ANTI-RETROVIRAL THERAPY OF HIV INFECTION

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INTRODUCTION

Since the identification of HIV-1 as the causative agent of AIDS, there has been much progress in the treatment in this infection. The introduction of zidovudine in 1985 was associated with improved survival in patients with AIDS[1]. Triple anti-retroviral therapy with protease inhibitors has had a major impact of the disease with decreased rates of opportunistic infections and dramatically increased the survival of HIV-1 infected individuals[2]. There are now 15 anti-retroviral agents available with many more in development. Unfortunate, the initial promise of these drugs has remained unfulfilled; a high proportion of patients fail therapy after as little as a year and a cure of HIV-1 is unlikely in the foreseeable future[3, 4]. In addition, anti-retroviral regimens are complex, difficult to adhere to and are associated with long-term complications[5, 6]. This article examines the current state of the art of anti-retroviral therapy and focuses on the promise as well as the peril of treating patients with HIV-1 infection.

PATHOGENESIS

VIRAL REPLICATION AND DYNAMICS

HIV-1 is a retrovirus and thus stores its genetic information in the form of single stranded RNA[7, 8]. Structurally the virus consists of protein core that contains RNA, regulatory proteins and reverse transcriptase, an RNA-directed DNA polymerase. The core is surrounded by a glycoprotein – lipid membrane that contains two critical proteins, GP 120 and GP 41 through which HIV infects CD4 bearing cells. GP120 binds to the CD4 molecule and fusion occurs after an additional interaction between GP41 and CCR5 or other chemokine receptors. Once in the cytoplasm, HIV-1 reverse transcriptase synthesizes double stranded DNA using the single strand of RNA as a template. The newly synthesized DNA is transported to the nucleus of the cell where it is integrated into the host DNA by an HIV-1 encoded integrase. The HIV-1 RNA is then transcribed. HIV-1 proteins are synthesized as a single long proto-protein that is cleaved into active molecules by an HIV-1 encoded protease. The virus then buds through the membrane of the CD4 cell into the plasma where it then infects other CD4 cells.

Immediately after the acute HIV infection, there is a burst of viral replication resulting in very high plasma concentration of the virus[8, 9]. An intense immune response drives HIV into the lymphoid tissue where it infects follicular dendritic cells, which act as a sync for HIV to infect other cells. Local cytokine response by follicular dendritic cells in response to HIV infection recruits CD4 cells into the lymphoid tissue, where these cells are then infected. Continuous viral replication takes place in the lymphoid tissue, spilling HIV into plasma. The level of HIV replication remains relatively constant for a given patient, but varies greatly among individuals. Cytotoxic T lymphocytes responses are directed against the virus, however this response is lost after approximately 6 months after acute infection[10, 11].

Viral production proceeds at the rate of 10^{10} new virus particles/day; 99% of the daily production of HIV derives from recently infected, activated CD4 cells[12, 13]. These activated CD4 cells have a half-life of 1-3 days. The remaining 1% of HIV production takes place in longer-lived pools of monocytes/macrophages and/or chronically infected, non-activated CD4 cells. The life span of these CD4 cells can be as long as 70 years.[14-16] Although the proportion of chronically infected cells with extended life spans contribute $\leq$0.01% of the total daily viral production in untreated patients, the long life of these infected cells make attempts to eradicate HIV from infected persons less likely.

HIV-1 infection is characterized by the progressive loss of CD4 bearing lymphocytes[17]. These are primarily destroyed through cell lysis during viral replication but other mechanisms such as accelerated apoptosis and decreased thymic output also contribute to CD4 count decline[7]. In response to this loss, there is an increase in production of CD4

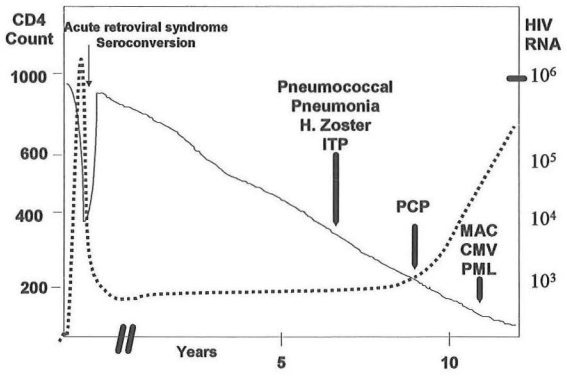
cells[18, 19]. However, the rate of production does not match the rate of destruction and there is a net loss in the number of circulating CD4 cells. Despite the fact that this is a highly dynamic process, the net decrease in circulating CD4 cell is actually quite slow. Typically, ten years pass before an infected individual's CD4 cell count falls below 200 cells/mm3, the clinical definition of AIDS[17].

