IVC. PROTEASE INHIBITORS

i. Nelfinavir mesylate / Viracept® (Agouron Pharmaceuticals)

by Mark Harrington

BACKGROUND

Nelfinavir mesylate, an inhibitor of the HIV protease enzyme, was the first protease inhibitor simultaneously approved for adults and children with HIV.

Indication. The Viracept® labeling indication is broad and vague, as has become typical for an antiretroviral drug licensed through accelerated approval: "Viracept is indicated for the treatment of HIV infection when antiretroviral therapy is warranted. This indication is based on surrogate marker changes in patients who received Viracept in combination with nucleoside analogues or alone for up to 24 weeks. At present, there are no results from controlled trials evaluating the effect of therapy with Viracept on clinical progression of HIV infection, such as survival or the incidence of opportunistic infections." (Agouron 1997). For adults the recommended dose is 750 milligrams (mg) taken three times daily (TID). For children over two years old the dose is 20-30 mg/kg, not to exceed 750 mg.

About the sponsor. Agouron Pharmaceuticals was founded in 1984. In 1987 it was awarded an NIH grant to determine the structures of HIV proteins. It began working with Eli Lilly in 1988 on drug discovery. By 1989, investigators had resolved the structure of the HIV protease complexed with (bound to) lead compound protease inhibitors. First named AG-1343 and identified as a development candidate in 1993, nelfinavir mesylate was licensed by Lilly to Agouron (Appelt 1993, Babine 1995), which initiated clinical trials in 1994 with funding from Japan Tobacco, Inc. (JTI) (Agouron 1996). Agouron filed for approval on Christmas Eve, 1996, and the drug received accelerated approval from the US Food & Drug Administration (FDA) on 14 March 1997. Its development time was just 38 months -- the quickest yet for an HIV protease inhibitor. Viracept® is Agouron's first FDA-approved drug. Other compounds in the pipeline target protease enzymes of cytomegalovirus (CMV), hepatitis C virus (HCV) and rhinovirus.

Agouron company has a partnership with Roche to market Viracept in Europe. The European Commission's European Medical Evaluation Agency (EMEA) approved nelfinavir on January 22, 1998, but the drug is not yet available in Italy, Portugal and Spain due to pricing and reimbursement negotiations between the relevant national health authorities and Hoffmann-LaRoche (Hollander 1998).

Mechanism of activity. Protease is an enzyme which enables HIV to cleave its proteins from the *gag-pol* polyprotein-- a long precursor protein chain -- into smaller functional units essential to HIV infectivity. All inhibitors of HIV protease block the action of this enzyme through binding within the active proteolytic (protein-cleaving) site. If you imagine the protease enzyme as a pair of hands joined at the wrists whose fingers open and shut like crab's claws, you can envision a protease inhibitor as a long protein which jams the claws and prevents them from slicing into their prey -- the *gag-pol* polyprotein -- rendering the virions non-infectious. Protease inhibitors are bigger molecules than the nucleoside

analogue or non-nucleoside reverse transcriptase inhibitors (RTIs), which means they must be taken in larger amounts to be active inside the body.

ANTIRETROVIRAL POTENCY

Test-tube studies. *In vitro*, nelfinavir is active against both laboratory and clinical (wild-type) HIV-1 strains and against the HIV-2 strain ROD. Its 95% effective concentration (EC_{95}) ranges from 7-196 nanomolars (nM). *In vitro* it is synergistic with the nucleoside analogue reverse transcriptase inhibitors (NRTIs) AZT, 3TC and ddC, and additive with ddI and d4T. Test tube interactions with other protease inhibitors were more variable, ranging from antagonistic to synergistic. These may not be relevant *in vivo*, since test tube cultures lack the liver cytochrome P450 system through which all protease inhibitors (as well as the non-nucleoside RTIs delavirdine, nevirapine, and efavirenz) are metabolized, leading in some cases to *in vivo* synergy or antagonism which would not be predicted *in vitro*.

Clinical trials. Nelfinavir has been studied clinically in over 1,500 individuals. The three pivotal studies enrolled 696 individuals. The pediatric trial enrolled 38 children aged two to thirteen.

Monotherapy. In a study of thirty people who received either 500, 750, or 1000 mg of nelfinavir monotherapy TID after a two-week antiretroviral washout period, mean HIV RNA reductions were 75%, 94% and 97% (0.6 1.2 and 1.5 logs) respectively after sixteen weeks of therapy (Agouron 1996a); these results were described in Vancouver as reductions of 1.4, 1.9 and 1.7 logs using the cutoff of 500 RNA copies/ml (bDNA), and 1.5, 2.4 and 2.3 logs using the cutoff of 100 RNA copies. 20% on 500 mg TID and 50-60% on 750 and 1000 mg TID fell below the limit of detection. "The most frequently reported adverse events were loose stool and mild to moderate diarrhea" (Conant 1996). Agouron study 505 randomized 97 (or 91; the Retrovirus Conference abstract says 97, the FDA package insert 91) HIV-infected individuals to receive either 750 or 500 mg nelfinavir TID (one third of the participants received placebo for the first four weeks). Of these, 37 were antiretroviral naive and 57 were experienced. The median baseline CD4 was 264 and HIV RNA was 5 logs (Powderly 1997).

Nelfinavir's lucky debut. Nelfinavir first made its dramatic debut in the collective consciousness of the global AIDS research elite on July 11, 1996, when it was lucky enough to have been included in the triple-combination study at Aaron Diamond AIDS Research Center in which David Ho for the first time showed that viral levels in eleven individuals which were undetectable using a lower threshold of detection of 400 copies/ml were also undetectable using an even lower threshold, measured with a new, super-sensitive bDNA assay, of 25 HIV RNA copies/ml. The trial, Agouron 509, enrolled twelve chronically-infected, treatment-naive individuals with baseline CD4 counts of 245 (range 26-501) and viral load of 56,000/ml (range 14,000-618,000). One patient was lost to follow-up. Within two weeks, viral levels dropped 99%, and descended further towards 400 copies/ml over time. CD4 counts rose by an average of 100 cells. At eight weeks, all eleven participants still on study had plasma RNA below 400 copies/ml, and HIV could not be cultured from up to 10 million peripheral blood mononuclear cells (Ho 1996, Markowitz 1996).

In Chicago during February 1998, Dr. Markowitz presented an update on the twelve individuals in this study. All twelve subjects achieved undetectable RNA levels (<500 copies) by week 12. Subsequently, three of 12 (25%) rebounded virologically at months 7, 15 and 17, as defined by two consecutive RNA

measurements above the limit of detection. All three nelfinavir failures had the RT M184V mutation characteristic of 3TC resistance; two had the protease D30N mutation and the third had the protease L90M mutation. In several other instances, there were "isolated, non-sustained increases in HIV RNA of persistent responders." There were no mutations found in this group's viral isolates, suggesting that the transiently detectable viral load was a burst of viral expression from latently-infected CD4 cells which were carrying archival, pre-treatment, wild-type, drug-susceptible virus. Of the three failures, one switched to d4T/ddI/ritonavir/saquinavir and one to d4T/ddI/ritonavir/saquinavir/delavirdine. Their viral loads at the time of the switch were 4,232 adn 2,330 respectively. One subject did not return to the Diamond Center for follow-up, but reported similar response to a second-line regimen. Of note, nelfinavir trough levels did not predict treatment failure. After twenty months of triple therapy, all twelve initial subjects have viral load below the limit of detection; 9/12 (75%) are still on the original regimen. The mean CD4 cell increase was 160. In addition, there was an apparent rise in the number of naive CD4 cells (CD45RA+62L+) from 63 to 120/mm^3, suggesting at least partial immune reconsitution (Markowitz 1998).

Acute primary infection. Another study carried out at the Aaron Diamond Center enrolled twelve recently-infected individuals with acute primary infection. The median entry CD4 count was 253 and viral load was 81,000 copies. One patient developed a grade 4 CPK elevation and withdrew from the study. The remaining eleven all had viral loads become undetectable within 12 weeks. The median CD4 count rose by 109 cells (Markowitz 1996).

Pivotal studies. In presentations at the Fourth Retrovirus Conference in January 1997, Agouron scientists described 2.5 log viral load reductions observed among individuals receiving nelfinavir in combination therapy regimens. Pooling data from three randomized pivotal studies (505 -- two doses of nelfinavir; 506 -- d4T/nelfinavir vs. d4T; and 511 -- AZT/3TC vs. AZT/3TC/nelfinavir), the sponsor claimed that nelfinavir-containing combination regimens reduced HIV RNA an average of 2.5 logs in over 700 individuals entering with a baseline viral load of just under 100,000 copies/ml. In those on triple therapy in study 511, the sponsor stated that the average HIV RNA reduction was 2.5 logs, and that 65-81% of recipients' viral levels became undetectable ($\leq$500 copies/ml). Reductions on d4T/nelfinavir were said to be 2.5 logs and 76% became undetectable.

In the package insert, these reductions have been described more conservatively. Agouron used a detection threshold of $\leq$400 copies/ml with the Chiron 2.0 bDNA assay. The FDA, however, insisted on using a higher cutoff of $\leq$1200 copies/ml, stating that "values below an estimated 1,200 copies/Ml could not be reliably quantified," resulting in apparently less dramatic viral load reductions. This, however, is an artifact of whichever cutoff is used:

Agouron 511: Impact of Viral Load Detection Threshold on Reported Potency of Nelfinavir

	Definition of "Undetectable": Six Month Data		
HIV RNA (copies/ml, bDNA)	***<1,200***	***<500***	***<100***
Agouron 511: AZT/3TC/nelfinavir 750	-1.7 $\log_{10}$	-2.0 $\log_{10}$	-2.5 $\log_{10}$

Mike Saag and colleagues reported longer-term follow-up from Agouron 511 at the 37th ICAAC in fall 1997. According to the Viracept Cooperative Study Group, 80% the patients who received 52 weeks

of nelfinavir/AZT/3TC sustained their viral load below limits of quantification. Mean CD4 increase over baseline was 180 cells at ten months (Saag 1997).

Double therapy in treatment-experienced individuals. Agouron study 506 randomized 308 HIV-infected individuals to two doses of nelfinavir (750 or 500 mg TID) plus d4T versus d4T alone. 89% of the participants were male and 75% were white. 20% were antiretroviral naive. The mean duration of antiretroviral experience in the previously-treated individuals was two years, eight months. Mean baseline CD4 count was 279 and mean plasma HIV RNA was 141,396 copies/ml (4.86 $\log_{10}$). By 24 weeks, 43 of 109 (39.4%) d4T monotherapy recipients switched to combination because of inadequate surrogate marker responses (Pedneault 1996, Agouron 1997a).

Agouron 506: HIV RNA Changes from Baseline ($\log_{10}$)

		Weeks of Therapy			*BLQ*[2]
Regimen	***N***	***Two***	***12***	***24***	***at w24***
d4T	109	-0.6	-0.5	-0.6 log	13 (12%)
d4T/NFV 500	99	-1.4	-1.1	-0.9 log	24 (24%)
d4T/NFV 750	107	-1.45	-1.25	-1.0 log	22 (21%)

Agouron 506: CD4 Cell Changes from Baseline

		Weeks of Therapy		
Regimen	***N***	***Two***	***Twelve***	***24***
d4T	109	+35	+ 30	+ 40
d4T/NFV 500	99	+75	+110	+ 95
d4T/NFV 750	107	+75	+120	+100

The data on nelfinavir plus a single nucleoside RTI are not as impressive as those with two RTIs.

Triple therapy in treatment-naive individuals. Agouron study 511 randomized 297 antiretroviral-naive, HIV-infected individuals to AZT/3TC alone or with either 500 or 750 mg TID of nelfinavir. The median age was 35. 89% were male and 78% white. Mean baseline CD4 count was 288 and plasma HIV RNA was 153,044 copies/ml (4.86 $\log_{10}$).

Agouron 511: HIV RNA Changes (bDNA, $\log_{10}$) from Baseline

		Weeks of Therapy				*N/% BLQ (<1200/ml)*
Regimen	***N***	***2***	***12***	***24***	***52***	***at 24 weeks***
AZT/3TC	101	-1.5	-1.25	-1.3	-1.6	30 (30%)
AZT/3TC/NFV 500	97	-1.7	-1.6	-1.9	-1.7	59 (61%)
AZT/3TC/NFV 750	99	-1.6	-1.6	-1.0	-2.0	73 (74%)

[2] BLQ = below the limit of quantification, which for the Chiron bDNA Chiron version 2.0 bDNA assay was $\leq$1,200 HIV RNA copies/ml.

A more useful metric is the proportion of patients with a viral load below the limit of quantification (BLQ). In Chicago, Agouron presented the proportion of patients from study 511 who went BLQ according to three different assays: the Chiron bDNA (limit <500 copies/ml, or 1,200 copies according to the FDA), Roche's standard, FDA-approved Amplicor RT-PCR (limit <400 copies), and Roche's new Ultra-Sensitive kit (lower limit <20 copies):

Agouron 511: Proportion with Viral Load BLQ* at 6 and 12 Monthts

	Six months			*Twelve months*		
	bDNA	*PCR*	*Ultra-S*	*bDNA*	*PCR*	*Ultra-S*
AZT/3TC	21%	8%	5%	53%	38%	23%
AZT/3TC/NFV 500	67%	62%	37%	58%	54%	35%
AZT/3TC/NFV 750	83%	81%	66%	79%	76%	62%

* Below limit of quantification

The mean log change in RNA at 6 months, using the Ultra-Sensitive kit, was -1.3 log for the control arm, -2.7 log for the 500 mg nelfinavir arm, and an impressive -3.0 log for the 750 mg nelfinavir arm. [Obviously the AZT/3TC patients added nelfinavir at six months.] It certainly appears -- especially at one year or using more sensitive assays -- that the higher dose of nelfinavir -- 750 mg as opposed to 500 mg TID -- is more potent (Clendeninn 1998).

Agouron 511: CD4 Cell Changes from Baseline

		Weeks of Therapy		
Regimen	***N***	***Two***	***Twelve***	***24***
AZT/3TC	101	+80	+ 80	+ 80
AZT/3TC/NFV 500	97	+80	+140	+130
AZT/3TC/NFV 750	99	+80	+120	+140

Triple-therapy with Bristol-Myers Squibb nucleosides. At the Fourth Retrovirus Conference, Bristol-Myers Squibb researchers presented preliminary data from a pilot study of ddI/d4T/nelfinavir in 22 protease-naive HIV-infected individuals, of whom 11 were antiretroviral naive. Median baseline CD4 count was 315 and viral load was 4.75 $\log_{10}$. Changes over first eight weeks of therapy were:

ddI/d4T/nelfinavir: Pilot Data

Parameter	***2 weeks***	***4 weeks***	***8 weeks***
CD4 change	+ 75 cells	+103 cells	+218 cells
HIV RNA change	-1.4 log	-1.7 log	-2.1 log

The lower limit of detection in this study was 500 HIV RNA copies/ml. After eight weeks viral load had become undetectable in three of eight (37.5%) participants. Seventeen participants (77.3% -- note the higher figure cited by a company which doesn't manufacture the drug in question) reported "occasional

episodes of loose stools," and there was one case each of grade 3 thrombocytopenia and one of grade 3 allergic reaction to nelfinavir (Pedneault 1997).

Nelfinavir with AZT and 3TC. The AVANTI 3 study compared AZT/3TC alone with AZT/3TC/nelfinavir. 102 antiretroviral-naive Australians, Canadians and Europeans were randomized and followed for a year. Unsurprisingly, the triple therapy group did considerably better than the double nucleoside group. At 28 weeks, the median viral load decrease was -0.98 log in the double therapy group and -1.85 log in the triple therapy group ($p<0.001$). Seven of forty individuals (18%) had a viral load below 500 copies on double therapy, versus 34/41 (83%) on triple therapy (Clumeck 1998).

