Exhibit 7 – Part 1 of 2

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PERSPECTIVE

Anti-Retroviral Therapy of Human Immunodeficiency Virus Infection: Current Strategies and Challenges for the Future

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THE LAST SEVERAL YEARS have seen anti-retroviral therapy of acquired immunodeficiency disease (AIDS) and related diseases move from a theoretical possibility to established clinical practice. One drug, 3'-azido-2',3'-dideoxythymidine (AZT, zidovudine) has been conclusively proven to prolong survival and to reduce morbidity in patients with established AIDS,[1-4] and a number of other drugs are in various stages of development.[5-9] In March 1987, AZT was approved in the United States and a number of other countries for the treatment of severe human immunodeficiency virus (HIV) infection. As of the spring of 1990, AZT is still the only anti-retroviral drug approved for the treatment of AIDS in the United States. However, several other agents found to have activity in early clinical trials are now being tested in large efficacy studies.[10-15] Indeed, the question of whether anti-retroviral therapy would be possible has been replaced by the question of how to best prioritize testing of possible therapeutic modalities.

The urgency of finding effective therapies for AIDS, coupled with inherent features of this disease, has created a unique clinical research environment. On one hand, clinical drug development in AIDS is being scrutinized by many parties to determine how the process can be accelerated. On the other hand, the complexity of the disease itself requires attention to scientific principles and to controlled trial methodology.[16] It is hoped that clinical studies can move quickly while preserving accuracy and safety.

One notable feature setting clinical research in AIDS apart is the intensity with which it is being pursued. Many experimental protocols are now available, and the majority of AIDS patients will at least consider experimental treatment at some time in the course of their illness. In AIDS as perhaps in no other disease, the line between approved and experimental therapy is difficult to draw. In this spirit, in addition to discussing established therapies, we will in this report summarize some of the novel approaches to anti-HIV therapy now under investigation. In addition, we will discuss several hematologic issues in the anti-retroviral therapy of HIV infection and identify certain target areas for future research.

REVERSE TRANSCRIPTASE INHIBITORS

The rationale for anti-retroviral therapy for AIDS is based on several premises[17]: (1) that HIV is the etiologic agent of AIDS; (2) that the development and maintenance of the disease state requires the continued infection of new cells by HIV; (3) that HIV brings about damage to the immune system either directly or indirectly; (4) that a significant proportion of infected cells are killed; and (5) that the principal target organ(s) (eg, the immune system) are able to reconstitute themselves or at least stabilize after therapy. It is now almost universally accepted that HIV is the etiologic agent of AIDS,[18,19] and, indeed, the recent finding of decreased CD4 counts or frank AIDS in a cohort of recipients of HIV-infected blood has tragically added to the fulfillment of Koch's postulates for HIV as the etiologic agent of this disease.[20] As will be detailed below, the demonstration of the clinical efficacy of AZT provided evidence that these premises were substantially correct. However, it is now apparent that they are oversimplifications. Macrophages, for example, may remain productively infected with HIV for a long period without dying[21] and, as will be discussed below, there appear to be limitations on the ability of the immune system to reconstitute itself in patients with advanced AIDS. Advances in therapy may depend in part on successfully addressing these issues.

HIV is one of the most complex retroviruses studied. Thus, in addition to the *gag* (group antigen), *pol* (polymerase), and *env* (envelope proteins precursor) genes that are common to all retroviruses, at least six additional viral genes have been identified in HIV. A number of discrete stages in viral replication have now been described, all of which may be potential targets of therapy (Table 1).[5,7,8] Much of the early work in devising therapy against HIV has focussed on inhibiting viral DNA polymerase (reverse

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Table 1. Steps in the HIV Replicative Cycle That May be Targets for Therapy

Step	Possible Intervention
Binding to target cell	CD4 analogues
	Antibodies to HIV or receptor
Fusion to target cell	Nonspecific inhibitors
Entry and uncoating of RNA	Anti-gp41 antibodies or drugs that block fusion
RNA to DNA transcription by reverse transcriptase	Drugs to block this step of HIV replication may be found
	Many active drugs (eg, AZT and other dideoxynucleosides, phosphonoformate, TIBO compounds) act at this step
Degradation of RNA by RNase	Specific inhibitors of HIV RNase may be found
Migration to nucleus and integration into host DNA	Agents that inhibit these steps may be found
Transcription and translation	Inhibitors of Tat or Rev activity
	Anti-sense constructs (eg, against *tat* or *rev*)
	TAR decoys (which may bind Tat)
Ribosomal frameshifting	Ribozymes may destroy HIV mRNA
Cleavage of Gag and Pol polyproteins	Inhibitors of frameshifting may be found
Protein modification	Inhibitors of HIV protease
	Glycosylation inhibitors (eg, castanospermine)
Viral packaging	Myristoylation inhibitors may be found
Viral budding	Antisense constructs against the packaging sequence
	IFNs (may work at other steps as well)
	Antibodies to viral release antigens
	Agents that selectively kill cells expressing HIV antigens (eg, CD4-toxin fusion proteins)

transcriptase).[1,5,22] Once HIV enters a cell, this viral *pol* product catalyzes the production of a single complementary strand (first strand) DNA copy of the viral RNA genome, and then catalyzes production of a second (positive strand) DNA copy so that the genetic information exists in a double-stranded DNA form (provirus).[23] Reverse transcriptase activity is essential for HIV replication and can for all practical purposes be considered a unique viral enzyme. Moreover, there is evidence that if a DNA copy of uncoated HIV RNA genome is not made promptly, the RNA is susceptible to degradation by cellular enzymes. The virus also degrades genomic RNA (using RNase H) as part of the process of reverse transcription. Therefore, inhibition of reverse transcriptase activity can potentially prevent (rather than simply delay) infection of a cell by HIV.[24]

In the spring of 1985, scientists in our group made the observation that certain dideoxynucleosides were potent inhibitors of HIV replication in vitro.[1,22] Dideoxynucleosides differ from normal deoxynucleosides (the building blocks of DNA) in that the hydroxy (-OH) group in the 3′ position of the sugar ring is replaced by hydrogen or another group that cannot form phosphodiester linkages (Fig 1). Once dideoxynucleosides enter cells, they are activated (anabolically phosphorylated) to a 5′-triphosphate form by cellular enzymes (kinases) (Fig 2).[25-28] It is believed that as 5′-triphosphates, these compounds act at the level of reverse transcriptase to inhibit HIV replication. As will be discussed below, the enzymes responsible for the phosphorylation of dideoxynucleosides vary from compound to compound, and this is a basis for the substantial differences in activity and toxicity profiles of these drugs. Thus, although they may have a common mechanism of action, each must be considered as a separate agent with an individual profile of activity and toxicity. There is no reliable algorithm for predicting the activity and toxicity of individual agents.

As 5′-triphosphates, dideoxynucleosides are believed to inhibit the replication of HIV by at least two mechanisms.[25,29-31] The first mechanism is through a process called chain termination. Once they are added on the 3′-end of a growing chain of viral DNA, no further nucleotides can be added (because of the 3′-modification), no excisional repair mechanism is available to the virus, and HIV replication is halted. The second mechanism is by acting as competitive inhibitors of the endogenous nucleoside-5′-triphosphates. Viral reverse transcriptase is approximately 200 times more sensitive to these compounds than is cellular DNA polymerase α, and this is probably one basis for their selective anti-HIV activity. However, DNA polymerases β and γ are somewhat sensitive to these drugs,[25,30] and this may be a basis for certain toxicities.[32,33] In particular, the myositis that is observed after long-term AZT therapy is associated with evidence of mitochondrial damage, presumably from inhibition of DNA polymerase γ.[34]

The antiretroviral activity of dideoxynucleosides (including AZT) is not restricted to HIV. Indeed, these compounds have potent activity against a broad spectrum of human and animal retroviruses, including HIV type 2, human T-cell lymphotropic virus type-1, animal-lentiviruses, and murine retroviruses,[32,35-37] provided that they are adequately phosphorylated in the target cell. However, there is profound species to species (and cell type to cell type) variation in regard to the anabolic phosphorylation of these drugs. Some congeners also appear to have activity in animal models of hepatitis B virus, which, although it is a DNA virus, replicates through an RNA intermediate using a reverse transcriptase-like DNA polymerase.[38] Based on this observation, Hoofnagle et al in the National Institute of

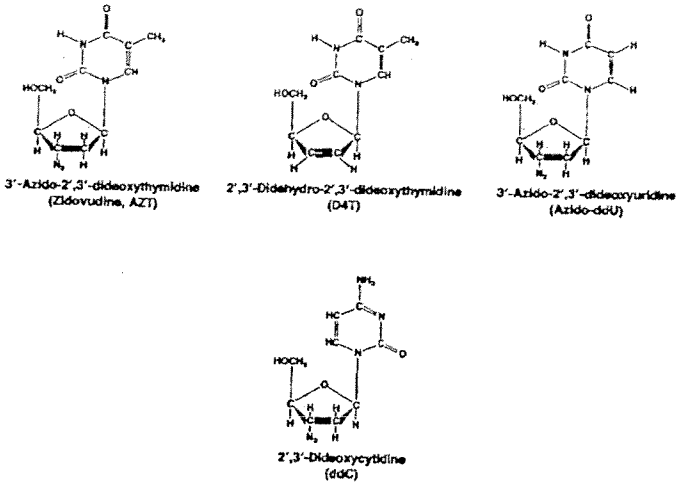

Fig 1. Structure of the first six dideoxynucleosides to enter clinical trial for use in AIDS. The three on the top line are thymidine analogues, the one in the center is a uridine analog, and the two on the bottom are purine analogues. ddA is rapidly deaminated to form ddI by the ubiquitous enzyme adenosine deaminase.

