HIV ANTI-RETROVIRAL TRIALS: A PARADIGM FOR CLINICAL RESEARCH?

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1. INTRODUCTION

Since the discovery of HIV as the causative agent of AIDS in 1983, significant progress has been made in the treatment of this disease. Effective therapy in the form of Zidovudine (ZDV) , also known as AZT, was available by 1987, only two years after its identification as an antiviral agent. Other ZDV analogues soon followed. The promise of a quick cure for HIV infection offered by these drugs was soon dashed as it became apparent that these agents could only retard the progression of HIV. In addition, flaws in the design of some of the clinical trials of these drugs allowed for ambiguities regarding their use. Application of new techniques to HIV clinical trials, as well as new knowledge of viral pathogenesis, has led to a better understanding of how antiretroviral medication work, and why they fail. The history of the currently used antiretroviral medications, their present uses and the development of clinical failure of antiretroviral agents is discussed below.

2. STRUCTURE OF HIV

HIV is composed of a lipoprotein coat envelope and a protein core containing genetic material (1). The envelope consists of a lipid bilayer and a large, external protein, gp120, that is non-covalently linked to a transmembrane protein, gp41. Within the envelope there is a core of structural proteins that surround two positive strands of RNA and several important enzymes including reverse transcriptase, an integrase and a protease.

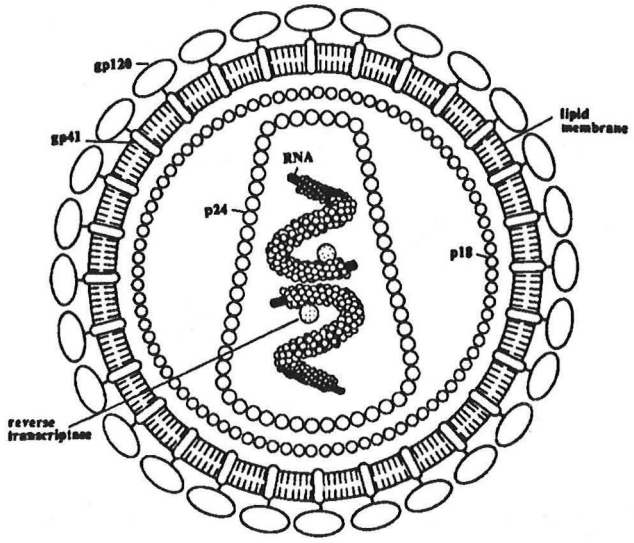

Figure 1. Structure of HIV

3. LIFE CYCLE OF HIV

The primary target of HIV infection is the CD4 bearing lymphocyte, though other cells including macrophages and neuroglial cells can be infected (1-3). HIV gp120 binds to the CD4 molecule after which the viral envelope fuses with the cellular membrane through an interaction with the transmembrane protein, gp41. The core and its contents are released into the cytoplasm where reverse transcriptase, an RNA directed DNA polymerase, synthesizes double stranded HIV DNA using the RNA as a template. HIV reverse transcriptase is characterized by a high error rate and poor proof reading ability allowing for the introduction of mutations at this step. The double stranded viral DNA is then transported to the nucleus where it is integrated into the cellular DNA by an HIV encoded

integrase, creating an HIV provirus. Once integrated, the HIV provirus permanently infects the cell. Major HIV gene products are synthesized from the proviral DNA as a single large protein that is clipped into its component parts by an HIV encoded protease and glycosylated by a cellular glycosidase. Assembly of the virus occurs in the cytoplasm after which mature viruses bud through the cell membrane. With high levels of viral replication, disruption of the membrane becomes extensive and cellular lysis occurs.

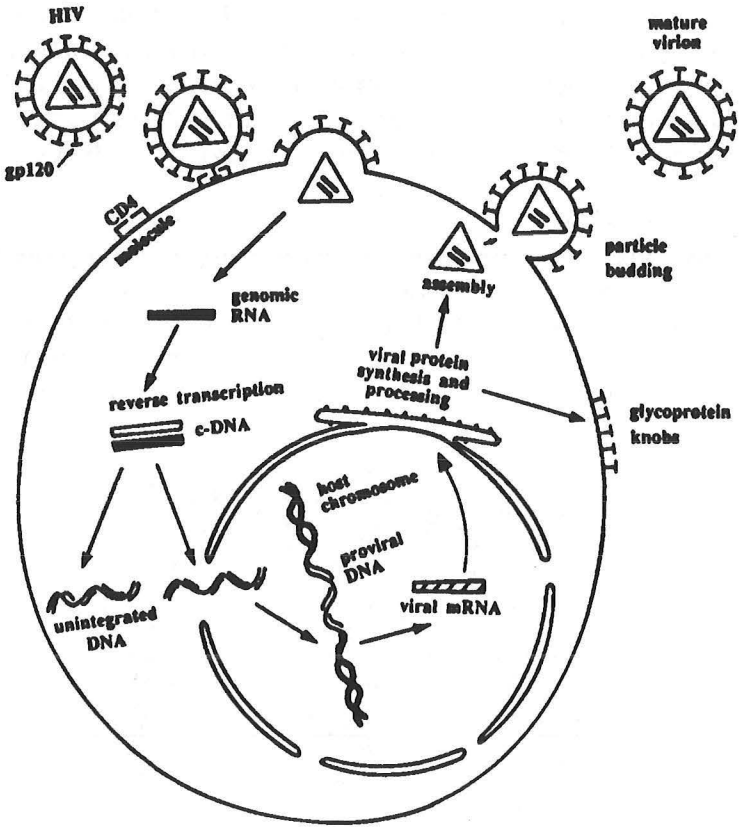

Figure 2. HIV Life Cycle

Several HIV gene products are important for completion of viral replication (4). Two early products, known as REV and TAT, up-regulate HIV transcription. REV also increases the transportation of HIV mRNA to the cytoplasm. A third protein, NEF, is necessary for viral replication and decreases the expression of CD4 on the cell surface. Additionally, this latter function may protect the cell from further infection by other viruses. Two proteins found later in the life cycle, Vpr and Vpx, assist in the assembly of mature viral particles while Vpu helps in the release of viral particles from the cell. These regulatory proteins are attractive targets for therapeutic intervention and are the focus of active research.

4. NATURAL HISTORY OF HIV INFECTION

HIV infects a host through sexual contact with another infected individual or through direct inoculation of infected blood or body fluids (5). After an incubation period of six to eight weeks, a burst of viral replication occurs rapidly disseminating HIV throughout the body (6-8). At this time, high titers of virus can be cultured from the blood.