Pediatric indication. 38 children ranging from two to 13 years of age were given nelfinavir in an open-label, uncontrolled trial. The recommended pediatric dose is 20-30 mg/kg thrice daily, not to exceed 750 mg TID. Similar toxicity was seen in children and adults. Oral clearance appears higher in children than in adults, which is seen with other drugs metabolized by cytochrome P450 (Krogstad 1997). Antiviral activity analyses are ongoing.

At the Fifth Retrovirus Conference in February 1998, two studies reported on the use of nelfinavir in HIV-infected children. Krogstad and colleagues followed sixty HIV-positive infants ranging from 3 months to 13 years of age (median 5 years) who were given nelfinavir with various NRTIs. Three patients (5%) diarrhea; one withdrew from the study as a result. At ten weeks, median viral load decreased by 1.3 log for the cohort as a whole. The decline was greater and more sustained if the child had switched at least one NRTI while starting nelfinavir. At 34 weeks, viral load was undetectable in 10/18 (55%) including 8 of 11 (73%) subjects who had switched at least one NRTI. CD4 cell increases were greater in those with undetectable viral loads. The drug was well-tolerated and appears comparable in virologic activity in children as in adults (Krogstad 1998).

In Chicago, Martel and colleagues reported on a dose-ranging study of nelfinavir in 17 nucleoside-experienced, protease-naive children ranging from 4 to 13 years of age (median 8). All but one child received at least one new RT inhibitor. Baseline RNA and CD4 values were compared with the first two post-nelfinavir visits. The median CD4 percentage went from 3% to 6.5% at the first post-nelfinavir visit and to 11.5% at the second. Median CD4 count went from 32 to 153 after the introduction of nelfinavir, and continued up to 181 at the second visit. Median HIV log change was from 5.2 at baseline to 3.5 at the first post-switch visit and 3.47 at the second. Fewer children developed undetectable viral load than has been seen in adult studies, either because the children in this study had advanced disease or for other, unknown factors (Martel 1998).

At the time of its approval, nelfinavir had not yet been evaluated in children less than two years old. In response to an inquiry from TAG, Agouron's Joy Schmitt wrote that "At the time the NDA was submitted, no children... [younger than 2] had been enrolled. Study 524 is ongoing and has since [NDA approval] recruited infants as young as 3 months. Data are being collected on the approximately 50 children now enrolled with the intent of eventual marketing clearance in children less than two years of age. Based on our experience to date, the current powder formulation, which may be combined with water, milk, and formula, is suitable for neonates... We are currently working with the PACTG to finalize Study 353, which will evaluate the safety, tolerance, and antiviral efficacy of the triple drug regimen, nelfinavir/AZT/3TC." (Schmitt 1997)

THE SEQUENCING CONTROVERSY

Much effort has been spent by various pharmaceutical sponsors convincing consumers and clinicians that *their* protease inhibitor should be used first. Arguments are based on competing claims about ease of use, tolerance, potency, resistance profile or market share. Sponsors are rarely willing to subject their drugs, however, to direct head-to-head comparisons of various first-line strategies. CPCRA 042, the nelfinavir vs. ritonavir study, was an exception, although Agouron had to buy the ritonavir for the study because Abbott refused to provide it. (Agouron got back at the public sector by declining to provide drug to some subsequent ACTG studies in treatment failures.)

Thus, there is very little reliable data to make firm conclusions about whether it is desirable to use nelfinavir in a first-line regimen or save it, perhaps for use among people whose viral load has returned on indinavir therapy.

Observational reports of salvage therapy for individuals failing nelfinavir. Keith Henry and colleagues reported at the 37th ICAAC on the use of ritonavir/saquinavir/d4T/3TC among nine nelfinavir failures. Their median duration of nelfinavir use had been 9.5 months. The median time on the new quadruple regimen was 2.2 months. Seven of the 9 (77%) achieved a viral load below the detection limit, the rest remained detectable. However, the preliminary results suggest that a four-drug regimen including two protease inhibitors may work in nelfinavir failures (the study authors include two Agouron scientists) (Henry 1997).

In Chicago, the same team reported on a by now larger group of 27 nelfinavir failures who were switched to RTV/SQV/d4T/3TC. The median prior nelfinavir use was one year. Nine of 27 (33%) had experienced a complete virologic response to nelfinavir but subsequently rebounded. Failure was defined conservatively as a viral load over 5,000 copies. Nineteen patients were evaluable. All of them (100%) reached undetectable viral load (<500 copies), and 9/10 (90%) sustained this for four months. However, only 3/7 (43%) of those with extensive prior antiretroviral treatment went undetectable. Genotypic resistance analysis at baseline showed that 17/25 (68%) had the D30N mutation, and 5/25 (20%) had L90M. The authors conclude that baseline genotypic resistance is not a good predictor of subsequent clinical response in this case, and that durable suppression is achievable with a four-drug, two protease regimen after virologic failure on a three-drug, nelfinavir-containing regimen (Tebas 1998).

Mike Barr presented an ICAAC late breaker in September 1997 which summarized data on 22 patients who lost control of viral load after at least twelve weeks of nelfinavir (average length, 8.6 months). Mean baseline RNA and CD4 counts were 58,875 and 180. Half of the participants switched to indinavir and half to ritonavir/saquinavir-containing regimens. Of eight patients available for analysis at four weeks, four experienced an *increase* in viral load after switching and four experienced a decrease; only one went undetectable (<400). The authors concluded that "treatment with a second protease inhibitor resulted in a smaller than expected reduction in plasma viral load or none at all." (Sampson 1997).

Observational reports of nelfinavir use in protease failures. One clinic reported its experience at the 37th ICAAC in late 1997. 37 patients with CD4 counts below 100 who had failed ritonavir or indinavir-containing regimens were switched to nelfinavir (the abstract did not say whether they were able to switch underlying nucleosides). The median baseline CD4 count was 37 (range 4-258) and baseline viral

load averaged 5.0 log. Outcomes were measured a month after starting nelfinavir. Median changes included an increase of 25 CD4 cells and an HIV RNA increase of 0.5 log. "Discordant responses on CD4 and viral load were observed in 52% of patients." The authors concluded that "this sample of antiretroviral-experienced patients, 92% of whom had failed at least one other PI, experienced over the short term a modest CD4 response, no viral load response, and no detectable quality of life impact" from the nelfinavir expanded access program [NEAP]." It is unclear whether this sample reflects today's approach to HAART, or whether the patients simply added nelfinavir to a failing regimen. Thus, it is hard to extrapolate too much from this study (Ballard 1997).

At Stanford, 16 individuals who had received saquinavir for at least six months and whose viral load returned to above 5,000 copies were switched to nelfinavir 750 mg TID plus two RTIs. Median baseline CD4 was 156 (range 21-306) and viral load was 16,716 (range 2,915-878,461). The addition of nelfinavir produced only a transient reduction in viral load (median 0.59 log drop at two weeks). Most patients'viral loads returned to baseline by three months. Eleven of the 16 patients (69%) subsequently failed the nelfinavir regimen and went on to indinavir/nevirapine and two RTIs (Lawrence 1998).

The Kaiser Permanente group in Denver also studied 16 patients in the nelfinavir expanded access program. All had received at least one protease inhibitor before enrolling in the NEAP. Nine of the 16 stayed on nelfinavir. They experienced a >0.3 log drop in viral load, compared with a half log increase in those who stopped taking the drug. Of the 8 patients (50%) whose RNA decreased, the mean decrease was 0.7 log and the CD4 cell increase was a modest 28 cells. Twelve of 16 patients (75%) reported side effects, mainly diarrhea (McNicholl 1997).

RESISTANCE & CROSS-RESISTANCE

Nelfinavir-resistant HIV strains were selected by *in vitro* passage. Observed point mutations were compared with point mutations observed in isolates drawn from nelfinavir-treated individuals. After 22 passages, the D30N mutation was observed to confer a nine-fold increase in the effective dose (ED_{90}). No cross-resistance was observed with other licensed protease inhibitors. Genotypic resistance analysis was performed on samples from 55 individuals treated with nelfinavir alone or with other antiretroviral agents, and phenotypic analysis was performed on 19 such individuals. The percentage of patients with genotypic resistance after 16 weeks of treatment was 56% on monotherapy, 6% on AZT/3TC/nelfinavir and 0% on AZT/3TC. Among the HIV protease mutations observed in more than 10% of individuals with evaluable isolates were amino acid substitutions at positions 30, 35, 36, 46, 71, 77 and 88. Among the 19 individuals from whose clinical isolates both genotypic and phenotypic analysis was performed, 9/19 (47.4%) showed five-to-93-fold reduced susceptibility to nelfinavir *in vitro*. All nine had at least one mutation in their protease gene. The most frequent mutation site was at position 30 (Patick 1997). Subsequently the researchers looked for the D30N mutation in 64 individuals on monotherapy and 49 individuals on AZT/3TC/nelfinavir combination:

D30N Mutation at 12-16 Weeks of Therapy

	N	*Total (%) with D30N mutation*
Nelfinavir monotherapy	64	36 (56%)
AZT/3TC/nelfinavir	49	3 (6%)

Of note, some individuals were undetectable at 12-16 weeks of therapy -- particularly, one presumes, in the triple-therapy group -- and by definition virus could not be isolated and amplified from these individuals.

Clinical viral isolates from five nelfinavir-treated individuals exhibiting five-to-93-fold reduced susceptibility to nelfinavir remained susceptible to indinavir, ritonavir, saquinavir and amprenavir *in vitro*.

A single isolate from a saquinavir-experienced individual which showed seven-fold decreased susceptibility to saquinavir *in vitro* remained sensitive to nelfinavir *in vitro*. However, six of seven HIV isolates which exhibited eight-to-113-fold decreases in susceptibility to ritonavir also exhibited decreased five-to-40-fold susceptibility to nelfinavir *in vitro*. The company did not report on experiments with isolates from individuals receiving indinavir. However, since indinavir is generally cross-resistant with ritonavir, it may be expected that indinavir-resistant HIV is likely to be resistant to nelfinavir as well.

While Agouron is to be commended for performing these resistance analyses, which are certainly more detailed than those shown at the time of approval for indinavir, ritonavir or saquinavir, the number of isolates sampled is small -- particularly phenotypically -- and the need for clinical studies of virologic responses to various protease sequencing regimens is critical.

Nelfinavir Cross-Resistance: Phenotypic Analysis of 13 Clinical Isolates

Protease Exposure	***N***	***Resistant to***	***Susceptible to***
Nelfinavir	5	NFV (100%, 5-93-fold)	IDV, RTV, SQV, 141
Indinavir	-	Not reported	Not reported
Ritonavir	7	NFV (85%, 8-113-fold)	Not reported
Saquinavir	1	Not reported	NFV (1 of 1)
Amprenavir	-	Not reported	Not reported

In a table published in the Viracept "Backgrounder", Agouron presented handy chart proclaiming that nelfinavir-resistant HIV strains remained susceptible to all three other licensed protease inhibitors. The total number of individuals from whom viral isolates were drawn was six (Agouron 1997b). Agouron also presented its analysis of mutational overlap between protease inhibitors:

Protease Cross-Resistance: Agouron's Version

HIV Protease Point Mutation Site

Saquinavir	10				***48***		63	71			***90***
Ritonavir	10	20	36	46	54		63	71	***82***	***84***	***90***
Indinavir	10	24	36	46	54		63 65	71	***82***	***84***	***90***
Nelfinavir		***30***	35 36	46				71	77		88

[Clinically observed mutations correlating with phenotypic resistance are shown in ***bold italics***.]

With so much hype, such pressure for market share, and so little clinical data, it is good to remain skeptical about pharmaceutical sponsor claims about resistance, as Mike Barr reminds us:

When it comes to corporate positioning for protease inhibitor market share, every company has a yarn to spin. Merck loyalists insist that theirs be used first-line because it's so powerful and, "after all, really requires *multiple mutations* in order to significantly alter viral sensitivity." Roche (and later, Agouron, in lock step) claim that their protease is the only one to deserve a first-line indication because the mutations elicited with saquinavir and nelfinavir are unique and not nearly as predisposing to cross-resistance as are, say, the indinavir mutations. Since scientists at all the protease outfits seem capable of pulling whatever color rabbit out of their hats is deemed most conducive to a successful marketing campaign (and since all cross-resistance analyses to date have been conducted in test tube experiments), trying to sort through the morass of claims and counter-claims has been at times Herculean; at others, Sisyphean (Barr 1997).

Many studies including nelfinavir are being conducted in saquinavir and indinavir failures; see below under "current & planned studies." Agouron's resistance work to date has largely focused on genotypic analyses. It should expand this work to cover phenotypic analyses in the future.

ADVERSE EVENTS & TOXICITY MANAGEMENT

Just 11% of patients discontinued nelfinavir in the two pivotal studies -- a proportion which Agouron claims is "a very low incidence for clinical trials" (Agouron 1997). Just 1.6% discontinued for diarrhea, and 4% for side effects overall.

Agouron 511 + 506: Pooled Data on Moderate or Severe Adverse Events

	Agouron 506 (Naive)			*Agouron 511 (Experienced)*		
	Placebo AZT/3TC	***NFV 500 AZT/3TC***	***NFV 750 AZT/3TC***	***Placebo/ d4T***	***NFV 500/d4T***	***NFV 750/d4T***
N	101	97	100	109	98	101
Abdom. pain	1%	0	0	3%	2%	4%
Asthenia	2%	1%	1%	4%	3%	1%
Diarrhea	3%	14%	20%	10%	28%	32%
Nausea	4%	3%	7%	1%	3%	2%
Flatulence	0	5%	2%	4%	8%	3%
Rash	1%	1%	3%	0	4%	3%

Clearly diarrhea is the most common serious toxicity, occurring in between 20-32% of individuals receiving the FDA-approved dose. (Anecdotes from people actually on the drug report a much higher occurrence of less severe diarrhea, which the sponsor likes to refer to as "loose stools", and which investigators are wont to dismiss with an airy, "Take Imodium!")

All the licensed protease inhibitors cause some degree of gastrointestinal discomfort, with symptoms ranging from mild GI upset to gastric reflux (heartburn) to gas and flatulence to "loose stools" to severe nausea and diarrhea. Little study has occurred into the cause of these GI toxicities, which impair quality of life and reduce adherence, risking the emergence of resistant HIV.

Few laboratory abnormalities were seen among individuals taking nelfinavir, with the most frequent

abnormalities including decreased neutrophils in 5% of participants receiving AZT/3TC/nelfinavir -- probably due to the AZT -- and elevated creatine kinase seen in 2-6% of participants.

On 11 June 1997 the FDA released a public health advisory warning that 83 cases of diabetes mellitus or hyperglycemia (elevated blood sugar) had been reported among individuals receiving protease inhibitors. Additional cases may be reported to FDA's MEDWATCH program at 1.800.FDA.1088 or faxed to 1.800.FDA.0178 (FDA 1997).

Expanded access program. In September 1996 Agouron opened an Expanded Access Program to provide nelfinavir free of charge to HIV-infected individuals who for whom approved, available protease inhibitors were failing, unacceptably toxic, or contraindicated. Originally the program was open only to those with fewer than 50 CD4 cells. In January 1997 the entry criteria were liberalized to include those in whom fewer than 100 CD4 cells had been measured at any time point. Also in January, Expanded Access was extended to children over two years of age. About 3,000 people enrolled in the program by the time of approval. Those in the Program will receive one month of free nelfinavir after approval, and assistance in transition to third-party payment. A resistance sub-study of the Expanded Access Program will continue for one year. Safety data from the Expanded Access Program were presented at the 37th ICAAC in fall 1997. 3,125 patients enrolled; their mean baseline CD4 was 41. Safety data were given for the first 1,532 patients.