Numerous studies have demonstrated that the absolute level of HIV-1 plasma RNA is predictive of the rate of CD4 cell loss, of the progression to AIDS, and of death [20-22]. Persons with no detectable virus have the slowest rate of progression, while those with the highest levels of virus have the highest rates of progression. Patients with ≥10,000 copies of HIV-RNA/mL of blood are more likely to develop AIDS and die than those who had less than this number. In addition reduction of HIV-1 plasma RNA with anti-retroviral therapy is associated with decreased loss of CD4 cells counts, progression to AIDS and death. Those individuals who achieve an HIV-1 plasma RNA that is below the level of detection through anti-retroviral therapy, have virtually no progression of disease as long as viral replication remains suppressed[23-25].

Clinical Syndromes

Within 6-8 weeks of exposure, 50%-70% of newly infected individuals develop an acute retroviral syndrome[26]. The acute illness lasts from few days to >10 weeks, but the duration is usually <14 days. The most common signs and symptoms include fever, fatigue, rash (40-80%), headache, lymphadenopathy and pharyngitis. A morbilliform rash, usually involving the trunk, occurs in 40-80% of persons with symptomatic acute HIV-1 infection. Mucocutaneous ulceration, involving the buccal mucosa, gingiva, palate, esophagus, anus, or penis, is also highly suggestive of acute infection in a person at risk.

After acute infection HIV-1 plasma RNA decreases to a set point, which remains fairly constant throughout the infection. During the acute seroconversion illness, anti-bodies to HIV develop[8, 27]. This is part of a generalized immune reaction to HIV-1 infection that includes both a humoral and a cellular response to the infection. Equilibrium between HIV-1 and the immune system develops where viral replication decreases from the peak at the onset of symptoms to a lower level of continuous viral replication that has been termed a virologic set point. Viral replication continues at this level until late stage AIDS where the immune response is on longer sufficient to even partially suppress viral replication. There is great variability in the rates of viral replication among individuals. The median HIV-1 plasma RNA level is 20,000 copies/ml but this number varies greatly[20]. There are some individuals with confirmed HIV-1 infection that never have detectable plasma HIV-1 RNA while others consistently have millions of copies of HIV-1 RNA in their plasma.



Clinical symptoms of HIV–1 occur with declining CD4 cell counts. There is a long clinically latent period between the acute seroconversion illness and development of significant immunosuppression[17, 28]. The mean time from infection with HIV to the diagnosis of AIDS is approximately 11 years. There is very little risk of opportunistic infections until CD4 cell count falls below 350 cells/mm3. Below this level, individuals at risk for bacterial pneumonia, oro-pharngeal candidiasis, and herpetic infections. A CD4 count of less than 200 cells/mm3 is the criterion for an AIDS diagnosis. Below this level,

Pneumocystis carinii pneumonia occurs at a great frequency. At CD4 counts of 100 cells/mm3 or less, individuals develop active infections with cytomegalovirus retinitis, cryptococcoccal meningitis, cerebral toxoplasmosis, disseminated mycobacterium avium complex or a host of other opportunistic infections.

ANTIRETROVIRAL AGENTS

Nucleoside analogues (NRTIs)

Nucleoside Analogues
Zidovudine (ZDV,AZT)
Didanosine (ddI)
Zalcitibine (ddC)
Stavudine (d4T)
Lamivudine (3TC)
Abacavir (ABC)
Combivir (zidovudine/lamivudine)
Trizivir (zidovudine/lamivudine/abacavir)

Nucleoside reverse transcriptase inhibitors are synthetic analogues of naturally occurring nucleosides. Currently there are 6 drugs available in this class of agent. These include zidovudine (AZT) and stavudine (d4T) both thymadine analogues; didanosine (ddI) an inosine analogue that is converted intracellularly to an adenosine analogue; zalcitabine (ddC), and lamivudine (3TC) both ctyosine analogues; and abacavir a guinosine analogue. Nucleoside analogues are well absorbed and are rapidly converted to dideoxynucleoside triphosphates intracellularly. The intracellular phosphorylation traps the nucleoside analogues in CD4 cell, allowing for accumulation of the active agents within these cells[29]. This feature of these drugs allow for longer dosing intervals than would be predicted by serum pharmaco-kinetics. Nucleoside analogues are eliminated by hepatic glucoronidation.

