PROTEASE INHIBITORS

Recent clinical findings with candidate protease inhibitors were discussed at the New York meeting by Michael S. Saag, MD, from the University of Alabama at Birmingham.

The HIV-1 protease performs a critical function in the life cycle of the virus, cleaving polyprotein precursors into enzymes (such as integrase, reverse transcriptase, RNAse H, and protease itself) and structural proteins (such as the nucleocapsid, capsid, and matrix) that make up the central viral core. The process begins during the final stages of viral assembly and continues as the virion buds through the host cell membrane. A number of pharmaceutical companies have designed and developed molecules that fit into and bind in the cleavage site of the protease enzyme to inhibit its function in producing the core components. HIV-1 cell lines exposed in vitro to protease inhibitors produce noninfectious virions characterized by the absence of the dense central viral core (Figure 1). Problems with protease inhibitor development have included increasing the water solubility of the molecules to ensure and enhance oral bioavailability and an expensive, demanding, and laborious production process.

Saquinavir (Ro31-8959)

Four protease inhibitors have undergone evaluation in HIV-infected subjects. Saquinavir (initially Ro31-8959), the most extensively evaluated, is now undergoing phase III testing. Initial evaluation of the compound consisted of three European 16-week phase I studies in which the compound was found to be well-tolerated and to have minimal side effects. In two monotherapy studies, saquinavir at dosages of 75 to 1800 mg/d was found to be associated with dose-related CD4+ cell responses. In one combination study in zidovudine-naive patients, the combination of saquinavir and zidovudine was associated with greater CD4+ cell response than either saquinavir or zidovudine monotherapy.

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Subsequently, saquinavir was evaluated in ACTG 229, the largest trial of a protease inhibitor in humans to be completed to date. Patients with CD4+ cell counts of 50 to 300/μL and extensive prior zidovudine treatment (median >24 months) were randomized to receive saquinavir 600 mg tid plus zidovudine 200 mg tid, zidovudine plus zalcitabine 0.75 mg tid, or a combination of the three agents. After 24 weeks of treatment, the triple-drug regimen was associated with greater reductions in viral load and a greater and more enduring CD4+ cell response. Figure 2 shows the change in mean CD4+ cell count over 24 weeks. CD4+ cell counts increased by at least 50/μL in 39% of the triple combination group, 28% of the zidovudine-saquinavir group, and 21% of the zidovudine-zalcitabine group. Decreases in plasma viral RNA were 50% (approximately 0.3 logs) in the triple combination group, 30% in the saquinavir-zidovudine group, and 28% in the zidovudine-zalcitabine group. The responses may not have been as robust as expected due to the fact that many of the patients had extensive experience with zidovudine, as well as other agents. No evidence of increased toxicity with the triple drug combination was observed.

