Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Strategies for the Future Chemotherapy of Human Immunodeficiency Virus (HIV)

S. Broder [1]

Acquired immune deficiency syndrome (AIDS) is caused by the third known human T-lymphotropic virus [1, 2]. One designation for the virus is HIV. In order to develop therapeutic strategies for the treatment of AIDS and related disorders, one must first consider the life cycle of the etiologic agent. As we review this life cycle, we will touch upon certain stages of particular interest for the development of new therapies. HIV (also called HTLV-III, LAV and ARV) belongs to the family of RNA viruses known as retroviruses [3–6] which must replicate through a DNA intermediate (i.e., at one step in their cycle of replication, genetic information flows from RNA and DNA, a reverse or "retro" direction).

The first step in infection of a cell by HIV is the binding to the target cell receptor. In the case of helper-inducer cells, this receptor is thought to be on or near the CD4 antigen [7–9], but other receptors may possibly be used by HIV in infecting different cell types. This binding step may be vulnerable to attack by antibodies either to the virus or to the receptor, and one can speculate that in the future, certain defined substances could be designed to occupy the receptor and accomplish the same thing.

It is conceivable that an experimental agent could alter the properties of the viral surface itself (e.g., by altering the lipid composition), and this might be one mechanism by which a new lipid agent could act [10]. It is known that there is some variation in the surface envelope from one viral isolate to another [1]. The range of possible alterations in the envelope binding site is, however, most likely limited by the need to bind to CD4 (which is relatively constant), and antibody directed against this site would probably bind to (and neutralize) most strains of HIV.

Thus, monoclonal antibodies to HIV may have a therapeutic role in patients with AIDS or related diseases. A potential difficulty of this approach, however, is that virally infected cells could make infectious cell-to-cell contacts. In this regard, the recognition that macrophages can harbor the virus and can infect T cells through cell-to-cell contacts makes it important to include assays of infectivity that test this route of transmission in testing new agents in vitro. It is also worth testing whether antibodies can gain access to relevant epitopes under such circumstances. Also, it has been shown that AIDS can occur in the face of neutralizing antibodies to HIV. Whether this occurs because the titers of such antibodies are low is a topic for ongoing research [11, 12]. After binding to a cell, HIV enters the target cell by a poorly defined mechanism, perhaps by a fusion process. It is conceivable that drugs which block this step could be developed.

After it enters a target cell, the virus loses its envelope coat and RNA is released into the cytoplasm. (Pharmacologic agents which block this "uncoating" might be developed in future strategies for the experimental treatment of AIDS.) HIV uses a lysine transfer RNA as a primer and its special DNA polymerase, i.e., the reverse transcrip-

[1] Associate Director, Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20892, USA

tase (RT), to copy itself, employing the viral RNA as a template. The viral DNA polymerase is encoded in a genetic region denoted as the *pol* gene. Eventually, the genetic information encoded by the virus as a single strand of RNA is transcribed into a double-stranded DNA form. RT is the enzyme that characterizes the entire family of retroviruses. Because of its unique role in retroviral replication and because a great deal is known about it [13], RT is a high-priority target for antiviral therapy, and, as noted below, a number of drugs which inhibit RT have been shown to block infection of cells with HIV in vitro. Recently we have been particularly intrigued by the capacity of oligonucleotides (which are linked by phosphorothioate esters) to inhibit retroviral replication, perhaps by serving as competitive inhibitors of primer for reverse transcription (Matsukura et al., unpublished).

The DNA copy of the virus is circularized soon after its formation, and it can either remain in an unintegrated form or become integrated into the host cell genome. It is not known whether the circularized form of the virus is biologically important. It is possible that chemicals could be developed to interfere with the viral "integrase" (thought to be a function of the *pol* segment) which mediates this integration step.