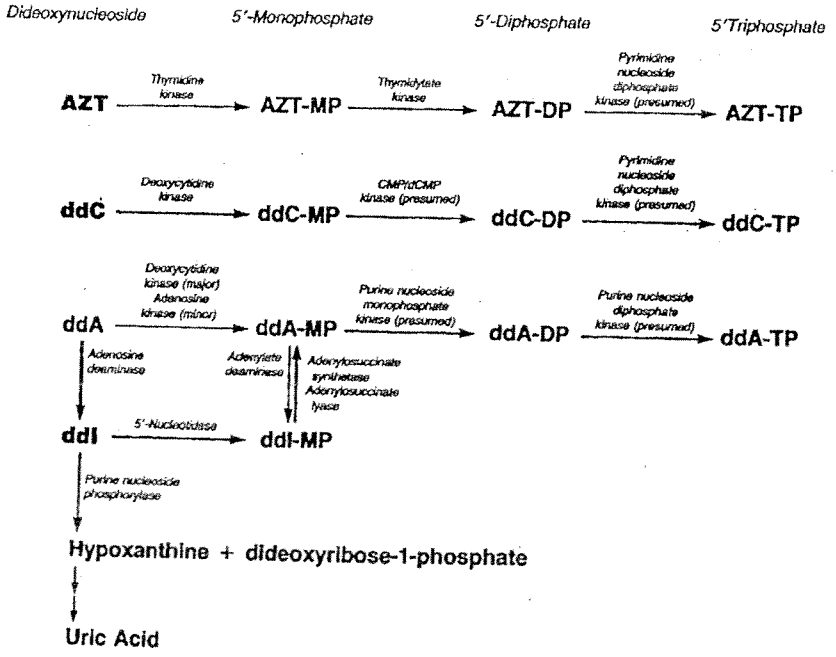

Fig 2. Phosphorylation pathways or AZT, ddC, ddA, and ddI. MP, monophosphate; DP, diphosphate; TP, triphosphate. (Modified and reprinted with permission.[6])

Diabetes and Digestive and Kidney Diseases, Bethesda, MD, in collaboration with our group, are now conducting a trial of dideoxyinosine in patients with hepatitis B infection.

Clinical Activity of AZT

AZT was synthesized by Horwitz et al in the early 1960s,[39] and in 1974, Ostertag et al in Germany first reported on its activity against a murine retrovirus.[35,40] Thus, the discovery of AZT may be properly ascribed to these workers. Its ability to inhibit HIV replication was first observed by Mitsuya et al in February 1985.[1] A phase I study conducted at the National Cancer Institute (NCI) and Duke University was initiated 5 months later.[2] This trial demonstrated that AZT was well tolerated in short-term therapy and had clinical activity against HIV.[2] In particular, patients with AIDS or AIDS-related complex (ARC) were observed to have increases in their CD4 cells, reversal of cutaneous anergy, increased energy and appetite, and weight gain. Subsequently, in a randomized phase II trial organized by Burroughs Wellcome Co Research (Triangle Park, NC) in 12 medical centers around the United States, AZT (at a dose of 1,500 mg/d) was shown to increase survival and reduce the incidence of opportunistic infections in AIDS patients who had had *Pneumocystis carinii* pneumonia or who had severe ARC; after 6 months, only one patient receiving AZT had died, compared with 19 patients receiving placebo.[3,41] At this time, all the patients originally randomized to the placebo arm were offered AZT. In an extension of this study, it was observed that the patients receiving AZT continued to do comparatively well. At 12 months, 84.5% of the patients originally randomized to receive AZT were alive.[42] This result is substantially better than the 60.9% survival at 9 months observed with the patients originally randomized to placebo.[3] The death rate in the placebo arm would have almost certainly been higher if the patients had not been switched to AZT after 6 months. Thus, although the trial was only randomized for 6 months, a beneficial effect of AZT could be observed for at least 1 year of therapy. As a result of this trial, AZT was approved as a prescription drug in the United States for HIV-infected patients who either had had *Pneumocystis carinii* pneumonia or who were symptomatic and had less than 200 CD4 cells/mm^3. The original recommended dose was 200 mg every 4 hours (1,200 mg/d). Subsequent research has led to recommendations for a lower dose (vide infra).

AZT can penetrate into the cerebrospinal fluid,[2,43] and in both the phase I and subsequent trials, it has been observed to improve HIV-related dementia.[4,44,45] In the absence of therapy, HIV-induced dementia is generally a progressive disorder, and one can argue that reversal of this process may be used as a measure of drug efficacy even in the absence of a control group. The effect of AZT on AIDS dementia is most evident in children with HIV infection. In such patients, neurologic dysfunction is a particularly prominent clinical feature, and the reversal induced with AZT by continuous intravenous infusion is sometimes striking.[4] There is some evidence that continuous infusion of AZT is superior to intermittent administration in children with advanced HIV dementia, and the optimal dose and schedule of this drug will be an important issue for further study. Monocyte-derived cells (eg, microglial cells) are believed to be important targets for HIV infection in the central nervous system,[21,46,47] and, in fact, high levels of unintegrated HIV DNA (one measure of active viral replication) can be found in the brains of patients with AIDS dementia.[48] It is possible that the effects of AZT on HIV dementia are related to its ability to protect macrophage-like cells from infection by HIV.[49,50] However, the pathogenesis of HIV dementia is still imperfectly understood. Thus, AZT may theoretically affect other mediators of this process.

The clinical development of AZT was exceedingly rapid; it was approved for clinical use in the United States about 2 years after the first in vitro observation of its activity against HIV. A number of clinical trials have been conducted since that time and these have refined our knowledge of its use. One study, a multicenter trial organized by the AIDS Clinical Trials Group (ACTG) of the National Institute of Allergy and Infectious Diseases (NIAID), demonstrated that a lower dose of AZT (100 mg every 4 hours after a 1-month initiation period of 200 mg every 4 hours) is as effective as the originally tested dose in patients with AIDS, but less toxic.[51] As a result of this study and another that will be described below, the recommended regimen for patients with symptomatic disease and less than 500 CD4 cells/mm^3 is now 200 mg of AZT orally every 4 hours for 1 month, followed by 100 mg every 4 hours (eg, a maintenance dose of 600 mg/d). However, it is unclear whether even lower doses of AZT may be preferable. Both in the original phase I study[2] and in a recent pilot study of patients with AIDS-related complex conducted by Collier et al,[52] some individuals were found to respond to a total dose of approximately 300 mg of AZT per day.[2,52] It is possible that this will be found to be the optimal dose, at least in certain patients. Whether such lower doses will be effective in patients with HIV dementia, however, remains to be seen.

Two other recently completed studies have shown that AZT may be useful in patients with earlier manifestations of HIV infection. In one study, 500 mg/d of AZT reduced the progression to AIDS or ARC over a 2-year period in a group of asymptomatic patients with 200 to 500 CD4 cells/mm^3.[53] In a related study, 600 mg/d of AZT reduced the progression to AIDS or ARC over the same time period in mildly symptomatic patients with 200 to 500 CD4 cells/mm^3.[54] It should be stressed that the beneficial effect of AZT in these studies was observed for at least 2 years. AZT is now approved for such patients. However, it is not known whether the overall survival of such patients will be enhanced by such early initiation of AZT therapy. Is it better to treat all patients early (when their CD4 cell count decreases below 500 cells/mm^3) or to wait until the count decreases below 200 cells/mm^3? Two ongoing studies, a cooperative European trial and a Veterans Administration trial, are presently studying this issue.

