with progressive infection; as noted by Dr Ho, the absence of waning of the humoral response indicates continued priming by viral proteins and, thus, persistence of some level of viral replication. Additional studies have suggested that there is a very potent cellular immune response and that no inherent resistance of CD4+ cells to infection is present. In studies in which virus was added in vitro to activated mononuclear cells isolated from the subjects and from normal donors, it was found that virus growth over 2 weeks, measured by antigen production, reached very high levels in normal donor cells, whereas nonprogressor subject cells exhibited a smaller initial peak antigen response followed by decline. Contrary to the notion that this might indicate inherent resistance of nonprogressor CD4+ cells to infection, it was found that depletion of CD8+ cells from nonprogressor samples resulted in marked growth of virus in the CD4+ cells (Figure 2). Sequential readdition of each subject's own CD8+ cells to the CD8+ cell-depleted cultures resulted in dramatic return of suppressive effect. As related by Dr Ho, identical effects have been observed in samples from each of six subjects studied thus far; the magnitude of the suppressive effect has been observed to be greater than that observed in samples from individuals with progressive disease.

Other researchers have found that infection of rhesus monkeys with simian immunodeficiency virus (SIV) in which the *nef* gene has been deleted results in controlled infection, with administration of SIV in this form being found to serve as a vaccine protecting the animals from subsequent challenge with wild type SIV. Given these findings, Dr Ho and colleagues performed PCR analysis of HIV *nef* sequences in isolates from nonprogressor subjects. No gross defects have thus far been observed and no clustering of the identified sequences has been identified in analysis including sequences from virus isolated from patients with AIDS. Dr Ho suggested that although the precise role of *nef* as a virulence factor remains to be determined, it would not be surprising if infected individuals with *nef*-defective virus were eventually identified and found to exhibit enhanced control of infection. He maintained that it is likely that defects of another portion of the viral genome are operative in the nonprogressor subjects that have thus far been analyzed.

In summarizing results so far obtained in these ongoing studies, Dr Ho stated that the findings indicate that there is a remarkably low level of HIV in the blood of nonprogressors, with work being done by others suggesting that nonprogressors also exhibit low levels of replication in lymphoid tissue. With regard to the potential mechanisms of enhanced control, the findings suggest that CD4+ lymphocytes in the nonprogressors are not atypically resistant to infection, that these individuals mount particularly strong cellular and humoral immune responses to infection, and that there appears to be some attenuation of viral replicative ability in infecting strains, although the precise viral defects remain to be defined. ■

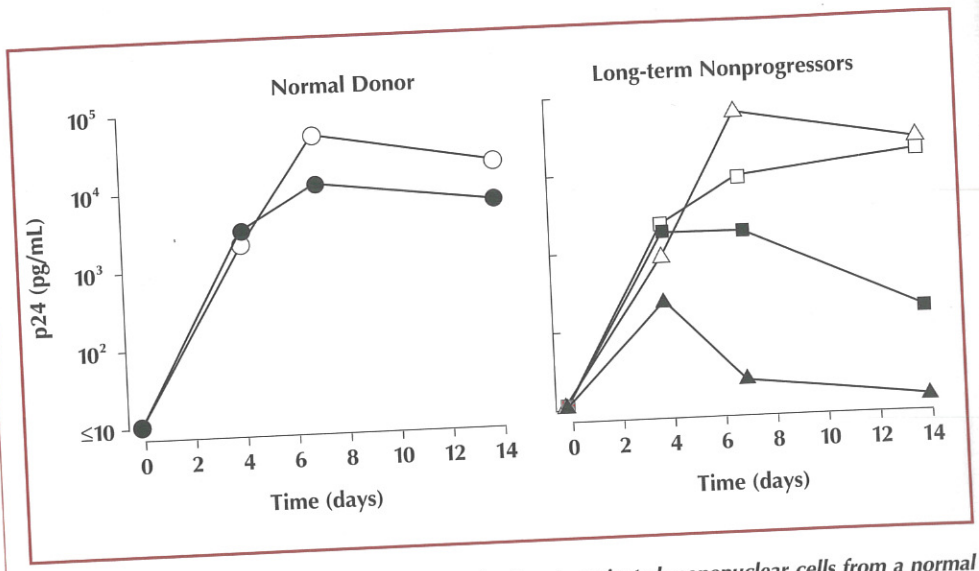

Figure 2. Viral growth, measured as antigen production, in activated mononuclear cells from a normal donor (left) and from two long-term nonprogressors (right). Closed symbols = growth in culture; open symbols = growth after CD8+ cell depletion.

Continuing Antiretroviral Therapy

Strategies for continuing antiretroviral therapy were discussed at the Los Angeles meeting by Michael S. Saag, MD, from the University of Alabama at Birmingham.

