their expression, which may be found in patients treated for HIV infection, may reduce drug absorption from the intestinal tract and enhance drug elimination in bile and urine.[3,5,12–14] P-glycoprotein and MRP channels in endothelial cells of the blood–brain barrier may also prevent transport of PIs into the central nervous system.[12,13,15] Although evidence has been presented suggesting that ritonavir inhibits the functional activity of P-glycoprotein and MRP channels[3,15–17] allowing a second PI to pass through cellular boundaries, one *in vitro* study found that concomitantly administered PIs did not inhibit P-glycoprotein mediated efflux.[18] Thus, whereas ritonavir inhibition of efflux channels combined with availability of higher levels of the second PI in the blood appears to facilitate penetration into HIV sanctuaries (such as the brain, cerebrospinal fluid and testis), more evidence is needed to support this benefit of ritonavir boosting.[3,5,12,14]

Although less well established, it has been suggested that concomitant ritonavir administration may also boost the unbound fraction (the only therapeutically active form of a PI) of the second PI in the systemic circulation.[3,6] Protein binding of the individual PIs varies, with ritonavir one of the most highly protein bound at 98–99%, indinavir the lowest at approximately 60%, whereas the other PIs are intermediate (86–99%).[3,6] Ritonavir may boost levels of the second PI through saturation of protein binding sites or through competition with the second PI for protein binding sites if present at significant levels, thus increasing levels of the unbound fraction of the second PI.[3,5,6] Additional research is needed in this area to confirm that ritonavir boosting impacts the level of unbound drug and the mechanism behind this effect.[19]

Once a PI is available in the systemic circulation and reaches an infected CD4 cell, the quantity of the PI that enters and remains in the cell determines its ability to suppress HIV replication. There is variability between the different PIs in their level of intracellular accumulation and intracellular $t_{1/2}$, which, as mentioned above, appears to correspond to the degree of P-glycoprotein and MRP channel expression present in the cell membrane.[13,20,21] Limited data indicate that ritonavir inhibits these channels on CD4 cells allowing greater intracellular accumulation of the second PI, but more conclusive evidence is needed to define the role these channels play in virological failure of PI regimens and the exact benefit provided by concomitant administration of ritonavir.[3,5,12,22–24]

In summary, ritonavir's inhibition of CYP3A4 is the most clearly defined of its mechanisms for improving the efficacy of the second PI in a boosted PI regimen. The resultant increases in AUC and C_{min} of the second PI have therapeutic implications related to increased bioavailability. A potentially lower proportion of patients receive subtherapeutic dosing, thus the level of HIV suppression is increased in the clinical setting. Effects on efflux channels and protein binding require further research to more accurately define their role in PI boosting.

Clinical experience

Saquinavir was the first licensed PI (1995), after a randomized clinical trial demonstrated significant increases in CD4 count and reductions in vRNA levels when the drug was added to a regimen of two NRTIs.[25] Since then, single PI-based regimens have demonstrated the ability to suppress vRNA below the level of detection in most PI naïve patients.[1] Saquinavir and indinavir were first studied in combination twice a day regimens with ritonavir in patients who failed one or more single PI-based regimens and required salvage therapy.[4–7] Lopinavir/ritonavir was coformulated and approved as a twice a day combination regimen. Amprenavir labelling includes recommendations for twice a day and once daily dosing when used with ritonavir. Atazanavir is approved for daily dosing, while improved bioavailability at a lower dose is attainable with the addition of ritonavir.