Nelfinavir Expanded Access Program

Contraindicated protease inhibitor	*Failed*	*Intolerant*
Saquinavir	1,187 (39%)	256 (8%)
Indinavir	2,176 (73%)	641 (21%)
Ritonavir	1,256 (42%)	1,343 (45%)

The most frequent adverse event in the first 1,532 patients was diarrhea (16%), followed by rash, nausea, headache and asthenia, all in fewer than 5% of participants. Overall, 5% of patients discontinued nelfinavir due to adverse events (Becker 1997). A contrasting report from a health maintenance organization reported that 12 of 16 (75%) of individuals who received nelfinavir as part of a salvage regimen complained of adverse reactions, mostly diarrhea (McNicholl 1997).

Note on hemophilia. Special caution may be warranted when administering protease inhibitors, possibly including nelfinavir, to people with hemophilia. "There have been reports of increased bleeding, including spontaneous skin hematomas and hemarthrosis, in patients with hemophilia type A and B treated with protease inhibitors. In some patients, additional factor VIII was given. In more than half of the reported cases, treatment with protease inhibitors was continued or reintroduced. A causal relationship has not been established." (Agouron 1997). TAG wrote to Agouron asking the company to carry out safety studies to ensure that nelfinavir is safe for use among individuals with type A or B hemophilia. In response, Agouron confirmed that "the safety data to date with hemophiliacs has been limited. There was [only] one hemophiliac enrolled in the US pivotal trials. Of the 39 patients currently enrolled in the clinical trials conducted by Japan Tobacco, 23 are hemophiliacs... We are currently unaware of any increased bleeding episodes attributable to nelfinavir in patients with hemophilia." (Schmitt 1997).

PHARMACOKINETICS, FOOD & DRUG INTERACTIONS

Pharmacokinetics is the study of how a drug is absorbed from the stomach, processed through the liver, transported through the body and into cells by the bloodstream, and excreted by the kidneys (through urine) or the GI tract (through feces). There are two ways of measuring how much of a drug gets into the body -- how *long* it stays there [its half-life in the blood, or plasma area under the curve (AUC)], and how *much* of it gets into the blood at its peak (maximal concentration, or C_{max}). Nelfinavir has a longer half-life and a greater area under the curve (AUC) than other protease inhibitors. Peak plasma concentrations occur after two to four hours when 500 to 750 milligrams (mg) of nelfinavir is taken with food. The approved dosing regimen is 750 milligrams thrice daily (750 mg TID). After four weeks of this regimen, peak plasma concentrations (C_{max}) averaged 3-4 micrograms per milliliter (μg/ml. Plasma concentrations before the morning dose were 1-3 μg/ml (drawn an average of 11 hours after the previous evening dose).

It is essential to eat nelfinavir with food, which increases the drug's C_{max} and AUC by two-to-three-fold. The impact of food on absorption was assessed in 14 individuals who ate meals containing 517-759 kilocaolories (Kcal), with 153-313 Kcal derived from fat. Agouron is very happy that its drug can be taken with food, and on a less restrictive time schedule (with meals thrice daily -- the drug's half-life is longer than indinavir's, and so adhering to a strict every eight hourly regimen is not as critical with nelfinavir, as long as three doses are taken each day). "Viracept's half life is between 3.5 and 5 hours which means that blood levels stay elevated long after eight hours. Comparatively, Crixivan's half life is 1.8 $\pm$ 0.4 hours, making it critical for patients to take their medications on time... Taking Viracept with food, as opposed to an empty stomach as recommended with Crixivan, may aid in the difficult task of adhering to a dosing regime," hints Agouron, helpfully (Agouron 1997b). On the other hand -- who knows? -- perhaps the hunger pangs associated with Crixivan dosing actually stimulate the brain to remember "Time for my Crix! In an hour I can eat!"

In the blood, nelfinavir is highly protein-bound. 82-86% of the drug in the plasma is unchanged. Its terminal plasma half-life is 3.5 to five hours. 87% of an oral 750 mg dose containing radioactive (Carbon 14, C^{14}) labeled nelfinavir was excreted in the stool. Only one to two percent of the dose was recovered in urine. Nelfinavir pharmacokinetics have not been measured in individuals with liver or kidney dysfunction. Because just two percent of the drug comes out in the urine, kidney dysfunction should not affect drug metabolism. The company studied between-gender differences in pharmacokinetics and found none. It did not study racial or ethnic differences in pharmacokinetics.

Agouron "currently [has] no human data on CNS penetration with nelfinavir. However, tissue distribution studies were performed in rats... After a six hour infusion... at a dose of 40 mg/kg, penetration into the brain was found. The brain levels recorded for this study were higher than required for antiviral activity of the drug." (Schmitt 1997). Great news for rats with AIDS dementia, but human data are still needed.

The main physiological interaction of nelfinavir is with the family of liver enzymes known as human cytochrome P450 isoforms, which include the proteins CYP3A, CYP2C19, CYP2D6, CYP2C9 and CYP2E1. Only CYP3A was inhibited by nelfinavir at concentrations in the therapeutic range. K_i is a measure of enzyme inhibition. A higher K_i concentration means a lower inhibition. Compared with

ritonavir, which has a K_i of 0.1, and indinavir, which has one of 0.7, nelfinavir is a milder inhibitor, with a K_i of 4.8 (Agouron 1997b). Because this liver enzyme system is also responsible for metabolizing a number of other commonly-used drugs, nelfinavir has significant effects on their plasma half-life (AUC) and plasma concentration (C_{max}):

Effect of Nelfinavir (750 mg TID) on Concomitant Drug Plasma AUC + C_{max}

Concomitant drug	*N*	*AUC (95% CI)*	*C_{max} (95% CI)*
3TC 150 mg	11	Up 10% (1-20%)	Up 31% (5-62%)
d4T 200 mg	8	No change	No change
AZT 200 mg	11	Down 35% (28-41%)	Down 31% (8-49%)
IDV 800 mg	6	Up 51% (25-83%)	No change
RTV 500 mg	10	No change	No change
SQV 1200 mg	14	Up 392% (271-553%)	Up 179% (105-280%)
Ethinyl estradiol 35 μg	12	Down 47% (41-63%)	Down 28% (14-39%)
Norethindrone 35 μg	12	Down 18% (12-27%)	No change
Rifabutin 300 mg	10	Up 207% (151-276%)	No change
Terfenadine 60 mg	12	Transiently measurable	Transiently measurable

Effect of Concomitant Drug on Nelfinavir (750 mg TID) Plasma AUC + C_{max}

Concomitant drug	*N*	*AUC (95% CI)*	*C_{max} (95% CI)*
ddI 200 mg	9	No change	No change
AZT 200 mg / 3TC 150 mg	11	No change	No change
IDV 800 mg	6	Up 83% (34-150%)	Up 31% (13-52%)
RTV 500 mg	10	Up 152% (86-242%)	Up 44%
SQV 1200 mg	14	Up 18% (5-33%)	No change
Ketoconazole 400 mg	12	Up 35% (21-49%)	Up 25% (8-44%)
Rifabutin 300 mg	10	Down 32% (10-48%)	Down 25% (6-38%)
Rifampin 600 mg	12	Down 82% (77-86%)	Down 76% (67-83%)

These pharmacokinetic interaction data -- more than we have ever had for any HIV protease inhibitor at the time of approval -- raise some safety concerns and suggest several follow-up studies for enhancing protease inhibitor efficacy through synergy.

Safety considerations. **Several drugs should *NOT* be taken with nelfinavir:**

* The antihistamines astemizole (Hismanal) and terfenadine (Seldane)
* The antimycobacterial rifampin (Rifadin, Rifamate, Rifater, Rimactane)
* The benzodiazepines midazolam (Versed) and triazolam (Halcion)
* The GI motility agent cisapride (Propulsid)

Use of these drugs in combination with nelfinavir may cause "serious and/or life-threatening cardiac arrhythmias or prolonged sedation" (Agouron 1997, Kerr 1997).

* **Persons on nelfinavir should *CUT THEIR DOSE OF RIFABUTIN (Mycobutin) IN HALF.***

Use of full-dose rifabutin with nelfinavir may increase the risk of uveitis (eye inflammation).

* The anticonvulsants carbamazepine (Atretol, Tegretol, Epitol), phenobarbital (Arco-Lase, Bellergal, Donnatal, Quadrinal, Mudrane, Solfoton) and phenytoin (Dilantin) may decrease nelfinavir plasma concentrations, leading to resistance and/or no efficacy
* Nelfinavir may decrease plasma concentrations of the oral contraceptives ethinyl estradiol and norethindrone (two drugs sold together as Brevicon, Demulen, Levlen, Lo/Ovral, Modicon, Nordette, Norinyl, Ortho-Cept, Ortho-Cyclen, Ortho-Novum, Ovral, Tri-Levlen, Tri-Norinyl, Triphasil, Nelova, Norethin), rendering them ineffective in preventing conception.

Efficacy considerations

* The protease inhibitors indinavir (Crixivan) and ritonavir (Norvir) may increase nelfinavir half-life by 83-152% and its plasma concentrations by 31-44%, respectively (Yuen 1997).
* Nelfinavir may increase the half-life of indinavir by 51% (Yuen 1997).
* Nelfinavir may increase the half-life of saquinavir (Invirase) by up to 400%, and its plasma concentration two-fold (179%) (Kravcik 1997).

For information on combinations of nelfinavir with either indinavir or saquinavir, see Spencer Cox's chapter on "New Treatment Strategies" (above).

Nelfinavir/NNRTI interactions. Not listed in the package insert, but tantalizingly hinted at in presentations at the Fourth Retrovirus Conference in January 1997 is the possibility of positive interactions between nelfinavir and the non-nucleoside reverse transcriptase inhibitor delavirdine (Rescriptor, a CYP3A inhibitor) . Note: the other NNRTIs, nevirapine (Viramune) and Efavirenz are CYP3A *inducers* and so *speed* metabolism of protease inhibitors, shortening their half-life and reducing their plasma concentration. Studies of nelfinavir with nevirapine, Efavirenz and delavirdine are all underway, with under 50 patients each. Several larger planned studies -- e.g., ACTG 364 and 374 -- involve nelfinavir/NNRTI combinations (Schmitt 1997).

Methadone. Not addressed in the package insert was the concomitant use of nelfinavir and methadone. All the protease manufacturers have been negligent in studying this interaction and, as a result, many drug users taking methadone are prohibited from taking protease inhibitors (Ken Fornataro, personal communication). In Europe, some researchers in France have undertaken to study the interaction of ritonavir and indinavir, respectively, with methadone (ARCAT SIDA 1997), but they are not studying nelfinavir, and ultimately this should be the responsibility of the sponsor. Agouron dodged TAG's request that the company "should assess the pharmacokinetic interaction of nelfinavir and methadone" by claiming that "conducting a small study is currently being *considered* [emphasis added]. However, CYP2E1, CYP3A4, and possibly CYP2D6 are involved in the metabolism of methadone. While possible that nelfinavir may inhibit methadone metabolism by CYP3A4, the extent of inhibition will be limited, since nelfinavir would not impair metabolism of methadone by CYP2E1 and CYP2D6" (Schmitt 1997).

CURRENT & PLANNED POST-MARKETING STUDIES

Agouron 509 (the Aaron Diamond study conducted by Martin Markowitz) continues. Updated results are given above.

Protease-Protease Studies

Agouron 534 will enroll 60 women, give them d4T/3TC, and randomize them to receive nelfinavir and saquinavir twice or thrice daily, along with d4T and 3TC. Twenty one women had enrolled by October 1997. Of seven patients treated for over three months, six (83%) went undetectable with a median CD4 increase of 100 (Zorrilla 1998).

Agouron 535 will randomize at least 160 people to nelfinavir + 2 NRTIs, saquinavir + 2 NRTIs, and nelfinavir/saquinavir + 2 NRTIs vs. the two proteases alone.

Agouron 547 is looking at 500 and 750 mg of nelfinavir with 1,000 mg of indinavir.

Protease/NNRTI Studies

NFV/delavirdine. Pharmacia & Upjohn is studying this combination.

NFVefavirenz. DuPont Pharma has two studies of this combination.study 019 and 30 will enroll in 024.

NFV/nevirapine. Boehringer Ingelheim is studying this combination.

Studies with Nucleosides

Agouron 542, a triple combination study with d4T/3TC/nelfinavir. 240 patients will enroll.

ATLANTIC will compare ddI/d4T/3TC to ddI/d4T/indinavir to ddI/d4T/nelfinavir, N=?.

BMS 062/063 is looking at ddI/d4T/nelfinavir/hydroxyurea in 30 individuals.

CPCRA 042 is comparing nelfinavir plus nucleoside analogues versus ritonavir plus nucleoside analogues in 1,300 treatment-experienced, HIV-infected adults with CD4<100. The study began enrollment in January 1997. Endpoints are progression to AIDS and death.

NV15436A is a Roche study randomizing 150 patients to receive saquinavir enhanced oral formulation (EOF, N=25), nelfinavir (N=25), SQV EOF + NFV (N=100), all with combination nucleosides. The main endpoint is RNA PCR at 16 weeks. Follow-up will be for 48 weeks. The study is taking place in Belgium, Germany, Holland, Switzerland and the UK (ARCAT SIDA 1997).

Abacavir/NFV. A 48-week, open-label study is comparing abacavir (1592) plus either indinavir, saquinavir, ritonavir, nelfinavir, amprenavir or efavirenz (ATDN 1997).

Resistance. A substudy of the Expanded Access Program followed 100 protease-experienced individuals to assess their response to nelfinavir after failing other protease inhibitors. Agouron claims that, "Anecdotally, many patients have responded," without giving qualitative or quantitative specifics (Agouron 1997b). Which drugs were the responders taking previously? How *much* resistance did they have? Which mutations were associated with a response, or with failure?

Salvage therapy. Several studies are giving nelfinavir to protease failures: 1) treatment of saquinavir failures with nelfinavir or ritonavir, each with nucleosides, in CPCRA 042; 2) treatment of indinavir failures with abacavir/efavirenz/nelfinavir in ACTG 372; 3) treatment of indinavir failures with ddI/d4T/nelfinavir/saquinavir or adefovir/nelfinavir/nevirapine/saquinavir in a Stanford study; 4) treatment of indinavir failures with abacavir/d4T/nelfinavir/saqunavir or ddI/d4T/nelfinavir/saquinavir in a study conducted by Steven Deeks; 5) treatment of indinavir failures with ddI/d4T/nelfinavir in a Bristol-Myers study carried out by Martin Hirsch and Douglas Richman; 6) treatment of amprenavir failures with a nelfinavir-containing regimen in an ACTG study; and 7) ongoing follow-up of study 511 (Schmitt 1997).

PRICE & ACCESS

Viracept costs $15.48 per day or $5,650 per year at the recommended dose *wholesale*. This is more expensive than Crixivan (indinavir) and less expensive than Invirase (saquinavir) or Norvir (ritonavir). Still, the price is too high. While it's Agouron's first drug, it reached market in unprecedented time, so development costs were surely much lower than the typically-cited figure of $500-700 million, which reflects an industry average assuming *ten years* of development time and several large, long phase III trials. Viracept took just 38 months from phase I testing to FDA approval. When TAG suggested that "Agouron should consider a price for Viracept more in line with that for Crixivan, the market leader, if it wants to be a widely-used contender for first-line protease therapy," the company responded, "While we appreciate your comments, current pricing will remain. Please remember that this is Agouron's first commercially available product after being in existence for thirteen years. We have additional products in our pipeline that obviously require developmental dollars..." (Schmitt 1997).

