The Use of Anti-retroviral Drugs in the Treatment of HIV Infection

Philip Keiser MD
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Philip Keiser MD

Assistant Professor of Medicine

Medical Director, Parkland Memorial Hospital HIV/AIDS Clinic

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2.Use of biologic modifiers in the treatment of HIV and its related infections

Introduction

Infection with HIV-1 is associated with progressive immune dysfunction, opportunistic infections and death. Treatment with anti-retroviral agents such as zidovudine (AZT, ZDV) can slow the course of this disease but do not alter the ultimate outcome. Recent studies into the pathogenesis demonstrated that HIV-1 replicates at extraordinarily high rates and it is the rate of viral replication that drives the illness. A new class of agents known as protease inhibitors can suppress viral replications to unprecedented levels. In addition, application of PCR techniques to the measurement of HIV-1 replication has provided clinicians with a powerful tool to monitor therapy.

The effectiveness of these new agents has been highly publicised in the media. Despite the theraputic advances made by protease inhibitors, there are significant barriers to long term suppression of HIV-1 infection. Anti-retroviral regimens are complex with numerous toxicities, resistance develops quite readily and these drugs are very expensive.

I will discuss the current state of the art of the therapy of HIV-1 infection, focusing on viral dynamics and the use of protease inhibitors to suppress viral replication.

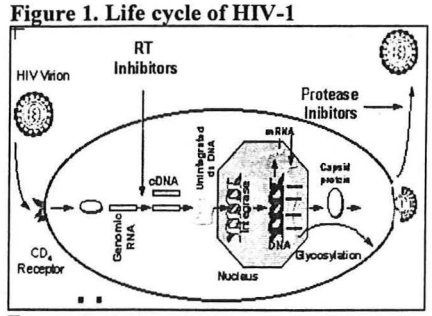
Structure of HIV-1

HIV-1 is composed of a lipoprotein coat envelope and a protein core containing genetic material [1]. The envelope consists of a lipid bi-layer and a large, external protein, gp120, that is non-covalently linked to a transmembrane protein, gp41. Within the envelope there is a core of structural proteins that surround two positive strands of RNA and several important enzymes including reverse transcriptase, an integrase and a protease. Currently available drug therapies target reverse transcriptase and the protease. An integrase inhibitor is currently under development.

Life Cycle of HIV-1

The primary target of HIV-1 infection is the CD4 bearing lymphocyte. though other cells including macrophages and neuroglial cells can be infected [1-3]. HIV-1 gp120 binds to the CD4 molecule,

Figure 1. Life cycle of HIV-1



Targets of anti-retroviral therapy are reverse transcriptase (RT) and the protease

after which the viral envelope fuses with the cellular membrane through an interaction with a fusin molecule now renamed CXCR4 [4-6]. In addition, CXCR4 binds directly to the HIV-1 gp120/CD4 complex, raising the possibility that CXCR4 mediates the second phase of viral entry. The core and its contents are released into the cytoplasm where reverse transcriptase, an RNA directed DNA polymerase, synthesizes double stranded HIV-1 DNA using the HIV-1 RNA as a template. HIV-1 reverse transcriptase is characterized by a high error rate and poor proof reading ability allowing for the introduction of mutations at this step. The double stranded viral DNA is then transported to the nucleus where it is integrated into the cellular DNA by an HIV-1 encoded integrase. Once integrated, the HIV-1 provirus permanently infects the cell. Major HIV-1 gene products are synthesized from the proviral DNA as a single poly-protein Assembly of the virus occurs in the cytoplasm after which viruses bud through the cell membrane. After release of the virus into the serum, the HIV-1 protease ligates the poly-protein into its component parts, forming a mature virus. With high levels of

viral replication, disruption of the membrane becomes extensive and cellular lysis occurs.

Several HIV-1 gene products are important for completion of viral replication [7]. Two early products, known as REV and TAT, up-regulate HIV-1 transcription. REV also increases the transportation of HIV-1 mRNA to the cytoplasm. A third protein, NEF, is necessary for viral replication and decreases the expression of CD4 on the cell surface. Additionally, this latter function may protect the cell from further infection by other viruses. Two proteins found later in the life cycle, Vpr and Vpx, assist in the assembly of mature viral particles while Vpu helps in the release of viral particles from the cell. Defects in these genes can effect the course of HIV-1 infection. Infection with a defective virus strain lacking a functional NEF gene appears to correlate with slow progression.

Natural History of HIV-1 Infection

HIV-1 infects a host through sexual contact with another infected individual or through direct inoculation of infected blood or body fluids [8]. After an incubation period of six to eight weeks, a burst of viral replication occurs rapidly disseminating HIV-1 throughout the body [8-10]. At this time, high titers of virus can be cultured from the blood. Infected individuals can have a mononucleosis like illness that may include fever, lymphadenopathy, pharyngitis, rash and diarrhea during this period [11,12]. The number of CD4 bearing cells in the peripheral blood quickly falls with the rapid viral replication. A vigorous immune response that includes both the humoral and cellular arms then develops. IgM antibodies directed against HIV-1 are initially found but are soon replaced with IgG antibodies directed against the viral core and envelope proteins. Similarly, T cell responses against viral antigens can also be detected at this time. This immune response drives HIV-1 into the lymphoid tissue where it infects follicular dendritic cells. The dendritic cells then act as a sync for HIV-1 infection of further infection. Local inflammatory response recruits uninfected CD4 cell into the lymphoid tissue where they become infected.

After development of the host immune response, HIV-1 enters a clinically latent period that lasts on average 11 years. Infected individuals may have little or no symptoms [13,14]. Despite this relative lack of illness, persistent viral replication occurs and CD4 counts continue to decline at a rate of 40 to 80 cells/mm^3 per year. There is an increased incidence of certain bacterial and viral infections once the CD4 count falls below 500 cells/mm^3. When the CD4 counts reaches 200 cells/mm^3, infected individuals are susceptible to the host of opportunistic infections that characterize the Acquired Immunodeficiency Syndrome. Death usually ensues within two to three years after an AIDS diagnosis. During the latent period little virus can be cultured from the blood. As the disease progresses, the peripheral blood viral titers gradually increase until the late stage of disease when large amounts of the virus can be found.

Viral Dynamics

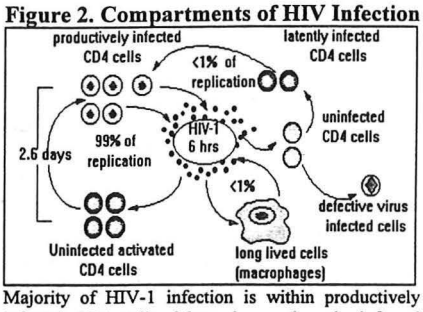
Measures of viral replication are now available through nucleic acid amplification techniques such branch chain DNA (b-DNA) and HIV-1 RNA Polymerase Chain Reaction (HIV-1 RNA PCR) [14,15]. The number of HIV-1 RNA copies/ml of blood are thought to be proportional to the total amount of HIV-1 in an infected persons body and is thus termed the "viral load". In the b-DNA test, HIV-1 RNA in the serum is annealed to a complementary nucleotide sequence in a microtiter plate. A second, complementary sequence that is attached to a DNA chain with multiple branches sandwiches the HIV-1 RNA. An alkaline phosphatase detector is then annealed to the branches on the DNA chain. The amount of HIV-1 RNA per unit of volume is proportional to the intensity of the chemiluminescent reaction. HIV-1 RNA PCR utilizes reverse transcriptase to amplify the HIV-1 genome in serum. The most

popular form of this assay uses non-radiometric detection techniques where the concentration of HIV-1 RNA can be determined with an ELISA reader. Both of these techniques can detect as little as 500 copies per milliliter of serum in an HIV-1 infected person. New generations of these tests can detect as low a 20 copies of viral RNA.