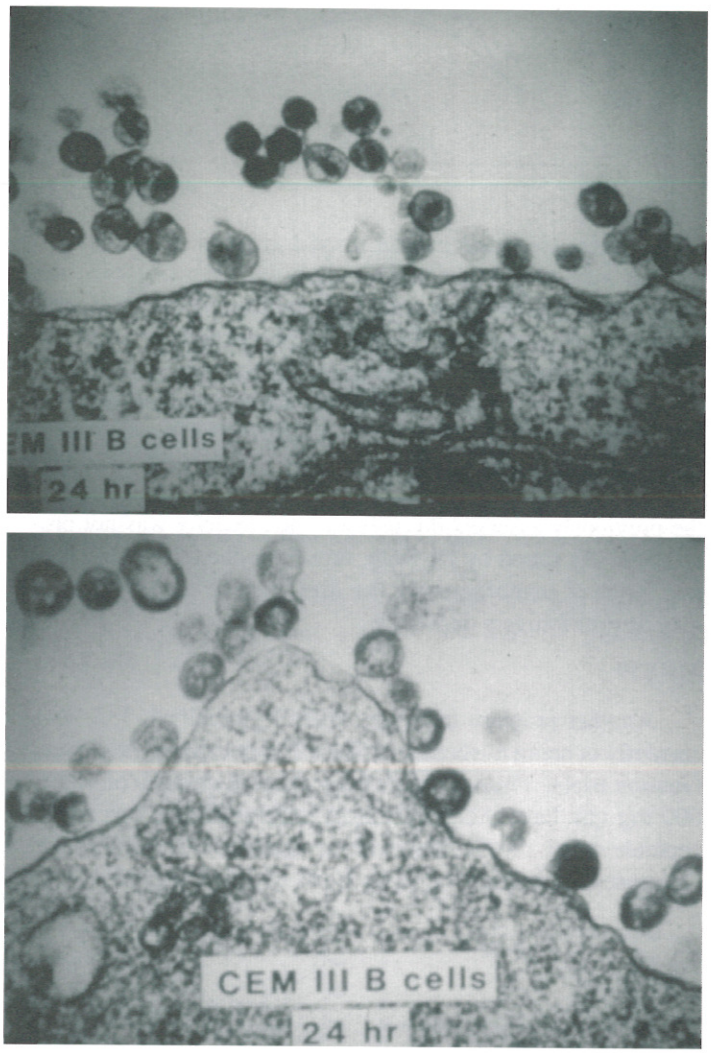

Figure 1. Top. HIV (HIV IIIB) budding from infected CEM cells show presence of dense viral core. Bottom. Virions budding from cells after exposure to 100 nM of protease inhibitor saquinavir are characterized by absence of core components.

The relative absence of significant toxicity associated with saquinavir at dosages of up to 1800 mg/d has prompted evaluation of higher dosages. A group of investigators at Stanford University recently evaluated the safety and antiviral effects of saquinavir at dosages of up to 7200 mg/d. They found that the viral load suppressive effect was greater at these dosages than at

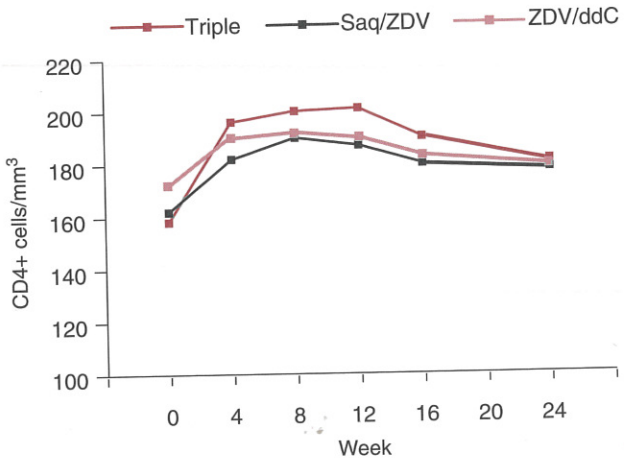

Figure 2. ACTG 229: mean CD4+ cell count response in patients receiving zidovudine (ZDV) plus saquinavir (Saq), zidovudine plus zalcitabine (ddC), or triple therapy with zidovudine plus saquinavir plus zalcitabine. Adapted with permission from A. Collier for the AIDS Clinical Trial Group, Executive Summary, ACTG 229, May 26,1994.

the previously assessed dosages and that toxicity was not prohibitive. As stated by Dr Saag, however, due to the high cost and labor of manufacturing the drug, use of higher dosages may be prohibitively expensive.

MK639

Another protease inhibitor, MK639 (initially L-735,524), currently is being evaluated in phase II monotherapy and combination trials. Phase I trials of the agent at dosages of 400 to 600 mg q6h have indicated that it is bioavailable, has an acceptable safety profile, and exerts a significant antiviral effect. Decreases in plasma viral RNA levels of 1 to 3 logs have been observed, although levels generally return to baseline with continuation of monotherapy for >12 to 24 weeks. Nevertheless, sustained elevations of CD4+ cell count have been observed to persist beyond 24 weeks. As with other protease inhibitors evaluated, viral isolates exhibit reduced susceptibility to MK639 after relatively short-term treatment. Figure 3 shows the HIV p24 antigen response and plasma HIV RNA response to MK639 administration in four patients.