Initial studies of dual PI combinations used a 400 mg dose of ritonavir with either saquinavir 600 mg or indinavir 400 mg plus two NRTIs.[6] Saquinavir/ritonavir 600/400 mg provided greater efficacy than ritonavir 600 mg as a single PI (68% versus 40% vRNA < 200 copies/mL), when both were given twice a day with two NRTIs in PI naïve patients over 48 weeks.[26] Saquinavir/ritonavir 400/400 mg twice a day plus one NRTI was less efficacious than indinavir 800 mg three times daily plus two NRTIs in a 48-week study of PI-naïve patients (43% versus 63% vRNA < 400 copies/mL).[27] Saquinavir/ritonavir 600/400 mg provided modest efficacy (0.2 $\log_{10}$ reduction in median plasma vRNA) in a study of prior indinavir-treated patients,[7] but produced greater viral suppression in patients previously treated with nelfinavir.[6,7] Indinavir/ritonavir 400/400 mg plus two NRTIs demonstrated efficacy in antiretroviral-naïve patients over

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Table 1. Protease inhibitor pharmacokinetic and pharmacological characteristics

Characteristics of single PI regimens	Saquinavir 1200 mg three times daily	Ritonavir 600 mg twice daily	Indinavir 800 mg three times daily	Nelfinavir 1250 mg twice daily	Lopinavir 400 mg (fixed combination with ritonavir 100 mg) twice daily	Amprenavir 1200 mg twice daily	Atazanavir 400 mg once daily
Plasma $t_{1/2}$ (h)	1.5–2	3–5	1.8	3.5–5	5–6	7.1–10.6	7.9
C_{min} (μg/mL)	0.16	3.7	0.15	1.4	5.5	0.32	0.22
C_{max} (μg/mL)	0.2	11.2	7.7	3.0	9.6	7.7	5.4
Protein binding (%)	97	98–99	60	>98	98–99	90	86
Oral bioavailability (%)	4 (hard gel capsule); not determined (soft gel capsule)	66–75	60–65	20–80	NR	NR	NR
Food effect (change in AUC)	increase six- to seven-fold	increase 13%	decrease 77%	increase two- to three-fold	moderate fat meal increases capsules 48% and solution 80%	high fat meal decreases 21%	light meal increases 70%, high fat meal increases 35%

Data obtained from the manufacturers' package inserts and refs 3 and 5–7. AUC, area under the curve; NR, not reported.

72 weeks with suppression of vRNA < 500 copies/mL (100% completers and 63% non-completers equal failures) and <80 copies/mL (95% completers and 60% non-completers equal failures).[28] The 400 mg dose of ritonavir used in these studies may act as a fourth active agent (providing what is better considered dual PI therapy) as well as boosting the second PI but has been associated with an increased incidence of side effects.

There are a limited number of studies that compare single PI-based regimens with regimens that include lower ritonavir doses used solely to boost the second PI. Indinavir/ritonavir 800/100 mg twice a day demonstrated similar efficacy to indinavir 800 mg three times daily, both with two NRTIs, in a study of PI-naïve patients, as well as in a study of patients responsive to treatment with a conventional indinavir 800 mg three times daily regimen.[4] Lopinavir/ritonavir twice a day demonstrated greater efficacy over 48 weeks than nelfinavir (vRNA < 400 copies/mL: 75% and 63%, respectively, and vRNA < 50 copies/mL: 67% and 52%, respectively), both combined with two NRTIs in antiretroviral naïve patients.[29] Lopinavir/ritonavir twice a day demonstrated greater efficacy over 24 weeks than atazanavir 400 mg daily (vRNA < 400 copies/mL: 75% and 54%, respectively, and vRNA < 50 copies/mL: 50% and 34%, respectively), both combined with two NRTIs in patients who failed only one prior PI-containing regimen.[30]

The lower doses of ritonavir used for boosting were also evaluated in combination with indinavir or lopinavir in open-label studies of antiretroviral naïve patients. A study of indinavir/ritonavir 800/100 mg plus two NRTIs produced viral suppression over 48 weeks (vRNA < 400 copies/mL: 95% completers, 45% non-completers equal failures and <50 copies/mL: 88% completers, 42% non-completers equal failures).[31] Likewise, lopinavir/ritonavir 200–400/100–200 mg achieved viral suppression over 48 weeks (85% vRNA < 400 copies/mL and 78% <50 copies/mL, both non-completers equal failures).[32]