Applications of viral amplification techniques has provided new insights into the pathophysiology of HIV-1 infection. Using DNA PCR, RNA PCR, and in situ hybridization, Pantaleo et al. found that individuals who were asymptomatic had lymphoid viral load five- to 10-fold higher than peripheral blood viral load[16]. In individuals with advanced disease, there was involution of lymph node germinal centers and equal amounts of infected cells were found in both compartments. Overall, viral replication was higher in the lymph nodes than in the blood at all stages of disease. Pantaleo and his co-workers concluded that a state of true microbiological latency does not exist during the course of HIV-1 infection.

Further studies revealed the extent of viral replication. In 1995 Wei et. al and Ho et. al. measured viral load in HIV-1 infected patients who were participating in trials of a then new class of anti-retroviral agents known as protease inhibitors [17,18]. These studies reported that 99% of the virus is produced by acutely infected CD4 T lymphocytes, and that when potent therapy is administered, viral levels decline in the peripheral blood by 100-fold (2 logs) over a two-week period. The rate of decline in the viral load was the same in each patient regardless of the stage of the disease of the person receiving the new agent. In addition, this rate of decay was independent of baseline viral load; that is, those subjects with high viral loads cleared HIV-1 with initiation of a protease inhibitor as well as those who had a low viral load prior to therapy. Applying a mathematical model to the changes in viral load and CD4 counts, Perelson et al. estimated that productively infected CD4 cells have an average life span of 2.2 days (half-life of 1.6 days), and that plasma virions have an estimated life-span of 0.3 days (half-life of 0.24 days).[19,20]. They then estimated that 10.3 billion virions are produced daily and that it takes 2.6 days from virion release from an infected cell to productive infection of a second cell. Further experiments suggested that there are two phases of viral decay, the first with a half life of 1.25 days and the second with a half life of 14 days.

Figure 2. Compartments of HIV Infection



Majority of HIV-1 infection is within productively infected CD4 cells. Macrophages, latently infected CD4 cell and other cells represent reservoir sites, allowing HIV-1 to attack uninfected CD4 cells.

These results are best explained by a five compartment model. The first compartment is the serum which contains free HIV-1 virions, whose half-life in the blood is about five hours. The second compartment are acutely-infected CD4 T cells. The half-life of these CD4 bearing cells in the blood is about 1.1 days (a reduction from the earlier estimate of 1.6 days). Compartments 3, 4 and 5 are infected macrophages, latently infected CD4 cells and follicular dendritic cells (FDC) in lymphoid tissues such as tonsils, adenoids, gut, and spleen. Compartments 1 and 2 (free virions and infected T cells) account for about 99% of the plasma viremia in an untreated infected individual. Collectively, these first two compartments account for the rapid (two week), 2-log (99%) reduction in virus seen in patients initiating therapy. Compartments 3,4, and 5 account for the slower (months to years) reduction in viral

load as macrophages and latently infected cells burn out, die, or express virus and are killed, and as FDC-trapped HIV-1 particles are cleared from lymphoid tissue.

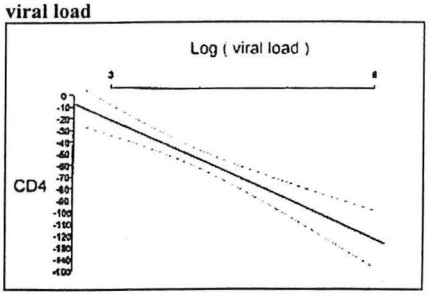
Assuming a quasi-steady state for HIV replication, one can use this model to predict the time required to clear HIV-1 infection from the body if an agent were able to totally suppress viral replication. Significant variables in the clearance time are the number of infected cells per compartment, and the measured half-life of the various compartments. Because of the large number of infected macrophages and latently infected cells, the rate-limiting step is the time that it would take for these cells to die. Thus, it would take from 9 to 36 months of total viral suppression to clear HIV-1 [20].

These estimates must be interpreted with caution. There are potential protected sanctuary sites, particularly in the central nervous system, where HIV-1 could continuously replicate and re-infect other compartments. It is also possible that two defective proviruses could recombine and produce a hybrid infectious HIV-1. This could then infect the other compartments when anti-viral therapy is withdrawn.

Viral Load And Prognosis

There is now evidence that viral load as measured by either HIV-1 RNA PCR or by branch chain DNA correlates highly with disease progression. Mellors et. al. retrospectively measured viral RNA levels from serum samples drawn from 1,601 men who participated in the Multicenter AIDS Cohort Study (MACS). [21-23] This is a natural history study of HIV-1 infection, where gay and bisexual men have been followed longitudinally since 1985. Participants periodically undergo clinical evaluations and have serum stored for future studies. Baseline HIV-1 RNA, defined as the RNA that was measured on the first serum sample test drawn when the subject entered the cohort, was a predictive factor

Figure 3. CD4 decline vs. log of baseline viral load



Yearly decline in CD4 cell count is directly proportional to the log of the baseline viral load value

for progression of disease. There was a linear relationship between the rate of CD4 count decline and the log of the baseline viral load. Viral load was also predictive of survival. Of the 855 men who died of AIDS by 1995, the baseline RNA count was 24,200; of the 993 men who developed AIDS, the baseline viral load was 19,145; for the 749 men remained alive it was 4,426; and for those men who did not develop AIDS, it was 3,636. Those subjects with an entry viral load over 30,000 copies/ml had a 13 times the risk of developing AIDS and an 18 times the risk of dying as those whose entry viral load was below this value. Dividing the entry viral load into quintiles, the investigators showed that those with the lowest viral loads on entry had the lowest risk of progression and those with the highest viral loads had the greatest risk of progression, while those with intermediate levels had an intermediate risk of disease.

The predictive value of HIV-RNA levels has been confirmed in at least 5 other prospective studies [24-28]. There is considerable variation in the range of viral load values observed in these trials, however. Hogg et. al. showed that HIV-1 RNA values in a large population of HIV-1-infected British Colombians, yield much higher apparent RNA counts than those observed in the Mellors MACS data [29]. In this study, 25% of participants had over 100,000 HIV-1 RNA copies per cubic milliliter. This disparity is best explained by

the fact that the MACS samples were collects in heparinized tubes and frozen, a process that can degrade HIV-1 RNA. The values obtained in these stored samples may be a full 50% lower than those obtained on properly collected, fresh serum. Thus clinicians and patients should interpret the Mellors risk thresh holds cautiously.