Several epidemiologic studies have shown that the survival of patients with AIDS has improved since the end of 1986 (the time that AZT began to be widely used).[55-57] A number of factors have probably contributed to this trend, including widespread prophylaxis and treatment of opportu-

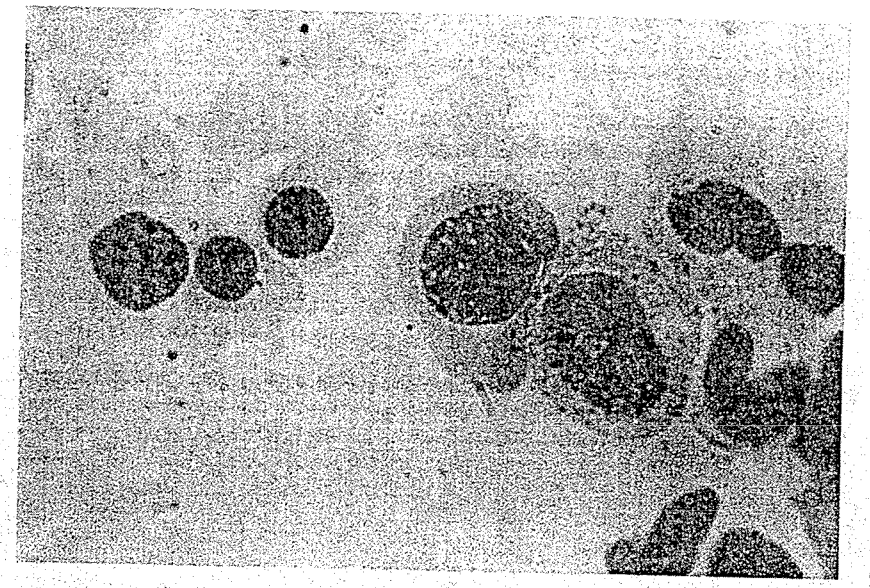

Fig 3. Representative bone marrow of a patient with AZT toxicity. Some megaloblastic changes can be seen.

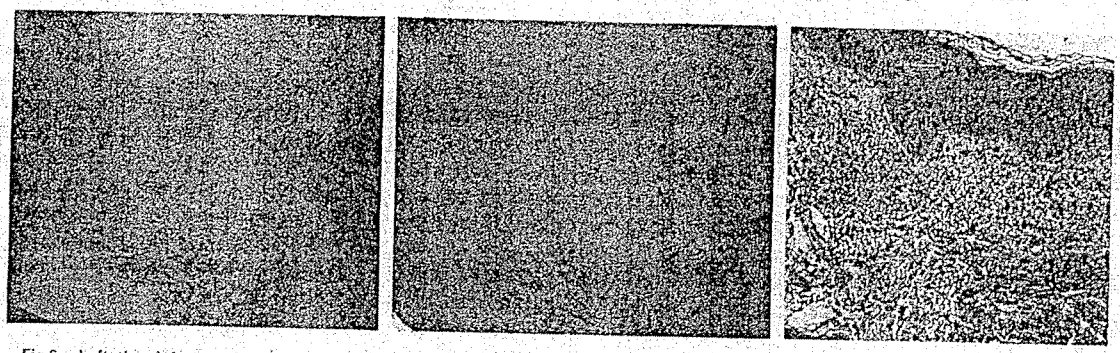

Fig 6. Left, the abdomen of a patient with a ddC-induced cutaneous eruption. Middle, photograph of the same patient 2 weeks later while still on the same dose of ddC, showing clearing of the cutaneous eruption. Right, hematoxylin-eosin stain of a different patient with a ddC-induced cutaneous eruption showing perivascular lymphocytic infiltration (×100).

nistic infections, increased physician experience, and, perhaps, changing demographics of the disease. However, the widespread use of AZT has been linked to this trend even if factors such as *Pneumocystis* prophylaxis are taken into account.[56,58] For example, in a large trial conducted in the San Francisco area comparing different doses of aerosolized pentamidine in patients with AIDS or ARC, all patients received some regimen of aerosolized pentamidine, but could either receive or decline AZT. At the end of 9 months, 15% of the AZT recipients had died, compared with 53% of the patients who had declined AZT therapy.[59] It is worth noting that aerosolized pentamidine, while decreasing the number of opportunistic infections due to pneumocystis, has not formally been shown to increase the survival of patients with AIDS.

There is also recent evidence suggesting that the widespread use of AZT in patients with AIDS-related complex has, by delaying disease progression, caused a recent reduction in the apparent incidence of fulminant AIDS.[59]

Toxicity of AZT

The use of AZT may be associated with substantial toxicity, especially in patients with established AIDS.[2,34,41,60,61] Bone marrow suppression is the most common dose-limiting toxicity.[2,41,60,61] The red cell lineage is most often affected, but leukopenia and thrombocytopenia can also occur. Many patients receiving AZT develop macrocytosis, and red blood cell mean corpuscular volumes of 110 μm^3 or greater are not uncommon.[2,41,62] In addition, megaloblastic changes can sometimes be observed (Fig 3). The extent of the macrocytosis in patients receiving AZT is not directly predictive of the subsequent development of anemia,[61,62] and it is possible that the drug has two or more separate effects on erythrocyte precursor cells.

Interestingly, while thrombocytopenia can sometimes be observed as a late complication of AZT therapy, patients with HIV-induced thrombocytopenia often have an increase in their platelet count upon being administered

AZT.[63,64] Thus, AZT can, depending on circumstances, either increase or decrease platelet counts. Indeed, AZT is now viewed by many as a front-line therapy for AIDS-related thrombocytopenia. There are several mechanisms by which HIV infection may cause thrombocytopenia, including infection of megakaryocytes, anti-platelet antibodies, nonspecific binding of immune complexes to platelets, complement activation, and splenomegaly,[65-69] and it is not clear which of these are the most important in causing disease. However, the rapid response of some patients to AZT would suggest that HIV may play a direct role in this process.

Bone marrow toxicity from AZT is more frequent in patients with AIDS (rather than ARC), with pretreatment anemia, with low CD4 counts, with neutropenia, or with fever.[41,60] Low or low to normal serum vitamin B_{12} levels may also predispose to AZT-induced anemia. In the phase II study of AZT, 45% of patients who had had *Pneumocystis carinii* pneumonia required transfusions or a reduction of dosage during the first 6 months of therapy. By contrast, bone marrow toxicity is substantially less frequent in patients with 200 to 500 CD4 cells/mm^3 receiving lower doses (500 to 600 mg/d) of AZT.[51,53] However, even at these doses, bone marrow toxicity may develop in more than 30% of patients with advanced HIV infection.[60]

The pathogenesis of AZT-induced bone marrow suppression is not completely understood. It has been observed that AZT-5′-monophosphate binds efficiently to thymidylate kinase (the enzyme that catalyzes the phosphorylation of thymidine-5′-monophosphate to thymidine-5′-diphosphate), but comes off slowly (Fig 2).[25] It can thus theoretically tie up this enzyme. This has been hypothesized to cause the decreased levels of thymidine-5′-triphosphate (a normal DNA building block that competes with AZT-5′-triphosphate) observed in certain T-cell lines after exposure to very high concentrations of AZT (over 50 μmol/L).[25] However, decreased levels of thymidine-5′-triphosphate have not been found in other cell lines exposed to AZT or with lower concentrations of AZT,[70,71] and the potential contribution of this process to either AZT-induced bone marrow suppression or macrocytosis is unclear at this time. It is noteworthy that macrocytosis is not observed with two other dideoxynucleosides, 2′,3′-dideoxycytidine (ddC) and 2′,3′-dideoxyinosine (ddI), both of which have undergone extensive phase I testing.[10-15]

Recently, Brunetti et al have suggested a means by which the specifics of AZT metabolism might be put to clinical advantage outside the clinical setting of AIDS.[72] They observed that the combination of AZT and 5-fluorouracil was more cytotoxic to cells than either agent alone. This cytotoxicity apparently occurs because 5-fluorouracil, through its metabolite fluorodeoxyuridine monophosphate, inhibits thymidylate synthase. This is a key enzyme in the de novo synthesis of thymidine-5′-monophosphate, and its inhibition causes cells to depend on the salvage pathway for thymidine. This in turn apparently leads to increased phosphorylation of AZT and increased incorporation of AZT into cellular DNA.[72] A simple mutation of thymidine kinase would not be a strategy open to such tumor cells, as this would theoretically make them even more vulnerable to depletion of thymidine pools. The combination of these drugs might conceivably have use in colon cancer chemotherapy. As an extension of this line of attack, it may be worthwhile exploring the combination of AZT, 5-fluorouracil, and leucovorin calcium. Interestingly, Horwitz et al initially synthesized AZT as a cancer chemotherapeutic agent,[39] and this initial vision may still be realized.