At some later time, perhaps after activation of the infected cell, the DNA is transcribed to messenger RNA using host RNA polymerases, and this RNA is then translated to form viral proteins, again using the biochemical apparatus of the host cell. However, within any given cell, the retrovirus could either remain latent (perhaps for the life of the cell) or begin another cycle of replication, sometimes in response to T-cell activating stimuli. It has recently been shown that HIV has a transcriptional and/or translational activating gene which makes a product (called the *tat*-III protein) that markedly enhances the production of viral proteins. All of the known pathogenic human retroviruses have special transcriptional or translational activating (*tat*) genes; but there are significant differences [1, 14–18], and certain ideas about these *tat* genes are still evolving.

The *tat*-III protein is thought to increase both translation of the viral RNA and its transcription, although this point requires further research. It is also thought to provide the virus with a positive-feedback loop in infected cells in which a viral product can in turn increase production of new virions. One of the hallmarks of HIV is its capacity to replicate within and destroy target T cells. This capacity seems to require a functional *tat*-III gene, although it is likely that genes outside the *tat*-III region will also be found to play a role in the cytopathic effect.

The *tat*-III protein is small (86 amino acids), with a cluster of positively charged amino acids, and it is thought the *tat*-III gene product influences the translation of other proteins by binding to critical regulatory sequences at the 5′-end of messenger RNA. It is possible that drugs or other agents may be found which inhibit either the *tat*-III product itself, a crucial nucleic acid binding site for this protein, or both.

Recently, Sodroski et al. have provided data for the existence of a seventh gene in the genome of HIV [19]. The data suggest that a product of this gene provides a *second* post-transcriptional kind of *trans*-regulatory function for the efficient synthesis of HIV *gag* and *env,* but not *tat*-III proteins. The segment of DNA required for this novel second *trans* function partly overlaps the *tat*-III and *env* genes in an alternate reading frame. It is apparent that this new gene could also be an important target for new therapies.

It is conceivable that a class of agents which interfere with the structure and function of retroviral messenger RNA in infected cells could have a role in AIDS. One drug, ribavirin, is believed to act as a guanosine analogue that interferes with the 5′-capping of viral messenger RNA in other viral systems, and perhaps this activity could be useful in retrovirally induced disorders [19, 20]. To date, ribavirin has not been shown to exert an in vivo effect against the AIDS virus.

One novel approach that may conceivably inhibit the translation (or transcription) of viral products would be the use of "antisense" oligodeoxynucleotides. Basically, these could be short sequences of DNA (or DNA which is chemically modified to enable better cell penetration and resistance to enzymatic degradation) whose base pairs are complementary to a vital segment of the viral genome [21–25]; by binding to this seg-

ment, such oligonucleotides could theoretically block expression of the viral genome through a kind of hybridization arrest or possibly by interfering with the binding of a regulatory protein such as *tat*-III or the second *trans*-regulatory gene already discussed, or with both. In principle, it might be possible to achieve the same goal by constructing an "anti-sense" virus (i.e., a retrovirus which has been genetically engineered to produce a stretch of messenger RNA that will bind to the messenger made by the wild-type virus).

The final stages in the replicative cycle of HIV involve the secondary processing of viral proteins by cleavage and glycosylation, assembly of the virus, and finally viral budding. Interferons may act to interfere with this stage of HIV replication. Other strategies for interfering with retroviral proteases and glycosylation could be explored in the future experimental treatment of AIDS.

While we have focused the discussion on how to suppress HIV, it might be worth noting that the virus could theoretically set off a chain of secondary events in vivo (autoimmune reaction, toxic lymphokine production, etc.) that is necessary for the expression of clinical disease. However, we will not be able in this article to discuss strategies for intervening against potential secondary events.

I would now like to turn to a discussion of a broad family of 2′, 3′-dideoxynucleoside analogues that can be potent inhibitors of the RT of HIV. From one perspective, these are certainly not new chemicals, and in several cases pioneering studies have been accomplished over the past 20 years or so [26–32]. However, their application as potential antiretroviral chemotherapeutic agents in human beings will require an expansion of how these agents might have previously been categorized. It is worth stressing that work with animal viruses often fails to provide a good model for how these drugs act against human viruses. Moreover, these drugs illustrate the need to combine virologic, immunologic, and pharmacologic perspectives in AIDS drug development. They are of special interest because they underscore the fact that a simple chemical modification of the sugar moiety can predictably convert a normal substrate for nucleic acid synthesis into a potent compound with the capacity to inhibit the replication and cytopathic effect of HIV, at least in vitro.