Infected individuals can have a mononucleosis like illness that may include fever, lymphadenopathy, pharyngitis, rash and diarrhea during this period (8,9). The number of CD4 bearing cells in the peripheral blood, on average 1000 cells/mm^3, quickly falls with the rapid viral replication. A vigorous immune response that includes both the humoral and cellular arms then develops. IgM antibodies directed against HIV are initially found but are soon replaced with IgG antibodies directed against the viral core and envelope proteins. Similarly, T cell responses against viral antigens can also be detected at this time.

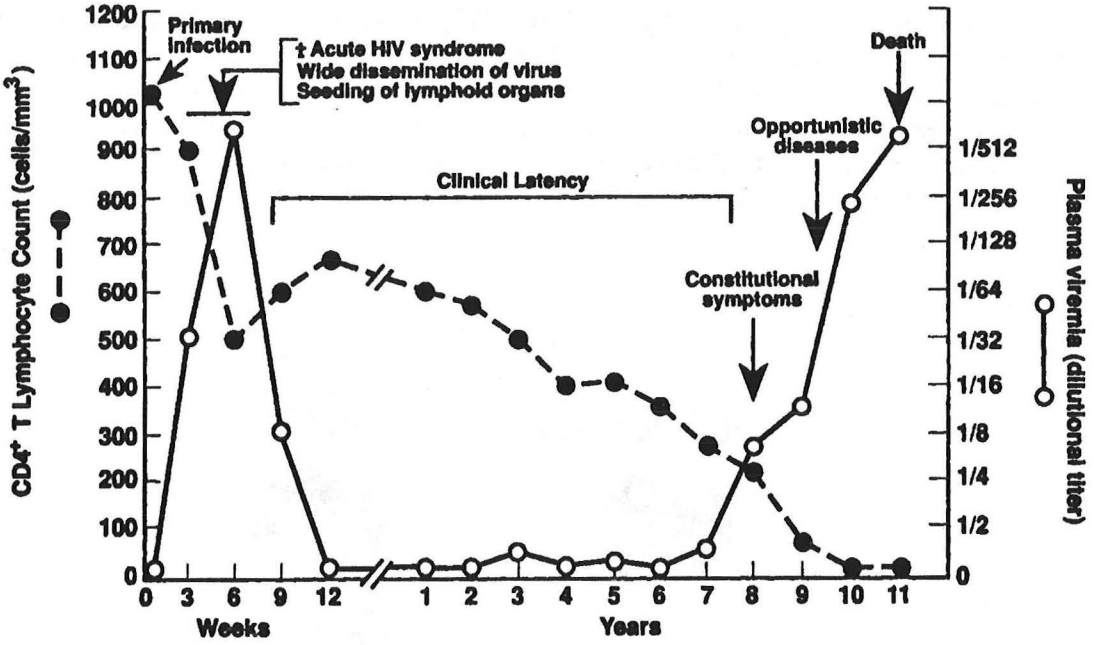

Figure 3. Natural History of HIV Infection

After development of the host immune response, HIV enters a clinically latent period that lasts on average 11 years. Infected individuals may have little or no symptoms (10,11). Despite this relative lack of illness, CD4 counts continue to decline at a rate of 40 to 80 cells/mm^3 per year. There is an increased incidence of certain bacterial and viral infections once the CD4 count falls below 500 cells/mm^3. Infected individuals are susceptible to the host of opportunistic infections that characterize the Acquired Immunodeficiency Syndrome when the CD4 counts reaches 200 cells/mm^3. Death usually ensues within two to three years after an AIDS diagnosis. During the latent period little virus can be cultured from the blood. As the disease progresses, the peripheral blood viral titers gradually increase until the late stage of disease when large amounts of the virus can be found.

Despite the difficulty in detecting HIV during the "latent" period, there is evidence that active viral replication occurs during the entire course of infection Using in vitro hybridization techniques, Palentalo et. al. demonstrated viral replication in the lymph nodes of HIV infected patients at all stages of disease (12). These investigators proposed that the vigorous immune response accompanying primary HIV infection drives the virus into the lymph nodes' follicular dendritic cells which act as a reservoir of infection. Local cytokine production recruits CD4 cells into lymph nodes where they are infected with HIV from the dendritic cells. Local viral replication continues over the course of the illness, gradually

destroying the architecture of the node. As immune function wanes, the virus escapes from the lymph nodes and spills over into the blood where high titers can be cultured.

5. SURROGATE MARKERS OF HIV INFECTION

Clinical disease with HIV is evident only very early and very late in the course of this infection. Thus indirect or surrogate markers are necessary to accurately determine the stage of the disease. In addition, surrogate markers have the potential to determine if patients are responding to anti-HIV therapy. Measurement of CD4 cell concentration in peripheral blood has become a universally accepted marker of the stage of HIV infection. The number of circulating CD4 cells consistently predicts the risk of death and of developing specific opportunistic infections (13). In addition, this test is easily performed by flow cytometry and is routinely available throughout the United States. Unfortunately, changes in CD4 counts with antiretroviral therapy are not predicative of a modified risk of mortality associated with antiretroviral therapy (14-16). Similarly, correlates of clinical progression such as beta-2 microglobin or neopterin are valuable in prognosis of HIV infection but do not predict increased survival in patients treated with antiretroviral drugs (15).

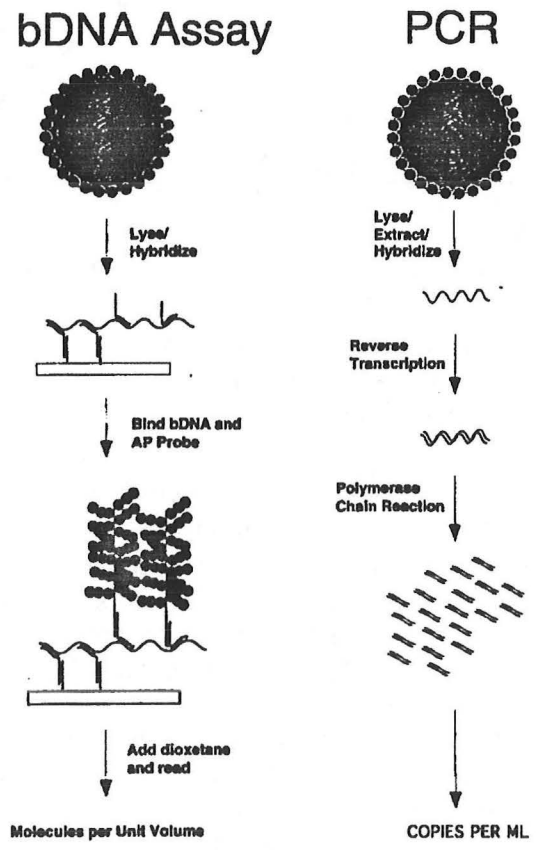

Figure 4. Schematic of HIV b-DNA and RNA PCR

Direct measures of the amount of virus in an infected individuals blood is now available through techniques such branch chain DNA (b-DNA) and HIV RNA Polymerase Chain Reaction (HIV RNA PCR) (15,16). In b-DNA, HIV RNA in the serum is annealed to a complementary nucleotide sequence in a microtiter plate. A second, complementary sequence that is attached to a DNA chain with multiple branches sandwiches the HIV RNA. An alkaline phosphatase detector is then annealed to the branches on the DNA chain. The amount of HIV RNA per unit of volume is proportional to the intensity of the a chemiluminescent reaction. HIV RNA PCR utilizes reverse transcriptase to amplify the HIV genome in serum. The most popular form of this assay uses non-radiometric detection techniques where the concentration of HIV RNA can be determined with an ELISA reader (16). These techniques can detect as little as 200 copies per milliliter of serum in an HIV infected person (15). While still experimental, they will be available for clinical use within several years. As discussed below, the change in the copy number per milliliter of HIV RNA may be predictive of response to antiretroviral therapy.