Some patients receiving AZT experience nausea, headaches, or malaise.[2,41,60,73] High doses are sometimes associated with anxiety or confusion, and rare patients have developed seizures (occasionally fatal) or a Wernicke's type encephalopathy.[60] Hepatitis and Stevens Johnson syndrome have also been reported to be associated with AZT therapy,[60] and some patients (particularly blacks) develop bluish fingernail pigmentation. Myalgias are commonly observed, and frank myositis can be a dose-limiting toxicity for long-term AZT therapy.[34] As noted above, AZT-induced myositis appears to be related to mitochondrial damage in muscle cells. This condition also has an inflammatory component. HIV infection itself can cause an inflammatory myositis, and these two conditions can be difficult to distinguish. The myositis caused by AZT will generally respond to discontinuation of drug or dose reduction, and this observation may be used to distinguish it from HIV-induced myositis. AZT myositis sometimes also responds to non-steroidal anti-inflammatory drugs.

Approximately 75% of an administered dose of AZT is metabolized by hepatic glucuronidation to form 3′-azido-2′,3′-dideoxy-5′-glucuronylthymidine, an apparently inert metabolite that is subsequently excreted in the urine.[74] Most of the rest is excreted directly into the urine. The enzyme or enzymes that catalyze this glucuronidation may be inhibited by certain other medications, and these may prolong the half-life of AZT and thus enhance toxicity. In particular, probenecid has been found to inhibit both the hepatic metabolism and renal excretion of AZT[75,76]; overall, the total body clearance is reduced by 65%. Other drugs that undergo hepatic metabolism are now being investigated for possible interactions with AZT. There was some suggestive evidence from the phase II trial that patients who received acetaminophen had increased AZT toxicity,[41] and it was thought that this might have been due to a similar interference with AZT glucuronidation. However, subsequent studies have shown that acetaminophen has no such effect on AZT metabolism.[77] Most likely, the apparent increased toxicity seen in the phase II trial was because patients who received acetaminophen had symptomatic HIV infection and were thus at higher underlying risk for AZT toxicity.

Patients with advanced AIDS often have underlying bone marrow suppression from HIV infection, opportunistic infections (especially with cytomegalovirus or *Mycobacterium avium-intracellulare*), or other drugs.[78] (Also, parvovirus B19 has recently been reported to be a treatable cause of severe chronic anemia in the setting of HIV infection.)[79] It is not uncommon for patients with underlying bone marrow suppression to be completely intolerant of AZT or to tolerate only very low doses.[60] Patients with low serum

vitamin B_{12} (or folic acid) levels may be at higher risk for developing AZT toxicity,[41,80] perhaps because deficiencies of these vitamins result in decreased production of thymidine-5′-monophosphate via de novo synthesis. It may therefore be prudent to measure serum vitamin B_{12} and folic acid levels in patients on AZT therapy and to institute vitamin replacement therapy if the levels are low. However, such therapy cannot reverse established toxicity.

Another strategy is the use of bone marrow-stimulatory cytokines.[81] Erythropoietin (Epo), granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte-CSF (G-CSF) have been studied in HIV-infected patients receiving AZT, and there is some evidence that these factors can increase the numbers of the respective blood elements.[82-85] Epo has been the most extensively evaluated cytokine in this setting. Many AIDS patients have relatively low Epo responses to AZT-induced anemia, and a controlled trial has shown that exogenously administered Epo can reduce the need for transfusions in anemic AZT recipients with less than 500 IU/L of serum Epo.[84] Epo is now available for this indication.

In an initial trial of GM-CSF in patients with HIV-induced leukopenia, Groopman et al observed an increase in neutrophils, eosinophils, and monocytes over a 6-month period of time.[82] There was apparently no change in the ability to culture HIV from peripheral blood cells obtained from GM-CSF–treated patients.[82] However, subsequent in vitro studies have shown that GM-CSF could enhance HIV replication in monocyte/macrophages.[86-88] A small trial conducted by Pluda et al showed that HIV-infected patients not receiving anti-retroviral therapy had increased serum HIV p24 antigen (a measure of viremia) during a 2-week course of GM-CSF therapy (Fig 4).[83] This was not simply a rebound from stopping AZT, as the patients either had never received AZT or were off it for 4 weeks before starting GM-CSF.[83] While only a few patients were studied, these results suggest that patients receiving GM-CSF without anti-retroviral therapy should at a minimum be monitored closely for an increase in HIV replication.

Additional studies by Perno et al in our group showed that the anti-HIV activity of AZT was enhanced in peripheral blood monocyte/macrophages exposed to GM-CSF.[87] A similar effect is observed in the U937 monocytoid cell line.[89,90] Metabolic studies suggest that this effect occurs both because the entry of AZT is enhanced in the presence of GM-CSF and also because its phosphorylation to 5′-triphosphate is increased.[87] Several trials of AZT used in conjunction with GM-CSF are now underway. Interestingly, the anti-HIV activity of other thymidine or uridine anti-HIV agents in monocyte/macrophages is also increased by GM-CSF, while that of other nucleoside analogues (such as 2′,3′-dideoxycytidine or 2′,3′-dideoxyinosine) is not (Fig 5).[87] Another cytokine, macrophage–CSF (M-CSF), also increases HIV replication in monocyte/macrophages.[86,87,91] However, preliminary studies suggest that, unlike GM-CSF, this agent does not potentiate the anti-HIV activity of AZT.[92] These results serve as a reminder that unexpected interactions among active agents may occur and each combination of agents must be studied

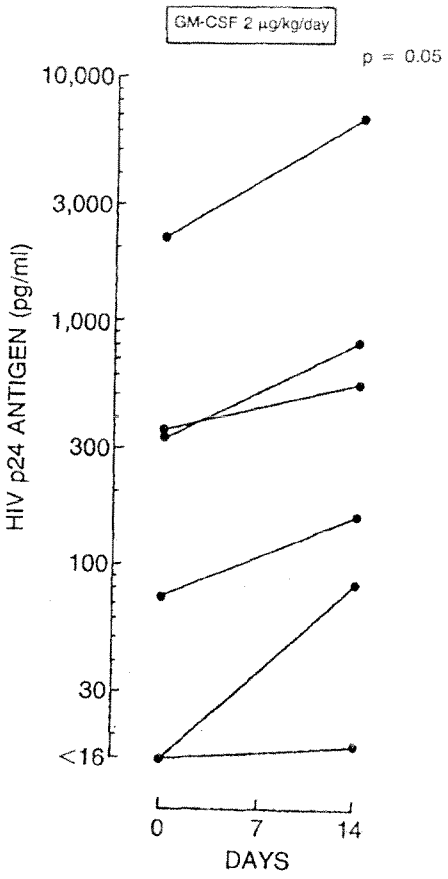

Fig 4. Serum HIV p24 antigen in six patients with AIDS or severe ARC receiving 2 mg/kg/d of GM-CSF for 2 weeks in the absence of anti-HIV therapy. Data from Pluda et al.[83]

in its own right. Finally, G-CSF, a cytokine that does not affect HIV replication in monocyte/macrophages in vitro,[86,92] has been found in a pilot study to ameliorate granulocytopenia in AIDS patients receiving AZT or other hematotoxic therapy.[85] It is not clear at this point what the ultimate value of these agents will be in the treatment of AIDS and controlled trials will be needed to sort these issues out. Other dideoxynucleosides, such as ddC or ddI, which have little hematologic toxicity,[10-15] may be found to offer an alternative therapeutic strategy in certain patients with AZT-induced bone marrow toxicity (vide infra).