More extensive clinical experience is available with the boosted PI regimens in salvage therapy use. An indinavir/ritonavir 800/200 mg plus two NRTIs study reported vRNA reductions over 24 weeks (vRNA < 400 copies/mL: 76% completers, 56% non-completers equal failures and <50 copies/mL: 50% completers, 37% non-completers equal failures) in patients who previously failed a saquinavir, indinavir, or nelfinavir-based regimen.[33] Lopinavir/ritonavir has also demonstrated efficacy following 12 months of prior PI therapy (48% vRNA < 500 copies/mL and 39% <50 copies/mL, non-completers equal failures).[34] One small, 24-week amprenavir salvage therapy study reported reductions in vRNA < 200 copies/mL among 9/17 (53%) patients.[35]

Three direct comparator studies of boosted PI regimens have been reported in the literature.[36–38] MaxCmin 1 included primarily patients (approximately 75%) who had previously received antiretroviral therapy.[36] This 48-week, 306 patient study compared saquinavir/ritonavir 1000/100 mg to indinavir/ritonavir 800/100 mg twice a day plus two or more NRTIs/NNRTIs and found that 94% versus 90% of patients (P = NS), respectively, in the completers analysis and 68% versus 53% (P = 0.014) of patients, respectively, in the non-completers as failures analysis achieved vRNA < 400 copies/mL. The difference in the non-completers analysis appeared to be primarily the result of a higher percentage of indinavir patients switching therapy. Results of the 48-week MaxCmin 2 study of 326 patients (50% PI naïve) treated with saquinavir/ritonavir 1000/100 mg or lopinavir/ritonavir 400/100 mg twice a day plus two or more NRTIs/NNRTIs found that 75% versus 70% (P = NS) of patients, respectively, in the completers analysis and 52% versus 60% (P = NS) of patients, respectively, in the non-completers as failures analysis

Table 2. Percentage change in AUC, C_{max} and C_{min} of the boosted PIs in ritonavir-boosted PI regimens

Regimen	Percentage change in pharmacokinetic characteristic		
	AUC	C_{max}	C_{min}
Saquinavir (soft gel capsules) plus ritonavir 800/200 mg twice daily versus saquinavir 800 mg twice daily[45]	1622%	753%	1875%
Saquinavir (hard gel capsules) plus ritonavir (400 or 600 mg/400 or 600 mg twice daily) versus saquinavir 600 mg three times daily	~1700%	~1400%	N/A
Indinavir plus ritonavir 800/100 mg twice daily versus indinavir 800 mg every 8 h (fasting state)[46]	LF meal 169%; HF meal 126%	LF meal 60%; HF meal 32%	LF meal 999%; HF meal 980%
Indinavir plus ritonavir 800/200 mg twice daily versus indinavir 800 mg every 8 h (fasting state)[46]	LF meal 254%; HF meal 229%	LF meal 77%; HF meal 59%	LF meal 2356%; HF meal 2483%
Nelfinavir plus ritonavir 1250/100–200 mg twice daily versus nelfinavir 1250 mg twice daily[10]	morning 20%; evening 39%	morning 13%; evening 25%	morning 29%; evening 91%
(Nelfinavir metabolite M8) nelfinavir plus ritonavir 1250/100–200 mg twice daily versus nelfinavir 1250 mg twice daily[10]	morning 74%; evening 86%	morning 55%; evening 68%	morning 108%; evening 132%
Amprenavir plus ritonavir 600/100 mg twice daily versus amprenavir 1200 mg twice daily[47]	38%	–21%	253%
Atazanavir[a] plus ritonavir 300/100 mg once daily versus atazanavir 400 mg once daily[31]	103%	18%	671%