6. ANTIRETROVIRAL NUCLEOSIDE ANALOGUES

The antiretroviral effect of nucleoside analogues was discovered in 1985 when Perno and his coworkers found that these agents could suppress the replication of HIV in monocytes/macrophages in vitro (17). This led to the rapid testing and licensure of Zidovudine (ZDV), then known as AZT. Subsequently, numerous agents have been tested and four antiretroviral drugs have been approved by the Food and Drug Administration. All currently used antiretroviral agents are 2 ' ,3 ' -dideoxynucleoside analogs. These drugs are successively phosphorylated in the cytoplasm of a target cell to form 2',3'-dideoxynucleoside-5'-triphosphate (17,18). These dideoxynucleoside-5 ' -triphosphates then compete with native nucleotides for reverse transcriptase binding and incorporation into the viral DNA. The incorporation into the DNA chain causes chain termination because a normal 5' ~ 3' phosphodiester linkage cannot be completed.

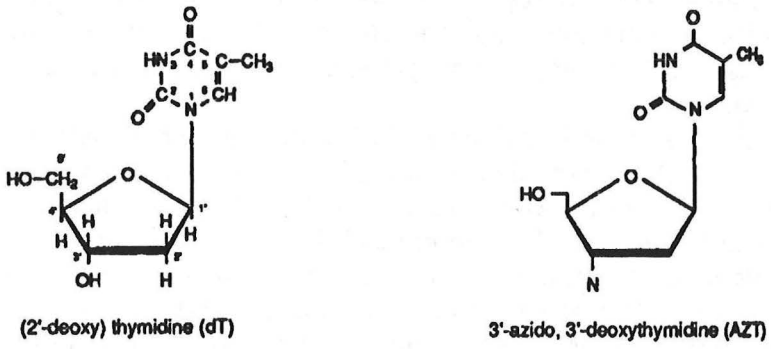

Figure 5. Comparison of ZDV and Thymidine

7. CLINICAL TRIALS OF ZIDOVUDINE

Because of its in vitro efficacy, a phase I clinical trial of ZDV was initiated in 1985 (19). This study showed that ZDV was well absorbed orally, that it had a half-life of 1 hour, and that it entered the cerebrospinal fluid (19,20). Subjects in the trial had transient CD4 increases on receiving ZDV as well as improved delayed-type hypersensitivity

reactions and weight gain (19). HIV culture of peripheral blood however, did not show a consistent reduction in viral titers. (50).

In 1986, a randomized, multicenter, placebo-controlled phase Il trial enrolled 280 individuals with advanced HIV infection (21). These subjects were randomized to receive either 1500 mg of ZDV per day or placebo. At the interim analysis, 19 patients receiving placebo had died compared with only one patient receiving ZDV. Based on this observation, the trial as terminated and all patients on this study were offered ZDV. Additionally, ZDV was made widely available through a Treatment Investigational New Drug (IND) program. In March 1987, ZDV became the first drug approved by the Food and Drug Administration for the treatment of HIV infection at a dose of 1200 mg per day. Since then, retrospective studies have provided additional evidence that ZDV improves survival in advanced HIV infection (22).

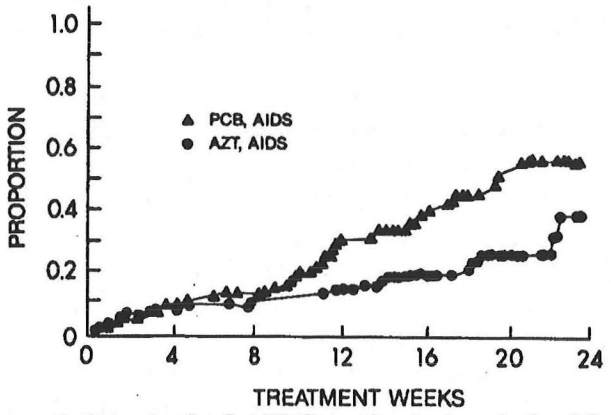

Figure 6. Survival of AIDS patients treated with ZDV or placebo

In addition to these beneficial effects, ZDV has significant toxicity (18,23). Hematopoietic suppression, particularly anemia is a frequent dose-limiting toxicity in patients with established AIDS taking large doses of ZDV (1200-1500 mg/day). Most patients receiving ZDV develop an increase in the erythrocyte mean corpuscular volume, and some also develop megaloblastic changes. Other toxicities associated with ZDV include malaise, nausea, and, in some patients, myositis, seizures, esophageal ulcerations, and cardiomyopathy.

The success of the initial trials of ZDV in symptomatic AIDS patients provided an impetus to extend the use of ZDV to individuals with asymptomatic HIV infection and to reduce its toxicities. In response, the National Institutes of Health developed the AIDS Clinical Trials Group (ACTG) to expand the scope and number of HIV therapies. In addition several other groups within the United States and Europe undertook large, multicenter trials of ZDV to determine if treatment of HIV infection at an earlier stage was more beneficial than waiting until patients developed symptoms of the disease. By 1989 no less than five such trials were underway (14,24-27).

The first AIDS Clinical Trials Group study of ZDV , designated ACTG 002 , was initiated to determine whether lower doses of ZDV could reduce toxicity without sacrificing clinical efficacy (24). In this study, 524 patients with AIDS were randomized to receive either 1500 mg, 600 mg or 300 mg of ZDV daily. Over a two-year follow-up period, improved survival, equivalent delays in disease progression, and similar increases in CD4+ cell number were observed in each group, but hematologic toxicity, particularly

neutropenia, was significantly reduced in the low-dose group (5). This study led to changes in prescribing recommendations, greatly reducing the toxicity associated with ZDV therapy.

