

considered a likely candidate for use. Atovaquone, which recently has been approved for treatment of mild to moderate PCP, is to be compared with dapsone in a large-scale prophylaxis study in TMP-SMX-intolerant patients; Dr Murphy cautioned that since atovaquone is poorly absorbed in its current formulation, requiring administration along with a high-fat snack, prophylactic use is difficult and probably requires full treatment doses.

Fungal infection

Cryptococcosis develops in 5% to 10% of AIDS patients not receiving prophylaxis and generally occurs at CD4+ cell counts $<100/\mu\text{L}$. In ACTG 981, involving 428 of the patients enrolled in ACTG 081 PCP study, patients were randomized to fluconazole 200 mg/d or clotrimazole troches 10 mg five times daily for prevention of fungal infection. Fluconazole was associated with significant reductions in the incidence of invasive fungal disease (9 vs 23 patients) and cryptococcosis (2 vs 15 patients); the 24-month risk of invasive disease was 2.8% in the fluconazole group and 9.1% in the clotrimazole group, and the CD4+ cell count-adjusted relative risk for invasive disease in the latter group was 3.25. Fluconazole was also associated with significant reductions in the incidence of esophageal candidiasis (3 vs 20 patients) and superficial fungal infection (10 vs 36 patients). No differences in mortality were observed, however. Dr Murphy maintained that such findings leave it unclear whether fluconazole prophylaxis should be utilized, given the absence of survival benefit, the low lethality of properly managed first-episode cryptococcosis, and the potential for development of resistance to an otherwise highly useful agent; he stated, however, that if prophylaxis is contemplated, fluconazole appears to be the azole of choice, since it does not have the absorption problems associated with ketoconazole or itraconazole, and suggested that fluconazole prophylaxis would be optimally begun at CD4+ cell counts below $100/\mu\text{L}$ and at a dosage lower than that investigated in the study (eg, on the order of 100 mg/d).

CMV infection

Recent data indicate that approximately 40% of patients with CD4+ cell counts below $50/\mu\text{L}$ develop CMV retinitis within a 27-month period. Currently, no agents suitable for prophylactic use are available. Trials of an oral form of ganciclovir are under way, and study of oral forms of foscarnet has been initiated in the United Kingdom. One European-Aus-

tralian study evaluating high-dose acyclovir (3200 to 4000 mg/d) found no protective effect against CMV disease, but documented an increase in survival in acyclovir-treated patients; according to Dr Murphy, recent MACS data may provide some support of an acyclovir-associated survival benefit.

MAC infection

MAC infection has been observed to occur in more than half of patients within 24 to 30 months following diagnosis of AIDS. On the basis of two studies involving approximately 1000 patients showing that rifabutin provided significant protection from development of MAC bacteremia, it was recommended in June 1993 that prophylaxis at a dosage of 300 mg/d be considered for patients with CD4+ cell counts below $100/\mu\text{L}$. Azithromycin and clarithromycin have activity against MAC and a large-scale trial comparing rifabutin plus clarithromycin and clarithromycin in MAC prophylaxis currently is under way. In noting that many practitioners are al-

ready using clarithromycin for prophylaxis, Dr Murphy suggested a more prudent current course might be to reserve the agent for treatment.

As noted by Dr Murphy, daily doses of rifabutin should not exceed 300 mg given recent reports of uveitis in higher-dose trials. Although uveitis was not observed at dosages of at or below 600 mg/d in initial studies, 23 cases have been reported in a Canadian treatment study in which patients are receiving rifabutin 600 mg/d, clarithromycin 1000 mg bid, and ethambutol 15 mg/kg/d; uveitis also has been observed in 11 of 1215 patients enrolled in an ongoing US ACTG/Community Programs for Clinical Research on AIDS trial. The uveitis has been reported to resolve with drug withdrawal or dose reduction. It is believed that there may be a bidirectional drug interaction between clarithromycin and rifabutin that increases blood levels of both agents. Further, it is known that fluconazole may increase rifabutin levels by as much as 80%, warranting caution in the concomitant use of these agents. ■

Future Directions for Anti-HIV Therapy

Future directions for anti-HIV therapy were discussed at the Chicago meeting by Douglas D. Richman, MD, from the University of California San Diego and the San Diego Veterans Affairs Medical Center, and at the Los Angeles meeting by Steven A. Miles, MD, from the University of California Los Angeles.