Data obtained from the manufacturer's package inserts (saquinavir hard gel capsules) and references cited above. AUC, area under the curve; C_{max}, maximum concentration; C_{min}, minimum concentration; N/A, not available; LF, low fat; HF, high fat. Table includes data from studies with boosted PI regimens (low dose ritonavir, 100 or 200 mg) and single PI comparator arms analysed using geometric means. Lopinavir is not included in the table as it is only available in combination with ritonavir.
[a]Atazanavir is only approved for once daily dosing.

achieved vRNA < 50 copies/mL.[37] More saquinavir (29%) than lopinavir (13%) ($P = 0.001$) patients switched therapy. The third study compared treatment with atazanavir/ritonavir 300/100 mg once daily, atazanavir/saquinavir 400/1200 mg once daily, or lopinavir/ritonavir 400/100 mg twice daily, each administered with tenofivir and one NRTI in patients who failed multiple antiretroviral regimens.[38] Preliminary results from this 16-week, 358 patient trial demonstrated that similar percentages of patients in the atazanavir/ritonavir (64%) and lopinavir/ritonavir (65%) groups, but a lower percentage of patients in the atazanavir/saquinavir (48%) group achieved vRNA < 400 copies/mL (*P* value not provided).

Dosing and tolerability

Dosing requirements, including multiple daily dosing and food and drug interactions, as well as side effects are major impediments to single PI-based therapy. Boosted PI regimens offer the convenience of less frequent dosing. In addition, concomitant ritonavir greatly reduces food effects of the second PI, allowing dosing regardless of meals and potentially increasing adherence to treatment. To minimize the potential for ritonavir-associated drug interactions and side effects, lower boosting doses of ritonavir (100–200 mg) are preferred. Optimal dosing of the boosted PI continues to be evaluated as well. For example, the BEST and MaxCmin 1 studies using indinavir/ritonavir 800/100 mg and the work of French researchers studying indinavir/ritonavir 400/100 mg twice daily suggest that lower indinavir doses may provide pharmacokinetic and tolerability advantages and comparable efficacy to a regimen including indinavir 800 mg three times daily.[36,39–41] Indinavir, saquinavir and other PIs are also being studied as once daily regimens in combination with ritonavir. Amprenavir/ritonavir 1200/200 mg was approved for use once daily. Atazanavir 400 mg was approved for use as a once daily, single PI regimen and has demonstrated potentially greater efficacy as a once daily combination regimen with ritonavir 100 mg.[30,38]

The type of side effects encountered with boosted PI regimens are generally similar to those associated with PI regimens that include just the second PI. The addition of ritonavir has the potential to increase the incidence of side effects, in a dose-dependent manner.[5] Side effects associated with the entire PI class include nausea, vomiting, glucose intolerance, elevated lipids, and fat redistribution, while additional side effects are associated with individual PIs. Lipodystrophy is a class effect that requires monitoring and possible treatment. Among the PIs, ritonavir has the greatest association with lipodystrophy, particularly hypertriglyceridaemia.[42–44] Diarrhoea is associated with the use of the PIs, although less commonly with indinavir.[5] Fluid requirements should be adhered to with indinavir to help prevent nephrolithiasis.[4,5]

Clinical studies have demonstrated effective HIV suppression with twice a day ritonavir-boosted PI regimens in both treatment naïve patients and in patients who have failed prior antiretroviral

therapy including single PI regimens. In addition, these regimens improve dosing convenience relative to frequency and timing of dosing around meals. Tolerability remains a limiting factor in treatment adherence that appears related to the dose of ritonavir. Lower doses of twice a day regimens and once-daily dosing regimens are being studied and appear to be the next step in expanding patient treatment options. It is yet to be seen if these regimens will provide comparable or greater HIV suppression than the presently used twice daily regimens.

Conclusion

The primary role of ritonavir in boosted PI regimens is to improve the pharmacokinetics of the second PI. Ritonavir's interaction with CYP3A4 makes twice daily dosing possible as the second PI has improved bioavailability. Boosted PI regimens have demonstrated high levels of viral suppression among both antiretroviral naïve and prior PI-treated patients. As a result, these regimens should be among the first options considered for use in clinical practice.