Dr Saag began his presentation by contending that from a virologic viewpoint, including the evidence that HIV continues to replicate during the period of clinical latency and that viral burden remains high throughout the earlier course of infection in untreated patients, very early institution of treatment makes eminent sense. The problem with this strategy, he maintained, is that currently available agents offer a time-limited benefit, citing a maximal 3- to 4-year period during which monotherapy with nucleoside analogue reverse transcriptase inhibitors (RTIs) can be expected to reduce replication; the issue is thus posed of when best to utilize the time-limited benefit. He suggested that if one does not believe that additional or better agents or treatment options will be available within the coming few years, it makes sense to delay institution of treatment until more advanced stages of disease develops (eg CD4+ cell count <300/μL). However, if one shares his own anticipation of the development of new agents and treatment approaches, one would be encouraged to start treatment early with the hope that some of these options will be available in the near future.

Viral quantitative techniques and viral replicative characteristics

In discussing the potential importance of viral markers in treatment, Dr Saag presented data on quantitative competitive PCR measurement of plasma HIV RNA levels in three previously untreated patients before, during, and after a 6-week period of zidovudine administration. Viral RNA levels decreased markedly with the start of zidovudine, remained suppressed during zidovudine administration, and exhibited a steep increase during the 1-week interruption of treatment (Table 1). In one patient with a baseline level of 173,600 HIV RNA copies/mL, a maximum decrease to 9200 HIV RNA copies/mL at week 6 of zidovudine administration was followed by an increase to 136,300 HIV RNA copies/mL in the week during which no zidovudine was given. Immune complex-dissociated (ICD) p24 antigen was undetectable in two patients before, during, and after drug administration and standard p24 antigen assay

was negative in each of the patients at all time points; each of the patients was plasma-culture negative prior to administration, with one becoming culture positive during interruption.

Dr Saag stated that accumulating findings such as these indicate that PCR and branched-DNA techniques for assessing plasma viral RNA provide a sensitive indicator of viral replication and virologic response to treatment. He also suggested that the strength of the viral prime directive, to survive and replicate, is indicated by the rapid resurgence of viral replication immediately upon withdrawal of the suppressive effects of zidovudine. He noted that although agents such as zidovudine are not all that could be desired in terms of magnitude of anti-HIV effect, producing a 1- to 2-log decrease in viral levels, or of duration anti-HIV effect, they nevertheless provide meaningful levels of suppression while they remain effective.

In discussing the replicative characteristics of HIV, Dr Saag related findings showing that more than 7 to 10 viral genotypes can develop in a patient within the first 2 to 3 weeks of infection. According to Dr Saag, RT exhibits a transcription error rate of approximately 1 per every 2000 to 3000 base pairs in the context of a viral genome that contains approximately 9000 base pairs, suggesting that several mutations may occur with each round of genome replication. Although the majority of these mutations are likely to be lethal to the virus, those that are not add to a growing 'library' of genotypes. Recombination between viral genotypes may also occur. Some mutants with drug resistance traits are subsequently selected for under antiretroviral therapy; some may be the syncytium-inducing HIV variants that are observed to gain prominence in many patients as infection advances. Dr Saag suggested that the evolution of genotypic diversity, as well as the progressive increase in viral burden, may in fact underlie the finding that isolation of zidovudine-resistant virus occurs markedly earlier after start of zidovudine treatment in patients with advanced disease than in those with relatively early disease. He also suggested that the rapid turnover and high mutation rate of the virus provides additional rationale for the use of combination therapy because it provides coverage of viral subpopulations not covered by a single agent. Such an approach theoretically would provide a more 'even' and consistent suppressive effect that might enable the immune system to recover or improve its functional abilities.

Recent combination studies

With regard to currently available treatment options, Dr Saag discussed findings in recent sequential or combination treatment trials. In Community Programs for Clinical Research on AIDS (CPCRA) protocol 002, patients with advanced disease who had failed on zidovudine monotherapy (intolerance or clinical progression) received either didanosine or zalcitabine; the two agents were found to have equivalent effects, although a potential survival advantage was associated with zalcitabine. In AIDS Clinical Trials Group (ACTG) protocol 155, patients with advanced disease who were receiving zidovudine continued on zidovudine therapy or were administered a zidovudine-zalcitabine combination; it was found that the combination was associated with a reduction in the incidence of clinical events among patients with CD4+ cell counts ≥150/μL but no additional benefit was found in patients with lower cell counts. As related by Dr Saag, a potential explanation for the lack of benefit of the combination in patients with more advanced disease is that benefit of such a combination may be restricted to earlier disease in association with the decreased overall viral replication occurring at earlier stages; in addition, he stated that since patients with CD4+ cell counts <50/μL who developed grade III toxicity were taken off treatment, many patients with advanced disease were not receiving therapy throughout the study, raising questions about the role of therapy in these patients. The patients with low CD4+ cell counts may have received therapy with zidovudine for a longer period and had more zidovudine-resistant virus at baseline. Dr Saag stated that, on balance, such findings indicate a benefit of switching treatments or initiating combination treatment when monotherapy begins to fail and maintained that his preference, while awaiting definitive data, is to institute combination treatment. He also suggested that in addition to being unable to produce profound suppression of viral replication and to maintain suppression for prolonged periods, the nucleoside analogues, when used as monotherapy, have diminishing effects in producing meaningful viral suppression as disease advances.