According to Dr Richman, the conclusion beckoning from the current status of antiretroviral therapy is that combination therapy will be required to improve effectiveness of treatment and that agents in addition to the nucleoside analogues are needed. Dr Richman identified the NNRTIs and protease inhibitors as classes of newer agents that show promise of utility.

NNRTIs: nevirapine

As stated by Dr Richman, data on the several NNRTIs under development indicate that the compounds have potent anti-HIV activity but rapidly select for drug resistance. Data from a group of patients receiving nevirapine at Dr Richman's institution show that resistance appears rapidly in virtually all patients, regardless of whether the drug is administered alone or in combination with zidovudine, and is evident as early as 1 week after the start of treatment. Several specific RT mutations associated with resistance have been identified, with those observed under monotherapy generally differing in distribution from those observed under combination therapy with zidovudine. Typical responses to low-dose

nevirapine monotherapy have consisted of an impressive increase in CD4+ cell count within a week of treatment but a return to baseline levels within 1 month due to acquisition of drug resistance. However, the use of higher doses in an attempt to achieve plasma levels sufficient to exceed viral resistance – an attempt encouraged by the absence of dose-limiting toxicity at lower doses – has shown that many patients have markedly prolonged response to high-dose monotherapy. The median CD4+ cell count in patients with higher baseline CD4+ cell counts receiving a dosage of 400 mg/d increased about 75 cells/ μL for 9 to 12 months. A similar maintenance of decreases in p24 antigen levels and PCR quantitated HIV RNA levels has been observed in patients.

Dr Richman emphasized that such a response is not seen in all patients receiving a higher dosage and that factors predictive of such response remain undetermined. Nevertheless, he stated that these findings and data from another group utilizing monotherapy and combination therapy in newborns indicate that the agent may have a role in some patients. The rationale for use despite the observation of resistance is that

- **Nevirapine: rapid development of resistance, maintained response in some patients at 400 mg/d despite resistance**
- **Protease inhibitors: activity, resistance, design-based and other potential strategies**
- **Early experience with Ro31-8959**

a drug selecting for virus with reduced susceptibility may be clinically effective if it has a therapeutic index and pharmacokinetic properties sufficient to allow achievement of plasma concentrations exceeding the viral susceptibility and if there are constraints on the mutability of the virus. Dr Richman suggested that there is at least some evidence that the mutability of HIV is limited. He also stated that any potential exploitation of apparent limitations of mutability in the context of combination therapy remains the object of further study. This principle should also apply to the utility of protease inhibitors in the face of the inevitable development of some level of resistance.

Protease inhibitors

As explained by Dr Miles, protease inhibitors act by blocking the activity of the HIV aspartyl protease, which is responsible for cleaving precursor gag and gag/pol polyproteins into the functional proteins p17, p24, p9, protease, RT, and integrase; there is also evidence that protease inhibitors may inhibit the viral preintegration complex from gaining entry to the host cell nucleus. A potential advantage of protease inhibitors over nucleoside analogue RTIs, for example, is that they appear to be active in chronically infected host cells, as well as acutely infected and activated cells.

Among the types of protease inhibitors that have thus far been developed are C₂-symmetric inhibitors and transition state mimetic inhibitors, analogues of the protease substrate that substitutes nonhydrolyzable elements for the amide bonds normally cleaved by the enzyme. Such inhibitors bind to the active site of the enzyme, preventing production of active proteins and resulting in immature, noninfectious virus. As related by Dr Miles, characteristics of the C₂ symmetric inhibitor, A80987, as an example of this class of agent, include activity against both HIV-1 and HIV-2 at nanomolar to micromolar concentrations, absence of cellular toxic-

ity, and activity against both zidovudine-susceptible and zidovudine-resistant strains. Sterilization of HIV in culture has also been observed with sufficient concentrations, although the mechanism of this phenomenon remains unclear. Another class of inhibitor is represented by the nonpeptidyl inhibitor DM323; this cyclic urea compound prevents polypeptide cleavage by excluding the water molecule used by the protease to actually perform cleavage. Characteristics of the compound include very low molecular weight, as well as the spectrum of activity and absence of cellular toxicity observed with A80987.