Nucleoside analogues act by inhibiting reverse transcriptase. This is accomplished by incorporation of the analogue into the HIV-1 DNA strand by the HIV-1 encoded reverse transcriptase. The incorporated nucleoside analogue then stericly blocks further progression down the HIV-1 RNA template by reverse transcriptase, knocking off the template and terminating chain prolongation. Nucleoside analogues inhibit HIV-1 replication *in vitro.*

In infected individuals, nucleoside analogues reduce HIV-1 plasma RNA by approximately 0.5 logs[1, 24, 30]. When used as monotherapy, there is a CD4 count increase averages 20 cells/μL in ~12 weeks. Survival benefit is approximately 18-24 months regardless of the stage of disease at the time of initiation of therapy. Sequential monotherapy with nucleoside reverse transcriptase inhibitors is associated at each change with the emergence of drug resistance. Patients treated with AZT typically develop resistant clones within weeks of initiation of therapy and have clinically resistant HIV-disease within 6 months.

The best results with nucleoside analogues are achieved when they are used with at least two other agents. Combining drugs reduces viral replication to a point where it is difficult for the virus to develop resistance. Most anti-retroviral regimens consist of two nucleoside analogues and protease inhibitor or a non-nucleoside reverse transcriptase inhibitor[31, 32]. However, there is evidence that three nucleoside reverse transcriptase inhibitors can be effectively used to treat HIV infection. Combinations of zidovudine, lamivudine and abacavir have demonstrated similar reduction in HIV-1 plasma RNA as a zidovudine, lamivudine and indinavir in several studies[32].

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

Non-nucleoside Reverse Transcriptase Inhibitors
Nevirapine (NVP)
Delavirdine (DLV)
Efavirenz (EFV)

Non-nucleoside reverse transcriptase inhibitors are synthetic inhibitors of reverse transcriptase. Currently there are 3 drugs available in this class of agent. These are nevirapine, delavirdine, and efavirenz. Non-nucleoside reverse transcriptase inhibitors are rapidly absorbed and are metabolized by the liver by the ctyochrome P-450 system. Non-nucleoside reverse transcriptase inhibitors require sustained serum levels in order to maintain activity against HIV-1. These agents have a high affinity for the active site of HIV-reverse transcriptase. A chemical reaction occurs between reverse transcriptase and the non-nucleoside reverse transcriptase inhibitors, irreversibly inhibiting reverse transcriptase. These agents are very potent inhibitors of HIV-1 replication *in vitro.*

Non-nucleoside reverse transcriptase inhibitors are associated with marked suppression of HIV-1 plasma RNA in infected individuals and with increases in CD4 cell counts. Like nucleoside analogues, they are more effective when used in combination with other antiretroviral agents. The combination of zidovudine, didanosine and nevirapine were associated with undetectable HIV-1 plasma RNA in 70% of antiretroviral-naive subjects who were treated with this regimen[33]. Similar results have been reported with the combination of zidovudine, lamivudine and delavirdine. In contrast, the combination of zidovudine, lamivudine and efavirenz resulted in over 90% of subjects achieving an undetectable level of plasma HIV-1 RNA[34]. This result was superior to that achieved by a combination of indinavir, a protease inhibitor and efavirenz or by the combination of zidovudine, lamivudine and indinavir.

Resistance to non-nucleoside reverse transcriptase inhibitors develops rapidly when these agents are used mono-therapy. Typically, resistance occurs with the development of one of several amino acid substitutions within the HIV-1 reverse transcriptase genome. These mutations confer cross-resistance to all of the agents within this class.