Table 1. Virologic markers in previously untreated patients receiving zidovudine for 6 weeks followed by a 1-week discontinuation

	HIV RNA (copies/mL)	HIV p24 antigen (pg/mL) ICD	HIV p24 antigen (pg/mL) Standard	Plasma culture (TCID/mL)
Patient 1				
Week 0: no zidovudine	84,900	0	0	0
Week 1: zidovudine	18,000	0	0	ND
Week 2: zidovudine	33,500	0	0	ND
Week 6: zidovudine	28,100	0	0	ND
Week 7: no zidovudine	72,700	0	0	0
Patient 2				
Week 0: no zidovudine	49,100	0	0	0
Week 1: zidovudine	7300	0	0	ND
Week 2: zidovudine	6500	0	0	ND
Week 6: zidovudine	11,200	0	0	ND
Week 7: no zidovudine	58,400	0	0	0
Patient 3				
Week 0: no zidovudine	173,600	79	0	0
Week 1: zidovudine	21,900	28	0	ND
Week 2: zidovudine	10,900	24	0	ND
Week 6: zidovudine	9200	31	0	ND
Week 7: no zidovudine	136,300	47	0	25

TCID = tissue culture infectious dose.

Adapted from Piatak et al, *Science*, 1993;259:1749-1754.

Nevirapine in combination and viral quantitative findings

With regard to the potential inclusion of non-nucleoside RTIs (NNRTIs) in combination therapy, Dr Saag related data from a study by his group showing that, after an impressive initial virologic response, resistance to the NNRTI L697,661 developed within 6 weeks after the start of high-dose monotherapy. Resistance was associated with mutation at RT codon 181, producing a change in susceptibility of as great as from 50 nM to >12,000 nM in isolates from some patients. According to Dr Saag, the emergence of resistance likely reflects selection of subpopulations of preexisting resistant mutants through the pressure of a strong antiviral effect on drug-susceptible subpopulations. In other studies, treatment with the NNRTI nevirapine has also been associated with emergence of resistance, although there are data to indicate that

some patients receiving high-dose monotherapy exhibit a sustained response despite development of resistance. According to Dr Saag, although current data suggest general unsuitability of NNRTIs for monotherapeutic use, there are some data indicating a prolonged beneficial effect in some patients when such agents are added to nucleoside RTI treatment.

Experience of Dr Saag's group with combination therapy has included an uncontrolled open-label extension of a pharmacokinetics study in which nevirapine (200 mg q12h) was added to treatment in a small group of patients who had received regimens of didanosine, zidovudine plus zalcitabine, or zidovudine plus didanosine for an average of 25 weeks. Monitoring of virologic response included use of quantitative competitive PCR and branched-DNA assessment of viral load and ICD p24 antigen testing. As related by Dr Saag, all patients demonstrated a substantial decrease in viral burden upon the addition of nevirapine. Yet, the long-term effects of adding nevirapine were variable. Although all patients exhibited initial CD4+ cell count increases and decreases in plasma viral RNA levels, time to return to baseline values varied from weeks to months. Dr Saag noted that even though rapid offset of effect of nevirapine was observed in some patients, it was heartening to find that additional suppressive effects could be obtained in patients already receiving RTI treatment. Figure 3 depicts the response in one patient. One case identified by Dr Saag as intriguing is that of the patient responding to zidovudine after rapid offset of effect achieved by the addition of nevirapine to didanosine. Consultation with this patient regarding potential alternative treatment revealed that the patient had been taking zidovudine but had discontinued it a month previously in order to be eligible for the study.