Clinical experience has thus far been greatest with the oral transition-state analogue Ro31-8959. In two small early-phase trials in patients with advanced (CD4+ cell count 50 to 250/ μ L) or early untreated (\leq 500/ μ L) HIV disease, maintenance of CD4+ cell counts over baseline levels and positive slopes of CD4+ counts were observed with a 600 mg tid dose, the highest dosage tested, over a 16-week treatment period (other dosages were 25, 75, and 200 mg tid). In patients with advanced disease, the 600 mg dosage was associated with a median maximum CD4+ cell count increase from baseline of 56/ μ L at week 2 and a median increase of 17/ μ L at week 16. In patients with early disease, the 600 mg dosage was associated with a median maximum increase of 104/ μ L over baseline at week 6 and a median increase of 36/ μ L at week 16. Of adverse effects observed, only abnormal LFTs were considered possibly related to Ro31-8959 treatment; no dose-related adverse effects were observed. In an additional study, untreated patients with advanced disease (CD4+ cell count \leq 300/ μ L) received zidovudine 200 mg tid alone or combined with Ro31-8959 75 mg, 200 mg, or 600 mg tid or Ro31-8959 600 mg tid alone for 16 weeks. All groups exhibited maintenance of median CD4+ cell counts over baseline for 16 weeks, with Ro31-8959 alone appearing to be more active than zidovudine alone and the greatest responses being observed in the combination groups receiving the highest Ro31-8959 dosages. Median increases from baseline CD4+ cell counts associated with the regimens at 16 weeks were 24/ μ L for zidovudine alone, 32/ μ L for Ro31-8959 alone, and 24/ μ L, 73/ μ L, and 56/ μ L for the combination regimens including 75 mg, 200 mg, and 600 mg Ro31-8959 doses, respectively; respective median maximum increases from baseline were 66/ μ L, 113/ μ L, 91/ μ L, 121/ μ L, and 147/ μ L. Response of at least a 50/ μ L or 50% increase in cell count

maintained for at least 4 weeks was observed in eight of 12 patients in the highest-dose combination group, five of 12 in the middle-dose combination group, three of eight in the lowest-dose combination group, four of 12 in the zidovudine monotherapy group, and five of 13 in the Ro31-8959 monotherapy group.

Some data are also available for the compound L735,524, which is active against multiple HIV strains at nanomolar concentrations and possesses the potential advantage of having greater absorption than Ro31-8959. According to Dr Miles, the latter agent has bioavailability of approximately 5% to 6%, whereas that of L735,524 is approximately 17%. Findings in a small group of p24 antigen-positive patients have included suppression of p24 antigen to undetectable levels at week 6, decrease in viral RNA by PCR and bDNA measurements with return to baseline values at approximately 12 weeks, and a dramatic increase in p24 antibody levels. Large scale trials of the agent have been put on hold, with the development of resistance being suspected. Some small scale trials involving higher doses and combination treatment are ongoing.

Dr Miles stated that resistance is likely to be a generic problem with the protease inhibitors. The HIV protease point mutations that have thus far been associated with resistance include: codon 8, 32, 46, and 82 mutations in vitro conferring 4- to 100-fold reduction in sensitivity to A80987; codon 48 and 90 mutations in vivo conferring \geq 60-fold reduction in sensitivity to Ro31-8959; and codon 82 mutation in vitro conferring 4- to 5-fold resistance to DM323. According to Dr Miles, although no in vitro resistance to L735,524 was found during initial development, cross resistance with the agent has since been demonstrated by several groups. However, Dr Miles maintained that one of the advantages posed by the protease inhibitor approach is the ability to design or redesign specific agents based on needed properties. In this regard, he indicated that once mutations associated with in vivo resistance are identified, it may be possible to develop or exploit inhibitors without cross resistance for use in combination. Further, it may be possible to produce directed mutations, with use of agents that force known mutations to occur being followed by use of agents specifically designed to be active only when such mutations are present. Other areas of protease inhibitor research identified by Dr Miles include: potential use of the agents as natural vaccines (exploiting a new antibody response observed with protease inhibitor adminis-

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tration by augmenting immune response with IL-4 to further increase antibody response or IL-2 to augment CTL activity); potential immunologic priming of the host with gene therapy to recognize protease mutations (with subsequent treatment with the mutation-inducing agent resulting in enhanced immunologic clearance of virus); focus on development of small-molecule agents (eg, DM323) that would be easier and cheaper to design and produce; and investigation of combination therapy – eg, with protease inhibitors and NNRTIs. Dr Miles noted that protease inhibitors currently are under development at more than 15 pharmaceutical companies.