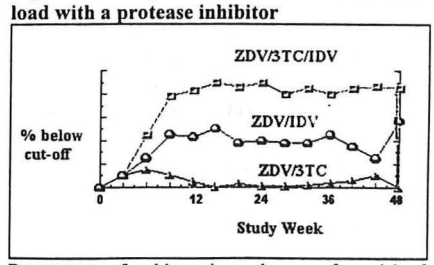
In addition to predicting progression to AIDS and death, high viral loads are associated with the development of specific opportunistic infections. For example, in a study of over 700 patients participating in AIDS Clinical Trials Group studies of opportunistic infection, Swindells et. al. found that viral RNA independently correlated with the risk of developing PCP, CMV and MAC, even in those subjects with very low CD4 counts [30].

Changes in Viral Load

There is also evidence that changes in viral load with initiation of anti-retroviral therapy are associated with a change in the course of disease. Using samples obtained from the Department of Veterans Affairs study of early zidovudine therapy, O'Brien et. al. demonstrated that those subjects with a 0.5 log, (a three fold reduction) in viral load with initiation of therapy had a more sustained CD4 count elevation and prolonged survival than those who did not have decrease in viral load [31]. Since then there have been 4 other prospective trials that have demonstrated that a 0.5 log decrease in viral load or greater with initiation of antiviral therapy was associated with better clinical outcomes [32-35]. Through out these studies, reduction in viral load was the single most significant factor in outcome. Consequently, those regimens that were most effective in reducing viral load were associated with the best clinical results.

It is now possible to lower the viral load to below the tests limits of detection. In a study of triple anti-retroviral therapy that included the protease inhibitor indinavir 92% of the developed undetectable viral loads [36]. This result added a new dimension to anti-retroviral trial, with investigators reporting the percentages of subjects who had undetectable viral loads. In addition, it prompted many clinicians to view an undetectable viral load as the goal of anti-retroviral therapy. Such interpretations are fraught with danger. Direct comparisons of numbers of patients who achieve an undetectable viral load in different studies cannot be used to determine the superiority of a given regimen because of variation in the patient populations. In addition, undetectable viral load does not mean that viral replication is not occurring, only that it is occurring below the levels of detection of the test. No studies have yet demonstrated that maintaining viral load below a certain critical threshold (such as undetectable levels) results in better clinical outcomes. Similarly, there are no studies that have shown that better outcomes are achieved by using viral load tests to initiate or change anti-retroviral therapy. Such viral load-based strategy trials are now an important research priority

Figure 4. Ability to achieve undetectable viral load with a protease inhibitor



Percentages of subjects in each arm of a trial of indinavir (IDV), zidovudine (ZDV), and lamivudine (3TC).

While anti-retroviral agents can lower viral load and improve outcome, there is evidence that opportunistic infections can elevate viral load. Bacterial pneumonia, tuberculosis, *Pneumocystis carinii* pneumonia, disseminated *Mycobacterium avium* complex infection and sexually transmitted diseases have all been associated with increases in viral load

[37,38]. The mechanism of this enhancement of viral load is unclear but is thought to be due to stimulation of HIV-1 infected CD4 cells and macrophages by these infection, resulting in more active viral replication. It is also possible that antigenic stimulation with vaccines may increase the viral load in HIV-1 infected patients [39,40]. Early studies demonstrated an increased viral load in patients receiving influenza vaccine. The increase in viral replication was not prolonged and the clinical significance of this finding is not clear. In addition, later studies did not observe an increased viral load with influenza immunization.

Collectively, studies utilizing the viral load assays have dramatically altered the concept of HIV-1 infection. Instead of being viewed as a static, slowly progressive disease, HIV-1 is now understood to be a highly dynamic infection, with enormous amounts of virus being produced. Large numbers of CD4 cells are produced in response to this infection. Unfortunately, the response in not sufficient to keep up with viral production. Over the course of years, CD4 counts decline and the individual progresses to AIDS. Reduction in the rates of viral replication with anti-retroviral agents can improve outcomes but the optimal use of these tests to guide therapy remains to be delineated.

Antiretroviral Nucleoside Analogues

The antiretroviral effect of nucleoside analogues was discovered in 1985 when Perno and his coworkers found that these agents could suppress the replication of HIV-1 in monocytes/macrophages in vitro [41]. These agents are 2 ' ,3 ' -dideoxynucleoside analogues of naturally occurring nucleotides. Nucleoside analogues are successively phosphorylated in the cytoplasm of a target cell to form 2',3'-dideoxynucleoside- 5'- triphosphate [42]. These dideoxynucleoside-5 ' -triphosphates then compete with native nucleotides for reverse transcriptase binding and incorporation into the viral DNA. The incorporation into the DNA chain causes chain termination because a normal 5' ~ 3' phosphodiester linkage cannot be completed. They also stericly block the movement of RT, knocking it off the HIV-1 DNA chain.

Nucleoside analogues can increase the CD4 count, decrease viral loads and prolong survival in HIV-1 infected patients when they are prescribed as mono-therapy [43-60]. Maximal CD4 count increase is about 20 cells and occurs at 12 weeks. Average viral load reduction is 0.5 logs when these agents are used as mono-therapy. Thus substantial viral replication occur with nucleoside mono-therapy. Survival benefit is approximately 18-24 months regardless of what stage of disease these agents are initiated. There are currently 5 nucleoside agents licensed by the Food and Drug Administration for the therapy of HIV-1. The first of these agents to become available was zidovudine (ZDV or AZT). This was followed by didanosine (ddI), zalcitibine (ddC), stavudine (d4T) and lamivudine (3TC).

Although numerous studies compared the relative efficacies of nucleoside analogues in various clinical scenarios, mono-therapy with any of these agents has limited clinical benefit. Because of this, sequential mono-therapy with nucleoside analogues was often employed. In this therapeutic schema, patients were initially treated with a nucleoside agents until there were signs of clinical failure (i.e. a fall in CD4 count or a new opportunistic infection). A new agent would be substituted at this point. There would be an initial increase in CD4 counts but ultimately the patient would have progression of disease.

The failure of nucleoside analogues to suppress HIV for long periods of time is due to the emergence of resistance [61-64]. Because nucleoside agents only partially suppress viral replication, there is selective pressure for the emergence of resistant mutants within weeks to months of initiation of anti-retroviral therapy. Clinical failure occurs 18 to 24 months after the initiation of

the agent. There have been various mutations in the HIV-1 reverse transcriptase genome that have been associated with the emergence of resistance. Fortunately, there is little overlap between these resistant mutation patterns among the various nucleoside agents. Thus patients whose virus had developed resistance to a given nucleoside would be expected to response to a new agent.

Combination Nucleosides

Because of emergence of resistance associated with nucleoside mono-therapy, the utilization of dual nucleoside therapy became an active area of investigation. The purpose of these investigations was to determine if two agents could suppress viral replication more than a single anti-retroviral drug, resulting in higher and more sustained elevations in CD4 counts, decreased progression of disease and prolonged survival. Because of the lack of cross resistance between these agents, it was hoped that dual nucleoside therapy would also result in less emergence of resistance and prolonged duration of clinical effect.