AZT Resistance

In many patients, particularly those with established AIDS, the increase in CD4 cells upon initiating AZT therapy is only transient.[3] In addition, some patients have late increases in serum HIV p24 antigen after an initial decrease.[93] The late decreases in CD4 cells may in part be a result of AZT toxicity, as they occur even earlier in patients on very high doses.[2] However, HIV isolates from the majority of patients on long-term AZT therapy have a

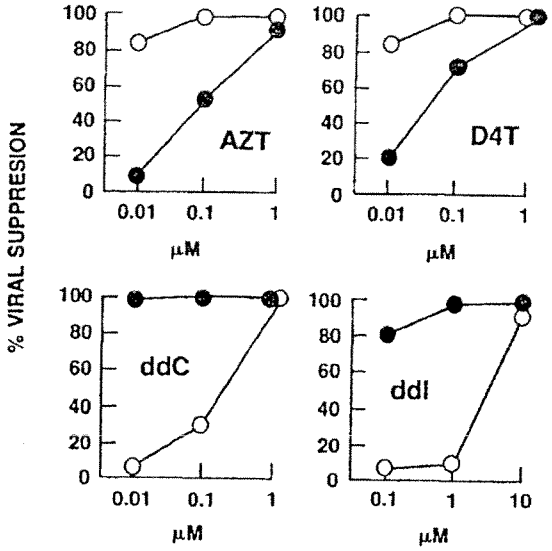

Fig 5. In vitro anti-HIV activity of AZT, D4T, ddC, and ddI in peripheral blood monocytes exposed to a monocytotropic strain of HIV in the presence or absence of GM-CSF. (●) No GM-CSF. (○) GM-CSF 100 U/mL. (Modified and reprinted with permission.[97])

decreased sensitivity to AZT,[94,95] and this probably contributes to an apparent late decline in efficacy. As recently reported by Richman et al, AZT resistance develops more rapidly in patients with advanced AIDS than those with earlier HIV infection.[96] This group also did not find an effect of AZT dosage on the development of resistance. Thus, while the potential development of resistance is a theoretical concern in the treatment of early HIV disease with low doses of AZT, it is possible that drug resistance occurs only comparatively slowly in this setting.

The biochemical mechanism for AZT resistance is presently under investigation. HIV replication is notably error prone,[97] and specific point mutations have been identified in the *pol* gene of certain AZT-resistant strains of HIV (particularly $Asp^{67} \rightarrow Asn$, $Lys^{70} \rightarrow Arg$, $Thr^{215} \rightarrow Phe$ or Tyr, and $Lys^{219} \rightarrow Gln$).[98] These changes are believed to be a basis for resistance. The mutations would likely affect the charge or α helix content of the probable catalytic site on the reverse transcriptase. Surprisingly, the reverse transcriptase from resistant strains has an unchanged susceptibility to inhibition by AZT-5′-triphosphate[98,99]; in particular, there were no changes in the 50% inhibitory dose (ID_{50}), the relevant Michaelis constant (K_m), or the inhibition constant (K_i). Additional studies will be needed to explain this paradox. One must keep open the possibility that an unidentified metabolite of AZT contributes to its effect at the level of reverse transcriptase.

Such point mutations might also provide a useful tool for screening for AZT resistance.[100] However, only a limited number of resistant strains have been sequenced at this time, and it will be useful to gather more sequence data to determine whether other mutations may also confer a resistant phenotype. Also, it has not been formally shown that the development of resistance is the cause of a decreased response to AZT. While we believe this is likely, additional studies will be needed to determine the clinical significance of this laboratory observation.

Interestingly, HIV strains that are resistant to AZT generally preserve their sensitivity to a number of other dideoxynucleosides, such as ddC or ddI, although exceptions certainly occur.[94,95,99] However, there does appear to be cross-reactive resistance to dideoxynucleosides with an azido group in the 3′-position such as 3′-azido-2′,3′-dideoxyuridine (azidouridine).[94,99] Thus, patients whose HIV becomes resistant to AZT may theoretically still respond to therapy with one of these other non–cross-reacting dideoxynucleosides. Studies are now underway to determine if combination regimens with different dideoxynucleosides can delay the development of resistance. This strategy will be discussed in greater detail below.

Clinical Studies with ddC

The second dideoxynucleoside to undergo clinical testing was ddC. In vitro studies by Mitsuya and Broder had shown that this compound is about 10 times more potent than AZT on a molar basis.[22] Additional studies showed that ddC had relatively less toxicity for bone marrow progenitor cells than AZT.[101] A phase I study of ddC conducted at the NCI showed that it was well absorbed by the oral route and penetrated into the cerebrospinal fluid (although not as well as AZT).[10,102,103] In addition, in this and in another study conducted by Merigan et al[11] in the ACTG, it was observed that nearly all patients receiving ddC had declines in their serum HIV p24 antigen and most had initial increases in their CD4 cells.[10,11] Thus, the phase I studies suggested that ddC had activity against HIV.

These studies also indicated that the toxicity profile of ddC was very different from that of AZT. The dose-limiting toxicity of ddC was found to be a reversible painful peripheral neuropathy in a glove-stocking distribution.[10,11] This was seen in most patients who received 0.09 mg/kg/d of ddC for 14 weeks or longer. More severe cases of the neuropathy were associated with decreased vibratory sensation or a decreased amplitude on nerve conduction studies.[104] Nerve biopsy in one patient showed axonal degeneration (R. Yarchoan, M. Dalakos, S. Broder, unpublished observation, January 1988). The neuropathy was clinically similar to the HIV-associated neuropathy often seen in patients with advanced AIDS.[105] However, patients with HIV-induced neuropathy generally have more hypesthesia than pain, and they have a relatively more pronounced decrease in their vibratory sensation than patients with ddC neuropathy. Finally, patients with ddC neuropathy generally had a gradual decrease in their symptoms starting several weeks after the drug was withdrawn. Other toxicities, generally seen with higher doses of ddC (0.18 mg/kg/d or greater) included stomatitis, transient cutaneous eruptions (ranging from macular to papular to pseudovesicular) (Fig 6), fevers, and edema.[10,11,106] Occasional patients at the highest doses tested (up to 0.75 mg/kg/d) developed thrombocytopenia or leukopenia. Bone marrow biopsies from patients at very high doses were generally normocellu-

lar. In some, vacuolization of erythroblasts was observed, the significance of which is unclear at this time. Finally, rare patients receiving ddC have been observed to develop hearing loss from VIII nerve damage or postural hypotension from autonomic neuropathy.[107]

The toxicities described above were generally observed in patients who received at least 0.09 mg/kg/d of ddC. However, there is preliminary evidence that a lower dose (0.03 mg/kg/d) can maintain an anti-viral effect in most patients with little or no short-term toxicity.[11,108] While the declines in HIV p24 antigen and increases in CD4 cells occurred relatively slowly on this dose, they appeared to be sustained for 6 months or longer in many patients. A randomized phase II trial using this dose schedule is now underway. In addition, ddC is being made available in the United States for certain patients who cannot tolerate AZT under the mechanism of an "open label protocol." Finally, as will be discussed in more detail below, several studies are now exploring the combined use of ddC with AZT.

Clinical Studies With ddI (Didanosine)

Both AZT and ddC are pyrimidines. The next dideoxynucleosides to be studied in clinical trials were the related drugs 2′,3′-dideoxyadenosine (ddA) and ddI. These compounds are closely related, and, in fact, ddA is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase,[28,109] so the two can be considered alternate forms of the same drug for many purposes. Either compound is phosphorylated in human cells to form ddA-5′-triphosphate (ddA-TP), which is believed to inhibit HIV replication at the level of reverse transcriptase.[24,71] The initial phosphorylation step of ddI is interesting in that it is catalyzed by 5′-nucleotidase with inosine-5′-monophosphate as the phosphate donor (Fig 2).[110] Both ddA and ddI had several features that made them candidates for clinical testing. Both had favorable in vitro therapeutic indices in T cells.[22] Their anti-HIV activity was not inhibited by either 2′-deoxyadenine or 2′-deoxyinosine.[22] They both had anti-HIV activity in monocyte/macrophages.[49,87] They had little in vitro toxicity for bone marrow progenitor cells.[101,111] Finally, once metabolized to a TP, they remained in cells for a long time (half-life greater than 12 hours).[109] This result is in contrast to AZT or ddC, whose triphosphates have half-lives of 3 hours or less,[25,30] and it suggested that an anti-HIV effect might be attained with relatively infrequent dosing of ddA or ddI.

Under acid conditions such as are found in the stomach, ddA and ddI are cleaved into 2′,3′-dideoxyribose and the free base. Adenine, the free base of ddA, is subsequently metabolized to 2,8-dihydroxyadenine, which is insoluble and can cause renal failure.[112] By contrast, hypoxanthine, the free base of ddI, is catalyzed to uric acid, a physiologic waste product. Thus, ddI seemed the more attractive candidate for oral dosing. However, it was still necessary to address the problem of acid instability, and this is done by administering ddI with buffers.