Other promising agents

Other promising agents under develop-

ment include nonimmunosuppressive cyclosporin analogues. As related by Dr Richman, recent studies have shown that the gag proteins of the HIV ribonucleic protein complex bind to host cell components and are active in transporting the complex to the host cell nucleus, with a similar phenomenon likely occurring in transport of virion components from the nucleus and cytoplasm to the budding membrane. The finding that cyclosporin can inhibit these processes has led to identification and development of nonimmunosuppressive analogues – eg, SDZ 811 – as potential antiretroviral agents. SDZ 811 has been found to bind to cyclophilin in the host cell and inhibit the HIV gag-cyclophilin interaction, inhibiting transport of reverse transcripts to the nucleus at micromolar concentrations. Further, it inhibits the infectivity of virions

at later steps of replication at 10- to 100-fold lower concentrations. Marked inhibitory effects have been observed in vitro in CD4+ lymphocytes and in monocytes at achievable drug concentrations.

Another class of compounds that have been shown to have potent activity in vitro is the bicyclams. These agents prevent uncoating of the virus after cell entry; the exact mechanism of action of the agents remains unclear, although it is known that they do not bind to the virion. Dr Richman anticipated that some of the bicyclams may enter development within the year. Other types of agents that have engendered interest but that are not likely to enter advanced phase clinical trials in the near future include the antisense oligonucleotides, ribozymes, and integrase inhibitors. ■

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variety of patient courses and associated prognoses after the start of zidovudine treatment; stratification of best and worst responses based on a baseline CD4+ cell count of 300/ μ L and CD4+ cell count increase or decrease of at least 50/ μ L, serum neopterin level changes, and increase or decrease in physical symptoms over 1 year of treatment indicated a 17-fold difference between best-case and worst-case response in risk for mortality within the following year. CD4+ cell count data from patients who had experienced rapid cell count declines for 5 to 6 years of follow-up prior to the start of zidovudine treatment similarly indicate heterogeneity of cell count response in association with time to clinical progression to AIDS: some exhibited continued steep cell count decline and rapid progression to AIDS, some exhibited reduced rate of cell count decline and progressed to AIDS after 2 years, and some exhibited relative maintenance of cell counts and did not progress to AIDS during the observation period. Dr Phair emphasized the variability of course of disease by presenting a histogram of 6-month changes in CD4+ cell count among individuals in a

MACS cohort followed by his group for 8 years. Although most patients have decreases in cell count, with the majority experiencing 6-month decreases of 30 to 40/ μ L, some 15% have exhibited increased cell counts over the course of infection (Figure 4); similar observations

have been made in San Francisco cohorts and in an Australian transfusion cohort. According to Dr Phair, although there is some evidence that such patients exhibit reduced viral load, how they differ from other patients in their response to infection remains unexplained. ■

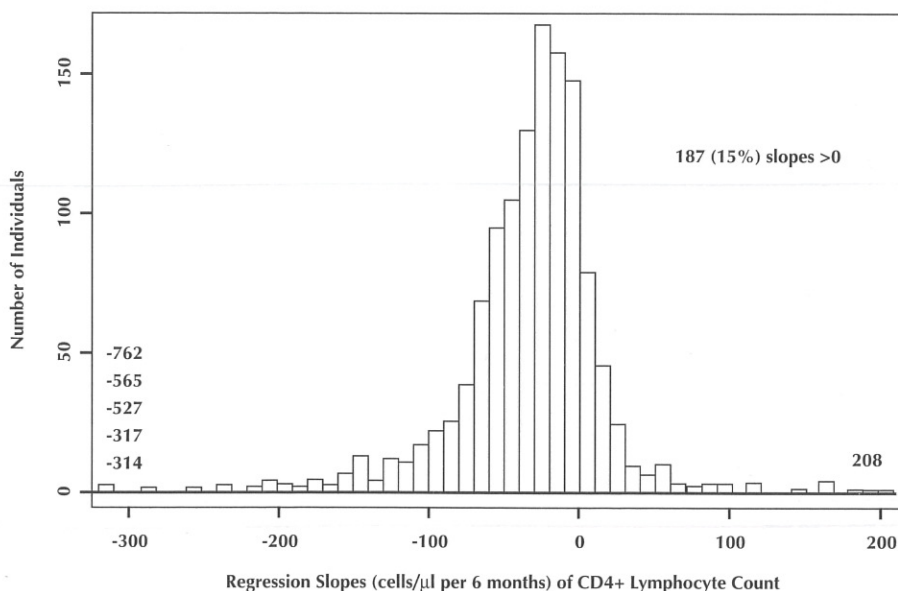


Figure 4. CD4+ cell count changes over 6-month intervals in patients with HIV infection followed for up to 8 years. Figure from Kirby et al (submitted for publication).