Patient assistance program. Agouron established a patient assistance program to assist people in obtaining reimbursement for nelfinavir. The company says it will "provide drug free of charge to people who are unable to pay for drug or find appropriate reimbursement sources." Agouron has also agreed to provide Medicaid and state AIDS Drug Assistance Programs (ADAPs) the standard Medicaid discount (17.5% off), and will provide nelfinavir free of charge to all children under the age of 12 not covered by public or private health insurance. "No child will go without drug." (Agouron 1997b). The patient assistance program for adults and children can be reached at 1.888.777.6637. Product information can be obtained at 1.888.847.2237.

COMMENT

Agouron conducted what was in many respects a model antiretroviral drug development program, with rapid development and a database which clearly merits accelerated approval. Particularly praiseworthy were the development of nelfinavir for children and the expanded access program. Its postmarketing development plan seems both ambitious and reasonable. Its marketing campaigns have been no more

egregious than those of its competitors[3], and yet one cannot help wishing that they would all subject their products to head-to-head and sequencing studies, with standardized resistance assays carried out by academic researchers not beholden to individual sponsors, rather than engaging in the currently fashionable sport of rival assay-bashing and rampant speculation. This impedes the development of studies to answer public health questions like the optimal starting regimen (protease-including or protease-sparing) and the optimal first- and second-line and salvage regimens.

*

REFERENCES

Agouron Pharmaceuticals, Inc. Investigators present results of Viracept studies at international AIDS meetings. Press release, Jully 1996a.

Agouron Pharmaceuticals, Inc. The Agouron Approach; A Timetable to Success, The shaping of a commercial pharmaceutical company. Undated materials, 1996b.

Agouron Pharmaceuticals, Inc. Viracept[R] (nelfinavir mesylate) TABLETS and ORAL POWDER. FDA package insert, 14 March 1997a.

Agouron Pharmaceuticals, Inc. Viracept[R] (nelfinavir mesylate) Backgrounder, March 1997b.

Agouron Pharmaceuticals, Inc. ***http://www.agouron.com*** website, March 1997c.

ARCAT SIDA. HIV and AIDS treatment directory France, 5th edition, 1997.

ATDN, AIDS Treatment Data Network. The experimental treatment guide, spring 1997.

Ballard C, Toerner JG, Colwell B, Mathews WC. Early CD4, viral load, and quality of life response to salvage treatment with nelfinavir: the UCSD Owen Clinic nelfinavir expanded access experience. Abstract I-192, 37th ICAAC, Toronto, 1997

Barr M. Sweating the Switch, *TAGLine 4:5*, May 1997.

Becker MI, Wilson D, Yuen N, et al. An expanded access program with Viracept (r) (nelfinavir mesylate). Abstract I-102, 37th ICAAC, Toronto, 1997

Broekhuizen A for the European AIDS Treatment Group (EATG), letter to David Feigal, 8 July 1997.

Clendeninn N, Quart B, Anderson R, et al. Analysis of long-term virologic data from the Viracept (R) (nelfinavir, NFV) 511 protocol using 3 HIV RNA assays. Abstract 372, Fifth Conference on Retroviruses and Opportunistic Infections, February 1998

Clumeck N. AVANTI 3, a randomized, double blind, comparative trial to evaluate the efficacy, safety and tolerance of AZT/3TC vs. AZT/3TC/nelfinavir in antiretroviral-naive patients. Abstract 8, Fifth Conference on Retroviruses & OIs, 1998

Conant M, Markowitz M, Hurley A, et al. A randomized phase II dose range-finding study of the HIV protease inhibitor Viracept[TM] as monotherapy in HIV positive patients. Abstract Tu.B.2129, XI International AIDS Conference, Vancouver, 1996.

---

[3] FDA scolded Agouron for a press release released on April 11 which was "misleading... lacks balance and implies that the results of this study are more representative than they really are," (*Wall Street Journal* 1997); perhaps this slap on the wrist reflects Agouron's relative inexperience with advertising, since it's never before had a licensed product.

FDA Public Health Advisory, Reports of diabetes and hyperglycemia in patients receiving protease inhibitors for the treatment of human immunodeficiency virus (HIV). 11 June 1997.

Gathe J, Burkhardt B, Hawley P, et al. A randomized phase II study of Viracept™, a novel HIV protease inhibitor, used in combination with stavudine (d4T) vs. stavudine (d4T) alone. Abstract Mo.B.413, X Intl AIDS Conference, Vancouver, 1996.

Kenry K, Kane E, Melroe H, et al. Experience with a ritonavir/saquinavir based regimen for the treatmetn of HIV infection in subjects developing increased viral loads while receiving nelfinavir. Abstract I-204, 37th ICAAC, Toronto, 1997]

Henry K, Lamarca A, Myers R, Chapman S. The safety of Viracept (TM) (nelfinavir mesylate, NFV) in pivotal phase II/III double-blind randomized controlled trials as monotherapy and in combination with either d4T or AZT/3TC. Abstract 240, 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

Ho DD, Perelson AS, Essunger P, Markowitz M. How long should treatment be given if we had an antiretroviral regimen that completely blocks HIV replication? Abstract Th.B.930, XI International Conference on AIDS, Vancouver, B.C., 1996.

Hollander N. It happens again. Europe yes, southern Europe no? The launch report -- almost live from Seville. European AIDS Treatment News, March/April 1998, p. 25.

Kerr B, Yuen G, Daniels R, et al. Strategic approach to nelfinavir mesylate (NFV) drug interactions involving CYP3A metabolism. Abstract 373, 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

Kravcik S, Sahai J, Kerr B, et al. Nelfinavir mesylate (NFV) increases saquinavir-soft gel capsule (SQV-SGC) exposure in HIV+ patients. Abstract 371, 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

Krogstad P, Kerr B, Anderson R, et al. Phase I study of the HIV-protease inhibitor nelfinavir mesylate (NFV) in HIV+ children. Abstract 721, 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

Krogstad P, Wiznia A, Luzuriaga K, et al. Evidence of potent antiviral activity in a phase I/II study of nelfinavir mesylate (Viracept) in HIV infected children. Abstract 270, Fifth Retrovirus Conference, February 1998

Lawrence J, Schapiro J, Winters M, et al. Salvage therapy with indinavir plus nevirapine in patients previously treated with two other protease inhibitors and multiple reverse transcriptase inhibitors. Abstract 422, Fifth Retroconference, 1998.

Markowitz M, Cao Y, Hurley A, et al. Triple therapy with AZT and 3TC in combination with nelfinavir mesylate in 12 antiretroviral-naive subjects chronically infected with HIV-1. Abstract LB.B.6031, XI Intl AIDS Conf, Vancouver, B.C., 1996.

Markowitz M, Cao Y, Hurley A, et al. Virologic and immunologic response to triple therapy with nelfinavir in combination with AZT/3TC in 12 antiretroviral naive HIV-infected subjects at 20 months. Abstract 371, Fifth Retrovirus Conference, 1998

Martel L, Valentine M, Ferguson L, et al. Virologic and CD4 response to treatment with nelfinavir in therapy experienced but protease inhbitor naive HIV infected children. Abstract 233, Fifth Retrovirus Conference, February 1998

Mascolini M. Nelfinavir is number 4 -- but should it bat cleanup, or lead off? *Journal of the International Association of Physicians in AIDS Care* 1997;3(6):17-21.

McNicholl IR, Mogyoros M. A descriptive report on 16 protease inhibitor (PI) experienced HIV positive patients receiving nelfinavir. Abstract I-196, 37th ICAAC, Toronto, 1997

Moyle GJ, et al. Extended follow-up of safety and activity of Agouron's HIV proteinase inhibitor AG1343 (Viracept®) in virological responders from the UK phase I/II dose finding study. Abstract Mo.B.173, XI Intl AIDS Conf, Vancouver, 1996.

Patick AK, Duran M, Cao Y, et al. Genotypic analysis of HIV-1 variants isolated from patients treated with the protease inhibitor nelfinavir, alone or in combination with d4T or AZT/3TC. Abstract 10, 4th Retrovirus Conference, Washington, D.C., 1997.

Pedneault L, Elion R, Adler M, et al. A pilot study of safety and activity of the combination of stavudine, didanosine and nelfinavir in HIV-infected subjects. AIDS 1996; 10 (Suppl 2):17.

Pedneault L, Elion R, Adler M, et al. Stavudine (d4T), didanosine (ddI), and nelfinavir combination therapy in HIV-infected subjects: antiviral effect and safety in an ongoing pilot study. Abstract 241, 4th Retrovirus Conference, Washington DC, 1997

Physicians' Desk Reference, 51st Edition, Medical Economics Co., Inc., Montvale, NJ, 1997.

Powderly W, et al. The efficacy of Viracept (TM) (nelfinavir mesylate, NFV) in pivotal phase II/III double-blind randomized controlled trials as monotherapy and in combination with d4T or AZT/3TC. Abstract 370, 4th Retrovirus Conference, 1997

Saag MS, Knowles M, Chang Y, et al. Durable effect of Viracept (nelfinavir mesylate, NFV) in triple combination therapy. Abstract I-101, 37th ICAAC, Toronto, 1997.

Sampson MS, Barr MR, Torres RA, Hall G. Viral load changes in nelfinavir treated patients switched to a second protease inhibitor after loss of viral suppression. Abstract LB-5, 37th ICAAC, Toronto, 1997

Schmitt J, Agouron Pharmaceuticals. Letter to S Cox + M Harrington, 3 June 1997

Tebas P, Kane E, Klebert M, et al. Virologic responses to a ritonavir/saquinavir containing regimen in patients who have previously failed nelfinavir. Abstract 510, Fifth Conference on Retroviruses and Opportunistic Infections, February 1998

Wall Street Journal, "FDA Faults News Release on Agouron's AIDS Drug," 29 April 1997.

Yuen G, Anderson R, Daniels R, Kerr B. Investigations of nelfinavir (NFV) pharmacokinetic (PK) interactions with indinavir (IDV) and ritonavir (RTV). Abstract, 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

Zorrilla C, Clark R, Currier J, et al. Women first: a study in HIV positive women of quadruple therapy: nelfinavir (NFV), saquinavir (SQV), stavudine (d4T) and lamivudine (3TC). Abstract 722, Fifth Retrovirus Conference, February 1998

*

IVC. PROTEASE INHIBITORS

A Tale of Two Saquinavirs

ii. Saquinavir Hard Gel Capsules / Invirase™ Saquinavir Soft Gel Capsules / Fortovase™ (Hoffmann-LaRoche Co.)

by Mark Harrington

BACKGROUND

Fortovase™ is the new soft gel capsule (SGC) formulation of saquinavir. For the purposes of this report, we are treating it as a qualitatively new and different drug from Invirase™ brand saquinavir mesylate, which was approved in a hard gel capsule (HGC) formulation at 600 milligrams (mg) thrice daily (TID) by the US Food & Drug Administration (FDA) in December 1995; it was the first licensed HIV protease inhibitor. Fortovase™ was approved by the FDA at a dose of 1,200 mg TID on November 7, 1997.

About the sponsor. This is the house that Valium built. Hoffmann-LaRoche Inc.is a Swiss-based global giant with far-flung investments in pharmaceutical and biotechnology research. Despite its size and wealth, however, Roche has seldom missed a chance to botch the development of any of its many AIDS-related drugs, from the fiasco that was ddC to the joke that was the Invirase to the more recent decision to slow down the development of valganciclovir, the oral ganciclovir prodrug which offers the hope for finally being an effective oral prophylaxis and maintenance treatment for cytomegalovirus (CMV) disease. As one high-placed Federal official noted (off the record, of course) of the latter decision, "Roche finally has a decent drug, and they're thinking of dropping it."

ANTIRETROVIRAL POTENCY

Saquinavir, like ddC, is the most potent drug of its class -- *in vitro*. However, in the originally licensed hard-gel capsule formulation, only 4% of the drug got into the bloodstream. Hoffmann-LaRoche was in such a hurry to get its drug licensed as the first protease inhibitor that it never bothered doing the dose-ranging studies which could have defined a maximum tolerated dose (MTD) for saquinavir.

CLINICAL TRIALS & TRIBULATIONS

I was a member of the ACTG's Primary Infection Committee in fall 1992 when Roche approached them to conduct the phase II study, dubbed ACTG 229. The dose chosen was 600 milligrams (mg) thrice daily, based, they claimed, on three European phase I studies, or, as others thought, on a limited drug supply which made higher doses impractical -- or not worth Roche's investment. While the Primary Infection Committee was never known as a bastion of open scientific debate, ACTG 229 was swaddled in a secrecy unusual even for them. Roche declined to present the results of its phase I studies to the

committee as a whole. Rather, they allowed Thomas Merigan of Stanford University and Ann Collier of the University of Washington at Seattle to take a peek at the alleged phase I virological response to saquinavir.

The study would take place in AZT-experienced patients, then the favorite population for trials of new antiretrovirals (remember ACTG 155?). They would be randomized to receive either AZT and ddC, AZT and saquinavir, or AZT, ddC and saquinavir. This was one of the first of the so-called "incestuous combination" studies pilloried in 1997 by Joep Lange, in which a company's own drugs are studied together as much as possible, regardless of the scientific rationale (or lack thereof) for doing so -- Glaxo can you hear me?

Preliminary review of the study design by the Division of AIDS (DAIDS) and Harvard's Statistics & Data Analysis Center (SDAC) raised several concerns, which I mentioned to Dr. Collier (the principal investigator of ACTG 229) in a letter on 30 September 1992:

> I remain perplexed about the current design of ACTG 229. *In particular, I share the CTRC's concern "about the selection of 600 mg TID as the dose of Ro 31-8959* [saquinavir] *since there is no established maximum tolerated dose*" [NIAID Clinical Trials Review Committee letter, 27 August 1992]. Doses as high as 1200-1800 mg TID have been tested in HIV-negative patients and found to be safe... but people with HIV have only been given doses up to 600 mg TID. I would concur with the CTRC that "the need for the pharmaceutical sponsor to be forthcoming with data from their European trials" is pressing as we proceed towards opening ACTG 229...
>
> I became even more concerned when I read David Schoenfeld's SDAC review. His bottom line was that "*the proposed study will not be able to detect whether Ro 31-8951 has moderate activity.*" (Harrington 1992)

Needless to say, the ACTG brushed aside the concerns of statisticians and activists and conducted the study as Roche wished it to. 300 AZT-experienced individuals were enrolled and followed for 18 months.

By June 1994, Roche had detected the surrogate marker response it hoped for (triple drug combination proved superior to either two drug combination as measured by CD4 cell response and, less impressively, by viral load). As Schoenfeld predicted, the study failed to show whether saquinavir was any more potent than ddC, the weakest of the nucleoside analogues *in vivo* (Collier 1996). Undeterred by this minor annoyance, Roche promptly petitioned the FDA to consider an accelerated new drug application (NDA) for saquinavir.

Worried by the precedent this would set for the protease inhibitors as a class, TAG joined several other AIDS organizations and wrote to FDA Commissioner David Kessler requesting that accelerated approval for saquinavir be placed on hold until a full and open public debate could take place to assess how much data would be required for accelerated approval of protease inhibitors, and how post-marketing confirmatory studies should be designed (Cox 1994).

In the controversy that ensued, Roche quietly agreed to double the size of its pivotal efficacy trials,

thereby increasing their ability to determine whether saquinavir provided any clinical benefit. Unfortunately, the study which was eventually to provide such evidence -- Roche NV14256B -- compared saquinavir to ddC to the combination in AZT-experienced patients. Since the role of ddC in this population is far from clear, and its benefit dubious in any population, such a control arm must be regarded as questionable. Nonetheless, to no one's surprise, the combination of these two drugs, each the weakest in its class, proved to be more potent than either one alone.