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References

1. National Institute of Allergy and Infectious Disease (NIAID). (2000). HIV infection and AIDS, an overview. NIAID Fact Sheet. [Online.] http://www.aegis.com/factshts/niaid/2000/niaid2000_fact_sheet_hivinf.html (1 April 2002, date last accessed).

2. Lucas, G. M., Chaisson, R. E. & Moore, R. D. (1999). Highly active antiretroviral therapy in a large urban clinic: risk factors for virological failure and adverse drug reactions. *Annals of Internal Medicine* **131**, 81–7.

3. Acosta, E. P. (2002). Pharmacokinetic enhancement of protease inhibitors. *Journal of Acquired Immune Deficiency Syndromes* **29**, S11–8.

4. Rathbun, R. C. & Rossi, D. R. (2002). Low-dose ritonavir for protease inhibitor pharmacokinetic enhancement. *Annals of Pharmacotherapy* **36**, 702–6.

5. Moyle, G. J. & Back, D. (2001). Principles and practice of HIV-protease inhibitor pharmacoenhancement. *HIV Medicine* **2**, 105–13.

6. Flexner, C. (2000). Dual protease inhibitor therapy in HIV-infected patients: pharmacological rationale and clinical benefit. *Annual Review of Pharmacology and Toxicology* **40**, 649–74.

7. Yu, K. & Daar, E. S. (2000). Dual protease inhibitor therapy in the management of the HIV-1. *Expert Opinion on Pharmacotherapy* **1**, 1331–42.

8. Kilby, J. M., Hill, A. & Buss, N. (2002). The effect of ritonavir on saquinavir plasma concentration is independent of ritonavir dosage: combined analysis of pharmacokinetic data from 97 subjects. *HIV Medicine* **3**, 97–104.

9. Condra, J. H., Petropoulos, C. J., Ziermann, R. *et al.* (2000). Drug resistance and predicted virological responses to human immunodeficiency virus type I protease inhibitor therapy. *Journal of Infectious Diseases* **182**, 758–65.

10. Kurowski, M., Kaeser, B., Sawyer, A. *et al.* (2002). Low-dose ritonavir moderately enhances nelfinavir exposure. *Clinical Pharmacology and Therapeutics* **72**, 123–32.

11. Sadler, B. M., Piliero, P. J., Preston, S. L. *et al.* (2001). Pharmacokinetics and safety of amprenavir and ritonavir following multi-dose, co-administration to healthy volunteers. *AIDS* **15**, 1009–18.

12. Fromm, M. F. (2000). P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *International Journal of Clinical Pharmacology and Therapeutics* **38**, 69–74.

13. Jones, K., Hoggard, P. G., Sales, S. D. *et al.* (2001). Differences in the intracellular accumulation of HIV protease inhibitors *in vitro* and the effect of active transport. *AIDS* **15**, 675–81.

14. Huisman, M. T., Smit, J. W., Crommentuyn, K. M. *et al.* (2002). Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. *AIDS* **16**, 2295–301.

15. Olson, D. P., Scadden, D. T., D'Aquilla, R. T. *et al.* (2002). The protease inhibitor ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1). *AIDS* **16**, 1743–7.

16. Drewe, J., Gutmann, H., Fricker, G. *et al.* (1999). HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporin analog SDZ PSC 833. *Biochemical Pharmacology* **57**, 1147–52.

17. Gutmann, H., Fricker, G., Drewe, J. *et al.* (1999). Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. *Molecular Pharmacology* **56**, 383–9.

18. Van der Sandt, I. C., Vos, C. M., Nabulsi, L. *et al.* (2001). Assessment of active transport of HIV protease inhibitors in various cell lines and the *in vitro* blood-brain barrier. *AIDS* **15**, 483–91.