Several very large clinical trials have confirmed the superiority of dual nucleosides over mono-therapy. The first of these was the ACTG 175 trial in which over 2000 asymptomatic adults with more than 200 CD4 cells were randomized to one of four treatment arms; ZDV/ddI, ZDV/ddC, ddI alone or ZDV alone [65]. After an average of 120 weeks of therapy, those subjects who received combination therapy had a 45% reduction in mortality compared to those who received mono-therapy with ZDV. There were was an average of 1.0 log decrease in viral load in subjects who received AZT & ddI, compared to 0.6 decrease in those who received ZDV mono-therapy. There was also prolonged CD4 count elevations and fewer opportunistic infections in the individuals who received combination therapy. These results were more pronounced in subjects who were anti-retroviral naive. This effect is best explained by the fact that subjects who received mono-therapy prior to entering the study, had already developed resistance to that agent. The combination of ZDV/ddI provided the best outcomes, though these results were not statistically significantly different than other combinations. Two additional studies, the so called DELTA trial and NuCombo trial, however, have confirmed the superiority of AZT/ddI over ZDV mono-therapy and reaffirmed that dual therapy provides the best results in patients who had not received prior anti-retroviral medications[66,67] . Taken in conjunction, these three trials changed the standard of care of HIV-1 infection to dual nucleoside therapy.

Since the results of these studies have become known, virtually every combination of nucleoside has been tested in clinical trials in HIV-1 infected individuals. Therapy with ZDV/ 3TC resulted in better suppression of viral load and prolonged elevation of CD4 counts when compared to ZDV alone in a series of studies collectively known as the NUCA trials [68-71]. Recent results from a Canadian-European trial have now demonstrated the 3TC containing regimens were associated with prolonged survival [72]. Preliminary results of a trial of ddI/d4T combination is associated with marked viral load decreases (approximately 2.0 logs) and elevation of CD4 counts though clinical endpoints are not yet complete [73]. Combinations of d4T/3TC have also been associated with improvement of surrogate markers [74]. Only one combination of nucleosides had been associated with poorer outcomes than mono-therapy. In a recent study of several combinations of nucleoside agents, the ZDV/d4T arm was discontinued after patients experienced a significant decline in CD4+ cell, as compared to patients treated with ZDV/3TC or d4T alone [75]. The poor result of this regimen is probably due to the fact that ZDV and d4T are thymidine analogues and thus compete for the same substrate.

Table 1. Summary of Reverse Transcriptase Inhibitors

Name	zidovudine (Retrovir, ZDV, AZT)
Mechanism	nucleoside analogue RT inhibitor
Clinical Efficacy	increased CD4, decreased VL, Prolongs survival ~18-24 months Best results when used with other agents (DDI,DDC, 3TC)
Toxicity	Anemia, Neutropenia, hepatitis, constitutional symptoms. Toxicity can be reduced through gradual acceleration of dosing
Dosage	300 mg B.I.D.
Name	didanosine (Videx, DDI)
Mechanism	nucleoside analogue RT inhibitor
Clinical Efficacy	increased CD4, decreased VL. Prolongs survival in patients pretreated with ZDV, Best results when used with ZDV, combination with D4T is promising
Toxicity	peripheral neuropathy, pancreatitis, hepatitis (rare)
Dosage	200 mg B.I.D.
Name	zalcitibine (Hivid, DDC)
Mechanism	nucleoside analogue RT inhibitor
Clinical Efficacy	increased CD4, decreased VL. Prolongs survival in patients pretreated with ZDV; Best results when used with ZDV in naive patients. May have limited use in advanced disease.
Toxicity	neuropathy, oral stomatitis.
Dosage	0.75 mg T.I.D.
Name	stavudine (Zerit, D4T)
Mechanism	nucleoside analogue RT inhibitor
Clinical Efficacy	increased CD4, decreased VL. Fewer opportunistic infections when compared to ZDV. Marked decrease in VL (~2.0 logs) when combined with DDI. Should not be used with ZDV
Toxicity	peripheral neuropathy
Dosage	40 mg B.I.D.
Name	lamivudine (Epivir, 3TC)
Mechanism	nucleoside analogue RT inhibitor
Clinical Efficacy	Rapid development of resistance to 3TC provides protection against ZDV resistance. ZDV/3TC combinations provide marked decreases in VL and sustained elevation of CD4 counts. D4T/3TC has good preliminary results.
Toxicity	anemia, neutropenia; toxicities may be synergistic with ZDV
Dosage	150 mg B.I.D.

Currently, there are few studies that compare the relative efficacies of each combination of nucleoside analogues. In those trials which did perform a head to head comparison, there was an advantage to ZDV/ddI compared to ZDV/ddC, but this benefit was not statistically significant [65,66]. Similarly, the combination of ZDV/3TC provided better results than ZDV/ddC in subjects with very low CD4 counts and prior therapy with ZDV [71]. Thus it is difficult to recommend one combination over another, particularly in patients who have never been treated before. Many practitioners prefer to use ZDV/3TC because of its relative efficacy and ease of administration.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

These anti-virals are synthetic agent with a high affinity to the active site of HIV-1 reverse transcriptase. A chemical reaction occurs between reverse transcriptase and the

Table 2. Summary of non-nucleoside reverse transcriptase inhibitors (NNRTI)

Name	nevirapine (Viramune, NVP)
Mechanism	non-nucleoside reverse transcriptase inhibitor
Clinical Efficacy	combination of NVP and ZDV better than a single agent. ZDV/DDI/NVP can achieve undetectable levels in naive patients
Toxicity	dose dependent rash, LFT abnormalities
Dosage	200 mg daily for 2 weeks; then 200 mg B.I.D.
Name	delavirdine (investigational) (Rescriptor, DLV)
Mechanism	non-nucleoside reverse transcriptase inhibitor
Clinical Efficacy	DDI/DLV no better than DLV alone in retroviral experienced patients. ZDV/DLV has better results but studies are on-going
Toxicity	dose dependent rash, LFT abnormalities
Dosage	No dosage recommendations yet

NNRTI, irreversibly inhibiting the enzyme. NNRTI's are associated with increased CD4 counts and suppression of viral loads [76]. Resistance develops in as little as four weeks in when they are used as mono-therapy and thus they should be used in combination with other anti-retroviral agents [77]. NNRTI's are generally safe, with the main reported side effect being a rash that is dose dependent and reversible with removal of the drug [78]. There have been several cases of Steven's-Johnson syndrome however. NNRTI's are metabolized by the cyp3a fraction of the cytochrome P450 system and thus can potentially interact with a wide variety of drugs, particularly HIV-1 protease inhibitors.

Nevirapine, is currently the only NNRTI approved by the Food and Drug Administration. The best results of this drug are seen when it is used in combination with other anti-retroviral agents. In a recent study, 70% of subjects receiving ZDV/ddI/ nevirapine had undetectable plasma HIV-1 RNA levels after 28 weeks of therapy, compared to 30% of the subjects receiving ZDV/ddI. None of the subjects receiving ZDV/nevirapine had undetectable levels of HIV-1 RNA [79].

A second agent, delavirdine, has been extensively studied in the treatment of HIV-1 infection. In a three clinical trials of over 2000 patients, subjects were randomized to receive delavirdine in combination with ZDV, 3TC or ddI. Patients in the delavirdine arms had better suppression of viral loads and more sustained elevation of CD4 counts than the control groups [80-82]. Triple anti-retro-viral therapy provides a more pronounced anti-viral effect: 60% of subjects treated with ZDV/ddI/delavirdine had undetectable plasma HIV-1 RNA levels after 52 weeks of therapy in an open label trial.[83] This effect may not translated into a clinical benefit, however. In a study of ddI/ delavirdine, there were equal number of deaths in the treatment and placebo groups, prompting the data safety monitoring board to halt the trial [82]. Because of these equivocal results, the Food and Drug Administration's advisory board did not recommend approval of delavirdine at this time.