The ACTG also initiated several trials to determine if treatment of early HIV infection had any benefit over deferring therapy until the disease had progressed to AIDS. Beginning in 1987, 711 patients with CD4 counts between 200- 800 CD4 cells/mm^3 were randomized to receive one of several doses of ZDV or placebo (ACTG 016) (25). By 1989, disease progression was observed in 34 subjects in the placebo arm and in 12 subjects receiving ZDV in the 200-500 CD4+ cells/mm^3 stratum. Too few subjects in the 500-800 CD4+ cells/mm stratum had disease progression to allow for adequate comparison ZDV versus no treatment. A second ACTG trial, (ACTG 019), randomized over 1300 subjects with asymptomatic HIV-l infection to receive either high-dose ZDV (1500 mg daily), low-dose ZDV (500 mg daily), or placebo (26). Among patients whose initial CD4 count was less than 500 cells/mm^3, clinical disease progression was observed in 38 of 428 of placebo recipients, compared to 17 of 453 recipients of low-dose ZDV, and 19 of 457 recipients of high-dose ZDV. Progression rates, as measured by clinical end points per 100 patient-years of treatment also showed a benefit to early ZDV and were 7.6 in the placebo group, 3.6 in the low dose ZDV group, and 4.3 in the high-dose ZDV group. As in prior studies, the lower dose of ZDV was associated with less toxicity and similar efficacy as the higher dose. A large trial involving over 900 subjects in Australia and New Zealand had similar results (27). Despite delaying progression to AIDS, neither of these two studies demonstrated an ultimate survival advantage of early ZDV administration. Disease progression, including changes in CD4 count, was the primary endpoint of each study and few deaths occurred leaving little power to detect a difference in mortality(18).

Despite the promising results of the ACTG and other trials, the results of several concurrent studies cast doubt on the clinical benefit of the early ZDV use. The Department of Veterans Affairs Cooperative Study Group on AIDS Treatment randomized over 330 patients with CD4 counts greater than 200 cells/mm^3 to receive either 1500 mg ZDV per day or placebo (28). Those who receive placebo crossed over to ZDV therapy when their CD4 counts fell below 200 cells/mm^3. As in the ACTG trials, clinical indicators of disease progression as measured by new opportunistic infections or a fall in CD4 count occurred later in early ZDV recipients than in those who received placebo. There was however, no difference in mortality in the early treatment group when compared to those who received late therapy. Because this study employed a relatively high dose of ZDV (1500 mg/day) and had a high drop out rate, its results were discounted until the results of a large European study was released in 1993.

Preliminary results from the so called the Concorde Study, (an English and French cooperative trial) demonstrated no survival advantage of early ZDV treatment when to compared late administration (14). In this randomized trial, more than 1700 HIV infected patients with CD4 counts over 300 cells/mm^3 either were treated with 1000 mg of ZDV per day or had therapy delayed until their CD4 count fell below 200 cells/mm^3. After the results of ACTG 019 were announced, subjects with CD4 counts between 200 and 500 cells/mm^3 were given the option of changing to open label ZDV therapy. The trial was then analyzed on an intention to treat basis. Like the ACTG trials, an interim analysis 18 months into the trial showed delayed progression of HIV infection among the subjects treated early with ZDV. After three years, however, there was no difference in survival

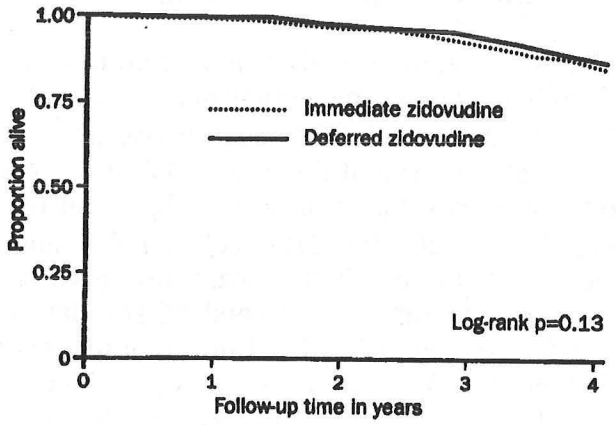

Figure 7. Survial in early vs late ZDV therapy in the Concorde study

among those patients who had received early ZDV treatment compared to those who started therapy later in their illness. In addition, changes in CD4 count with initiation of antiretroviral treatment was a poor predictor of response to therapy. In subjects receiving early ZDV, there was a small but statistically significant increase in CD4 count over those who randomized to delayed therapy. Despite this divergence of CD4 counts between these two groups over the course of the trial, mortality death rates were the same at three years.

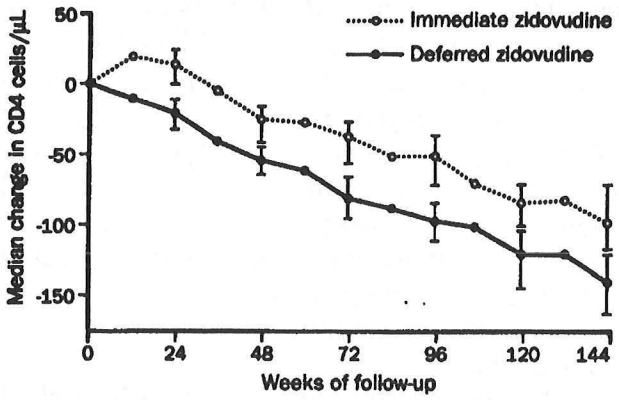

Figure 8. CD4 decline in early versus late ZDV in the Concorde study

Further insight into the these trials can be obtained from a four year follow-up study of ACTG 019 (29). This analysis showed that ZDV was associated with a significant but transient decrease in the rate of progression. In subjects with less than 300 CD4 cells/mm^3, ZDV benefits persisted for approximately 18 months. In those with greater than 300 CD4 cells/mm^3, the benefit was longer than two years. The duration and sample size of the study, however, were inadequate to determine a survival benefit.

Similar findings were noted with the long-term follow-up of patients enrolled in ACTG 016. Thus, ZDV therapy is associated with a delay in disease progression that is more sustained if treatment is started at higher CD4 cell counts.

Interpretation of these ZDV trials is made difficult by the fact that each used different entry criteria for "early" therapy and that each used different doses of ZDV. Each used different definitions of disease progression. In addition each had methodologic flaws which make generalization of results difficult. The ACTG trials relied heavily on changes in CD4 counts as a clinical endpoint and were not sufficiently powered to detect differences in mortality rates even when long term follow up was obtained. The VA cooperative trial had a high drop out rate and many of the deaths in this trial were unrelated to HIV infection. The Concorde trial allowed for crossing over to active treatment and used an intention to treat analysis, potentially diluting the effect of withholding therapy. Despite these difficulties, several clear conclusions emerge:

Early ZDV delays progression of HIV infection and is less toxic than when administered later in the disease.

Lower dose (500 mg daily) of ZDV is beneficial and less toxic the higher dose ZDV (1500 mg daily).

The benefits of ZDV are finite, lasting from several months to several years.