This led to accelerated approval for saquinavir, now dubbed INVIRASE™, by the FDA in November 1995. The drug was licensed at the dose studied in ACTG 229, 600 mg thrice daily, despite the fact that there was *already* evidence at the time that a dose twice as high was more potent and equally tolerable (Schapiro 1995). Moreover, it was already known at the time that suboptimal doses of protease inhibitors might predispose HIV towards the development of resistance and possibly even cross-resistance to other protease inhibitors (Condra 1995).

RESISTANCE & CROSS-RESISTANCE

Thus, ever since the licensure of saquinavir-HGC at the end of 1995, Roche has known that the licensed dose was suboptimal and that its use could well result in widespread cross-resistance to multiple protease inhibitors. Had saquinavir rapidly become the drug of choice for people who were failing on nucleoside analogue monotherapy or double therapy, a public health disaster might well have resulted. If cross-resistance became widespread through broad and prolonged use of saquinavir, many people would not have been able to benefit from the later introduction of more potent protease inhibitors.

Luckily, help was not long in coming. Within three months, both Abbott's NORVIR® brand ritonavir and Merck's CRIXIVAN® brand indinavir were licensed, at doses which were able, when given in combination with new reverse transcriptase inhibitors, to drive viral load beneath the limit of detection in over 75% of patients who could tolerate them for up to one year (Merck 035, etc.), and could prolong health and life when compared with standard of care (Abbott study, ACTG 320). Of note, Roche's survival study used ddC monotherapy, which no one, even then, regarded as standard of care.

Roche also completed an unexciting European study in antiretroviral naive patients, SV14604C (AZT vs. AZT/ddC vs. AZT/saquinavir-HGC vs. AZT/ddC/saquinavir). 3,485 antiretroviral naive (no more than 16 weeks AZT experience), HIV-infected individuals enrolled in 22 countries. Baseline CD4 was around 200 and median baseline HIV RNA was 5 logs. The triple drug regimen scored a 50% reduction in clinical endpoints compared with either two drug arm (Roche 1997b):

SV 14604: AZT/ddC vs. AZT/SQV-HGC vs. AZT/ddC/SQV-HGC

	AZT/ddC	*AZT/SQV-HGC*	*AZT/ddC/SQV-HGC*
AIDS or death	142	116	76

This failed to deter Roche from charging $5,800 wholesale for a year's supply of INVIRASE™, an inexplicably high price for such a weak drug. Yet Roche faced a quandary. Despite its slipshod, post-haste development plan, two other protease inhibitors reached the market within three months of its own NDA. Even those unversed in the intricacies of retrovirology could tell they were more potent.

How could Roche redeem its drug? Two opportunities presented themselves. One was boosting saquinavir exposure by co-administering saquinavir with ritonavir, which would inhibit cytochrome p450 metabolism, thereby increasing the bioavailability, exposure, half-life, and maximum concentration of saquinavir to therapeutic levels. The other, more prosaic, approach was to finally begin addressing the need for a more bioavilable formulation and higher dose of saquinavir itself, unassisted by complex hepatometabolic pathways. Roche proceeded to follow both leads.

As for those participants lucky enough to survive ACTG 229, they were given the chance to enroll in ACTG 333, the first-ever randomized study in protease failures. ACTG 333 randomized 72 SQV-experienced individuals to continue on hard gel cap (HCG) saquinavir at 1.8 grams/day, switch to the more bioavailable soft gel capsule (SGC) formulation at 3.6 grams/day, or switch to indinavir at 2.4 grams/day. They were asked *not to switch underlying nucleoside analogues* for the first eight weeks of the study. The primary endpoint was virologic response. The study would stop early if no arm achieved greater than a 0.7 $\log_{10}$ reduction in HIV RNA. After an interim analysis conducted when 72 patients reached 8 weeks of follow-up showed that no arm did in fact achieve such a reduction, ACTG 333 was terminated. Participants had received an average of 112 weeks of prior saquinavir therapy. 86% were male, 75% white, non-Hispanic, and the median age was 43. Median baseline HIV RNA was 20,911 copies/ml; 6% had fewer than 200 RNA copies/ml at entry. Median baseline CD4 was 220 cells/mm^3. Follow-up for the first 72 subjects was a median 18 weeks (range 12-22 weeks).

ACTG 333: 8 Week RNA and CD4 Results

	HIV RNA reduction	*Undetectable (<200/ml)*		*CD4 change (/mm^3)*
		*Ever**	*At week 8*	
SQV-HGC	+0.04 log	2/24 (8%)	2/22 (9%)	- 0.4 cells
SQV-SGC	-0.23 log	4/22 (18%)	2/20 (10%)	+ 37 cells
IDV	-0.58 log	9/21 (43%)	7/19 (37%)	+ 22 cells

(ACTG 1997)

* Undetectable at one or more of the week 2, 4, 6, or 8 timepoints.

The study team commented that "while there was variability in the RNA responses in individual subjects in both the IDV and SQVsgc arms, the mean decreases in RNA and mean CD4 cell increases in both arms was [sic] less than seen in other trials of protease inhibitor[s] used in combination with nucleosides." (ACTG 1997). Based on these disappointing results, accrual to ACTG 333 was terminated. Already enrolled patients were allowed to remain on assigned therapy or switched based on virological response.

Genotypic analysis of patients in ACTG 333 found no relationship between resistance mutations at study baseline and virologic outcome at weeks 8, 16 or 24. This finding raises serious concerns about the utlility of genotypic tests in assessing the likelihood of response to protease inhibitors after an initial treatment failure. Several other things are notable about ACTG 333:

1. These were sequential monotherapy patients, many given first AZT, then AZT/ddC or AZT/saquinavir (in ACTG 229), then given SQV-HCG, SQV-HCG or indinavir, without regard to treatment history or virological status at baseline. Certainly the trial would be designed differently if it were begun today.

2. ACTG 333 participants had almost two years (112 weeks) of previous saquinavir experience upon enrolling into 333.
3. Most participants switched to SQV-SGC did not experience much of an antiretroviral benefit. The minority who did probably had not been receiving therapeutic doses of SQV-HGC, and hence had not developed SQV resistance.
4. Most participants switched to indinavir experienced far less of a viral load reduction than typical with this drug when given as a first protease inhibitor[4]. However, results are given for indinavir patients *as a group*. They might fall into three subgroups: a) fully susceptible to indinavir; b) partially susceptible to indinavir, like the group average); and c) wholly resistant to indinavir.

After the ACTG 333 fiasco, Roche called various community groups in a series of anxious conference calls to try and squelch doubts raised by the study. Roche's whole marketing campaign for INVIRASE® was based on the drug's alleged tolerability and the presumption that you could use it as a first-line protease inhibitor and then go on to use others without fear of cross-resistance[5]. ACTG 333 called this notion into doubt. Moreover, on one of these calls, Roche representatives admitted that saquinavir HGC, when used with AZT and 3TC in antiretroviral-naive individuals, lowered viral load beneath the limit of detection in fewer than 40% of patients -- less than AZT/ddI/nevirapine in INCAS/BI 1046.

Of note, genotypic analysis from ACTG 333 was presented at the Fifth Retrovirus Conference in February 1998. Most subjects had protease-associated gene mutations at baseline; 42/81 (52%) had mutations at position 90 and 6/81 (7%) at position 48. The analysis did not indicate that the presence or absence of these mutations at baseline was clearly associated with response or lack thereof to the new treatment, once again calling into question the utility of current genotypic resistance assays in predicting response to treatment or, indeed, in helping to guide therapeutic decisions (Para 1998).

Roche's anxieties were deepened when it apparently received a preliminary draft of the HHS Clinical Practice Guidelines for Treatment of HIV Infection and discovered that -- *quelle surprise!* -- saquinavir did not make the cut as a first-line protease inhibitor.[6] Spurred by the prospect of being left off formularies across the country, Roche decided to accelerate its filing for FDA approval of the new saquinavir-SGC formulation.

---

[4] In Merck 028, protease-naive patients given indinavir as monotherapy experienced a 1 log reduction in HIV RNA at two weeks which was sustained for 24 weeks, by which point 37% of them had HIV RNA levels below 500 copies/ml. CRIXIVAN (indinavir sulfate) package insert, Merck & Co., 1996.

[5] Roche advertisements for INVIRASE™ ran until fall 1997, asking"When considering an HIV protease inhibitor... Consider a protease inhibitor you can live with," and "What's your strategy...?"

[6] The 1997 version of the *HHS Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* commented on the role of saquinavir with two nucleosides as first-line therapy with a footnote in Table VI which reads, "The current hard gel capsule formulation of saquinavir is not recommended do to poor bioavailability..." (HHS 1997). The company lobbied to change this rational and, indeed, restrained, comment. Its efforts failed. However, Roche put out a positive press release when Fortovase™ was approved by the FDA (Roche 1998).

Roche scientists have asserted that Fortovase™ the new (SGC) saquinavir provides *eight to nine times* the exposure of the earlier Invirase™ hard gel capsule (HGC) formulation (Roche 1997). Intriguingly, despite this improved bioavailability, the new formulation was approved at *twice* the dose of saquinavir-SGC, a tacit admission by the company that Invirase™ was licensed at *too low* a dose.

Recent Fortovase™ Clinical Data

The "SUN" study of saquinavir-SGC was an observational study of SQV-SGC in combination with AZT/3TC in antiretroviral naive patients. The combination produced fairly substantial reductions in plasma HIV RNA, with saquinavir-SGC behaving more like a potent protease inhibitor than saquinavir-HGC ever did:

SUN Study: SQV-SGC + AZT + 3TC

Log_{10} HIV RNA Change From Baseline (LOQ = 20 copies/ml)

	Week 20	*Week 24*	*Week 32*
N	24	21	20
Mean	-3.2	-3.2	-3.4
Median	-3.2	-3.3	-3.3
Range	-4.5 to -0.7	-4.8 to -0.4	-4.5 to -1.8

* LOQ = limit of quantification

At week 32, approximately 90% of study participants had plasma HIV RNA counts below the limit of quanitification on the standard Amplicor assay (<400 copies/ml). Approximately 70% of participants at week 32 had plasma HIV RNA levels that were less than 20 copies/ml. Moderate to severe side effects that occurred in more than five percent of participants included nausea (n=6), vomiting (n=4), diarrhea (n=4) and headache (n=5). Laboratory abnormalities included mainly liver enzyme elevations, although one case each of neutropenia and elevated triglycerides was seen.

Study NV15355 compared saquinavir-HGC to saquinavir-SGC, both combined with two nucleoside analogs, in treatment naive patients. Most study participants chose to take either AZT/3TC or d4T/3TC as their nucleoside analogs. 81 patients were assigned to receive saquinavir-HGC, and 90 patients to recevieve saquinavir-SGC. The comparative study lasted sixteen weeks, after which all patients were treated with saquinavir-SGC. At week sixteen, there was no significant difference in mean plasma HIV RNA reduction between saquinavir-SGC and saquinavir-HGC. However, significantly more patients had plasma HIV RNA levels below the limit of quantification at week sixteen on saquinavir-SGC (80% <400 copies, 47%<50 copies)) than on saquinavir-HGC (43% <400 copies, 28% <50 copies). At week 32, 79% of patients taking saquinavir-SGC were still below 400 copies/ml, and 66% were below 50 copies/ml. Clinical adverse events in this study were mainly nausea, diarrhea, flatulence and adbominal discomfort/abdominal pain (Hoffmann-LaRoche 1997).

The Cheese study ("**C**omparative trial in **H**IV infected patients **E**valuating the **E**fficacy and **S**afety of saquinavir **E**nhanced oral formulation") compared the safety and efficacy of saquinavir-SGC in

combination with AZT/3TC to that of indinavir/AZT/3TC in antiretroviral naive patients. After six months of therapy, 93% of patients taking saquinavir-SGC and 92% of patients taking indinavir had undetectable plasma HIV RNA levels (<400 copies/ml). Patients taking saquinavir-SGC also had a larger CD4 cell increase than those taking indinavir (124 cells on SQV-SGC vs. 49 cells on IDV at week 12), though this difference was not statistically significant. The main adverse event in patients taking saquinavir-SGC was "gastrointestinal complaints," which were identified in six participants (Borleffs 1997, 1998).

Adverse events: NV15182. At the 37th ICAAC in fall 1997, Roche presented safety data from NV15182, a 444 person study of saquinavir soft gel capsules plus other antiretrovirals. 10% of the participants were women and 27% were non-white. 95.5% were antiretroviral experienced and 18% were protease experienced.

Side Effects of Fortovase vs. Invirase from NV 15182 and NV 15355

	NV 15182	*NV15355*	
	Fortovase + TOC	***Invirase + 2 NRTIs***	***Fortovase + 2 NRTIs***
N	442	81	90
Duration of study	48 weeks	-- 16 weeks --	
Gastrointestinal side effects			
Diarrhea	19.9%	12.3%	15.6%
Nausea	10.6%	13.6%	17.8%
Abdominal discomfort	8.6%%	4.9%	13.3%
Dyspepsia	8.4%	--	8.9%
Flatulence	5.7%	7.4%	12.2%
Vomiting	2.9%	1.2%	4.4%
Abdominal pain	2.3%	1.2%	7.8%
Taste alteration	--	1.2%	4.4%
Constipation	--	--	3.3%
Other side effects			
Headaches	5.0%	4.9%	8.9%
Fatigue	4.8%	6.2%	6.7%
Insomnia	--	1.2%	5.6%
Depression	2.7%	--	--
Pain	--	3.7%	3.3%

(Hoffmann-LaRoche 1997)

TOC = treatment of choice; NRTI = nucleoside reverse transcriptase inhibitor

Fourteen percent of patients withdrew prematurely, 21 (5%) due to adverse events (mainly gastrointestinal). Marked laboratory abnormalities were seen with AST (3%), ALT (2%) and bilirubin (1%). Two serious adverse events were thought related to Fortovase™ by the investigator. One patient with hemophilia suffered a cerebral hemorrhage but was able to resume SQV-SGC without bleeding problems. A second patient was hospitalized for diarrhea. Overall, 43% of participants had a viral load beneath the limit of quantification at week 24, including 28% of the protease-experienced and 75% of the RTI naive patients.

Pharmacokinetic interactions with other drugs. Because Fortovase is so much more bioavailable than its predecessor, it is probably unwise to extrapolate drug interactions from what is known about Invirase. Among the interactions specifically mentioned in the Fortovase package insert are the following:

Effect of Fortovase on Concomitant Drug Concentrations and Vice Versa

Drug	*Effect of Drug on Fortovase*	*Effect of Fortovase on Drug*
Clarithromycin	177% increase in SQV AUC	45% increase in Clari AUC; 24% decrease in Clari 14-OH metabolite AUC
Indinavir	364% increase in SQV AUC	NG
Nelfinavir	392% increase in SQV AUC	18% increase in NFV AUC
Ritonavir	1700% increase in SQV AUC	NG
Delavirdine	500% increase in SQV AUC	NG
Nevirapine	24% decrease in SQV AUC	NG
Efavirenz	***60% decrease in SQV AUC***	NG
Ketoconazole	130% increase in SQV AUC	NG
Rifabutin	***43% decrease in SQV AUC***	NG
Rifampin	***84% decrease in SQV AUC***	NG

(Hoffmann-LaRoche 1997)

AUC = area under the curve; NG = not given; drugs which the sponsor recommends not be taken concurrently with Fortovase are in ***bold italics***..