Protease Inhibitors

The activity of the HIV-protease is crucial for the formation of mature viral particles [84]. HIV-1 viral proteins are transcribed as large, polypeptide chains known as polyproteins. These poly-proteins are assembled and packaged at the cell surface where immature viral particles bud off and are released into the plasma. Final viral maturation occurs within the serum through the action of the HIV-1 protease. The protease is a complex enzyme composed of dimers; the active site of this protein is within cleft formed by these dimers [85,86]. The protease cleaves the

polyproteins into their component parts, forming infectious viral particles.

HIV-1 protease inhibitors represent one of the first examples of rational drug design. The HIV-1 protease was purified and its three dimensional structure was determined through X-ray crystallography. Computer modeling was then used to identify compounds that specifically fit into the substrate binding pocket of the protease, thus potentially inhibiting its activity [86].

Since this time numerous compounds have been synthesized that have anti-HIV-1 activity [87]. Four agents, saquinavir, ritonavir, indinavir, and nelfinavir now been approved by the Food and Drug Administration for the treatment of HIV-1 infection in humans. Several other drugs from this class are in the late stages of development and will be available shortly.

Protease inhibitors are primarily metabolized by the liver through the cytochrome P450 system, particularly cyp3a isoform of this system [87] . Consequently, there is the potential for a variety of interactions between protease inhibitors and other drugs [88-91]. In general, medication that induce this system, such as rifampin and rifabutin, can reduce the bioavailability of these agents, while agents that block this enzyme, such as ketoconazole, can increase the levels of these medications. Finally HIV-1-1 protease inhibitors may inhibit the metabolism of terfenadine (Seldane), astemizole (Hismanal)) and cisapride (Propulsid), leading to increased plasma concentrations of these drugs and potentially serious cardiac arrhythmias.

The affinity of protease inhibitors for the cytochrome P450 enzymes allow for significant interactions between each of the protease inhibitors and other anti-retroviral agents. Because of its avidity for the cyp3a enzyme, ritonavir markedly increases the levels of saquinavir [92]. This interaction actually results in a therapeutic benefit because it increases the overall bioavailabilty of saquinavir. Nelfinavir can also increase levels of saquinavir but the therapeutic implications of this interaction remain untested [93]. The interactions between ritonavir, indinavir and nelfinavir are clinically insignificant [91]. Non-nucleoside reverse transcriptase inhibitors can also interact with protease inhibitors. Nevaripine reduces the levels of saquinavir, ritonavir and indinavir [94,95]. Because of low achievable serum levels of saquinavir, it should not be used with nevaripine. The interaction between indinavir and nevaripine can be successfully handled by increasing the dose of indinavir. The interactions between nelfinavir and nevaripine are unknown. Unlike nevaripine, delavirdine is a potent inhibitor of the cyp3a enzyme, elevating levels of saquinavir [96].

Saquinavir, ritonavir, indinavir, and nelfinavir are potent inhibitors of HIV-1-1 protease activity [97-102]. The concentrations required to inhibit laboratory and clinical isolates range from approximately 10 nmol/L to 100 nmol/L, concentrations readily obtained in vivo. In early phase 1 and phase 2 studies, each drug increased the level of CD4 cell counts and decreased the levels of plasma viral RNA. Although long-term efficacy trials are ongoing, preliminary data confirm that protease inhibitors delay clinical progression and prolong life. There are no head to head trials of protease inhibitors and thus one cannot compare the various drugs since each was studied in unique patient populations.

Saquinavir

Saquinavir was the first protease inhibitor to show clinical efficacy but its clinical benefits have been limited by its poor bioavailability. Only 4% of saquinavir achieves sustained serum levels [88]. A phase 2 study of 302 patients with advanced disease and extensive prior zidovudine treatment, subjects receiving the triple combination of saquinavir/ZDV/ddC had better surrogate marker response than those who received ZDV/ddC or ZDV/saquinavir [103]. These effects were modest, however, with only a 40- to 50-cell increase in CD4+ cell levels and a 0.5 log decline in HIV-1

Table 3. Summary of protease inhibitors

Name	saquinavir (Invirase, SQV)
Mechanism	protease inhibitor
Clinical Efficacy	up to 1 log VL reduction; best results when used with two nucleoside analogues. Combination of SQV/DDC provides better survival than either agent alone.
Toxicity	nausea, diarrhea, LFT abnormalities. Generally well tolerated.
Dosage	600 mg T.I.D.
Name	ritonavir (Norvir, RTV)
Mechanism	protease inhibitor
Clinical Efficacy	up to 2.5 log VL reduction; best results when used with two nucleoside analogues. RTV improves survival and decreases time to opportunistic infections in advanced AIDS.
Toxicity	nausea, vomiting, diarrhea, asthenia, LFT abnormalities, hyper-triglyceridemia. Up to 30 % of subjects in clinical trials discontinued RTV because of untoward effects.
Dosage	600 mg B.I.D.
Name	indinavir (Crixivan, IDV)
Mechanism	protease inhibitor
Clinical Efficacy	up to 2.5 log VL reduction; best results when used with two nucleoside analogues. Clinical endpoints not yet available.
Toxicity	nausea, vomiting, diarrhea, asthenia, hyper-bilirubinemia, renal stones in 10% of patients after 1 year.
Dosage	800 mg Q8 hours on empty stomach
Name	nelfinavir (Viricept, NFV)
Mechanism	protease inhibitor
Clinical Efficacy	up to 2.5 log VL reduction; best results when used with two nucleoside analogues.
Toxicity	diarrhea in up to 21% of patients, usually controlled with lomotil
Dosage*	750 mg T.I.D. with meals

*500 T.I.D. may be adequate in patients with low viral loads.

RNA at week 24. A phase 3 study of 978 patients randomized to saquinavir vs ddC vs saquinavir/ddC revealed minimal suppression of viral load with saquinavir mono-therapy and only a modest suppression with the saquinavir/ddC [104]. Despite this relatively weak activity of saquinavir against HIV-1, subjects randomized to the saquinavir/ddC arm had 46 AIDS-defining events or deaths in the combination group compared with 85 events or deaths in those treated ddC alone (P<.001). The current recommended dosing of saquinavir is 1800 mg/d. In a recent pilot study, increasing the dosage to 7200 mg/d was associated with a marked increase in anti-viral activity, suggesting that improvement in this drug bioavailabilty will enhance its clinical efficacy.[105] A new soft-gel formulation, with enhanced bioavailability, is currently undergoing clinical evaluation. Despite the modest effects of saquinavir, these studies set the stage for future therapy with protease inhibitors by demonstrating that these agents are not effective as mono-therapy and provide the best results when used with two nucleoside agents.

Because of its 4% bioavailability saquinavir is the most sensitive protease inhibitor to modifications in the cytochrome P450 system. Serum levels of this agent can be reduced by as rifampin or rifabutin while levels can be increased by concomitant ketoconazole administration [91].