PROTEASE INHIBITORS (PI)

Protease Inhibitors
Saquinavir (SQV)
Ritonavir (RTV)
Indinavir (IDV)
Nelfinavir (NLF)
Amprinavir (AMP)
Lopinavir/ritonavir (Kaletra)

Protease inhibitors are small, synthetic molecules that fit into the active site of the HIV-1 protease. Currently there are 6 drugs available in this class of agent. These are saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir. Protease inhibitors have variable absorption from the gastrointestinal tract with some agents achieving high levels while others have marginally effective levels. The liver metabolizes all HIV-1 protease inhibitors by the ctyochrome P-450 system. As in the case of non-nucleoside reverse transcriptase inhibitors, protease inhibitors require sustained serum levels in order to maintain activity against HIV-1. HIV-1 transcribes its proteins as a single polyprotein that is cleaved by an HIV-1 encoded protease. Protease inhibitors fit into the active site of the protease, resulting in the formation of immature, non-infectious viral particles. These agents are very potent inhibitors of HIV-1 replication *in vitro.*

Protease inhibitors are associated with marked suppression of HIV-1 plasma RNA in infected

individuals and with increases in CD4 cell counts. Like non-nucleoside reverse transcriptase inhibitors, they are more effective when used in combination with other antiretroviral agents. Protease inhibitors can reduce viral replication by up to 99% [13]. Combinations of protease inhibitors and nucleoside analogues can reduce plasma HIV-1 RNA to undetectable levels in up to in 80-90% of naive patients [31, 35-39]. It currently difficult to is to determine the relative efficacy of protease inhibitor containing regimens as there have been few comparative trials completed to date

When protease inhibitors are used as single agents, resistance can develop in as little as 6 weeks. However, when used in combination with nucleoside reverse transcriptase inhibitors, their effects can last for years. Prevention of resistance requires uninterrupted administration of protease inhibitors; thus patient compliance is critical to achieve and maintain efficacy[40]. There is significance cross-resistance between various protease inhibitors[41, 42]. This cross-resistance is not necessarily complete but, in general, patients who fail a protease inhibitor-containing regimen will have a less robust response to subsequent protease inhibitor regimens. [43].

TREATMENT GUIDELINES

Treatment of HIV infection is difficult because of the number of agents available, the complexity of the regimens and the overlapping toxicity of the agents. Numerous studies had demonstrated that patients treated with 3 anti-retroviral agents have superior outcomes that those treated with 2 or one drug. However, optimal regimens remain undefined. In addition, the optimal timing for initiation of anti-retroviral therapy remains unclear. Although therapy with anti-retroviral drugs can suppress viral replication below the limits of detection in a high proportion of subjects in clinical trials, there are high rates of failure in patients outside of studies because of difficulties in adhering to the regimens [3, 44, 45]. Because of the complexity of anti-retroviral therapy United States Public Health Service and the International AIDS Society-USA have issued guidelines for the treatment of HIV infection [46]. These treatment guidelines are similar and differ more in style than in substance. Both guidelines emphasize the need to treat HIV infection with two nucleoside analogues and a protease inhibitor. Both guidelines recommend that the goal of therapy should be the suppression of HIV-1 plasma RNA to undetectable levels.

Recommendations for Initiation of Therapy

CD4 Count	Viral Load	Recommendation
<200 cells/ml	Any	Treat
200-350 cells/ml	Any	Treat
>350 cells/ml	> 55,000 cpm	Treat
>350 cells/ml	<55,000 cpm	Observe

The decision to initiate therapy should be based on a patients CD4 cell count and the HIV-1 plasma RNA. In the past, therapy was recommended if the CD4 count is below 500 cells/mm3 or the HIV-1 plasma RNA is greater than 20,000 copies/ml. These recommendations were based in part on the assumption that it was better suppress viral replication before there was a significant loss of immune function. However with recognition of long-term adherence difficulties and the high rate of treatment failures to anti-retroviral therapy, a consensus has grown that therapy should be delayed until the CD4 count reaches lower levels. This consensus is based on several observations: Patients rarely have symptomatic disease before CD4 count falls below 350 cells/mm3: CD4 count is actually a better predictor than viral load of opportunistic infections and progression of disease in patients with lower CD4 counts; CD4 count response to anti-retroviral therapy is poorer in patients with CD4 counts under 50 cells/mm3 compared to those with CD4 counts over 200 cells/mm3 while those with CD4 counts greater than 200 cells typically have a robust response.