The transient nature of ZDV effectiveness has led some to argue that this drug should be withheld until late into the course of HIV infection when it may be most needed. However, the increased duration of ZDV benefit in subjects with CD4 counts higher than 300 cells/mm^3 as well as the development of other antiretroviral agents has persuaded many physicians to pursue a treatment strategy of early ZDV followed by other nucleoside analogues once disease progression occurs.

8. CLINICAL TRIALS OF OTHER NUCLEOSIDE ANALOGUES

In addition to ZDV, several other drugs active against HIV are now available for treatment of HIV infection. Like ZDV these drugs are nucleoside analogues and thus have a similar mechanism of action. As with ZDV, their use can be limited by toxicity, though these toxicities vary greatly from those of ZDV. In addition, clinical trials have shown that when used in sequential therapy or combination therapy with ZDV, these drugs may effect better clinical out comes when ZDV is used as the sole agent. Currently, three agents in addition to ZDV have been approved by the FDA for use in humans. These drugs, didanosine (ddI), zalcitibine (ddC) and stavudine (d4T), are discussed below.

DIDANOSINE (ddI)

Didanosine or ddI was the second antiretroviral drug approved for use in the United States. Phase I/II trials showed that ddI was effective in the treatment of HIV (30). In addition, the toxicities of this agent, a stocking glove peripheral neuropathy and pancreatitis, do not overlap those of ZDV(30-32). Unlike the case with ZDV, patients receiving ddI rarely develop hematologic toxicity, and indeed, parameters such as white blood cell count and hematocrit sometimes improve on initiation of ddI therapy. It was unclear from these trials however, how ddI compared to ZDV in the treatment of HIV infection. The AIDS Clinical Trials Group enrolled 913 patients who had previously received ZDV into several studies (ACTG 116B/117) that compared continued ZDV to switching therapy to ddI (33).

Subjects were randomized to either two doses of ddI (500 mg and 750 mg daily) or a 600-mg daily dose of ZDV. Patients who received the lower dose of ddI had a significant delay in the onset of new AIDS-defining events when compared with those who continued to receive ZDV. In another trial (ACTG 116A) in patients with relatively advanced HIV-l infection, the comparative effectiveness of ddI or ZDV depended on the duration of prior ZDV therapy (34). ZDV was more efficacious in subjects who had never been treated with antiretroviral therapy whereas those who had received 8 to 16 weeks of ZDV therapy did better when switched to ddI. A third study sponsored by the ddI's manufacturer, demonstrated a benefit of switching from ZDV to ddI in subjects who had signs of clinical deterioration such new opportunistic infections or weight loss (35). As in the trials of ZDV, these studies of ddI included CD4 count decline as a definition of clinical deterioration. Although these trials showed a decrease in all clinical endpoints including death with ddI therapy, there was no significant difference in mortality alone between the cohorts receiving ZDV or ddI. This is because these studies were not designed with sufficient power to detect mortality differences. These results suggest that switching from ZDV to ddI may be beneficial after a certain period of ZDV therapy in patients with advanced disease. The optimal time for the change in therapy, however, remains unclear.

ZALCITABINE (ddC)

Although Zalcitabine (ddC) was the second antiretroviral agent to be developed it only recently was licensed by the Food and Drug Administration. Like ddI, ddC can produce a painful peripheral neuropathy, primarily involving the feet. Other toxicities include aphthous stomatitis, fever, and rash that ranges from an erythematous morbilliform eruption to papulovesicular lesions.(18). These adverse effects as well as the failure of early trials to demonstrate a survival advantage of ddC therapy compared to ZDV delayed the appearance of this drug on the market. In a large trial sponsored by the ACTG, 635 subjects with CD4 counts less than 200 cells/mm^3 and less than three months of prior ZDV therapy were randomized to either continued ZDV or ddC (36). At the one year interim analysis, there were 59 deaths in the ddC group compared to 33 deaths in the ZDV group. Later studies showing a clinical benefit of ddC combined with ZDV (discussed below) subsequently led to its approval. A recently completed post-marketing study of ddC performed by the Community Program for Clinical Research on AIDS (CPCRA), demonstrated efficacy of this drug given as a single agent (37). In this trial, 467 subjects with fewer than 300 CD4 cells/mm^3 who had received an average of 17 months of prior ZDV therapy were randomized to change to either ddI or ddC. After a median follow up of 16 months, there was a slight survival advantage to those in the ddC group, though this difference was not statistically significant. This trial has led many to regard changing from ZDV to either ddI or ddC monotherapy as equivalent clinical options.

STAVUDINE (d4T)

The most recent addition to the antiretroviral armamentarium is stavudine or d4T. Of the currently available nucleoside analogues, d4T appears to be the least toxic, though future clinical experience may prove this assertion false. Stavudine may cause a painful peripheral neuropathy that is similar to that caused by ddI and ddC (38). In the case of d4T, however, the neuropathy is often less severe than with the other two agents. In addition, d4T has not been associated with severe pancreatitis, stomatitis or rash as have been ddI and ddC. Preliminary results of an ongoing clinical trial have demonstrated a clinical benefit of d4T in patients who have previously received ZDV (39). In a study designed similarly to those of ddI and ddC, 822 subjects with greater than 6 months of prior ZDV

therapy were randomized either to continue with ZDV or to switch to d4T. An interim analysis of the first 359 subjects to complete a median of six months of therapy revealed an increase in CD4 counts, amelioration of symptoms and weight gain in subjects treated with d4T compared to those who continued ZDV therapy. Survival, the primary clinical endpoint of this study, remained blinded at the interim analysis and is currently being evaluated. While the results of this study, as well as the toxicity data portray d4T in a favorable light, they must be interpreted with caution. The preliminary interpretation of the trial is based on short term results of less than half of the enrolled participants and thus may change when all of the subjects are finally analyzed. In addition, secondary endpoints such as change in CD4 counts have been shown to be poor predictors of ultimate clinical outcome in studies of antiretroviral drugs. Thus it is prudent to wait for the final results of this trial before concluding that d4T is efficacious in the treatment of HIV.

9. COMBINATIONS OF NUCLEOSIDE ANALOGUES

The clinical benefits of each of the nucleoside analogues against HIV led investigators to postulate that combinations of these agents may provide better therapy against HIV than each used separately (40). In addition, it was hoped that combination drug therapy would allow for lower doses of each nucleoside, resulting in lower toxicity rates for each drug. Thus far, combinations of ZDV and ddC as well as ZDV and ddI have been completed (41-47). Ongoing trials include a ZDV plus d4T combination and ZDV plus 3TC, an experimental nucleoside analogue.