Saquinavir, in its current formulation, appears to be the best tolerated of the available protease inhibitors. The

most common adverse effects are diarrhea, nausea, gastrointestinal discomfort, and rash [91]. These symptoms are uncommon and well tolerated.

Ritonavir

Unlike the saquinavir, ritonavir achieves high serum levels and has impressive anti-retroviral activity in humans In phase 2 studies, ritonavir therapy was associated with 1-2 log decreases in HIV-1 RNA levels with increases in CD4 counts of 150-200 cells/mm3 [106,107]. These antiviral effects were readily translated into a clinical benefit. In a large phase 3 trial, over 900 patients who were already on a stable anti-retroviral regimen were randomized to ritonavir or placebo. All subjects continued to take their existing antiretroviral regimen. Thus the subjects in the ritonavir arm received at least one drug in addition to the study medication. Within six-months, 33% of patients treated with placebo developed a new AIDS-defining event or died compared with only 16% treated with ritonavir, P<.001).[108]. This result was sustained through 9 months of follow-up.[109]. Preliminary viral load data from this study showed decreased antiretroviral activity at month 6, suggesting that the duration of effect of ritonavir may be limited.

Ritonavir can cause large increases in the plasma concentrations of long acting anti-histamines, various anti-arrhythmics (amiodarone, encainide hydrochloride, flecainide acetate, quinidine), and sedative-hypnotics (such as alprazolam, diazepam, flurazepam hydrochloride, midazolam, and triazolam). The full list of contraindicated concurrent therapies is quite extensive and is in the package insert.[92] Ritonavir has good bioavailability and can achieve therapeutic blood levels when taken twice daily.

Ritonavir can be associated with severe side effect, particularly within the first several weeks of administration [92]. Ritonavir causes diarrhea, nausea, vomiting, anorexia, headaches, asthenia, fatigue, circumoral paresthesia and taste disturbances. Seventeen percent of subjects in a large clinical trial of over 1000 individuals discontinued the medication because of side effects. [108]. In addition, ritonavir is associated with elevations of transaminases, creatinine kinase, and triglycerides. Clinical experience suggests that the side effects may be more severe than reported in the studies, with 30 to 40 percent of the patients stopping the medications. The severity of the side effects of ritonavir are directly related to the concentration of the drug. In addition, ritonavir induces its own metabolism, resulting in lower levels and fewer side effects once the patient has taken the drug for several weeks. Because of this, the side effects can ameliorated by acceleration of the dose of ritonavir over several weeks [110] .

Indinavir

Indinavir has potent anti-viral effects that are comparable to ritonavir. Data from the first 266 of 750 antiretroviral-naive patients randomized to ZDV, indinavir, or ZDV/indinavir demonstrated greater than a 1 log reduction in viral load for patients who received an indinavir-containing regimen [36,111]. In addition, patients treated with ZDV/ indinavir were less likely to develop genotypic resistance to zidovudine and to indinavir. In a 3 arm study of indinavir vs ZDV/3TC vs. indinavir/ZDV/3TC, 24 of 26 patients on the 3-drug combination had undetectable viral RNA levels (<500 copies/ml) at week 24 . In contrast, 13 of 26 patients treated with indinavir and 0 of 26 patients treated with zidovudine and lamivudine had undetectable levels.[36] In patients who complied with the 3-drug regimen, this potent effect was sustained through week 44. Despite these effects, data regarding reduction in AIDS events and deaths are lacking. Results from a clinical endpoint study of ZDV/3TC/indinavir vs ZDV/3TC will be available soon and are expected to

show clinical benefits with indinavir therapy.

Indinavir is metabolized by the cytochrome P450 cyp3a enzyme but about 20% of the drug is excreted unchanged in the urine [90]. Indinavir has similar interactions with rifabutin and ketoconazole as ritonavir. The degree of these interactions is less and thus these drugs can be concomitantly administered with dosage adjustments. As with ritonavir, long acting anti-histamines should be avoided. The bioavailability of indinavir is reduced with meals, so it should be taken without food. In addition, indinavir must be taken every 8 hours (not 3 times per day) in order to maintain adequate serum levels.

Indinavir is relatively safe and well tolerated. The nausea, vomiting, and diarrhea associated with indinavir do not require dosage adjustments or acceleration. Indinavir caused nephrolithiasis in 5% of patients taking this drugs. These stones are precipitates of indinivir and drinking large volumes of water can prevent this side effect. Indinavir related nephrolithiasis can be treated with hydration and analgesia. Discontinuation of the drug is usually not necessary. Mild bililrubin elevations with indinivir therapy are common but are not associated with hepatitis or hepatic damage [90].

Nelfinavir

Nelfinavir also has potent anti-retroviral activity in humans that is comparable to ritonavir and indinavir. In phase 2 dose-ranging clinical study, 33 subjects were randomized to receive received d4T or nelfinavir/D4T combination. At eight weeks, the D4T arm had a mean 0.9 log decline in viral load, compared with a mean 2.0 log decrease in viral load in the nelfinavir/D4T arm. The effect in the nelfinavir arm was sustained for over 5 months.[112]. In a large study of two dosages of nelfinavir combined with ZDV/3TC, there was an mean decrease in viral load of 2.0 logs, with 80% of the subjects having undetectable viral loads. There was no differences in the efficacy of the two dosages of nelfinavir except in those subjects whose baseline viral load was greater than 100,000 copies per ml. In this sub-group, the higher dose of nelfinavir suppressed viral load for longer periods of time [113] .

The drug interaction profile of nelfinavir is similar to that of indinavir [114]. Nelfinavir should not be used with terfenadine, astemizole, or cisapride. Rifampin will reduce nelfinavir plasma concentrations; therefore, these agents should not be used concurrently. Although ketoconazole increases plasma levels of nelfinavir, dose modifications are not necessary. Other drug interactions, including those with benzodiazepines, have not been fully evaluated. Unlike indinavir, nelfinavir can be taken 3 times daily with food.

Nelfinavir is most commonly associated with loose stools but clinical experience with this drug is currently limited [115].

Combination Protease Inhibitors

Use of two protease inhibitors simultaneously in the treatment of HIV-1 infection has several potential therapeutic benefits. First, there larger and longer reductions in viral loads may be achieved by combining two potent. In addition, utilization of two protease inhibitors with distinct, non-overlapping patterns of resistance theoretically can prevent the emergence of resistance. Finally, specific combinations of protease inhibitors may increase serum drug levels and allow for reduction in dosages without compromising the antiviral activity of these agents. This would allow for more convenient dosing regimens that would reduce the costs of protease inhibitor therapy and improve patient compliance.

The first combination regimen to be studied in detail involves saquinavir and ritonavir. Because of its interactions with the cyp3a enzyme, ritonavir inhibits the metabolism of saquinavir, resulting in

sustained, increased levels of the latter drug.[116] In study testing the feasibility of combining these two agents, 136 patients were randomized to receive 1 of 4 ritonavir-saquinavir combinations. At 26 weeks there was a mean 3.0 log decrease in viral loads across the treatment regimens. In addition, subjects who received reduced dosages of ritonavir-saquinavir had fewer side effects when compared to higher dosages of either drug.[117]. Seven subjects did not initially achieve undetectable levels of viral RNA but 6 of these 7 had undetectable viral load after the addition of nucleosides to their regimens. Based on these results, the "optimal" dosage of this combination is 400 mg/B.I.D. of ritonavir and 400 mg/B.I.D. of saquinavir [118]. This dosing regimen has the added advantage of lower cost and requires taking fewer pills than triple combination therapy using nucleosides and a protease inhibitor. Other potential protease combinations are under study [119].