We started a small intravenous phase I trial of ddA in February 1988.[113] In July 1988, permission was obtained to administer ddI, and we refocused our efforts on this analog.[12,114] The phase I trial of ddI involved patients with AIDS or poor-prognosis ARC who had had 4 or less months of prior AZT therapy. Two other studies of ddI were started soon afterward, one at the University of Rochester and New York University and a second at Boston University.[13,15] The NCI study found that ddI was well absorbed by the oral route if administered with antacids (mean bioavailability, 40%) and that it penetrated into the cerebrospinal fluid.[12,114,115] In all the trials, it was observed that patients who received 200 mg/d or more of ddI orally frequently had increases in their $CD4^+$ lymphocytes, their total lymphocytes, their CD4:CD8 ratio, and the absolute number of CD8 cells.[12,13,15]

The results from the phase I trials also indicated that some patients had sustained responses to ddI.[13,15,116] For example, in the NCI phase I trial, 11 of the first 58 patients (19%) were still receiving ddI and still had an increase of ≥ 20 CD4 cells/mm^3 over baseline values 1 year after entry into the study (Fig 7) (R. Yarchoan, J. Pluda, K. Wyvill, S. Broder, unpublished observation, June 1990). This group included both patients who were initially entered at very low doses as well as patients who developed toxicity upon receiving very high doses of ddI (vide infra), and it is possible that more consistent responses would have been obtained if all patients had taken what is now felt to be an optimal dose range. The 28 patients who remained on ddI for 1 year overall had an increase of 31 ± 14 CD4 cells/mm^3 (mean $\pm$ SEM) as compared with baseline ($P < .05$). Also, many patients receiving ddI had increases in their neutrophil counts, platelet counts, and hemoglobin.[15]

The CD4 increases were most consistently observed in patients who had previously received AZT for 4 months or less, but they were also seen in some patients who had received long-term AZT therapy before entry into the trial (Fig 8).[116] This effect was not simply because the patients with prior long-term AZT therapy had lower starting CD4 counts; indeed, in patients who had previously received less than 4 months of AZT, there was no correlation between the starting CD4 count and the percent CD4 cell increase

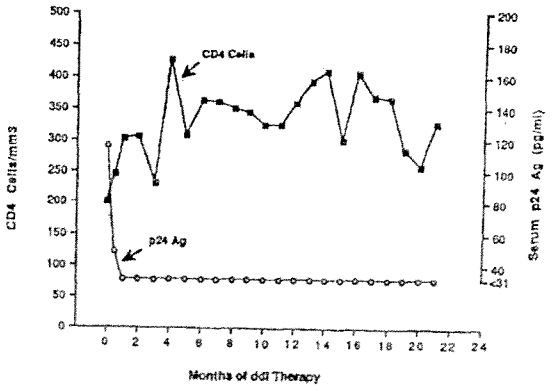

Fig 7. Course of an AIDS patient who previously had esophageal candidiasis upon starting ddI therapy. This patient had a sustained increase in CD4 cells and decrease in p24 antigen during treatment with 9.6 mg/kg/d of ddI divided into three daily doses.

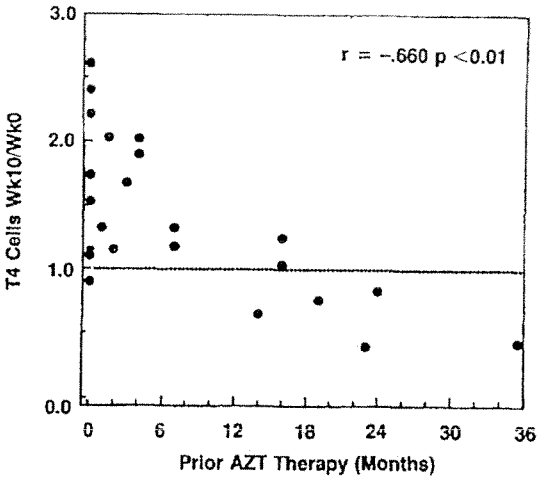

Fig 8. CD4 increases (expressed as a ratio) during the first 10 weeks of ddI therapy related to the length of prior AZT therapy. As can be seen, the greatest increases were generally observed in patients who had previously received 4 months or less of therapy (Yarchoan, Pluda, Wyvill, and Broder, unpublished observation, September 1990).

with ddI therapy (R. Yarchoan, D. Venzon, J. Pluda, S. Broder, unpublished observation, September 1990). Most patients receiving ddI, irrespective of prior long-term AZT therapy, also had a decrease in their circulating HIV p24 antigen.[12,13,15] The cause of the discrepancy between the serum p24 response and CD4 cell increase in some patients extensively pretreated with AZT is unknown and requires further research. Many of the patients receiving ddI reported increased energy and increased appetite, and they gained an average of 1.5 kg. Finally, certain patients in the NCI study receiving high doses of ddI were observed to have improvement in HIV dementia.[116]

These phase I studies, as well as a more recent study conducted in adults at the New England Deaconess Hospital, also showed that ddI was well tolerated by most patients at doses of up to 750 mg/d, even over a long period of administration.[13,15,116,117] Hematologic toxicity was rarely observed, even in patients who had previously been intolerant of AZT.[13,15,114,117] Because of this favorable activity/toxicity profile, ddI has been moved into phase II/II testing. Three randomized multicenter studies were initiated in October 1989; two comparing ddI with AZT and a third comparing three doses of ddI in patients who are intolerant of AZT. In addition, ddI, at doses of 334 to 750 mg/d, has been made available in the United States to patients with severe HIV infection who either cannot tolerate AZT or who have progressive disease despite AZT therapy under the regulatory mechanisms of a "treatment IND" and "open label protocol," respectively. As of this writing, more than 17,000 adult patients have received ddI. In our current studies, we generally avoid exceeding a dose of 500 mg/d, and even lower doses are probably appropriate during combination studies.

There now an interest in moving new AIDS drugs into children as early in the developmental process as possible. In a trial of ddI in children with symptomatic HIV disease conducted by Butler et al, an anti-HIV effect was observed at doses that were tolerated in most patients.[118] In particular, a number of children had improvement in HIV-induced dementia. The absorption of oral ddI was found to be more variable than in adults, and these investigators were able to relate the anti-HIV effects (including reversal of dementia) and toxicities to the area-under-curve (AUC) of plasma ddI levels.[118]

The main purpose of a phase I trial is to define the dose-limiting toxicity of a drug, and to this end, doses of ddI higher than 750 mg/d were initially studied. At doses up to 3,500 mg/d, ddI was found to cause peripheral neuropathy, sporadic acute pancreatitis, and sporadic hepatitis.[12,13,15,114] Some patients also developed headaches, insomnia, or clinically silent increases in serum amylase, triglycerides, and uric acid. As noted above, the increases in uric acid are most likely a direct result of ddI catabolism through hypoxanthine. Also some patients receiving a preparation of ddI mixed with a citrate/phosphate/sucrose vehicle (buffer/sweetener) have developed diarrhea, apparently from the vehicle rather than the drug itself.

The peripheral neuropathy from ddI is clinically similar to that of ddC, but is usually less severe. We have found that if the ddI is stopped when the pain or tingling in the feet becomes mild to moderate in intensity and lasts for several hours, the neuropathy will generally subside over the next several weeks. Patients can sometimes then be restarted at a lower dose without the neuropathy recurring. However, the most serious toxicity from ddI is acute pancreatitis. The mechanism for this toxicity is not known. Of the initial 58 patients enrolled in the NCI phase I study, three developed frank pancreatitis and two others had clinically ambiguous or mild cases.[116] Each case of frank pancreatitis occurred during the first 6 months of therapy, and this may conceivably be a high-risk period for this complication. In each case, the pancreatitis resolved rapidly after ddI was stopped. In the two instances where we challenged patients with a lower dose of ddI, pancreatitis did not recur. (However, we do not recommend rechallenging patients who have had a ddI-related episode of acute pancreatitis, except in an experimental protocol.) This is different from the experience with 6-mercaptopurine, another purine analog. 6-Mercaptopurine (or its alternate form azathioprine) can cause pancreatitis in up to 6% of patients with inflammatory bowel disease, and the pancreatitis will predictably reoccur if the patients are subsequently rechallenged with even very low doses.[119]

The development of pancreatitis (and the other toxicities) in the patients receiving ddI is dose related, and doses of 750 mg/d or lower are well tolerated in most patients. This toxicity is uncommonly observed in patients receiving 750 mg/d or less. Two of the three patients who developed pancreatitis in the initial phase I study had a history of substantial alcohol ingestion, and this may have been a contributory factor. Also, one of the patients had an elevation of his triglyceride level (eventually reaching over 1,000 mg/dL) starting several weeks before the development of pancreatitis, and this may have been a premonitory

sign of impending toxicity. In patients who develop high triglycerides while taking ddI, it may be worthwhile to temporarily hold the drug until the triglycerides become lower. We are also exploring whether stopping the ddI for 1 week in patients with serum amylase levels of 1.5 times the upper limit of normal can prevent the development of pancreatitis. Such transient asymptomatic elevations of serum amylase should not be scored as pancreatitis per se.[120] Interestingly, we have found that some ddI recipients with elevated serum amylases had predominantly salivary amylase, and in certain instances this may be associated with complaints of xerostomia. The significance of this finding is unclear. It is possible that fractionation of the circulating amylase into salivary and pancreatic components might help guide physicians in the management of ddI toxicity.