The current recommendations (as of February 5, 2001) suggest initiation of therapy in patients with CD4 counts below 350 cells/mm3 regardless of baseline HIV-1 viral load. Any patient with a high VL should be treated regardless of CD4 count. The most conservative recommendation calls for initiation of therapy at a VL ≥55,000 copies/ml by RNA PCR. There is no consensus for the initiation of therapy for patients with CD4 count ≥350cells/μL and lower VL (i.e. ≤ 55,000 copies/ml) Some experts would initiate therapy for lower viral loads, while others would withhold therapy until the CD4 count falls by 30% or the viral load is becomes elevated.

Recommended Initial Therapy

Nucleoside Combinations	Protease Inhibitors
zidovudine/didanosine	nelfinavir
zidovudine/lamivudine	indinavir
didanosine/stavudine	saquinavir/ritonavir
stavudine/lamivudine	indinavir/ritonavir
	lopinavir/ritonavir
	efavirenz

Once a decision to initiate therapy has been made, it is recommended that all patients be treated with two nucleoside agents and one other highly potent agent. Potential combinations are summarized in the table below. The backbone or foundation of therapy is the nucleoside analogues. Almost all of the other highly potent agents are protease inhibitors or combinations of protease inhibitors. The one exception to this is efavirenz, which has been shown to be superior to the protease inhibitor indinavir in a large clinical trial.

After initiation of therapy, patients should be monitored for changes in HIV-1 plasma RNA. In general, there should be at least a 1.0 log reduction in VL within 4 weeks. Most patients will have an undetectable viral load within 6 weeks. There are some patients in whom the HIV-1 plasma RNA does not become undetectable for almost 24 weeks. Monitoring of viral RNA should take place on a regular basis and therapy should be changed when a patient fails anti-retroviral therapy. Currently, there is no good definition of failure of anti-retroviral therapy but most clinicians would consider changing therapy when there are 2 or more consecutive detectable viral loads that are increasing in value. At this point, resistance testing should be performed to identify the failing components of the regimen and to substitute them with agents to which the virus is sensitive.

Effect of HAART on Viral LOAD AND OUTCOMES

There is ample data that the use anti-retroviral agents have a positive effect on patients with HIV infection. Numerous clinical trials have demonstrated that anti-retroviral therapy with two nucleosides and another agent can reduce viral replication to undetectable levels in 60-70% of all subjects[34-37, 39, 47, 48]. Accompanying this reduction in viral load is a marked increase in CD4 cell count that can be large as several hundred cells/mm3 in some patients. These changes are associated with decreased rates of opportunistic infections, HIV related neoplasms and death. Other improvements have also been noted: subjects treated anti-retroviral therapy have weight gain, better performance on functional and neuro-psychological testing and report better quality of life[49]. The suppression of HIV RNA can be prolonged. In some studies subjects have viral loads below the level of detection for up to 3 years. The relationship between suppression of HIV-1 viral load and clinical outcomes is so strong that the Food and Drug Administration now accepts changes reductions in plasma HIV-1 RNA levels as primary evidence of a agents efficacy in treating HIV-1 infection. Thus it is not surprising that all of the combinations of anti-retroviral agents currently available can reduce plasma RNA below the level of detection in a majority of subjects.

There is a dramatic increase in CD4 cells that occurs with 12 weeks after initiation of anti-retroviral therapy.[11, 50, 51] After this increase, there is a slow, but steady rise over the next two years. The initial increase in CD4 counts is due to clonal expansion of already existing CD4 cells. These cells are necessarily memory cell and are directed against antigens that have already been presented to the immune system. The second, slow phase of CD4 increase is due to increased thymic output of naïve CD4 cells.[52] These naïve cells can then respond to new antigens and thus increase the repertory of the immune system.

Effects of Combination Anti-retroviral Therapy
Decreased HIV-1 plasma RNA
Increased CD4 Cells Counts
Improvement in CD4 Cell Function
Decreased Opportunistic Infections
Decreased AIDS Related Neoplasms
Decreased Deaths
Decreased Hospitalizations

Large observational trials have demonstrated the efficacy of anti-retroviral therapy outside the realm of clinical trials[45, 53-55]. The Centers for Disease Control and Prevention monitors rates of progression to AIDS and death among HIV- infected individuals in the United States. From the beginning of the epidemic through 1995, there was a steady increase in the number of AIDS cases and deaths due to AIDS. In 1996, however (the year that triple anti-retroviral therapy became widely available) there was a 40% decrease in AIDS deaths. This trend continued until 1998, when the number of AIDS death leveled off to a new low rate. Other studies have demonstrated a relationship between the decrease in the number of AIDS deaths and the proportion of patients treated with 3 anti-retroviral agents.