Combination Therapy and 'Viral Eradication'

Therapy of newly infected individuals is an active area of research. The goal of this research is to change the course of HIV-1 infection, and if possible, eradicate the infection. Mathematical models suggest that if viral replication can be totally suppressed for up to three year, cure of HIV-1 is possible [20]. In a proof of concept study, Ho et. al initiated aggressive anti-retroviral therapy within 90 days of acquiring acute HIV-1 infection in 24 patients [120,121]. All subjects received triple combination therapy with ZDV/3TC and a protease inhibitor. The specific protease inhibitor varied and included ritonavir or nelfinavir. All of the twenty subjects who remained the trial had undetectable levels of virus in the serum as measured by HIV-1 RNA PCR for as long as 16 months after initiation of therapy. In addition semen and gut associated lymphoid tissue was examined for evidence of HIV-1 DNA and RNA. Specifically the investigators measured unspliced RNA which represents whole virus, such as that trapped on follicular dendritic cells and spliced messenger RNA, which reflects ongoing viral replication inside productively infected cells. None of the patients had HIV-1 RNA in their seminal fluid but HIV-1 DNA was detected in all patient semen samples. Similarly, analysis of GALT revealed no detectable unspliced RNA, but did demonstrate spliced RNA and HIV-1 DNA [20].

Thus aggressive treatment of HIV-1 is associated with no active viral replication in seminal fluid and lymphoid tissue. There remains evidence of inactive infection after up to 16 months of therapy. Further studies will determine if prolonged therapy will result in clearance of spliced RNA and HIV-1 DNA from these tissues. Future research strategies will focus on the effects of therapy on lymphoid biopsies and conducting assays in the cerebrospinal fluid (CSF). Ultimately, Ho and his colleagues will consider stopping treatment after the 2.5 to three years, the estimated time needed for infected cells to die.

Resistance to Protease Inhibitors

Emergence of resistance is a major problem with protease inhibitor therapy. The rate of viral replication, combined with the error prone HIV-1 reverse transcriptase results in a high mutation rate for HIV-1. Under the selective pressure of a protease inhibitor, there is rapid emergence of resistant mutants when protease inhibitors are used as mono-therapy. [122]. Specific mutations within the protease genome has been associated with resistance to each of the 4 currently available protease inhibitors [123-128]. Saquinavir resistance has been linked to amino acid substitution at codons 48 or 90 of the protease with a change at codon 90 being the most common. Indinavir resistance has been associated multiple mutations including substitutions at codons 32, 46, 71, 82, 84. High level resistance to indinavir requires the sequential development and persistence of these multiple mutations. The resistance pattern

for ritonavir is similar to that reported for indinavir; however, an initial mutation at V82 appears to be necessary for the subsequent development of other mutations. The resistance pattern for nelfinavir may be unique, with the critical mutation occurring at codon 30, although other mutations have been reported [128].

Based on the mutation patterns, it is expected that there will be substantial cross resistance between indinavir and ritonavir. There is a lower probability between cross resistance saquinavir and indinavir or ritonavir. Because of the unique mutation patterns associated with nelfinavir use, these may be little cross resistance between nelfinavir and the other protease inhibitors. Preliminary studies suggest that this may be the case. Viral isolates from 6 subjects who had failed nelfinavir retained sensitivity to indinavir, ritonavir and saquinavir in *in vitro* testing [128].

This data regarding cross resistance to protease inhibitors should be interpreted with caution as there remains limited clinical experience in treating patients who have failed a protease inhibitor with a second, similar agent. In addition, wide spread use of combination therapy may induce novel mutation which can confer resistance to the entire class of agents.

Prevention of resistance may be accomplished through several means. Protease inhibitors should always be prescribed with other agents because mono-therapy with these drugs leads to rapid emergence of resistance [129]. Reduction of viral replication to the lowest levels possible through individualized therapy using viral load tests will decrease the rates of mutations and development of resistant clones. The optimal level of viral load to achieve this is unknown, however. In addition, alterations in anti-retroviral therapy should always include the addition of two agents to which the individual has had limited exposure in order to reduce the chance of resistance developing to the new agents. Patient compliance with regimens is key as persistent viral replication in the face of inadequate drug levels may lead to the emergence of resistance. Despite these efforts emergence of resistance may be inevitable: Kozal et. al found that a high percentage of HIV infected patients in Iowa had de novo resistance to protease inhibitors [130].

Compliance to Protease Inhibitor Regimens.

Compliance with protease inhibitor regimens is important to achieve the maximal therapeutic benefit and to prevent the emergence of resistance. Unfortunately, the number of pills, side effects and dosing schedules of these regimens make compliance very difficult. A typically regimen of ZDV/3TC and indinavir requires taking 14 pills per day. This number only represents anti-retroviral therapy and does not include the medications an individual may take to prevent or treat opportunistic infections. In addition, indinavir must be taken on a Q 8 hour schedule on an empty stomach, thus adding to the difficulty in taking this regimen. Substitution of a ritonavir would allow for B.I.D. dosing but would increase the number of pills to 20 per day and would expose the patient to the added toxicity of this drug. Use of other agents are associated with other trade-offs.

Patients receiving protease inhibitor therapy are experiencing difficulty complying with the requirements of these regimens. In a review of pharmacy records at Parkland Hospital, only 79% of the patients refilled their protease inhibitors in a manner that they could be taking the drugs in the manner in which they were prescribed. This figure only reflects refill patterns and does not indicate how often the medication is taken once it has been refilled. Thus the actual amount of protease inhibitor taken by the patient may be significantly less than the 79% compliance observed in our hospital. Poor compliance may have significant clinical effects. In a study of ritonavir/saquinavir combinations, there were significantly higher viral loads in those subjects had less than 90% compliance with their regimen.

Costs of Protease Inhibitors

Protease inhibitors are very expensive. Public Health Services (PHS) prices, generally the lowest available prices in the United States are $360.00 per month for indinavir, $393 per month for saquinavir and $414 for ritonavir. PHS prices for nelfinavir are not yet available. The price paid by individuals and health care delivery systems may be substantially higher. When combined with nucleoside analogues, the total yearly cost of anti-retroviral therapy can range from $9000 to $15,000 per patient per year, depending upon the regimen used and the costs of each component. Monitoring therapy with viral load assays increase the costs even more. The impact of these costs on a health care system can be staggering. For example, it would cost $21 million per year to treat and monitor all 2500 patients enrolled in the Parkland Memorial Hospital's AIDS clinic with triple combination therapy that included a protease inhibitor. This figure represents almost two thirds of this hospitals entire pharmacy budget for the same period of time! Because a large portion of the nations HIV-1 infected patients have limited financial resources, public hospitals all over the nation are seeking ways to provide these medications to their patients.