Up to 40% of HIV-infected patients have been reported to have pancreatic abnormalities in the absence of ddI therapy[121] and a number of factors may contribute.[120,122-124] HIV infection itself can be associated with pancreatic abnormalities.[121] Also, opportunistic infections such as cytomegalovirus infection or mycobacterial infection can cause pancreatitis in patients with AIDS. A number of drugs used in AIDS, including pentamidine, sulfonamides, cimetidine, and ranitidine, can cause pancreatitis,[120] and such drugs should probably be avoided if possible in patients receiving ddI. In particular, until we know more about such drug interactions, ddI should probably be temporarily held in patients who receive intravenous pentamidine or sulfonamide-containing regimens for the treatment of *Pneumocystis carinii* pneumonia. Alcohol should be absolutely avoided for the same reason, and recent extensive alcohol ingestion should probably be considered a relative contraindication to ddI therapy. A prior history of pancreatitis from any cause should be considered virtually an absolute contraindication, until the results of further studies are available.

No patients died from ddI-related toxicities in the initial phase I studies.[13,15,116] However, pancreatitis can be a fatal complication; during the first 6 months of the expanded access program, 6 of 6,000 patients receiving the drug died following an episode of acute pancreatitis. It will be important to learn how the toxicities of ddI can best be prevented and managed. At the present time, it appears that the most important determinant of ddI toxicity is dose intensity,[116] and it will be important to learn the lowest dose that still maintains effective anti-HIV activity. The situation may in some ways be compared with the development of fludarabine phosphate, an anti-cancer purine analog.[125-127] In initial phase I and phase II trials, severe (and sometimes fatal) delayed neurotoxicity developed in some patients who received 96 mg/m^2/d for 5 to 7 days. However, the finding that this complication rarely occurred in patients receiving doses of less than 80 mg/m^2/d was important in its subsequent development as a clinically useful drug for the treatment of chronic lymphocytic leukemia and certain other lymphoproliferative disorders. Thus, finding the demarcating line for the safe administration of an agent is crucial in any drug development effort. In the case of ddI, avoiding its use in certain high-risk patients may also be important in reducing the incidence of pancreatitis. However, the potential for drug toxicities reminds us of the usefulness of controlled trial methodology to assess the relative benefits and risks of drugs in serious or fatal conditions such as AIDS or cancer.

Other Reverse Transcriptase Inhibitors

In addition to the above agents, a number of other dideoxynucleosides and closely related compounds have been identified as potent in vitro inhibitors of HIV replication,[7,9,22,31,95,128-134] and several are now either in clinical trial or have trials planned for the near future. Two of the dideoxynucleosides, 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) and 3′-azido-2′,3′-dideoxyuridine (azidouridine, AzdU, CS-87) entered phase I testing in 1989.[135-138] The first, d4T, is about as active as AZT in vitro on a molar basis.[31,128,130] However, unlike AZT, it appears to not inhibit the activity of thymidylate kinase. Preliminary results from the phase I trials suggested that an anti-HIV effect was observed and that anemia, peripheral neuropathy, and hepatitis were the dose-limiting toxicities.[135,136] AzdU is less active than AZT on a molar basis, but is reported to have a better in vitro therapeutic index.[129] Also, as noted above, strains of HIV that become resistant to AZT have cross-reactive resistance to AzdU.[94] Preliminary results from the phase I trials have so far shown very little toxicity; it is too early at this point to draw conclusions about its activity. Both of these nucleosides merit further study.

Certain dideoxynucleoside analogues may be of interest because of specific features. For example, a 2′-fluoro analogue of ddA with anti-HIV activity is relatively stable under acid conditions[139]; this may make it orally bioavailable without the need for buffering. In addition to dideoxynucleosides with an intact sugar ring, several acyclic compounds have recently been identified as having anti-HIV activity.[140,141] Such compounds may yield new knowledge regarding structure and activity, and may be of value in developing novel anti-HIV agents. Finally, several halogenated dideoxynucleoside prodrugs, which may yield increased penetration across the blood brain barrier, have recently been identified.[142] The hope, of course, is that such agents will be superior to the existing drugs.

Finally, we should mention several other compounds that act at the level of reverse transcriptase. Phosphonoformate, a pyrophosphate analogue originally developed for its anti-herpes activity, was found to also have activity against HIV.[143,144] This agent is now being studied for both its anti-HIV and anti-herpes effect in patients with cytomegalovirus retinitis. More recently, several tetrahydroimidazo[4,5,1,jk][1,4]-benzodiazepin-2(1H)-one and thione (TIBO) derivatives, which share structural characteristics with the benzodiazepines, have been reported to have potent anti–HIV-1 activity in vitro with very little cellular toxicity.[145] These compounds appear to work by an allosteric inhibition mechanism. Surprisingly, they have very little activity against HIV-2 in vitro. This is in contrast to the dideoxynucleosides, which have activity against a broad range of human and animal retroviruses, provided that they

can be phosphorylated by the relevant target cells.[32,36] Phase I studies of the TIBO derivatives are now in progress.

OTHER TARGETS FOR ANTI-HIV THERAPY

So far, we have focussed on agents that block reverse transcriptase. However, as we have seen above, there are a number of other steps that may be targets for therapy (Table 1).[5,7,8,23] Indeed, HIV is perhaps the most complex retrovirus studied to date. Such complexity may aid the virus in infecting immune cells. However, at the same time it also offers a wide variety of target points for attack.

Early Replicative Steps as Targets for Therapy

The first step in the life cycle of HIV is its binding to the surface of a target cell, usually by attachment of the HIV gp120 *env* glycoprotein to CD4. CD4 is an exceedingly interesting molecule. It belongs to the Ig superfamily,[146] whose members generally function in various recognition processes. CD4 is found on the surface of certain T lymphocytes ($CD4^+$ T cells), particularly those that recognize antigens associated with class II major histocompatibility (MHC) moities (primarly helper T cells). CD4 is also found on other cells such as monocyte-macrophages and some Epstein-Barr virus (EBV)-infected B lymphocytes. Clapham et al have recently found evidence that certain tumor cell lines lacking demonstrable CD4 can nonetheless still be infected with HIV, suggesting that alternate receptors for HIV infection may exist.[147] However, the significance of this observation requires further research.

There are a number of approaches that might be used to inhibit the binding of gp120 to CD4. For example, antibodies to relevant domains on gp120 or CD4 can block binding to cells by HIV. However, HIV-infected patients usually have only low titers of neutralizing anti-gp120 antibodies. There has recently been an interest in studying whether the administration of anti-HIV antibodies (passive immunization) will benefit patients with AIDS or ARC.[148] A theoretical issue is that very low concentrations of anti-HIV antibodies can sometimes enhance HIV infection.[149,150] Several groups have found evidence that both the Fc receptor and the CD4 receptor are involved in this process.[151-153] It is possible that binding of the HIV–anti-gp120 antibody complex to the Fc receptor brings the virus into proximity to CD4 on the cell surface. However, there is not universal agreement on this matter. Two groups have reported that under certain condition (eg, cytomegalovirus-infected fibroblasts) antibody-mediated HIV infection may occur by a CD4-independent mechanism,[154,154a] and further studies will be needed to assess these issues. In any case, anti-HIV–induced enhancement generally occurs at much lower concentrations of anti-HIV antibody than would be obtained by passive immunization.