Changing patterns of health care utilization also confirm the efficacy of anti-retroviral therapy [56-58]. Rates of hospitalization, numbers of hospital days, and numbers of emergency room visits have decreased concurrently with the use of protease containing regimens. There have been increases in outpatient visits to HIV clinics and other specialty clinics. Costs of caring for HIV infected patients have shifted from the in-patient services and emergency room to the out patient clinics and to the pharmacy. Currently, pharmacy costs represent the single expenditure in providing care for HIV infected patients. Overall costs, when adjusted for inflation, have remained constant while there has been an improvement in the quality of life of HIV patients.

Real World use of ART

Unfortunately, the performance of anti-retroviral therapy in clinical trials has not been duplicated in clinical practice[3, 43, 44, 59-64]. Numerous studies in large HIV clinics have demonstrated higher failure rates that those seen in clinical trials. Forty to sixty percent of individuals whose viral load became undetectable have a persistent rebound in HIV-1 plasma RNA after 6 months of therapy. After one year of therapy, the proportion of patients who have an undetectable viral load is as low as 20% in one study. Predictors of failure are a low CD4 count at baseline, high HIV-1 plasma RNA at baseline and poor adherence.

The baseline parameters of a high viral load as a predictor of failure are readily understandable given the current paradigm of HIV pathogenesis. Patients with high baseline viral loads may have continuing replication of virus that is below the limits of detection of the HIV RNA PCR test because the therapy is insufficient to halt all viral reproduction. Several studies using ultra-sensitive HIV-1 RNA PCR assays that have a limit of detection of 50 copies/ml have demonstrated this. Subjects who have a viral load below 50 copies/ml are less likely to fail than those who have a viral load between 50-400 copies/ ml. In addition, there are other reservoirs of HIV-1 where anti-retroviral drugs poorly penetrate, thus allowing on-going viral replication, despite good activity of these drugs in the plasma.

The major cause of treatment failure, however, is poor adherence[60, 65-67]. [17] Studies have

demonstrated that subjects must take 90-95% of their medication in order to maintain long-term viral suppression. Anti-retroviral regimens are complex, requiring patients to take as many as 20 pills per day. The size and number of the pills make ingestion of these medications unpalatable. Some of these drugs have differing food requirements, forcing the patients to take different drugs at different times. The high incidence of side effects contributes to patients being unwilling or unable to comply with the long-term requirements of anti-retroviral therapy.

There are major sociologic components to non-adherence to HIV medication[68-70]. Most individuals with HIV-1 in the United States are on the margins of society. Illicit drugs use and mental illness are common co-morbid conditions. Poverty is also common with almost 70% of HIV infected individuals nationwide qualifying for some form of government assistance. Access to care may be limited and individuals are being asked to pay for an ever-increasing proportion of their health care. Despite these barriers, the largest barrier to adherence is human nature. In a study of HIV patients who admitted poor compliance, the most common reason for not taking medicines was "I forgot".

Sub-optimal adherence inevitably leads to the emergence of resistance[65, 71]. HIV-1 reverse transcriptase is highly error prone and has no proof reading ability. Thus there is the potential for many transcription errors to occur each day. Sub-therapeutic levels of drugs allow for viral replication and provide selective pressure for mutations that confer resistance to these anti-retroviral drugs Once low level resistance occurs, addition mutations increase the ability of the virus to over come the drugs and cause failure of the medication. There is significant cross-resistance between the agents in each of the classes of anti-retroviral drugs. Thus treatment with a secondary regimen tends to be less successful as the initial regimen.

Side Effects of Anti-retroviral Medications

Drug Toxicity

Drug toxicities are common with all of the medications used to treat HIV infection. Often there is overlapping toxicity that limits the use of these agents in combination. Gastrointestinal side effects are, by far, most common and is seen across all classes of drugs. Other toxicity, such as neuropathy, may be seen only in a specific class of drugs.