A Phase I/II trial (ACTG 047) comparing alternating versus intermittent regimens of ZDV and ddC in 131 patients with AIDS or symptomatic HIV infection demonstrated a reduction in expected toxicities when ZDV and ddC were given as alternating therapy (41). A second phase I/II study, (ACTG 106), combined ZDV and ddC in 56 previously untreated patients with CD4+ cell counts of less than 200/mm (42). All toxicities observed had been previously described with either drug alone and no serious adverse events were seen with any of the combination regimens. Subjects in this trial gained weight, and experienced increased CD4 counts and decreased p24 antigen levels. The best overall responses were seen in patients treated with the combination of 600 mg of ZDV and 0.03 mg of ddC/kg daily. Finally, in a large phase III trial, 991 subjects were randomized to receive either ZDV or ddC alone or in combination. There was no difference in morality in each of the three groups but a post-hoc analysis showed a trend to slower disease progression in subjects with CD4 counts between 50 and 150 cells/mm^3 who received combination therapy (43).

Several combinations of ZDV and ddI have been compared to ddI alone in a phase a phase I/II, open-label, randomized trial (ACTG 143) involving 116 symptomatic HIV-positive patients (44). The combination of drugs was well tolerated; there was, however, an increased incidence hepatotoxicity in hemophiliacs receiving the ZDV-containing regimens. Preliminary results showed an increase in CD4+ cell counts from a mean of 340 cells/mm^3 to 403 cells/mm^3 among patients in all four treatment arms of the study. A second randomized trial of combined ZDV and ddI in 69 patients with CD4 count less than 400 cells/mm^3 and fewer than 121 days of prior ZDV therapy demonstrated a higher and more sustained increase in CD4 counts in subjects receiving both drugs compared to those who received ZDV alone (45,46). Toxicities were low in each group but those subjects treated with combination therapy tended to have a more pronounced drop in hemoglobin.

Taken in toto, these trials suggest that combination therapy of ZDV with other nucleoside analogues is safe. In addition trends toward better clinical outcomes have been

seen with combinations of ZDV/ddC while combinations of ZDV/ddI have produced sustained elevations of CD4 counts. Despite these findings, there is no conclusive proof to date demonstrating decreased rates of progression of HIV infection or differences in mortality in subjects treated with these regimens. On going trials of ZDV/d4T or ZDV/3TC combinations may provide more definitive results.

10. NIH CONSENSUS PANEL ON THE USE OF ANTIRETROVIRALS

Despite differences in entry criteria, dosing regimens, and definitions of endpoints, several consistent conclusions can be drawn from these multiple trials of nucleoside analogues. First, ZDV has limited effectiveness in preventing disease progression and this effectiveness is most prolonged when therapy is instituted earlier stages of disease. Second, ZDV is more effective as a primary agent against HIV infection than ddI, ddC or d4T but switching to one of these latter agents after a period ZDV therapy produces better short term clinical results than continued therapy with ZDV. Finally, therapy with combinations of ZDV and either ddC or ddI produces a more pronounced improvement in surrogate markers of HIV infection. Nevertheless, the optimal utilization of these agents in clinical practice remains unknown.

The National Institutes of Health convened an expert panel to review the all available data on the these agents in order to develop guidelines for clinicians in the use of antiretroviral drugs. In December 1993, this panel published a consensus statement that described 11 different potential clinical scenarios and made recommendations for antiretroviral therapy in each scenario (47). ZDV was recommended as the initial agent in

Clinical Status	CD4+ Range, Cell Count×10⁹/L	Recommendation
No Previous Antiretroviral Therapy		
Asymptomatic	>0.50	No therapy
Asymptomatic	0.20-0.50	Zidovudine or no therapy
Symptomatic	0.20-0.50	Zidovudine
Asymptomatic	<0.20	Zidovudine
Symptomatic	<0.20	Zidovudine
Previous Antiretroviral Therapy		
Stable	≥0.30	Continue zidovudine
Stable	<0.30	Continue zidovudine or change to didanosine
Progressing	0.05-0.50	Change to didanosine or zalcitabine
Progressing	<0.05	Change to didanosine or zalcitabine
Intolerant to Zidovudine		
Stable or progressing	<0.50	Change to didanosine or zalcitabine

Figure 9. NIH Consensus Panel Recommedations for Antiretrovrial Use

the therapy of HIV infection. It may be started in an asymptomatic patient with a CD4 count between 200 and 500 cells/mm^3. An option to withhold ZDV until symptoms of HIV develop was also permitted under this recommendation. All patients with CD4 counts less than 200 cells/mm^3 should be treated initially with ZDV. As the disease progresses or toxicity occurs, switching to either ddI or ddC was recommended. D4T may be used as salvage therapy for those who have failed other agents.

11. NUCLEOSIDE ANALOGUE RESISTANCE AND CLINICAL FAILURE

As stated above, clinical trials of nucleoside analogues showed that ZDV has limited effectiveness and that switching to another agent may be beneficial after a period of ZDV therapy. These findings suggest that ZDV is the most clinically potent antiretroviral agent. ZDV resistance develops after exposure to the drug and thus changing to a less potent agent such as ddI or ddC may provide a better, ongoing clinical result. Insight into the emergence of resistance by HIV to antiretroviral agents has been obtained through the application of techniques such a HIV RNA PCR and b-DNA to serum samples obtained from clinical trials and as well as the development of assays to measure viral resistance to these drugs.

Though techniques to measure HIV viral titers in patients serum have existed since early in the epidemic, standardized methods for quantifying HIV RNA in the serum or plasma were developed only in the last several years. These tests, b-DNA and HIV RNA PCR (described above) can detect and quantify HIV-l RNA in nearly all HIV infected individuals with CD4 cell counts below 500 cell/mm^3(15,16). They are based on the assumption that circulating viral RNA is proportional to the total viral burden and that the clinical effect of nucleoside analogues would be accompanied by a fall in serum HIV RNA and that clinical failure of these agents would be presaged by a rise in serum viral RNA.
Two studies to test this hypothesis have been completed to date. The first was a retrospective analysis of the VA Cooperative Trial of Early ZDV Therapy (15). Serum samples collected during this study were tested for HIV RNA level by quantitative PCR, and were assayed for P_{24} antigen and beta-2 microglobin. HIV copy number/ mm^3 decreased in those subjects treated with ZDV. The HIV copy number later increased prior to a fall in CD4 cell count in those subjects who subsequently developed clinical failure. Other markers of HIV progression such as P_{24} antigen and beta-2 microglobin did not predict clinical failure.