Certain relationships between the structure and activity of these nucleoside analogues have been explored in previous work [33]. It can be shown that a simple reduction (removal of the hydroxyl group) at the 3′-carbon of the sugar can convert a normal nucleoside into a potent agent against HIV in this system. A further reduction at the 5′-carbon, creating 2′,3′,5′-trideoxynucleoside, nullifies the antiretroviral effect. However, not all dideoxynucleosides have an antiretroviral effect, and a putative effect needs to be established on a case by case basis.

The National Institutes of Health, through the Developmental Therapeutics Program of the National Cancer Institute, is developing 2′,3′-dideoxycytidine and 2′,3′-dideoxyadenosine as possible experimental agents for HIV infections. Interestingly, deoxycytidine kinase can phosphorylate both drugs. The dideoxycytidine derivative is farthest along in its preclinical development, and preliminary studies in mice and dogs suggest that it will prove to have good oral bioavailability and to be comparatively nontoxic (Grieshaber, unpublished observations). This drug is now in phase I clinical trials. The dose-limiting toxicity appears to be a peripheral neuropathy, in the phase I study.

While there are several issues related to the antiviral effects of dideoxynucleosides which are as yet not resolved, it would appear that as 2′,3′-dideoxynucleosides are successively phosphorylated inside a target cell to yield 2′,3′-dideoxynucleoside-5-triphosphates, they become analogues of the 2′-deoxynucleosides that are the natural substrates for cellular DNA polymerase *and* viral DNA polymerase (RT). It is important to stress that the crucial phosphorylation reactions are catalyzed by host cellular kinases; the retrovirus does not provide these enzymes and, therefore, it (unlike *herpes* versus certain antiviral drugs) cannot adopt a simple strategy of mutating a kinase gene to develop drug resistance, although drug resistance (e.g., a mutation in the viral DNA polymerase) must always be among the reasons why an experimental agent could fail. Similar considerations apply to 3′-azido-3′-deoxythymidine (AZT) [34], a compound

which we will discuss in more detail below. In this context, the lack of activity against HIV that was observed using 2′,3′,5′-trideoxyadenosine probably related to the unavailability of the 5′-site to undergo phosphorylation. It is also worth noting that cells with different histologic or species origins may show different profiles of phosphorylating activity. Therefore, in testing these drugs in animals it is important to determine such phosphorylating profiles in advance.

We are now focusing our research efforts on various 2′,3′-dideoxynucleosides. There are data to suggest that the DNA polymerase (RT) of HIV is much more susceptible to the inhibitory effects of these drugs as triphosphates than is mammalian DNA polymerase alpha (Mitsuya and Broder, unpublished work), an enzyme which has key DNA synthesis and repair functions in the life of a cell. This parallels what has been learned in animal retroviral systems (see discussion in [33]). *One* explanation for the activity of these drugs is that following anabolism to nucleotides (triphosphates), they bring about a selective chain termination as the RNA form of the virus attempts to make DNA copies of itself, because normal 5′→3′ phosphodiester linkages cannot be completed. Thus, one model for the activity of these compounds is that the viral DNA polymerase is more easily fooled into accepting the dideoxynucleotide than is the mammalian enzyme counterpart, or that the viral DNA polymerase has less capacity to repair the incorporation of the false nucleotide, or both.

We have found that with the sugar in a 2′,3′-dideoxy configuration, almost every purine and pyrimidine tested suppresses HIV replication in vitro; however, dideoxythymidine had substantially less activity than the others [33]. This represents a drastic departure from what we had expected on the basis of observations in certain animal retroviral systems with this drug, and is another warning that one cannot extrapolate from animal models in developing drugs against pathogenic human retroviruses. Interestingly, the substitution of an azido group at the 3′-carbon of the sugar in place of a hydrogen (AZT) significantly restored the antiviral effect of the 2′,3′-dideoxythymidine against HIV [35]. This azido substitution yields AZT.