Drugs contraindicated with Fortovase™ include:

* Terfenadine, cisapride, astemizole, triazolam, midazolam, or ergot derivatives

Drugs which should be used with caution with Fortovase™, or for which substitutes should be sought, include:

* Rifabutin and rifampin

Other drugs which may decrease Fortovase™ plasma concentrations include:

* Carbamazepine, phenobarbital, phenytoin, corticosteroids including dexamethasone

DuPont Pharma recommends that Fortovase™ not be taken concurrently with Sustiva™ brand efavirenz. Recent data indicate that efavirenz lowers Fortovase™ plasma concentrations by about 60% -- enough to render the drug ineffective and thus to speed up the development of resistance (James 1998).

Liver enzyme levels should be carefully monitored in people who are taking Fortovase™ in combination with Rescriptor™ brand delavirdine, as 13% of subjects in a small preliminary study developed elevated liver enzymes, and 6% had grade 3 or 4 LFT elevations (Hoffmann-LaRoche 1997).

Pediatric study. Among HIV-infected children treated with saquinavir-SGC plus two NRTIs, 60% achieved viral load below 400 copies after 16 weeks; 50% achieved viral load below 50 copies.

Comparative resistance data. Patterns of genotypic resistance mutations that arise following treatment with saquinavir-SGC are similar to those arising after saquinavir-HGC therapy. Genotypic data from the early studies Fortovase™ were presented in Chicago during February 1998. Pre- and post-therapy protease genes were sequenced from viral isolates taken from 74 patients enrolled in three differentclinical trials. Protease sequences were not detected in 30 patient samples, and one patient had the 48V and 90M mutations at baseline. The relative frequency of 48V and 90M after therapy was 13/74 (17.6%) and 16/74 (21.6%) respectively, equivalent to a 48V:90M ratio of 0.81, compared with ratios of approximately 0.2 in trials of Invirase™. The investigators conclude that the soft gel capsule version of saquinavir increases the relative incidence of 48V mutations over 90M mutations compared with the hard gel capsule formulation. Another interesting difference was that a mutation at position 71 was present in 4/74 (5.4%) patients at baseline and in 13/74 (17.6%) after a median of 16 weeks treatment. Further genotypic analysis of samples from subsequent studies is underway (Craig 1998).

Fortovase in double-protease combinations. Fortovase is being combined with each of the other approved protease inhibitors in ongoing studies. For more information, see Spencer Cox's chapter on New Treatment Strategies, above. The dose of Fortovase™ when used in combination with ritonavir is the same as the Invirase™ dose (400 or 600 mg BID).

Fortovase makes the cut. Recently, the committee responsible for establishing the US PHS Guidelines for the Treatment of HIV Infected Adults and Adolescents reviewed available data on Fortovase and concluded that is was in fact a potent protease inhibitor which should be added to the list of "preferred" treatments. However, long-term data are still needed to ensure that saquinavir-SGC is as potent as other protease inhibitors.

*

REFERENCES

ACTG 333 study team, "Executive Summary of Interim Analysis of ACTG 333," 3 April 1997.

Borleffs JC, Boucher CA, Bravenboer B, et al. Saquinavir soft gel capsules versus indinavir as part of AZT and 3TC containing triple therapy. Abstract I-92, 37th ICAAC, Toronto, September 1997

Borleffs JC for the CHEESE Study Team. First comparative study of saquinavir soft gel capsules vs indinavir as part of triple therapy regimen (CHEESE). Abstracts 387b, 391, Fifth Conference on Retroviruses & Opportunistic Infections, February 1998

Collier AC, Coombs RW, Schoenfeld DA, *et al.* Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *N. Engl. J. Med.* 1996;334:1011-7.

Cox C, Davidson D, Gonsalves G, Harrington M, Hogan C & Pringle Smith R. *Rescuing Accelerated Approval: Moving Beyond the Status Quo*, Treatment Action Group, September 1994.

Condra JH, Schleiff WA, Blahy OM *et al. In vivo* Emergence of HIV-1 Variants Resistant to Multiple Protease Inhibitors. *Nature* 1995;374:569-571.

Craig C, O'Sullivan E, Cammack N. Increased exposure to the HIV protease inhibitor saquinavir (SQV) does not alter the nature of key resistance mutations. Abstract 398, Fifth Conference on Retroviruses and Opportunistic Infections, February 1998

Gill MJ, Beall G, Beattie D, et al. Safety of saquinavir soft gelatin capsule (SQV-SGC) in combination with other antiretroviral

agents: multicenter study NV15182: 24 week analysis. Abstract I-90, 37th ICAAC, Toronto, September 1997

Harrington M. Letter to Ann Collier re ACTG 229, 30 September 1992.

HHS, "Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents," June 1997.

Hoffmann-LaRoche, Fortovase brand saquinavir package insert, November 1997.

James J. Efavirenz (Sustiva™) and saquinavir (Fortovase®) combination: pharmacologic interaction. AIDS Treatment News 296, 5 June 1998, p. 2.

Opravil M, for the SPICE Study Team. Study of protease inhibitor combination in Europe (SPICE); saquinavir soft gelatin capsule (SQV-SGC) and nelfinavir in HIV infected individuals. Abstract 394b, Fifth Retrovirus Conference, February 1998

Para MF, Coombs R, Collier A, et al. Relationship of baseline genotype to RNA response in ACTG 333 after switching from long term saquinavir (SQVhc) to indinavir (IDV) or saquinavir soft gelatin capsule (SQVsgc). Abstract 511, Fifth Conference on Retroviruses and Opportunistic Infections, Chicago, February 1998

Roche News, "Roche submits new drug application for new formulation of HIV protease inhibitor -- Provides eight to nine times the exposure of current formulation at studied dose." Press release, 12 May 1997a.

Roche News, "Largest-Ever AIDS Study Finds Three Drug AIDS Cocktail Better Than Two", press release, 2 June 1997b.

Roche News, "New data from first head-to-head protease inhibitor study show Fortovase™ (saquinavir) challenges market leader, Crixivan (indinavir sulfate). News release, 3 February 1998

Schapiro JM, Winters MA, Kozal MJ, Merigan TC. Saquinavir monotherapy trial: prolonged suppression of viral load and resistance mutations with higher dosage. Abstract LB-5, 35th ICAAC, San Francisco, 1995.

Sension M, Farthing C, Siemon-Hryczyk, et al. Saquinavir soft gel capsules (SGC) in combination with AZT and 3TC in treatment native [sic] patients. Abstact I-190, 37th ICAAC, Toronto, September 1997

Vázquez E. Bad medicine: Why the old saquinavir (Invirase) may be bad for your health. *Positively Aware* July/August 1997.

*

IVC. PROTEASE INHIBITORS

iii. Amprenavir / 141W94 (Glaxo Wellcome, Vertex Pharmaceuticals)

by Paul Dietz

> *Most disturbingly, some AIDS doctors fear that even before GW141 hits the market, some patients may have acquired resistance to it because they have fared poorly with the already-available protease inhibitors.*
>
> -- Michael Waldholz (*WSJ* 1997)

BACKGROUND

Amprenavir is an experimental HIV protease inhibitor discovered by Vertex Pharmaceuticals and licensed for clinical development to Glaxo Wellcome (outside of Asia) and Kissei Pharmaceuticals (for the Asian market). Amprenavir offers twice-a-day dosing without dietary limitation, and a resistance profile that may be distinctive from one or more of the approved protease inhibitors. While this early lab data has lifted the hopes of Vertex's faithful cadre of researchers, a great multitude of questions remain unanswered in the clinic. Fortunately or not, depending on one's perspective, the course of clinical trials plotted out by Glaxo Wellcome is a long and winding road.

About the sponsors. Vertex Pharmaceuticals is a small drug discovery company started by a former lead chemist from Merck. The company uses structure-based "rational" design methods to create small molecule drug candidates for the treatment of HIV, multidrug-resistant cancer, autoimmune diseases, and hepatitis C. Amprenavir is currently Vertex's most advanced drug candidate.

"Structure-based" or "rational" drug design gained interest in the 1980s as the availability of powerful computer-based modeling tools offered an alternative to the traditional "trial and error" drug screening process. Structure-based design begins with the use of X-ray crystallography and nuclear magnetic resonance to establish the three-dimensional structure of a therapeutic target, most often a protein. Using this information, scientists "design" a chemical compound that binds with the target protein in a way that either blocks or enhances the protein's natural activity. Once a lead compound is identified, additional design work is performed in an iterative manner to optimize the drug's potency and pharmacokinetic attributes.

Given its dearth of resources and development experience, Vertex has partnered with Glaxo Wellcome and Kissei Pharmaceuticals to guide amprenavir through clinical trials. Glaxo and Kissei will fund a large portion of the development cost and will provide technical and marketing expertise in exchange for distribution rights in their respective territories. Glaxo Wellcome is one of the world's largest pharmaceutical companies, and through its ownership of rights to AZT and 3TC, has a leading market share in the market for HIV antivirals. Glaxo's license includes the US, Europe, and certain other territories outside of Asia. Kissei, a mid-size Japanese drug company, has the rights for Japan, China, and

other markets in the far east. Vertex will receive a royalty on the sales of amprenavir should the compound pass all regulatory hurdles.

As if three companies holding financial stakes in amprenavir aren't enough, Glaxo and Vertex deemed it prudent to acquire a license from Searle, filer of certain patent applications over a broad class of protease inhibitors that apparently covers amprenavir. Vertex and Glaxo paid Searle the astronomical sum of $25 million up-front, and will also pay a royalty on future sales. Though in theory, the drug's price (should it pass all regulatory hurdles) will be more a function of its competitive characteristics than its development cost, you can bet that the price will in some way reflect the succession of stakeholders associated its development.

Mechanism of activity. Protease is an enzyme that enables HIV to cleave its proteins from the *gag-pol* polyprotein precursor chain, into smaller functional units essential to HIV infectivity. All inhibitors of HIV protease alter the action of this enzyme by binding within the protein-cleaving site, resulting in genesis of morphologically-altered noninfectious virus.

ANTIRETROVIRAL POTENCY

In vitro studies. Amprenavir is a potent inhibitor of HIV *in vitro* with a mean IC_{50} of 0.08 uM in HIV-infected MT4 cells and peripheral blood lymphocytes, and 0.012 uM against a diverse set of clinical isolates (St. Clair 1996). The IC_{90} is 40uM in CEM cells infected by HIV-IIIB (Kim 1995). These inhibitory concentrations are known to increase by 1.5 fold in plasma due to the binding of amprenavir to alpha-1 acid glycoprotein (Livingston 1995). C_{min} levels above the IC_{90} (adjusted for plasma protein binding) can be achieved with doses as low as 300 mg twice per day (BID). Doses of 1200 mg BID result in plasma-protein adjusted trough concentrations of up to 15 times the IC_{90} (Murphy 1997). According to the protocol from an ongoing ACTG trial, "results of animal and single-dose human studies have led some researchers to believe that a 600 mg BID dose of amprenavir would result in blood levels that are comparable to indinavir in terms of their relationship to IC_{90} for the compound" (ACTG 347). *In vitro*, amprenavir is synergistic with the nucleoside analogues AZT, ddI and abacavor, and the protease inhibitor saquinavir. Amprenavir also shows additivity with indinavir and ritonavir (St. Clair 1996).

CLINICAL TRIALS EXPERIENCE

Monotherapy. Preliminary results of a dose-ranging study were presented at the 36th ICAAC (Schooley). The study included forty-two protease-naive patients with between 150 and 400 CD4 cellsat study entry.

4 Week HIV RNA and CD4 Changes on Amprenavir

Dosage (BID)	*300 mg*	*600 mg*	*900 mg*	*1200 mg*
N	9	12	9	7
Baseline HIV RNA	4.72 $\log_{10}$	4.84 $\log_{10}$	4.75 $\log_{10}$	5.04 $\log_{10}$
HIV RNA change	-0.58 $\log_{10}$	-1.025 $\log_{10}$	-1.69 $\log_{10}$	-1.95 $\log_{10}$
Baseline CD4	254	303	305	266
CD4 change	+64	+85	+35	+110

(Schooley 1996)

The viral load suppression and CD4 response at the 1200 mg BID dose is good for monotherapy. Three patients (7%) discontinued the study due to adverse events -- two with rash, one due to worsening chronic colitis. This trial likely influenced Glaxo Wellcome's choice of the 1200 mg BID dosing regimen.

Amprenavir and abacavir (1592U89) in protease-naive patients. Nine patients were enrolled in a four week pilot study testing the safety and activity of amprenavir (900 mg BID) in combination with abacavir (1592U89), an experimental nucleoside analogue also in development at Glaxo Wellcome. Patients began the study with between 150 and 400 CD4 cells.

Amprenavir (900 mg BID) plus Abacavir (300 mg BID): 4 Week HIV RNA and CD4 Changes

	N	*Baseline*	*Change*	
HIV RNA	7	4.17 $\log_{10}$	-2.08 $\log_{10}$	
CD4 cells	7	223	+79	*(Bilello 1997)*

In this study, five of seven patients had their viral load drop below the limits of detection (400 copies). Two of nine patients withdrew from the trial due to adverse events - one with dysarthria (difficulty with speech) and rash, and the other because of nausea.

In a more recent reporting of data, 13/17 patients treated with abacavir with amprenavir are still on therapy at week 16. All participants have plasma HIV RNA levels below 400 copies, and 10 have HIV RNA levels below 50 copies (Mellors 1998).

While these preliminary data justify the expanded study of amprenavir with abacavir, we should keep in mind that potent data reported from early pilot studies in the past has often led to disappointment when data from a larger body of patients has shown more modest effects. Cases in point include the preliminary data reported at the 1995 ICAAC for the d4T/ddI combination, and the early results reported on nelfinavir plus d4T (see "Nelfinavir", above).

Instincts compel us to question Glaxo's pretext in conducting a trial that employs two of its development drugs in combination. (This same nepotistic testing strategy with 3TC, another compound not developed but controlled by Glaxo, and AZT helped increase sales of AZT and boost its position relative to d4T which also works well with 3TC.) But the combination of amprenavir and abacavir is of interest in that it couples two of the seemingly more potent and best penetrating HIV drugs in their respective classes. In addition, the twice-per-day dosing schedule and potentially lower cost offered by the dual

combination would be a desirable addition to the HIV arsenal. Since it is hoped that both drugs may benefit some antiretroviral-experienced patients, a similar trial is planned for patients that have failed triple therapy. We encourage Glaxo to initiate this trial with urgency.

In addition, data from a Glaxo-sponsored study of AZT/3TC versus AZT/3TC with multiple doses of amprenavir were presented at the 6th European Conference on Clinical Aspects and Treatment of HIV Infection in Hamburg. All 80 study participants received AZT/3TC, and were then randomized to receive an amprenavir placebo, or a dose of 900 mg, 1050 mg or 1200 mg twice daily of amprenavir. Median baseline viral loads for the four study arms ranged from 4.65 to 5.15 logs of HIV RNA copies. CD4 cell counts ranged from 312 to 422, and all patients were naive to protease inhibitors and 3TC.

AZT/3TC ± 3 Doses of Amprenavir: 12 Week Results

	AZT/3TC/placebo	*AZT/3TC/900 mg*	*AZT/3TC/1050 mg*	*AZT/3TC/1200 mg*
HIV RNA	-1.33	-2.08	-2.7	-2.65
CD4 cells	+91	+158	+83	+95

(Clumeck 1997)

RESISTANCE & CROSS-RESISTANCE

In vitro resistance. It's become sadly evident that HIV can develop high level resistance to the approved protease inhibitors while maintaining, or regaining through compensatory mutations, most or all of its ability to replicate. While resistance patterns have yet to be well characterized in the clinic, lab studies suggest amprenavir is susceptible to resistance just like all the other drugs that have come before.