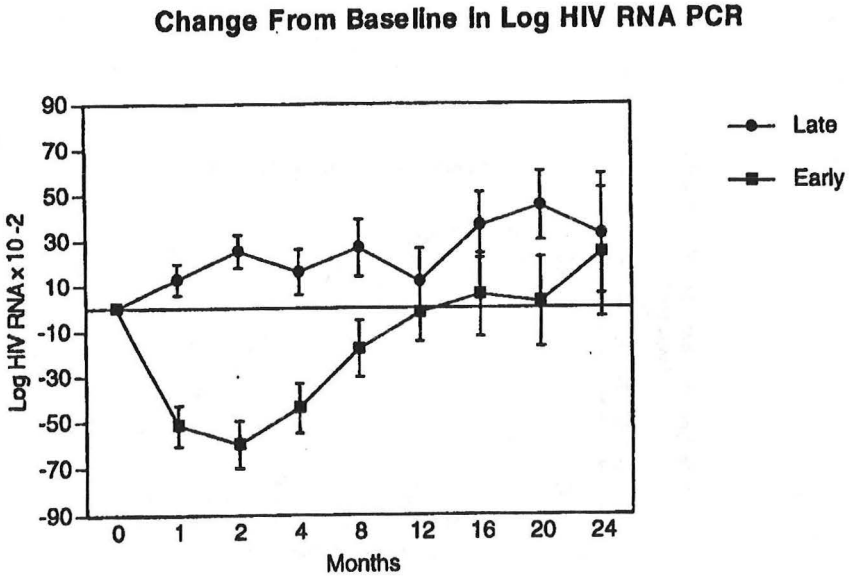

Figure 10. Change in HIV RNA in VA Cooperative Study

In a second trial, HIV RNA was measured retrospectively from plasma samples collected from patients enrolled in a trial of continued ZDV therapy versus switching to ddI

(ACTG 116B/117) (16) . Baseline plasma samples from 100 subjects in this trial were analyzed in parallel by the Chiron Quantiplex b-DNA assay and the Roche Molecular Systems reverse transcription-based PCR assay (RNA PCR). Both the b-DNA and RNA PCR assays yielded comparable HIV RNA results. Subjects with the highest HIV RNA levels had the greatest risk of disease progression, the lowest CD4 cell counts and the latest stage of HIV infection. The HIV RNA copy number decreased for those switched to ddI and increased for those subjects who continued to receive ZDV. In subjects with a 50 percent reduction in HIV-l RNA copy number by 4 weeks of therapy, there was a 32 percent reduction in disease progression. These two trials support the hypothesis that HIV RNA serum concentration correlates with the disease stage and that changes in serum HIV copies number, either upward or downward, is predictive of response to antiretroviral therapy.

HIV reverse transcriptase has a high error rate and no proof reading ability allowing for numerous transcriptional errors. Thus resistant strains can potentially emerge under the selective pressure of antiretroviral analogues. In vitro resistance has been demonstrated in HIV strains isolated from patients taking ZDV (48). Genetic sequencing of reverse transcriptase (RT) from these isolates has shown numerous mutations associated with ZDV resistance (49-53). The most common is a Thr =>Tyr,Phe substitution at codon 215). In many cases a Met =>Leu substitution at codon 41 is found as well. In addition, analysis of HIV isolates obtained from subjects in several antiretroviral trials have shown that the likelihood of developing resistance is a function of duration of treatment (54). Susceptibilities to zidovudine was measured for 55 isolates of HIV-l from 31 patients receiving zidovudine in one of four clinical trials. One year after starting ZDV, 89% of persons with late-stage disease had developed resistance, while only 31% of persons with early stage disease had resistant mutants. Not all patients develop high-level resistance, however, even after ZDV years of therapy.

Several studies have now been completed which associate the emergence of resistance to a poor clinical outcome. The emergence of the codon 215 mutations in HIV reverse transcriptase from patients

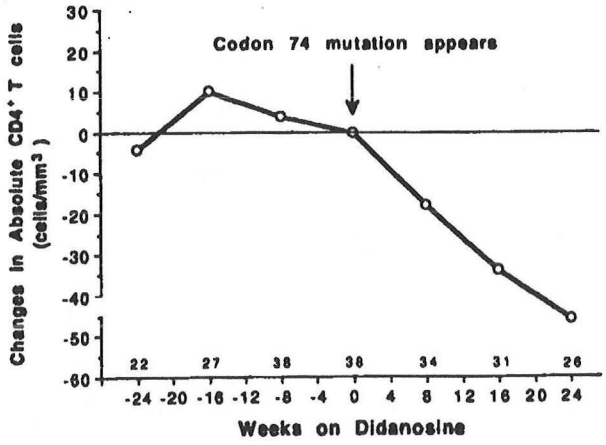

Figure 11. CD4 count decline with emergence of ddI resistance

enrolled in ACTG 016 and 019 who received over 2 years of ZDV was associated with

accelerated fall in CD4 cell counts (55) . Analysis of HIV isolates from a trial of continued ZDV versus switching to ddI (ACTG 116B/117) showed that high level resistance was present in 15% of the subjects at baseline. As in previous studies, the presence of zidovudine resistance correlated with the duration of zidovudine treatment prior to study entry (56).High-level zidovudine resistance at study entry conferred nearly two fold the risk of disease progression and an almost three fold risk of death. Subjects who were switched to ddI in this trial developed resistance to this agent as well. Resistance to ddI was associated with a mutation in codon 74 in reverse transcriptase (56). After 24 weeks of therapy, over half of the subjects who received ddI had HIV containing the 74 codon mutation. Those with ddI resistance had faster CD4 decline and a higher viral burden as measured HIV RNA PCR than those who had no evidence of ddI resistance. Similar studies of combinations of ZDV/ddC and ZDV/ddI also showed emergence of resistance to ZDV (57,58).

Two recently published studies have provide insight into the dynamics of HIV replication and the emergence of resistant strains. Measuring the amounts of virus circulating in the blood of HIV infected patients before and after the institution of antiretroviral therapy, Ho et. al. determined that the clearance of the virus was independent of the stage of disease (59). They calculated that the half life of HIV was approximately two days and while that of CD4 cells was approximately one week. Using similar methods, Wel et. al also calculated the half life of HIV to be approximately two days and showed that wild type virus was replaced by resistant mutants after 14 days of therapy with an HIV protease inhibitor (60). These experiments show that HIV infection is a dynamic process that is sustained by relentless viral replication. The rapid rate of viral turnover, combined with the high error rate of HIV reverse transcriptase, facilitate the emergence of resistant viral isolates. The observation that viral resistance is more likely in subjects with later disease is easily explained by fact that viral titers are higher than in the earlier stages of illness.