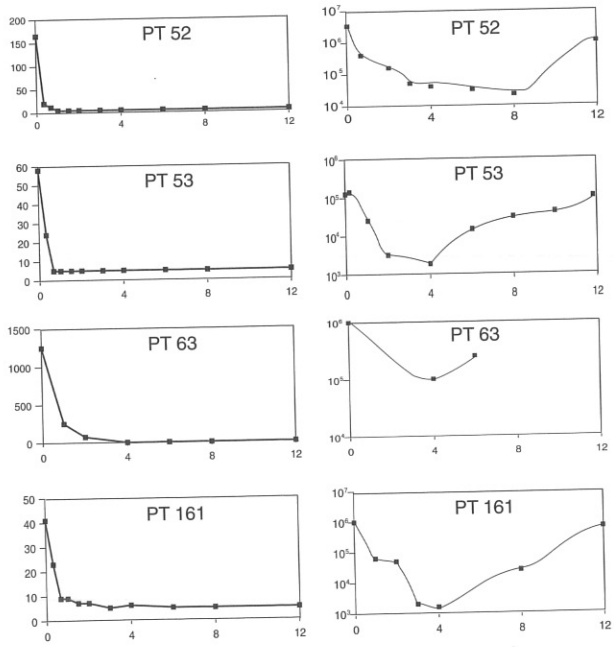

Figure 3. Left. HIV p24 antigen response to treatment with MK639 in four subjects. Right. Plasma HIV RNA response to treatment over same time in same four subjects.

Although HIV p24 antigen remains suppressed for the entire 12 weeks shown, viral RNA levels begin to return to baseline level. It has been noted that HIV p24 antibody levels increase dramatically in such patients. It is believed that an altered form of HIV p24 is produced under protease inhibitor treatment and that new HIV p24 antibodies produced in response mask the presence of HIV p24 antigen in the circulation as detected by p24 ELISA assay. Thus, whereas measurement of HIV p24 antigen level with current assays provides the impression of maintained suppression of replication, the viral RNA assays indicate a gradual return in replication. There have been cases of dramatic increases in CD4+ cell count under treatment with this protease inhibitor. In one case, a patient's count rose from 7/μL to 215/μL and the CD4+ cell percentage increased from 1% to 16%. After more than 1.5 years of treatment with the agent, the patient's count is at 30 to 40/μL. Results of phase II trials with this agent should be available in 1995.

ABT-538

Preliminary results of phase I trials with ABT-538 dosed at 200 to 300 mg q6h or q8h in the United States and 300 to 600 mg bid in Europe and Australia have documented 1 to 3 log decreases in viral load and 35% to 95% increases in CD4+ cell counts during 12 to 16 weeks of dosing. According to Dr Saag, the gradual return of viral replication after 6 to 12 months of treatment also has been observed with this agent. The formulation of the agent has a characteristic poorly tolerated taste, but few adverse events have been observed thus far. Dramatic CD4+ cell count elevations have also been reported with ABT-538—one Australian patient was reported to have an increase from approximately 70/μL to 550/μL.

SC-52151

As related by Dr Saag, some of the difficulties with development of protease inhibitors as therapeutic agents have been pointed out from the experience with SC-52151. This agent demonstrated potent in vitro activity, and was evaluated in a rapidly accrued phase I study of two formulations. This study (ACTG 282) demonstrated no measurable effects on HIV RNA levels, HIV p24 antigen levels, or CD4+ cell counts despite the achievement of adequate serum drug levels. Subsequent study revealed that a serum protein, alpha-1 acid glycoprotein, tightly bound the inhibitor and dramatically reduced the intracellular uptake of the compound. The manufacturer has halted further development of the compound.

Protease Inhibitor Cross-Resistance

Although it was initially believed that cross-resistance among different protease inhibitors was rare or unlikely, recent

Table 1. Protease-specific Amino Acid Substitutions (Serum Viral RNA) in a Patient Receiving MK639 Monotherapy

Week	Protease amino acid substitutions	IC_{95} (nM)
0	L10V, T12I, G16E, R57K, I64V	50
12	L10R, M46I, R57K, I64V	100
24	L10R, M46I, (L63P), (I64V), V82T	400/800
36	L10V, T12I, L24I, N37D, M46I, L63P, A71V, V82T, I84V	1500/3000

Parentheses indicate substitutions that occur less frequently. IC_{95} = 95% inhibitory concentration.

investigation has shown that with treatment of sufficient duration, decreases in susceptibility to most of the available inhibitors are observed. However, the decrease in susceptibility observed among the inhibitors was not uniform and the findings remain to be validated. Table 1 shows an example of the amino acid substitutions and associated decreases in susceptibility over time in isolates from a patient receiving protease inhibitor monotherapy. As stated by Dr Saag, what occurs in terms of resistance when combination treatment involving these agents is begun early in infection remains to be determined. He noted the possibility that use of combinations of protease inhibitors might forestall or prevent selection for some resistant mutants through interaction of resistance mutations. Ongoing and future studies will better define the potency, durability, and clinical utility of the protease inhibitors in combination with existing nucleoside analogues, NNRTIs, and with each other.

Recent data have shown that decreases in susceptibility to most of the available protease inhibitors will develop with treatment of sufficient duration; these findings remain to be validated, however.

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