Amprenavir's habitual triple mutation. *In vitro* serial passage of amprenavir has spotlighted a predominant triple-mutation at the 46, 47, and 50 residues. The mutation at codon 50, a key bonding point for the molecule, confers a moderate three-fold reduction in sensitivity to amprenavir. The addition of a second mutation, M46 to I/L, produces up to 7 times reduced sensitivity. Typically the third mutation to arise, I47V, yields a triple mutant with a 20-fold reduction in sensitivity. Despite improved growth properties over the mono-mutated virus, the trivariant is slightly growth impaired versus the wild type. To date, the 46/47/50 triple mutation has not been seen with other protease inhibitors, and such isolates remain susceptible *in vitro* to saquinavir and to a lesser degree indinavir (Painter 1996). We are obliged to caution, however, that the cross-resistance "equation" is more complex under real world conditions. Variables include, but are certainly not limited to, the duration of HIV infection, virulence and fidelity of the HIV strain, the intensity and duration of current and previous antiretroviral drug exposure, host factor variability, etc. As a result, promising early lab data is often contradicted in the clinic (for more information refer to "Saquinavir SGC" above). Clinical trials are in progress to study cross-resistance between amprenavir and other available protease inhibitors.

Other recurring mutations. Additional mutations have been observed with amprenavir *in vivo* including L10F, M36I, D60V, V77I, L184V and L101I/L (Painter 1996). While their significance is unclear at the moment, the fact that mutations at 10 and 84 are seen with other protease inhibitors is somewhat alarming. While some believe that they may have only compensatory effects on viral growth kinetics (Painter 96), the mutants arise rather quickly and reduce drug sensitivity *in vitro*.

Amprenavir Resistance Profile

Exposure*	Mutation	IC_{90}	*Fold Increase in IC_{90} After Serial Passage* AMP	IDV	SQV
0 / none	None	23	1	1	1
7 / 800	L10F, I84V	880	38	2	1
8 / 1600	L10F, I50V	2,000	87	NA	NA
9 / 1600	L10F, M46I, I47V, I50V	3,400	150	3	1
10 / 3,200	L10F, M46I, I47V, I50V, D60V	4,000	170	6	1

(Partaledis)

* Number of serial passages / selecting concentration (nM); NA = data not available assay not performed.

The mutation at codon 84, which emerges early *in vitro* but is then replaced by the more assertive I50V, is unsettling in that mutation at this residue is associated with resistance to indinavir and ritonavir.

Adding Amprenavir to the Protease Resistance Roster

HIV Protease Point Mutation Site

Saquinavir	10								***48***			63		71					***90***
Ritonavir	10	20				36	46				54	63		71		***82***	***84***		***90***
Indinavir	10		24			36	46				54	63	65	71		***82***	***84***		***90***
Nelfinavir				***30***	35	36	46							71	77			88	***90***
Amprenavir	10						46	47		50							84		

[Clinically significant mutations are shown in ***bold italic***; insufficient clinical data on amprenavir are currently available to determine what amprenavir-associated mutations may be clinically significant.]

Cross resistance to amprenavir after indinavir. HIV-infected cells that have been pretreated *in vitro* with indinavir are reportedly four fold less sensitive to amprenavir (Tisdale 1997).

Resistance in vivo. In January 1997, Glaxo Wellcome reported the results of genotypic (amino acid sequence) and phenotypic (drug sensitivity) resistance analyses from the previously-described four week dose-escalating trial of amprenavir monotherapy. Not surprisingly, some patients receiving low doses of amprenavir began to experience a rebound in viral load by week four, though no consistent pattern of amino acid substitutions was observed (Tisdale 1997 and D'aquila 98). Researchers dismissed the existence of sporadic mutations as the result of "natural genetic drift". With regard to the phenotypic analysis, one allegedly noncompliant patient demonstrated a 4-5 fold reduction in viral sensitivity to amprenavir during the study (Vertex 1997). Based on these data, Vertex issued a rosy press release stating that test results "showed that resistance does not appear to develop to amprenavir during four weeks of monotherapy, whether at the lower sub-optimal doses or at higher doses where potent antiretroviral activity was observed". In addition, the release stated that "phenotypic analyses showed no reduction in drug sensitivity after four weeks". Though the wording was carefully crafted to exclude the noncompliant patient, Vertex's statement borders on hyperbole. Rather than overstated fanfare, we

need the drug companies to direct resources and energy toward clinical trials that will measure the effect of amprenavir in patients that have failed other protease inhibitor treatments and vice versa. Toward this end, ACTG 373, a roll-over study from ACTG 347, will study the efficacy of an indinavir-containing regimen in patients with previous exposure to amprenavir monotherapy.

ADVERSE EVENTS & TOXICITY MANAGEMENT

Animal toxicology. Administration of amprenavir for 28 days was well tolerated in rats at doses up to 1,000 mg/kg/day, and in dogs at up to 400 mg/kg/day. Adverse events included reversible increases in the size of liver cells and liver weight in the rat, though this was not noted in the dog (ACTG 347).

Human toxicology. Doses of between 150-1200 mg BID were well tolerated in the Phase I/II dose-ranging study. Adverse events occurring in more than 10% of patients were rash, diarrhea / loose stools, and headache. The most frequent adverse experience associated with amprenavir is clearly rash. In pooled clinical trial data, approximately 20% of patients developed rash, with 3% experiencing severe (grade III/IV) rash. One case of Stevens Johnson syndrome was noted in a patient who was receiving a number of drugs in addition to his or her antiretroviral regimen, including one of a class of sulfa-based drugs that are known to induce Stevens Johnson Syndrome. The study drop out rate resulting from rash is said to be about 6%, though there are reports that use of antihistamines such as Benedryl have allowed a number of patients with less severe rash to be treated through until the condition resolves (personal conversation). The rash usually appears between day nine and twenty of starting the drug, but as the risk of rash does not appear to be dose-related, the use of a titration (dose escalation) schedule is not currently under consideration.

Note on hemophilia. Special caution may be warranted when administering protease inhibitors to people with hemophilia. "There have been reports of increased bleeding, including spontaneous skin hematomas and hemarthrosis, in patients with hemophilia type A and B treated with protease inhibitors. In some patients, additional factor VIII was given. In more than half of the reported cases, treatment with protease inhibitors was continued or reintroduced. A causal relationship has not been established." (Agouron 1997).

Note on diabetes. In June 1997 the FDA warned that approximately 80 cases of diabetes had been reported in patients taking HIV protease inhibitors, and advised doctors to monitor patients for abnormal blood sugar levels. Elevated blood sugar levels were found in some animal toxicology studies of amprenavir, so the situation will have to be closely observed as with other protease inhibitors.

PHARMACOKINETICS, FOOD & DRUG INTERACTIONS

Amprenavir is one of the smaller and more soluble protease inhibitors to reach clinical evaluation. Its bioavailability ranges between 40% and 90% in animals and is estimated to be 70% in humans, though the latter estimate is based upon algorithm rather than the complex tests required to achieve an accurate measure. According to Glaxo Wellcome, the C_{min} is 0.17 micrograms for the 900 g dose and the C_{max} is 5.00 micrograms / ml. The C_{max} for the 1200 mg dose reportedly ranges between 3.9 um and 18.0 um, but information on the C_{min} for this dose was not obtainable. The area under the curve (AUC) is said to be linear within the 300 mg to 1,200 mg dosing range, and interpatient variability for AUC, C_{max} and

C_{min} has been "minimal". The drug's half-life varies between seven hours at the 150 mg dose and ten hours at the 1200 mg dose. This compares favorably with indinavir's relatively short half-life of about two hours. Average plasma concentrations of amprenavir at eight and twelve hours after dosing were greater than 10 times the IC_{50} (Painter 1996). As a result, the twice daily 1200 mg dosing regimen is being employed in prospective clinical trials.

Dosing requirements. Fortunately, the absorption of amprenavir is not dependent on food intake and it may therefore be taken with meals or on an empty stomach. The disadvantage however, is that the 1,200 mg BID dose requires swallowing eight large capsules twice daily. Vertex is considering use of the pediatric liquid formation (at a 70 ml BID dose -- equal to roughly one-third of a soda can twice daily) as an alternative for adults who have difficulty swallowing the pills. They are also working to reduce the 16 pill daily requirement.

Protein binding. The binding of HIV protease inhibitors to human serum proteins has caused the failure of a number of promising drug candidates (remember the Searle, Dupont Merck, and early Upjohn drugs?). However, it is important to consider not only the drug's affinity for protein binding, but also whether the attachment is strong or weak -- the latter permitting the drug to free itself and impede the replication of HIV. This is illustrated by the cases of ritonavir and nelfinavir which are highly protein bound and yet have shown potent efficacy *in vivo*. In plasma, amprenavir is approximately 90% protein bound, mainly to alpha 1 acid glycoprotein. However, the off rate is extremely fast which suggests little effect on amprenavir's antiviral activity, though this could probably the reason that such a large dose of amprenavir is required. *In vitro* antiviral assays have shown that the addition of human plasma causes a modest two-fold increase in the IC_{90} (Livington 1995).

Lymph system penetration. The mesenteric (central body) lymph node tissue-to-blood concentration ratio in rats and mice was found to be more than 11 times based on AUCs with similar disappearance as in the blood (Painter 1996).

Central nervous system penetration. Tissue distribution studies indicate that the brain to blood AUC ratio is *about* 1.7 in the rat (Painter 1996). However, studies recently carried out at Vertex, also in the rat, indicate a lower brain tissue concentration. Brain tissue concentrations were found to be twice that of the cerebrospinal fluid (CSF) levels. ACTG 347 will assess the drug's penetration into the CSF. In addition, CSF data from a handful of patients in an ongoing four-drug study including amprenavir showed a 1.22 log drop in CSF HIV RNA after three to eight weeks of therapy (Kost 1998).

Seminal penetration. Semen is a major HIV transmission vector, and there is concern that penetration of protease inhibitors into the testes may be suboptimal. As a result, the ability of amprenavir-containing regimens to lower HIV levels in the semen is being examined in ACTG 347.

Drug interactions. In general, HIV protease inhibitors are inhibitors of the cytochrome-P450 family of liver enzymes. Such inhibition is known to alter levels of other protease inhibitors and nonnucleoside reverse transcriptase inhibitors in the human body. It appears that amprenavir, like indinavir and nelfinavir, is an inhibitor of CP3Y4A, and its drug interactions are therefore likely to be similar (personal conversation with Glaxo Wellcome). In a pilot study, coadministration of amprenavir and indinavir increased the C_{max} and AUC for amprenavir by 31% and 64% respectively. Indinavir levels appeared to

be unaffected. Circumoral paresthesia (mouth tingling) was reported by three of twelve patients (Sadler 1997).

The impact of coadministration of efavirenz and amprenavir is currently under study: the mean AUC, C_{max} and C_{min} of amprenavir were reduced respectively by 36%, 39% and 43% when the drug was administered with efavirenz, but the clinical significance of these change is unknown (Piscitelli 1998).

In addition, pharmacokinetic interaction studies suggest that doses of rifabutin should be decreased when the drug was given with amprenavir (rifabutin levels were increased by three- to six-fold), and rifampin should not be co-administered with amprenavir (Polk 1998).

No meaningful interaction was found with abacavir (Ravitch 1998).

ONGOING TRIALS

A wide array of clinical trials are underway or planned for amprenavir.

Phase II study with AZT and 3TC (PROA2002). In September, 1996, Glaxo initiated a Phase II open label dose ranging study of amprenavir in combination with AZT and 3TC in 80 patients. Patients in one of the four arms were given amprenavir placebo for the first 12 weeks of the study. The duration of this study has been extended from 12 to 48 weeks.

Phase III study with AZT and 3TC (PROAB3001). Without waiting for the results of the Phase II trial, Glaxo lunged amprenavir into a Phase III multinational white elephant that was intended to be pivotal but is now unlikely to provide a basis for FDA evaluation. In this trial, 240 adults were randomized to receive either amprenavir in combination with AZT and 3TC or to AZT and 3TC alone. You will recall that a similar indinavir trial, ACTG 320, was stopped early when the Data Safety Monitoring Board learned that a faster rate of disease progression occurred within the AZT/3TC control group. The use of the dual nucleoside arm in clinical trials is now regarded as unethical under the current treatment goal of suppressing viral load below the limits of detection. In study 3001, patients with two or more consecutive viral load measurements greater than 5,000 copies at week 16 are permitted to switch to open label amprenavir and add abacavir. The expected number of participating trial sites has been reduced from 30 to 13 and a sixteen-week data analysis is now being performed. For the moment, Glaxo has returned to the drawing board to begin a trial comparing triple therapy with amprenavir to indinavir in nucleoside-experienced patients.

Amprenavir monotherapy trial (ACTG 347 / 373 roll-over). The NIH-sponsored AIDS Clinical Trials Group (ACTG) conducted a perilous but interesting double-blind 24-week multi-center study that randomized 92 protease naive patients to either the triple combination of amprenavir with AZT and 3TC or amprenavir monotherapy with nucleoside placebo. The trial compared the relative safety, CD4 response, and proportion of patients in each arm whose plasma HIV RNA is suppressed below the limit of detection after 24 weeks of therapy. Genotypic and phenotypic resistance data were gathered from patients who do not achieve full viral suppression.

This author feels that the monotherapy application of amprenavir in this trial was a bit premature. The

use of a single agent is and should be discouraged under the presumption that monotherapy generally invites drug resistance and therefore treatment failure within a relatively short period of time; while antiretroviral drug combinations have been shown to slow resistance. However, in this case, the ACTG felt that the potent antiviral activity and lack of drug-induced mutations witnessed in the four-week monotherapy trial, along with trial safeguards, provided justification for the study to go forward. No doubt the prospect of a single drug able to achieve the same virological and immunological effects as triple therapy in all or a subgroup of patients provided a tempting rationale for this study.

As it turns out, the monotherapy arm was abruptly discontinued when the first 9 placebo patients rebounded from their virologic nadirs at week four ($p=0.0009$). Updated review showed that 13 patients on the amprenavir and one on the indinavir arm then experienced virologic failure ($p=0.0007$). Both groups experienced CD4 cell increases of about 50. Gastrointestinal side effects were the most common adverse events, and were more common in the triple therapy arm. After the premature termination of ACTG 343, all patients in the amprenavir monotherapy arm were offered open label indinavir/3TC/d4T/nevirapine (ACTG 373) or amprenavir/AZT/3TC (Murphy 1998).

We applaud trial administrators for showing the proper degree of concern and flexibility in putting a timely end to the suboptimal treatment arm in ACTG 347. The ACTG's use of "real time" viral load monitoring afforded this additional patient protection. According to the original protocol, subjects with detectable HIV RNA at weeks 16 and 20 were to be offered open-label triple drug therapy as a consolation though it is certainly questionable whether such "serial" triple therapy would be a wise choice at that point. However, in actuality, the monotherapy arm was discontinued sooner and failing patients are being encouraged to employ a more aggressive four drug indinavir-containing potpourri.

One could argue that the ACTG should have waited until the drug's long term efficacy in various combinations was first established. Moreover, it might have helped alleviate concerns if amprenavir's propensity to cause cross-resistance to other protease inhibitors had been ruled out prior to the trial as well. Also, like other studies, the viral load measurements for ACTG 347 incorporates a minimum level of detection of 500 copies. It is unclear why a lower minimum could not be employed given that more precise tests with minimums of 400, 200, and even 20 are available. Two interesting substudies will proceed in the open label phase of the trial:

* In substudy ACTG 847, a subset of volunteers will contribute cerebrospinal fluid (CSF) samples one hour after taking the first dose and at one other point during the 24 week treatment period. The patient's CSF will be evaluated for drug levels to assess the ability of the study drugs to enter and reduce HIV viral load in the CSF. It is hoped that HIV levels in the CSF are representative of brain tissue which is a known reservoir for HIV. Exaltations belong to the 35 trial participants who have volunteered for this intrusive substudy.