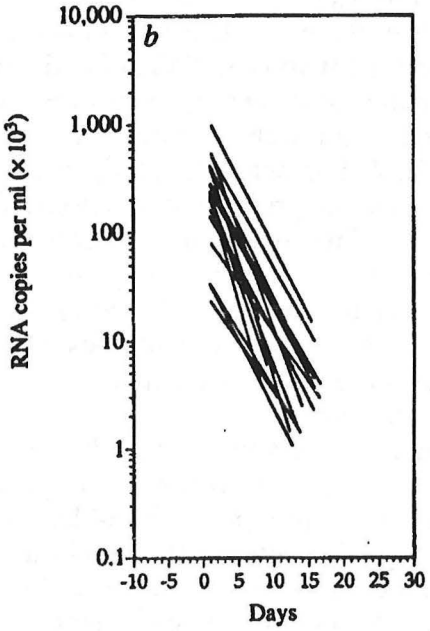

Figure 12. HIV RNA deline with antiretroviral therapy

12. FUTURE DIRECTIONS OF ANTIRETROVIRAL RESEARCH

All of the available agents for the treatment of HIV infection are nucleoside analogues. The complexity of the HIV life cycle, however, offers multiple targets for potiential antiretroviral agents. Three major new classes of antiretroviral drugs are currently in clinical trials. In addition, novel therapeutics are also being tested in humans. Future studies will focus on the use of these agents alone or in combination in subjects with little evidence of immune dysfunction.

Non-nucleoside reverse transcriptase inhibitors (NNRTI) binds irreversibly to the active site of HIV RT, blocking HIV replication (61,62). As with the nucleoside analogues, resistance develops rapidly, often within 6 weeks. Use of NNRTIs with a nucleoside analogue may delay emergence of resistance and thus may extend the benefit of both agents. Phase III clinical trials of NNRTIs in combination with nucleoside analogues are ongoing. HIV Protease inhibitors prevent proteolytic cleavage of HIV gene products, suppressing the formation of mature viral particle (63). Again, resistance develops in as little as two weeks. Synergistic effects of protease inhibitors with nucleoside analogues has been observed in humans. A phase II trial of ZDV/ddC/ saquinavir (a protease inhibitor) had more pronounced suppression of HIV RNA than any agent alone or in combination (64). As many as five other protease inhibitors are in development in phase I/II trials. As this saquinavir is thought to be the least potent protease inhibitor in development, it is hoped that these newer agents will provide better results. Glucosidase inhibitors attack a cellular enzyme and prevent post translational glucosylation of HIV gp120, causing the formation of defective viral particles. These drugs are synergistic with ZDV <u>in vitro</u>. In phase II trials, glucosidase inhibitors have shown increased CD4 counts and decreased P_{24} antigen when used in combination with ZDV (65). Further efforts are necessary, however, to determine whether any of these agents have a clinical benefit in HIV infected individuals.

In addition to developing drugs active against various portions of the HIV life cycle, novel methods of HIV therapy are currently being investigated. One of these methods, so called gene therapy, utilizes genetically engineered HIV gene products that target HIV regulatory genes, blocking expression of native products and inhibiting HIV expression. One such example of these agents is a *trans* dominant TAT mutant that binds more avidly to HIV TAR than the wild type TAT, effectively inhibiting high level HIV reproduction (66). Other potential gene therapy agents are targeted against the NEF and REV genes of HIV. A second major area of research are immunologic agents that can prevent HIV replication of HIV. For example, HIV, recent studies in long term non-progressors has demonstrated that the presence of cytotoxic T cells were associated with decreased viral replication (67). This finding has led some to speculate that infusion of cytotoxic T cells into HIV infected individuals may depress viral replication and slow disease progression. A second area of interest is based on the finding that activated CD4 cell support HIV replication better than quiescent ones (68). Thus immunosuppressive agents such as cyclosporin may have the paradoxical effect of preserving immune function by indirectly inhibiting viral replication.

In addition to utilizing new and novel agents, future trials will target new patient populations. Initial HIV clinical trials were performed in patients with advanced disease. Later studies used patients that were asymptomatic but had significant degradation of the immune system as measured by CD4 counts. The rapid turnover of HIV, as well as the faster emergence of resistance to ZDV at more advanced stages of illness, suggest that antiretroviral agent would have a more pronounced effect when given to individuals with the lowest viral burden, i.e. subjects with CD4 counts greater than 500 cells/mm^3. Zidovudine failed to have an impact in such subjects, it is postulated, because it was given

as a single agent and resistance developed before a clinical benefit could be measured. Newer agents such as NNRTI or protease inhibitors, are more active against HIV in vitro than the nucleoside analogues. In addition, their use in conjunction with individuals with high CD4 cells counts may prevent the emergence of resistance and may prolong the benefit observed with current antiretroviral agents.

SUMMARY

HIV clinical research has been characterized by stunning achievements and major disappointments. The development of ZDV as a drug for the treatment of HIV took less then two years from the recognition of its antiretroviral properties. This accomplishment was based on an understanding of the viral life cycle and on sound clinical trial design that utilized accepted clinical endpoints. Subsequent trials on early nucleoside analogue therapy have yielded more equivocal results, partly because the investigators relied on surrogate markers that had not been validated and because they failed to design these experiments with sufficient statistical power to detect differences in mortality. Studies that did use mortality as an endpoint, such as the VA and Concorde trials, produced results which, on the face of it, cast doubt on the utility of antiretrovirals. One cannot be too critical, however, as these trials were conceived in a relative vacuum; continuous viral replication throughout the illness was not understood and direct measures of viral replication such as HIV RNA PCR were not sufficiently standardized to be applied to large scale clinical endeavors. In addition, many investigators involved in these early trials naively hoped that the right combination of nucleoside analogues given at the right stage of disease would actually cure HIV infection.

One unintended consequence of this enthusiasm was a partial subversion of the peer review process. In an effort to inform physicians and patients, research results were often reported in press releases months before their appearance in medical journals. For example, the recommendation that ZDV be used in patients with CD4 counts of 200-500 cells/mm^3 was announced by the press in early 1990 while the initial studies were not published until the spring of that year. During that time, physicians involved in HIV care frantically read the New York Times medical section for information about the expanded use of ZDV. Results of other studies, such as ACTG 116A (which compared ZDV to ddI in subjects who had never received therapy) have yet to be published in a major medical journal despite being presented at international meetings and being widely disseminated by the lay press and pharmaceutical manufacturers for promotional purposes.

The limited success of early antiretroviral therapy has prompted a shift in thinking. Instead of seeking a quick cure, many investigators now view antiretroviral clinical trials as a means to better understand viral pathogenesis. This improved understanding may ultimately provide the information necessary to conquer HIV infection. For example, new drugs that attack different steps in HIV replication are already being developed because of a better grasp of the viral life cycle and its regulatory processes. The targets of these agents include reverse transcription (non-nucleoside RT inhibitors), regulation of HIV gene expression (TAT inhibitors, other gene therapy), the formation of mature viral particles (protease and glycosidase inhibitors) The work of HO et. al. and Wel et. al. determining viral kinetics utilized the new HIV protease inhibitors and is a elegant example of this new concept of clinical trials providing insights into pathogenesis. Future studies will undoubtedly follow this model by using novel therapies to answer questions about the nature of HIV infection.

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