An important observation in the study is that the PCR and branched-DNA findings appear to sensitively reflect the replicative behavior of the virus during treatment, showing marked declines in viral levels with initial administration of a potent antiretroviral agent and increases with apparent offset of activity, while inversely correlating with CD4+ cell count changes. He stated that the viral quantitation techniques are likely to become extremely useful in the guidance of day-to-day management of patients in the future as a means of indicating when therapy should be altered and assessing the response to treatment. He suggested that agents with a greater ability to maintain significant suppression for prolonged periods may soon exist to provide better options in responding to data produced by these techniques. In the latter regard, he identified the NNRTI delavirdine, which currently is entering clinical evaluation, as a promising agent with potent in vitro anti-HIV effects. He placed particular emphasis on the potential utility of protease inhibitors, suggesting that some candidate agents could be available for use in novel treatment strategies within the coming year or two. ■

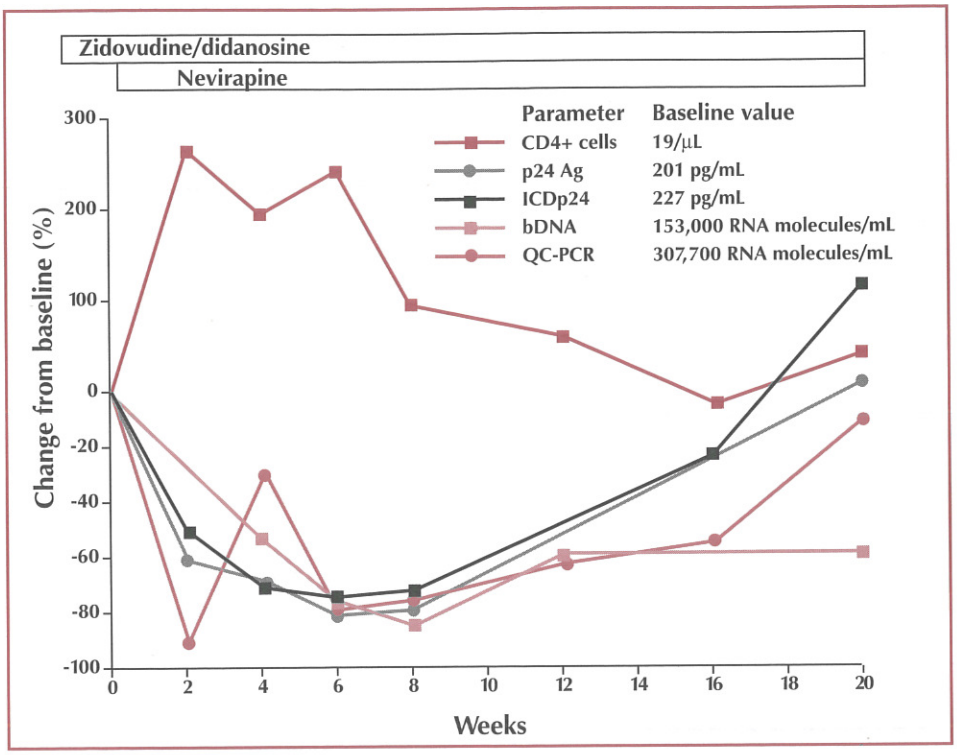

Figure 3. Virologic and CD4+ cell count response to addition of nevirapine to nucleoside analogue treatment in one patient. bDNA = branched-DNA. ICD p24 = immune complex-dissociated p24 antigen. QC-PCR = quantitative competitive PCR. p24 Ag = p24 antigen. Figure courtesy of Michael S. Saag, MD.

Antiretroviral Resistance

Aspects of HIV resistance to antiretroviral agents were discussed at the Atlanta meeting by Victoria A. Johnson, MD, from the University of Alabama at Birmingham, and at the Boston meeting by Clyde S. Crumpacker, MD, from Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts.

Factors in clinical drug failure

According to Dr Johnson, HIV resistance to antiretroviral agents is very likely unavoidable in the absence of complete suppression of viral replication, with the elements of chronic active infection, prolonged drug exposure, and underlying immunodeficiency combining to provide an ideal setting for development of resistance. She noted that virtually every agent that has been extensively evaluated has been associated with at least in vitro resistance. She also stressed, however, that clinical drug failure is multifactorial in nature. Although much attention has focused on resistance as a mechanism in failure, it is now evident that other factors contributing to disease progression during treatment include high and increasing viral load and conversion to SI phenotype, as well as potential cellular resistance to antiretroviral agents.

According to Dr Johnson, viral load may be considered the most fundamental determinant of progression, with optimal suppression of viral load forming the ideal objective of antiretroviral treatment. She suggested that the goal of suppressing viral load could be best achieved by early institution of treatment and use of combination regimens. The rationale for combination treatment is that it may provide more complete suppression of viral replication; limit emergence of drug-resistant virus by reducing replication; and provide treatment of established drug resistance, the latter on the view that viral populations in an individual harboring virus resistant to an agent are mixtures of susceptible and resistant viral strains. She also stated that better antiretroviral agents are needed. It is part of Dr Johnson's current practice to offer combination therapy to patients beginning