* In substudy ACTG 850, a subset of male patients will provide seminal fluid samples. The objective of this substudy is to assess the ability of the drugs to reach and reduce HIV levels in the semen. The testes are also a known biologic compartment for HIV and the semen is believed to be a medium for sexual transmission of the virus. Praise of a slightly different sort goes out to these unblushing patient volunteers as the seminal donations are donated on site.

Hopefully, the HIV isolated from CSF and semen can be analyzed for phenotypic and genotypic resistance to the study drugs.

According to ACTG 347 investigator Dr. Rob Murphy, preliminary results from the rollover study, in which failures on amprenavir monotherapy were treated with d4T/3TC/indinavir/nevirapine, "appear promising." (Murphy 1998)

Indinavir equivalence protocol (PROAB3006). Glaxo has launched a large scale pivotal study that will compare triple combinations containing amprenavir with those containing indinavir. The trial will enroll 460 PI naive patients with previous nucleoside exposure and will run for 48 weeks at 30 centers. In addition to measuring relative tolerance, viral load suppression and CD4 response, data will also be collected on resource utilization. To gauge the relative propensity of the drugs to induce cross resistance, failing patients will be permitted to change their protease inhibitor and/or the NRTI(s) and will be monitored for their response to subsequent therapy.

Double-protease combinations (PROA2001). In early 1997, Glaxo began a Phase II 24-week study to test the safety, pharmacokinetics and antiretroviral efficacy of amprenavir (800 mg *three-times-per-day*) in combination with either indinavir, nelfinavir or saquinavir. The trial is ongoing at three sites in the United States and will enroll a total of 48 patients. A fourth "control" arm will receive amprenavir monotherapy for three weeks then add AZT and 3TC. Entry criteria includes a CD4 count in excess of 200 cells and viral load over 10,000. Protease experienced patients are ineligible.

Phase III pediatric study (PROAB3004). While eleven anti-HIV drugs are licensed for the treatment of HIV-infected adults, only six are FDA approved for use in children. We are therefore relieved that a multinational, randomized, double blind study with a total of 210 pediatric patients (between the age of 6 months and 18) has started enrollment. The trial will compare the results of adding amprenavir or placebo to the child's current antiretroviral regimen, presumably a pair of nucleosides. Primary endpoints will be the proportion of subjects with viral load below 10,000 copies at weeks 16 and 48. As HIV is known to impair neurodevelopment, brain function assessments will also be made during the trial.

Adding a placebo or even amprenavir to ongoing nucleoside therapy in patients with HIV plasma levels greater than 10,000 copies is perplexing when viewed in comparison with adult trial designs. Unfortunately however, HIV is apparently much harder to control in very young children, and the difficult-to-tolerate protease-containing therapies are not yet to be regarded as the standard of care.

The dosage is being chosen from the results of an ongoing phase I pharmacokinetic trial in 20 HIV-infected children between the age of 6 and 13. Though an improved pediatric (sweet liquid) formulation is in development (the first was scrapped due to its bad taste), the trial will begin with 50mg capsules, thus delaying the enrollment of younger children. If the data warrants, Glaxo intends to file the pediatric and adult applications with the FDA simultaneously. According to Glaxo Wellcome, amprenavir will eventually have two pediatric formulations: a capsule and a solution.

Amprenavir quadruple-therapy in recent seroconverters (PROA2003). This study is being conducted in the Aaron Diamond Research lab of David Ho by Martin Markowitz. It will compare the combination of amprenavir, AZT, 3TC and abacavir with other so-called potential eradication arms in recent

seroconverters. A second arm will examine the same combination in those with chronic HIV infection but no prior exposure to protease inhibitors and 3TC. Seventeen week results were reported at Chicago in February 1998. Thirteen acutely infected and twelve chronically infected people enrolled and were given abacavir twice daily, amprenavir (1200 mg) with AZT and 3TC. The most common side effects were rash (7/25, 28%).

Amprenavir, Abacavir, AZT & 3TC: Glaxo's Gang of Four -- 12 Week Results

	Acute infection	*Chronic infection*
N	13	12
Baseline plasma RNA	192,651	57,174
Mean plasma RNA change (8w)	-2.61 log	-2.26 log
Plasma RNA went undetectable (8w)	14/20 (70%)	
Baseline CSF RNA	1,644	8,093
Median CSF RNA change (3-8w)	-1.22 log	
Baseline CD4	560	343
Mean CD4 change (12w)	+172	+126
Mean CD4+CD62L+RA+ change	+106	+ 29
Mean CD8+CD62L+RA+ change	+ 76	+ 61

(Kost 1998)

CSF = cerebrospinal fluid; CD62L+RA+ cells = naive cells

European study with abacavir. A study has recently been launched in Europe that tests the combination of amprenavir and abacavir in approximately 30 patients for 48 weeks.

PLANNED STUDIES

With abacavir in indinavir failures. This study will test the combination of amprenavir and abacavir in ten patients who have failed indinavir. This study will include 48 patients and run for 24 weeks.

AIDS dementia. As the design for this trial appears to be awaiting cerebro-pharmacokinetic data from ACTG 347, detailed information was not available. Its possible that this study will be conducted in combination with abacavir, which is known to have good CNS penetration.

Combinations With NNRTIs. Study 2007 will evaluate amprenavir in combination with NNRTIs in indinavir failures.

With so many unanswered questions, the lost time appears to be just that. We must not be tempted to grab too quickly for drug regimens with fewer pills and less frequent dosing intervals. Given HIV's hideous ability to outwit multiple drugs with one wave of mutations, patients may have only one or two shots at achieving maximal suppression of the virus. Therefore, the primary objective at this time should be drug efficacy and survival. With indinavir (Crixivan) and nelfinavir (Viracept) on the market, we will need a comprehensive and assuring body of data regarding the long term relative safety and durability of response for amprenavir before it can be regarded as an alternative first line therapy.

The issue of cross resistance is an entirely different matter. A fast track should be established for a new

drug that has shown the ability to help to people who have failed other protease inhibitors. Therefore, the most pressing open question surrounding amprenavir is its propensity to either cause or suffer from cross resistance. The expanded access program for amprenavir is only in early planning stages, and we do not know whether amprenavir will ever receive FDA approval. However, at the current pace, if all goes well, the NDA for amprenavir will be filed in the third quarter of 1998. As at least the twelfth AIDS drug to reach the market and the fifth protease inhibitor (counting saquinavir only once), it would behoove Glaxo to price amprenavir competitively.

One might think that increased sales volume resulting from more people seeking and staying on treatment would bring price reductions for HIV drugs. But this will need to be driven by competition. Should amprenavir and abacavir join AZT and 3TC in receiving FDA approval, Glaxo's shrewdly assembled Gang of Four could theoretically attain a tight grip on the market. While HIV drugs accounted for just 5% of Glaxo's 1996 revenues, this share is expected to grow to 12% of the total by 2000 (Business Week). Competition from the other nine drugs will be scattered among seven companies including: Roche (two), Bristol Myers Squibb (two), Merck (one), Abbott (one), Agouron (one), Boehringer Ingelheim (one), and Upjohn (one). (Gilead and Dupont Pharmaceuticals also have drugs in development). We can only wonder what commitment these companies will show in the face of Glaxo's fearsome machine.

*

REFERENCES

ACTG 347 protocol

Bart PA, Combination 1592/141W94 Therapy in HIV-1-Infected Antiretroviral Naive Subjects with CD4 Counts >400 cells/μl and Viral Load >5000 copies/ml, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 365

Bilello JA, et al. 1592U89, a novel carbocyclic nucleoside analogue with potent anti-HIV activity, is synergistic in combination with 141W94 an HIV protease inhibitor. [Abstract] 4th Retrovirus Conference, Washington, D.C., 1997

DePasquale MP et al, Mutations Selected in HIV Plasma RNA During 141W94 Monotherapy, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 406A

Drusano GL, Sadler BM, Millard J, et al. Pharmacodynamics of 141W94 as determined by short term change in HIV RNA: influence of viral isolate baseline EC_{50}. Abstract #A-16, 37th ICAAC, September 1997

Eron J et al, Preliminary Assessment of 141W94 in Combination With Other Protease Inhibitors, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 6

Kim EE, Baker CT, Dwyer MD, et al. Crystal structure of HIV-1 protease in complex with VX-478, a potent and orally bioavailable inhibitor of the enzyme. *Journal of the American Chemical Society*. 117(3): 1181-1182, 1995

Kost R, Cao Y, Vesanen M, et al. Combination therapy with abacavir (1592), 141W94, and AZT/3TC in subjects acutely and chronically infected with HIV. Abstract 363, Fifth Conference on Retroviruses and Opportunistic Infections, February 1998

Lazdins JK, et al. Effect of alpha-1-acid glycoprotein on CGP-61755. An in vitro comparative study with other relevant HIV protease inhibitors. [Abstract] 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 1996

Lederman M et al, Early T-Lymphocyte Responses to Antiretroviral Therapy with Abacavir (1592) and HIV Protease Inhibitors

(Pis), 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 364

Livinsgton DJ, Pazhanisamy S, Porter DJ, et al. Weak binding of VX-478 to human plasma proteins and implications for anti-human immunodeficiency virus therapy. *J Infect Dis*. 172(5): 1238-45, Nov 1995

Maschera B, et al. Human immunodeficiency virus. Mutations in the viral protease that confer resistance to saquinavir increase the dissociation rate constant of the protease-saquinavir complex. *J Biol Chem*. 271(52): 33231-33235, Dec 1996

Mellors J et al, Antiretroviral Effects of Therapy Combining Abacavir (1592) with HIV Protease Inhibitors, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 4

Murphy R, ACTG 347 Study Team. Interim Review and Update, Letter to ACTG 347 Study Participants. July 21, 1997

Murphy R, DeGruttola V, Gulick R, et al. 141W94 with or without zidovudine/3TC in patients with no prior protease inhibitor or 3TC therapy -- ACTG 347. Abstract 512, Fifth Conference on Retroviruses & Opportunistic Infections, February 1998

Navia MA, Sato VL, Tung RD: Design of VX-478, a potent inhibitor of HIV protease. *International Antiviral News*. p. 143-145

Painter GR, Ching S, Reynolds D, et al. 141W94 VX-478 KVX-478 *Drugs of the Future*. Prous Science 21(4): 347-350, 1996

Partaledis JA, Yamaguchi K, Tisdale M et al. 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. *Journal of Virology*. 69(9): 5228-5235, September 1995

Pazhanisamy S, Stuver CM, Cullinan AB, et al. Kinetic characterization of human immunodeficiency virus type-1 protease-resistant variants. *J Biol Chem* 271(3): 17979-17985, Jul 1996

Physicians' Desk Reference, 51st Edition, Medical Economics Co., Inc., Montvale, NJ, 1997

Piscatelli S et al, Effect of Efavirenz (DMP 266) on the Pharmacokinetics of 141W94 in HIV-infected Patients, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 346

Polk RE, Israel DS, Pastor A, et al. Pharmacokinetic interaction between ketoconazole (KCZ) and the HIV protease inhibitor 141W94 after single dose administration to normal volunteers. [Abstract A-61] 37th ICAAC, September 1997

Polk RE et al, Pharmacokinetic (PK) Interaction Between 141W94 and Rifabutin (RFB) and rifampin (RFP) After Multiple Dose Administration, 5th Conference on Retroviruses and Opportunistic Infections, February 1998, Abstract 340

Rao BG, Kim EE, Murcko MA. Calculation of solvation and binding free energy differences between VX-478 and its analogues by free energy perturbation and AMSOL methods. *J Comput Aided Mol Des*. 10(1): 23-30, Feb 1996

Sadler BM, Eron J, Wakeford J, et al. Pharmacokinetics of 141W94 and indinavir (IDV) after single dose coadministration in HIV-positive volunteers. [Abstract #A-56] 37th ICAAC, September 1997

Sadler BM, Millard J, Munnally P, et al. Evaluation of potential pharmacokinetic (PK) drug interaction between 141W94 and 1592U89 in HIV+ patients. [Abstract #A-62] 37th ICAAC, September 1997

Schooley RT. Preliminary Data on the Safety and Antiviral Efficacy of the Novel Protease Inhibitor 141W94 in HIV-infected Patients with 150 to 400 CD4 cells/mm^3. [Abstract] 36th ICAAC, September 1996

Schooley RT and the 141W94 International Study Group. Preliminary Data From a Phase I/II Study on the Safety and Antiviral Efficacy of the combination of 141W94 plus 1592U89 in HIV-infected patients with 150 to 400 CD4 cells/mm^3. [Abstract] 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997

Singh R, Chang SY, Taylor LC. In vitro metabolism of a potent HIV-protease inhibitor (141W94) using rat, monkey and human

liver. *Rapid Commun Mass Spectrom*. 9(10): 1019-1026, 1996

St.Clair MH, Millard J, Rooney J, et al. In vitro antiviral activity of 141W94 (VX-478) in combination with other antiretroviral agents. *Antiviral Res* 29(S-1), Jan 1996 [from abstract]

Stolberg SG. A revolution in HIV drugs excludes the tiniest patients. The New York Times. September 8, 1997, page 1

Symonds WT, Sadler BM, Chittick GE, Moss J. A clinical study of the HIV-1 protease inhibitor, 141W94 (VX-478) to evaluate a new soft gelatin capsule formulation and to determine the effects of food upon bioavailability. [Abstract] Ninth International Conference on Antiviral Research, May, 1996

Tisdale M. HIV Protease Inhibitors - Resistance Issues. *International Antiviral News*. p. 41-43

Tisdale M, Myers RE, Harrigan PR, et al. Analyses of HIV genotype and phenotype during 4 weeks dose-escalating monotherapy with the HIV protease-inhibitor 141W94 in HIV-infected patients with CD4 counts 150-400/mm^3. [Abstract] 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997

Vertex Pharmaceuticals. Resistance does not develop to HIV protease inhibitor 141W94 / VX-478 in a four week clinical study. [Abstract 593] 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997

Vertex Pharmaceuticals Press Release: Early Results Suggest Potential for New Combination of Investigational HIV Drugs. January, 1997

Vertex Pharmaceuticals Press Release: Glaxo Wellcome Begins Phase II Clinical Trial of 141W94 / VX-478 in Double HIV Protease Inhibitor Treatment Regimens. January, 1997

Vertex Pharmaceuticals Press Release: Resistance Does Not Develop to HIV Protease Inhibitor 141W94 / VX-478 in Four Week Clinical Study - Report at the Fourth Conference on Retroviruses and Opportunistic Infections - January, 1997

Vertex Pharmaceuticals Press Release: AIDS Clinical Trials Group (ACTG) Begins Clinical Study of 141W94 / VX-478, an HIV Protease Inhibitor. February, 1997

Vertex Pharmaceuticals Press Release: Phase III Pivotal Clinical Trial Begins with 141W94 / VX-478, a Protease Inhibitor for Treatment of HIV Infection and AIDS. February, 1997

Vertex Pharmaceuticals Press Release: Vertex and Glaxo Announce Pivotal Phase III Clinical Trial of HIV Protease Inhibitor 141W94 (VX-478) in Pediatric Patients. July, 1997

Vertex Pharmaceuticals ***http://www.vpharm.com*** website, June 1997

Vertex Pharmaceuticals Annual Report 1996

Vertex Pharmaceuticals S1 Registration March 1997

Waldholz M. *The Wall Street Journal*, 23 January 1997

Waldholz M. Why isn't Glaxo's New AIDS Drug Ready Yet? *The Wall Street Journal*, 23 January 1997

Wooley J, Studenberg S, Boehlert C, et al. Cytochrome P-450 isozyme induction, inhibition, and metabolism studies with the HIV protease inhibitor, 141W94. [Abstract #A-60] 37th ICAAC, September 1997

*