In the remaining portion of this article, I would like to summarize some of our preliminary clinical observations made when using AZT in patients with AIDS and related disorders [35]. This drug is an interesting compound that was synthesized over 20 years ago [27] and shown by Ostertag et al. more than 12 years ago [34] to inhibit C-type murine retrovirus replication in vitro; however, no application of the agent was found for the practice of medicine. It was recently developed as an experimental agent against HIV in a clinical collaboration between the Clinical Oncology Program of the National Cancer Institute, and Duke University. The drug is manufactured by the Burroughs-Wellcome company under the tradename Retrovir™. It was found to have in vitro activity against HIV as part of the Clinical Oncology Program's screening effort in February 1985, after it had been shown to have potent in vitro activity in a murine retroviral system at the Wellcome Research Laboratories. At that time, it had not been used as a drug in human beings. We gave the first patient the drug as part of a phase I AIDS therapy protocol in July 1985, at the Clinical Center of the National Institutes of Health [36].

Initially, the patients received AZT intravenously. It was subsequently shown that the drug was well absorbed when given orally (60% bioavailability), and each patient was then switched to receive oral AZT after an initial 2-week administration of AZT intravenously. Pharmacokinetic studies showed that peak levels of 1.5–2 μM were attained following a 1-h infusion of 1 mg/kg or oral administration of 2 mg/kg, and that the drug has a half-life of approximately 1 h. Increased doses of the drug yielded proportionally increased peak levels; for example, 5 mg/kg given intravenously over 1 h yielded a peak level of 6–10 μM and a concentration of 0.6 μM 4 h after the start of the infusion. In addition, sampling of the cerebral spinal fluid (CSF) showed penetration of AZT; CSF levels have ranged from 15% to >100% of simultaneously measured plasma levels ([36], and Klecker et al., unpublished observation). The excellent capacity of this drug to cross the blood-brain bar-

rier is a noteworthy feature, given the propensity of HIV to replicate within the central nervous system [37].

More than twenty-five patients have so far been studied in this phase I trial. This was an escalating-dose trial, and patients received 3, 7.5, 15, 30, or 45 mg/kg per day intravenously for 2 weeks, followed by twice that dose given orally for 4 weeks. The first four-dose regimens have previously been described [36]. For the first two-dose schedules, the drug was administered three times a day, and for the last three-dose schedules, it was divided into six doses spaced 4 h apart. This scheduling modification was made because of the relatively short half-life of AZT. Bone marrow suppression was observed in half the patients on the two highest doses, suggesting that these doses might not be suitable for prolonged therapy in most patients; however, this issue is still under study. Even at lower doses, certain side effects (especially anemia) occurred under conditions of long-term administration, and this will be discussed later.

While the primary purpose of this phase I study was to determine whether AZT could be tolerated over 6 weeks in patients with AIDS or AIDS-related complex, the results might indicate in addition that at least partial immunologic and/or clinical responses occurred in some of the patients during this short-term administration. In particular, a majority of the patients had increases in the absolute number of circulating helper-inducer T-lymphocytes, 6 of the 16 patients who were anergic at entry developed positive skin tests while on AZT, and the one patient who was serially studied had the restoration of an in vitro cytotoxic response to influenza virus-infected autologous cells. (This test requires an intact collaboration between helper cells and cytotoxic cells, and is almost always depressed in patients with AIDS). At least some immunologic improvement seemed to occur in patients receiving 7.5 or 15 mg/kg per day intravenously (followed by twice that dose given orally), and in fact, each of the 11 patients at these doses had an increase in their absolute number of helper-inducer (CD4+) cells ($p < 0.001$). At the highest dose tested (45 mg/kg on day IV), the data suggest that drug-induced bone marrow toxicity represented a significant side effect (Yarchoan and Broder, unpublished observations). However, some patients with fulminant AIDS have bone suppression even before experimental therapy is initiated. One must keep open the possibility that patients with early HIV infections will tolerate drugs better than patients with advanced disease.