19. Boffito, M., Hoggard, P. G., Reynolds, H. E. *et al.* (2002). The unbound percentage of saquinavir and indinavir remains constant throughout the dosing interval in HIV positive subjects. *British Journal of Clinical Pharmacology* **54**, 262–8.

20. Meaden, E. R., Hoggard, P. G., Newton, P. *et al.* (2002). P-glycoprotein and MRP1 expression and reduced ritonavir and saquinavir accumulation in HIV-infected individuals. *Journal of Antimicrobial Chemotherapy* **50**, 583–8.

21. Jones, K., Bray, P. G., Khoo, S. H. *et al.* (2001). P-glycoprotein and transporter MRP1 reduce HIV protease inhibitor uptake in CD4 cells: potential for viral drug resistance. *AIDS* **15**, 1353–8.

22. Lucia, M. B., Rutella, S., Leone, G. *et al.* (2001). HIV-protease inhibitors contribute to P-glycoprotein efflux function defect in peripheral blood lymphocytes from HIV-positive patients receiving HAART. *Journal of Acquired Immune Deficiency Syndromes* **27**, 321–30.

23. Chaillou, S., Durant, J., Garraffo, R. *et al.* (2002). Intracellular concentration of protease inhibitors in HIV-1-infected patients: correlation with MDR-1 gene expression and low dose ritonavir. *HIV Clinical Trials* **3**, 493–501.

24. Bossi, P., Legrand, O., Faussat, A. M. *et al.* (2003). P-glycoprotein in blood CD4 cells of HIV-1-infected patients treated with protease inhibitors. *HIV Medicine* **4**, 67–71.

25. Collier, A. C., Coombs, R. W., Schoenfield, D. A. *et al.* (1996). Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *New England Journal of Medicine* **334**, 1011–7.

26. Michelet, C., Ruffault, A., Sebille, V. *et al.* (2001). Ritonavir-saquinavir dual protease inhibitor compared to ritonavir alone in human immunodeficiency virus-infected patients. *Antimicrobial Agents and Chemotherapy* **45**, 3390–402.

27. Florence, E., Dreezen, C., Desmet, P. *et al.* (2001). Ritonavir/saquinavir plus one nucleoside reverse transcriptase inhibitor (NRTI) versus indinavir plus two NRTIs in protease inhibitor-naïve HIV-1-infected adults (IRIS study). *Antiviral Therapy* **6**, 255–62.

28. Lichterfeld, M., Nischalke, H. D., Bergmann, F. *et al.* (2002). Long-term efficacy and safety of ritonavir/indinavir 400/400 mg twice daily in combination with two nucleoside reverse transcriptase inhibitors as first line antiretroviral therapy. *HIV Medicine* **3**, 37–43.

29. Walmsley, S., Bernstein, B., King, M. *et al.* (2002). Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection. *New England Journal of Medicine* **346**, 2039–46.

30. Bristol-Myers Squibb. (2003). Reyataz (atazanavir) Bristol-Myers Squibb New Drug Application NDA 21–567. [Online.] Product Label Posted July 8, 2003 at http://www.fda.gov/cder/approval/index.htm, 12–3 (25 September 2003, date last accessed).

31. Young, B., Fischl, M. A., Wilson, H. M. *et al.* (2002). Open-label study of a twice-daily indinavir 800-mg/ritonavir 100-mg regimen in pro-

tease inhibitor-naïve HIV-infected adults. *Journal of Acquired Immune Deficiency Syndromes* **31**, 478–2.

32. Murphy, R. L., Brun, S., Hicks, C. *et al.* (2001). ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral-naïve adults with HIV-1 infection: 48 week results. *AIDS* **15**, F1–9.

33. Katner, H. P., Paar, D. P., Nadler, J. P. *et al.* (2002). Open-label study of a twice-daily indinavir 800-mg/ritonavir 200-mg regimen in HIV-infected adults failing a protease inhibitor regimen. *Journal of Acquired Immune Deficiency Syndromes* **31**, 483–7.