It has been shown by several groups that soluble forms of CD4 produced by recombinant DNA technology (called recombinant soluble CD4 or rCD4) can inhibit the infection of T cells by laboratory strains of HIV at concentrations of 1 to 5 μg/mL.[155-159] Soluble CD4 also can inhibit infection of monocytes/macrophages.[153,160,161] A potential advantage is that it does not cause bone marrow toxicity. However, it has a relatively short serum half-life and this poses a drawback.[162] Three phase I trials of rCD4 have been conducted.[162-164] Two trials showed no signs of anti-viral activity,[162,164] while a third showed a slight decrease in serum HIV p24 antigen.[163] To address the problem of short half-life, researchers are exploring altered forms of rCD4. One approach has been the creation of a CD4-Ig (rCD4-Ig) "chimera" or hybrid protein.[161,165,166] This is also known as an "immunoadhesin". Such hybrid proteins, which have similar anti-HIV activity to soluble CD4, have a substantially longer serum half-life.[166] Also, through the function of its Ig moiety, CD4-Ig chimeras may help kill cells infected with HIV by antibody-dependent cellular cytotoxicity. Finally, there is evidence from primate models that rCD4-IgG can cross the placenta.[166] Phase I studies of rCD4-IgG are now underway at the NCI and several other medical centers.

Recently, data has emerged to suggest that higher concentrations of rCD4 or rCD4-IgG might be required than originally considered. Gomatos et al and Daar et al have reported that certain monocytotropic or fresh isolates of HIV are relatively insensitive to inhibition by rCD4,[167,168] most likely because such strains have a lower affinity for CD4 than the T cell-tropic laboratory strains originally studied. Also, anti-gp120 antibodies have been reported to block rCD4-gp120 binding[169] and could thus potentially interfere with the activity of these analogs. It is hoped that the ongoing studies of rCD4-IgG will provide some information on whether this approach has use in patients.

Another approach being explored is the linkage of CD4 with potent cellular toxins such as ricin or *Pseudomonas* endotoxin.[170,171] Such hybrid proteins can selectively bind to HIV-infected cells expressing a gp120 on their surface, and because of the attached toxin then kill the cells. Thus, their main usefulness would perhaps be in destroying certain cells (such as macrophages) that are resistant to the cytopathic effects of HIV. Such constructs would probably best be combined with an anti-retroviral drug such as AZT. Clinical studies are expected in the near future. A variation of this approach is the delivery of therapeutic agents to cells that are prime targets for HIV infection. Zarling et al, for example, have recently reported on the selective targeting of pokeweed antiviral protein to $CD4^+$ T cells using monoclonal antibodies.[172]

In addition to these CD4-based agents, certain sulfated polysaccharides can block HIV replication in vitro, at least in part by inhibiting viral binding to the cell surface. The prototype compound for this type of viral inhibition is dextran sulfate (molecular weight, 7,000 to 8,000).[173-175] Other similar compounds shown to have such activity include the anticoagulants heparin and pentosan polysulfate.[174,176,177] In a phase I trial of oral dextran sulfate in patients with AIDS or ARC conducted by Abrams et al, the drug was found to be well tolerated.[178] However, no evidence of anti-HIV activity was observed. It has since been shown that dextran sulfate is poorly absorbed by the oral route,[179,180] and trials to study intravenous administration of this drug are underway. Sulfated polysaccharides may also be worth investigating for activity against Kaposi's sarcoma. There is evidence that a number of growth factors, includ-

ing basic fibroblast growth factor, are involved in the cascade of events that leads to Kaposi's sarcoma (vide infra).[181] Pentosan polysulfate has been found to inhibit basic fibroblast factor-induced growth of certain tumor cell lines,[182] and it is possible that it might inhibit the growth of Kaposi's sarcoma. A phase I trial to explore the use of pentosan polysulfate is now underway at the NCI.

After binding, the virion fuses with the cells (this is mediated by gp41 Env glycoprotein).[183-185] The viral contents then enter the cell and the outer proteins are removed to expose the viral RNA (as a ribonucleoprotein complex) and reverse transcriptase. It is at this point that reverse transcription occurs to form the provirus. Agents that specifically inhibit reverse transcriptase have been described above. In addition, as an essential part of this process, the *pol*-encoded protein ribonuclease H (RNase H) degrades the viral RNA strand of the RNA-DNA duplex hybrid in an orderly process. Any approach that interfered with RNase H activity would be expected to block viral replication. We are rapidly learning about the structure and function of RNase.[186,187] Recently, the HIV-1 RNase H has been crystallized and its structure determined. This is the first step in the rational design of inhibitors of this viral enzyme.

Double-stranded viral DNA can enter the nucleus and, through the function of viral integrase (also believed to be a *pol* gene product), become integrated into the DNA of the host cell. Again, it is possible that specific inhibitors of HIV integrase function may be identified in the future.[188-191]

Late Replicative Steps as Targets for Therapy

The viral DNA may remain as a latent provirus for some time without being activated. However, at some point, perhaps upon immunologic stimulation of the infected cell, the viral DNA is transcribed to RNA and viral polyproteins are translated from this RNA. This process uses the replicative machinery of the host cell.[23] However, several HIV-encoded proteins (such as Tat or Rev) are necessary for this process to occur efficiently, and several groups are searching for specific inhibitors to these compounds. The Tat protein is a particularly interesting target for therapy. Tat protein serves to enhance the expression of viral genes; for this to occur, a specific nucleotide sequence (designated TAR), within the 5′ long terminal repeat (LTR) must be present at the level of mRNA. The TAR region serves as a binding site for the Tat protein. As will be discussed below, there is recent evidence that Tat protein released from infected cells may be taken up by other cells and activate TAR sequences.[192] There is also evidence that released Tat protein may play a role in the pathogenesis of Kaposi's sarcoma.[193,194] Tat may bind to certain integrin receptors on cell surfaces[195]; however, the significance of this finding will require further study.

One possible approach to interfere with Tat function is to identify inhibitors of Tat-TAR binding. As another approach, Sullenger et al have recently reported that HIV replication can be blocked by the introduction of TAR sequences (TAR decoys) into cells using a tRNA (pol III) transcription system and retroviral gene transfer.[196] A cell making large quantities of TAR decoys would, in effect, be genetically resistant to HIV replication. Gene transfer is already a reality,[197] and such an approach could conceivably be tried in humans with HIV infection in the not too distant future.

An approach now being studied for inhibiting this step of viral replication is the use of "antisense" segments of modified DNA (eg, phosphorothioate oligodeoxynucleotides).[198,199] These are strands of DNA, modified so as to prevent degradation by cellular nucleases or to improve cell entry, with sequences complementary to that of the HIV RNA. It is believed that these constructs will bind to either genomic RNA or mRNA. They could cause a hybridization arrest at the level of ribosomes and thereby prevent viral proteins from being formed. Alternatively, they may make the viral RNA susceptible to degradation by RNaseH. Such constructs have been found to inhibit the production of HIV proteins in chronically infected H9 cells.[198,199] Phosphorothioate oligodeoxynucleosides may also inhibit HIV replication in a sequence-nonspecific manner, perhaps by interfering with HIV binding.[200] However, there are several problems in considering their clinical use. One problem is that relatively high concentrations (generally 5 to 25 μmol/L) are required to achieve suppression of infected cell lines in vitro, and even at these concentrations, the suppression is usually incomplete. Another is that there may be strain-to-strain differences in the response to these constructs, possibly because of varying expression of the target sequences or because of sequence differences in the target region (Kageyama and Mitsuya, unpublished results, December 1990). There may also be target cell differences in the response to these constructs. Finally, there are some technical (and financial) hurdles in scaling up for large quantity production of such agents for experimental therapy. These issues will have to be resolved before it would be appropriate to undertake clinical trials.

After viral polyproteins are formed, they must undergo a variety of modifications, including cleavage into smaller proteins, myristylation, and glycosylation.[23] A number of laboratories are studying these as targets for therapy. A particular focus of interest for many groups is the viral aspartyl protease, a *pol* gene product.[201] The Gag and Pol proteins are originally translated as a large polyprotein (Gag-Pol fusion polyprotein) that must then be cleaved by viral protease for proper viral function and assembly to occur. This 99 amino acid viral enzyme, which exists as a dimer, has been purified and its three-dimensional structure determined by X-ray crystallography.[202,203] This has permitted the rational design of inhibitors that are specific for HIV.[204-206] At least one such inhibitor has now entered clinical trial. This promises to be a fruitful area for research in the near future.

As noted above, viral proteins have sugar residues added to form glycoproteins. As a part of this process, specific terminal sugars are then trimmed off by trimming glucosidases. Castanospermine, an inhibitor of certain trimming glucosidases, and analogues of this compound can inhibit HIV-induced syncytium formation and the production of infectious virions from chronically infected cells.[207,208] As a final step in the replicative cycle, viral components assem-