In addition to the partial immunologic reconstitution observed in these patients, some short-term clinical improvement may have been seen in some. Two patients who had chronic nail-plate fungal infections at entry experienced clearing of these infections without specific antifungal therapy. In addition, 1 patient who had debilitating aphthous stomatitis before therapy had healing of the lesions, a majority of patients had weight gains of 2 kg or greater (not explainable by fluid retention and associated with an increase in appetite), and 6 patients noted that their fevers stopped or that they had an improved sense of well-being. One patient had lower extremity weakness and dysesthesia accompanied by electromyelogram abnormalities which were attributed to HIV infection. After receiving AZT, his symptoms resolved, and a repeat electromyelogram was normal. Finally, one patient with an expressive aphasia and one patient with severe impairment of cognitive functions improved on AZT therapy. It is worth emphasizing that phase I studies by definition do not have a special control arm, and in the absence of a control arm (see discussion later in this presentation), no definitive conclusions can be drawn from these results. This is because AIDS is an inherently variable disease, and for certain parameters (e.g., weight gain), it is virtually impossible to rule out the power of a placebo effect.

Four of the patients developed non-life-threatening infections (localized herpes zoster, sinusitis, pneumonia, and *Pneumocystis carinii* pneumonia, respectively) during the 6-week period of the initial protocol; each of these infections responded to appropriate therapy. The patient with *Pneumocystis carinii* pneumonia developed clinical evidence of infection 5 days after starting on the highest dose of AZT, and it is likely that this infection was present even before he started on therapy.

In spite of the possible clinical and immunologic improvements, HIV could be identified in several of the patients on the lower dose regimens by using the technique of phytohemagglutinin-stimulated lymphocyte culture to detect the virus. At the higher doses (e.g., $\geqq 30$ mg/kg per day intravenously followed by $\geqq 60$ mg/kg per day orally), however, the virus was generally not detected in the patients on therapy, in whom such an evaluation could be made, thus suggesting that a virustatic effect might have been attained with high-dose AZT.

One of the fundamental problems of clinical research involving HIV is that a reliable and quantitative assay for viral load is still not available. Most of the techniques in use were designed to detect and isolate HIV in experiments where the object was to *find* virus, however little there might be. At the beginning of such research, it was a technical breakthrough just to identify and propagate the retrovirus on a wide scale [38, 39]; quantifying the amount of virus present in vivo was never a primary goal. The current techniques usually depend on detecting RT or a structural protein of the virus in cultures of peripheral blood lymphocytes that have been activated by a polyclonal T-cell mitogen. It is difficult to distinguish de novo activation of viral replication in vitro (i.e., unmasking a previously latent state) from a previously established chain of viral replication which had been underway in the patient and is permitted to continue in tissue culture. Physicians should take these factors into consideration as they evaluate reports summarizing HIV-related treatment protocols, and it is especially important to recognize the pitfalls of relying on viral cultures alone as an end point. More recent techniques such as measuring p24 expression in the circulating plasma might be very useful.

The earliest patients entered on this trial were taken off AZT for about 1 month and then restarted on the drug. As more experience was gained, patients who were enrolled into the later phases of the study were sometimes continued on the drug after the initial 6-week course of treatment, and in several cases an escalating-dose regimen was used. At the National Cancer Institute, we are currently following 12 patients on extended AZT therapy. While it is too early to draw conclusions, preliminary results suggest that in patients with fulminant AIDS the number of helper-inducer T cells may reach a plateau after 6 weeks of therapy using the current regimens, and in some patients (particularly at higher doses), the number of helper-inducer T cells may show a decline. Such patients appear to remain at some risk for developing opportunistic infections. Seven of the patients being followed have Kaposi's sarcoma; of these, one has had a complete remission of his Kaposi's sarcoma, and three have had some clearing of their lesions (Yarchoan and Broder, unpublished observations). Interestingly, the patient with complete clearing had worsening of his lesions during the early part of the AZT regimen but had clearing starting in the 8th week of experimental therapy. At this time, we cannot say whether these changes are due to the antiviral effects of the drug or to an unanticipated antitumor effect. At the least, it would appear from the preliminary results that if one is following a response to an antiviral drug by monitoring the Kaposi's sarcoma lesions as an end point in patients with HIV infection, one should be prepared to wait at least 3 months before making a definitive assessment about a response.