Despite the expense of anti-retroviral medication, there are programs which can help offset the costs of these drugs. Nationwide, 60% of HIV-1 infected adults receive Medicaid. Although this program is financed by both federal and state governments, eligibility and limits of coverage are determined at the state level. Texas Medicaid provides only three drugs to covered individuals. Thus patients receiving medications can have their anti-retrovirals provided to them but will not receive other drugs that they may require. Thus this program may have little impact on the total expense of HIV-1 care by shifting the costs of non-anti-retroviral therapy to local areas. The AIDS Drug Assistance Programs are state operated programs that utilize both state and federal funds. The ADAP program was conceived as emergency funding to provide medications for individuals who would otherwise do without. Even prior to the advent of protease inhibitors, many of these programs were financially strained. The Texas State AIDS Drug Assistance Program is currently able to provide protease inhibitors to approximately 1400 individuals statewide, a number which is only a fraction of the estimated 40,000 HIV-1 infected persons living in Texas. In addition to these governmental programs, pharmaceutical firms are providing protease inhibitors at no charge to indigent patients with no other source of these drugs. While the total impact of these resources is substantial, the consensus is that more funds will be required to pay for these medications.

Table 4. Comparisions of cost efficacy of HIV therapy and other common treatments

Intervention	Cost/Life-Year Gained
Triple HIV Therapy	$15,000
Mammography (> age 40)	$30,000
PSA Screening (> age 50)	$113,000
CABG (> age 50)	$113,000

There is evidence by changing the course of HIV-1 infection, protease inhibitor therapy may reduce health care costs associated with HIV-1 [131]. There are several computer models based on decreased rates of opportunistic infections observed in clinical trials of protease inhibitors that project a cost savings of up to $6000 per patient per year. This projected saving is exclusive of drug acquisition costs and thus represent little net savings. Other models have compared the cost of life year saved by protease inibitors and compared these to other well accepted therapies for diseases ranging from cancer to heart disease. Triple combination therapy was far more cost effective in prolonging life than therapies or screening tests for other diseases [132]. Validation of these models is

ongoing but preliminary data from several hospitals in France showed a significant reduction in the cost of providing total HIV-1 care in those centers that used protease inhibitors. There is also evidence of increased survival [133]. Last month the CDC reported a 13% decrease in the number of AIDS deaths during the first 6 months of 1996. Large cities including New York and Dallas have found similar declines in mortality.

Treatment Recommendations

Currently there is no consensus on how to most effectively use anti-retroviral agents to treat HIV-1 infection. While most experts agree that the goal of anti-retroviral therapy is to push the viral load "as low as possible for as long as possible", there is considerable debate on how this end should be achieved. In 1996 the International AIDS Society recommended that individuals with a viral load greater than 5000-10000 RNA copies/ml and a CD4 count less than 500 cells/mm3 be treated with at least two drugs [134,135]. Those with greater than 30,000-50,000 RNA copies per ml should be treated regardless of CD4 count. Finally, protease inhibitor therapy should be instituted in those subjects who fail to have an adequate response to initiation of therapy. The IAS recommendations did not delineate a specific value of RNA copies number per ml of blood as the target of anti-retroviral therapy. Similar treatment algorithms that target specific levels of viral replication have been developed at VA and public hospitals around the country to guide anti-retroviral therapy. Since the results of the indinavir studies showing undetectable viral loads for prolonged periods of time became available, many clinicians now believe that the goal of anti-retroviral therapy should be "complete viral suppression," rather than simply low levels of viral replication [36]. Although there is indirect evidence to suggest that an undetectable viral load will result in better clinical outcomes and less emergence of resistance, there are no clinical trials demonstrating that an undetectable viral load is better than low, but detectable, levels of viral replication. In addition undetectable levels of HIV-1 replication is not achievable in all patients and may not be feasible in many other patients because of drug intolerability, poor compliance or expense of combination therapy. Currently, clinical trials are determining the "optimal" viral load required to prevent HIV-1 disease progression for prolonged periods of time.

Development of treatment guidelines must take into account all of the factors which may effect outcome, including the medications efficacy, tolerability, patient compliance and the cost of medications. At Parkland Memorial Hospital, we have developed a treatment algorithm that use a stepwise approach to initiation of anti-retroviral therapy [136]. In this schema all patients with a CD4 count below 500 cells/mm3 would be started on dual nucleoside therapy. The choice of these drugs depends on the patients treatment history, compliance and potential toxicities to anti-retroviral agents. Given the current state of the art, combinations of AZT/3TC, AZT/DDI, AZT/DDC or DDI/D4T are reasonable choices. Viral load measurements are performed prior to and 4-6 weeks after the initiation of therapy. Those patients with at least a 0.5 log decrease in baseline viral load and a post initiation of therapy viral load less than 5000 RNA copies per ml would continue on this therapy. Patients who do not have a 0.5 log decrease in baseline viral load and less than 5,000 RNA copies per ml after several weeks of therapy should have a protease inhibitor added to their regimen. Indinavir is a popular choice in this case because of its efficacy and tolerability. Nelfinavir is also a good choice for initial protease inhibitor therapy and may have an advantage over other drugs because resistance to it does not preclude the subsequent use of other agents. Nevaripine, the non-nucleoside reverse transcriptase inhibitor could be an alternative to a protease inhibitor for anti-retroviral naive patients who do not meet

the minimal treatment criteria with nucleoside analogues. Once a stable anti-retroviral regimen has been established, CD4 counts and viral load measurements should be repeated every 4 to 6 months.

Anti-retroviral medications should be changed for patients who have one of the following: greater than a 0.5 log increase in viral load toward baseline with a concomitant decline in CD4 cells; a new opportunistic infection; or intolerance to therapy. Changes in viral load without CD4 count changes should be interpreted with caution as transient viral load increases can occur in response to a variety of stimuli. Patients who fail therapy should be treated with at least two agents that they had not previously received. If a protease inhibitor has not been used, it should be started at this point. For patients who fail or did not tolerate other protease inhibitors, use of saquinavir as salvage therapy may be considered because of decreased chance of resistance. The combination of saquinavir and ritonavir may be a good choice in patients who have been heavily pretreated with nucleoside analogues but have few other treatment options.

There currently are many similar treatment algorithms in existence. They are popular because they allow for clinicians to target those patients who most need protease inhibitors and provide health care providers with a means of controlling costs.

Conclusions

Experiments into the pathogenesis of HIV-1 infection have demonstrated that rates of viral replication drive the progression of disease. Protease inhibitors, when used in combination with other agents, can decrease viral replication to unprecedented levels. Over the short term these regimens have decreased rates of opportunistic infections and prolonged survival. The durability of effect of these agents is unknown, however. Although there is potential for long term suppression of viral replication, there are significant barriers to this goal including emergence of resistance, drug toxicity, poor compliance and limited drug availability because of the expense of these drugs. Predictions of a cure in patients with early disease are premature and should be interpreted with caution. HIV-1 is an integrated virus and there is no precedent for eradicating such a virus once it has been incorporated into the host's genome. Ongoing research will focus on determining the best regimens for long term suppression of viral replication. In addition, new agents with potent antiretroviral activity and once daily dosing are currently being developed. These agents will simplify treatment regimens and may have unique resistance mutations. Thus in the future, anti-retroviral therapy may consist of a series of multi-drug regimens that results in long term stabilization of HIV-1 infection.

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