34. Gilleece, Y. C., Qazi, N. A., Morlese, J. F. *et al.* (2003). The efficacy of lopinavir in individuals experiencing protease inhibitor failure. *Journal of Acquired Immune Deficiency Syndromes* **32**, 238–42.

35. Arvieux, C., Tattevin, P., Souala, F. M. *et al.* (2002). Salvage therapy with amprenavir and ritonavir: prospective study in 17 heavily pretreated patients. *HIV Clinical Trials* **3**, 125–32.

36. Plosker, G. L. & Scott, L. J. (2003). Saquinavir: a review of its use in boosted regimens for treating HIV infection. *Drugs* **63**, 1299–324.

37. Youle, M., Gerstoft, J., Fox, M. *et al.* (2003). The final week 48 analysis of a phase IV, randomised, open-label, multi-centre trial to evaluate safety and efficacy of lopinavir/ritonavir(400/100 mg twice a day) versus saquinavir/ritonavir (1000/100 mg twice a day). In *Second IAS Conference on HIV Pathogenesis and Treatment, Paris, France, 2003*. Late Breaker Abstract LB23. International Medical Press, London, UK.

38. Badaro, R., DeJesus, E., Lazzarin, A. *et al.* (2003). Efficacy and safety of atazanavir (ATV) with ritonavir (RTV) or saquinavir (SQV) versus lopinavir/ritonavir (LPV/RTV) in combination with tenofovir (TFV) and one NRTI in patients who have experienced virologic failure to multiple HAART regimens: 16-week results from BMS AI424–045. In *Abstracts of the Second IAS Conference on HIV Pathogenesis and Treatment*, *Paris*, *France, 2003.* Abstract 118. *Antiviral Therapy* **8**, *Suppl. 1*, S212.

39. Ghosn, J., Lamotte, C., Ait-Mohand, H. *et al.* (2003). Efficacy of a twice-daily antiretroviral regimen containing 100 mg ritonavir/400 mg indinavir in HIV-infected patients. *AIDS* **17**, 209–14.

40. Arnaiz, J. A., Mallolas, J., Podzamczer, D. *et al.* (2003). Continued indinavir versus switching to indinavir/ritonavir in HIV-infected patients with suppressed viral load. *AIDS* **17**, 831–40.

41. Bani-Sadr, F., Perre, P., Peytavin, G. *et al.* (2001). Indinavir-ritonavir combination: pharmacologic results and tolerance in patients infected by HIV. *Presse Medicale* **30**, 731–5.

42. Saves, M., Raffi, F., Capeau, J. *et al.* (2002). Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. *Clinical Infectious Diseases* **34**, 1396–405.

43. Heath, K. V., Hogg, R. S., Singer, J. *et al.* (2002). Antiretroviral treatment patterns and incident HIV-associated morphologic and lipid abnormalities in a population-based cohort. *Journal of Acquired Immune Deficiency Syndromes* **30**, 440–7.

44. Tsiodras, S., Mantzoros, C., Hammer, S. *et al.* (2000). Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. *Archives of Internal Medicine* **160**, 2050–6.

45. Buss, N., Snell, P., Bock, J. *et al.* (2001). Saquinavir and ritonavir pharmacokinetics following combined ritonavir and saquinavir (soft gelatin capsules) administration. *British Journal of Clinical Pharmacology* **52**, 255–64.

46. Saah, A. J., Winchell, G. A., Nessly, M. L. *et al.* (2001). Pharmacokinetic profile and tolerability of indinavir-ritonavir combinations in healthy volunteers. *Antimicrobial Agents and Chemotherapy* **45**, 2710–15.

47. Sale, M., Sadler, B. M. & Stein, D. S. (2002). Pharmacokinetic modeling and simulations of interaction of amprenavir and ritonavir. *Antimicrobial Agents and Chemotherapy* **46**, 746–54.