Thus, experimental treatment with AZT was associated with objective responses of the Kaposi's sarcoma in certain patients on a short-term basis. However, the number of helper-inducer lymphocytes reached a plateau after an initial rise and often fell in patients whose daily dose was escalated. At the same time, as doses were increased, some patients developed megaloblastic changes in their bone marrows (not explainable by folate or vitamin B12 deficiencies which do, in fact, occur in this patient population), accompanied by falls in the total white blood cell counts. Some patients develop red-cell hypoplasia on long-term treatment. (Interestingly, in our studies, AZT seemed to spare platelets, and, indeed, some patients seemed to have significant increases in platelet counts. Therefore, perhaps patients with idiopathic thrombocytopenic purpura in the setting of HIV infection would be particularly interesting to study with this drug.)

Recent data suggest that certain changes in bone marrow function, including the late depression of white blood cells, are related

to a drug-induced depletion of normal pyrimidine pools. We have recently shown that T cells exposed to high concentrations of AZT in vitro have decreased levels of thymidine triphosphate, and AZT-induced pyrimidine starvation, due in part to an inhibition of thymidylate kinase, may be one factor responsible for this late toxicity (Balzarini et al., unpublished results). Therefore, one of the important challenges for future clinical investigation will be to develop approaches that minimize this depletion of normal pyrimidines, which if successful, might permit long-term administration without unacceptable toxicity. In patients known not to have other explanations such as folate or vitamin B12 deficiencies, it may also be possible to monitor the increase in the mean corpuscular volume of circulating red blood cells (a peripheral reflection of the megaloblastic changes in the bone marrow, which in turn appears to be a function of pyrimidine starvation) as an index of impending drug toxicity (Yarchoan and Broder, unpublished observations).

We are currently trying to explore regimens which take the above observations into consideration. In collaboration with the Wellcome Research Laboratories, we are exploring how to clinically modify hepatic glucuronidation since this is a major route of AZT elimination, particularly when the drug is given orally. (It is interesting to note that in preclinical testing, dideoxycytidine, the nucleoside analogue discussed earlier, is excreted into the urine essentially unchanged.) We are also planning regimens which might be able to test combinations of antiviral modalities in the future. An alternating regimen of AZT and dideoxycytidine may offer several pharmacologic advantages, and preliminary results are encouraging.

These initial results with AZT suggest that this drug can be administered with potentially interesting effects in patients in a short-term regimen. A randomized, double-blind/placebo-controlled trial of orally administered AZT was recently conducted. This was a multi-center study that involved approximately 280 patients. Roughly equal numbers of patients received drug and placebo. The study was initiated (using the pharmacokinetic and dosing data observed in our phase I study) in February of 1986, and patients were accrued over a four month period of time. By September of 1986, there were 19 deaths in the placebo arm and one death in the drug arm. There were a number of immunologic and clinical parameters that provided additional data that patients were deriving at least a short-term benefit from the administration of AZT, and accordingly on September 19, 1986, an independent data-safety monitoring board recommended that patients in the placebo arm begin to receive drug. AZT has now been approved as a prescription drug for the therapy of certain patients with AIDS and its related disorders in the United States and in several European countries.

More recent studies (Yarchoan and Broder, unpublished) suggest that AZT may have the capacity to at least temporarily reverse some of the dementias that are associated with AIDS. From a clinical point of view, some of the neurologic improvements may sometimes be more evident than other features of AZT therapy. From one point of view, the capacity of the drug to improve the neurologic function of certain patients is unexpected and should be the topic of continued basic and clinical research.

AZT is certainly not a final answer, and in some patients the drug may exhibit prominent bone-marrow suppressive effects. Nevertheless, the studies involving AZT have confirmed in a general way the hypothesis that an antiretroviral intervention can provide a clinical benefit to patients suffering from pathogenic retroviral infections, even in advanced disease.

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