Nucleoside Analogues

Nucleoside Analogue Toxicities

Nausea, GI distress	All
Hepatitis	All
Pancreatitis	ddI, d4T
Peripheral Neuropathy	ddI, d4T,ddC
Cytopenia's	ZDV,ABC
Hypersensitivity	ABC

Nucleoside analogues typically cause nausea and vomiting. Increases in transaminases are also common though a frank hepatitis is rare. Zidovudine can cause pancytopenia, with anemia being the most common manifestation of this side effect. It is usually dose related and is more severe in patients with advanced HIV disease. The neutropenia and anemia associated with zidovudine is readily reversible with discontinuation of drug, however some patients require on going support with G-CSF or erythropoietin in order to maintain therapy with this agent. Didanosine, stavudine and zalcitabine all cause peripheral neuropathy. This typically begins as painful paresthesias in the feet that progress up the legs in a stocking distribution. Discontinuation of the drug usually results in resolution of symptoms. Continuation of therapy once neuropathy has developed can result in permanent neurological damage and disability. Didanosine, and to a lesser extent stavudine, are associated with pancreatitis. This usually occurs in patients who are at risk for pancreatitis because of alcohol abuse or other metabolic abnormality. Pancreatitis associated with nucleosides can be severe and there have been deaths. Abacavir is associated with an idiopathic hypersensitivity reaction in 5% of patients taking this drug. Abacavir HSR occurs with 60 days of initiating therapy, often within the first

several days. The symptom complex of fevers, myalgias and rash defines abacavir hypersensitivity and can also include nausea, vomiting and diarrhea. Prompt discontinuation of the drug leads to resolution of symptoms while continued therapy results in worsening symptoms and a sepsis – like syndrome. Rechallenge in patients with hypersensitivity can result in death.

Non-Nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside Reverse Transcriptase Toxicities

Rash	All
CNS toxicities	Efavirenz
Severe Hepatitis	Nevirapine
Stevens-Johnson Syndrome	Nevirapine

Each of the non-nucleoside reverse transcriptase inhibitors is associated with a rash. This is typically maculo-papular in nature and occurs within several weeks of initiating therapy. In mild cases the rash will resolve without discontinuation of the drug. Efavirenz also is associated with central nervous system side effects such as dizziness, a feeling of drunkenness, lethargy and vivid dreams. It some times has been associated with worsening of depression and with suicide attempts in patients who had underlying psychiatric disease. These side effects usually resolve within a month though there are some patients who cannot successfully be established on this drug. In addition to rash, nevirapine is associated with a Stevens-Johnson syndrome. Nevirapine can cause hepatitis that can be more severe in women and in individuals with underlying liver disease. There have been reports of fatal hepatic necrosis and death with initiation of nevirapine prompting the recommendation that liver function tests be monitored every two weeks in patients starting this drug.

Protease Inhibitors

Protease Inhibitor Toxicities

Nausea	All
Diarrhea	All
Paresthesia's	RTV,IDV,LPR
Nephrolithiasis	IDV
Hyperbilirubinemia	IDV
Hypertriglyceridemia	RTV
Hypercholesterolemia	All*

**may be less common with AMP*

All of the protease inhibitors are associated with nausea, vomiting and diarrhea. High serum aminotransferase concentrations have been reported in association with these drugs, but symptomatic hepatitis is rare. Increased risk of bleeding has been reported in patients with hemophilia taking protease inhibitors, but mechanism of action of these drugs on bleeding diathesis is uncertain. Ritonavir and indinavir cause circumoral parestheisias and paresthesias of the hands and feet. Use of indinavir is associated with nephrolithiasis (3-15%) and with elevations in creatinine. Patients taking IDV should drink at least 48 oz of fluid daily in addition to their normal fluid intake. Reversible unconjugated hyperbilirubinemia is frequent in patients taking indinavir but is not usually associated with high serum aminotransferase concentrations or overt liver disease. Ritonavir causes hypertriglyceridemia in up to 50% of patients; serum triglyceride concentrations can exceed 1000mg/dL. This side effect has not been accompanied by complications such as pancreatitis.

Protease inhibitors also have significant interactions with other drugs that complicate their usage. These drugs are metabolized by ctyochrome p450 enzyme. Ritonavir binds most avidly to the ctyochrome P450 enzyme and thus has the most interactions. Protease inhibitors can increase the levels any drug that is also metabolized by the P450 system including benzodiazopines and statins. They also act as inducers of hepatic enzymes and can lower levels of certain estrogens used in oral contraceptives. Protease inhibitors can also inhibit the metabolism of other protease inhibitors. This has lead to the practice of combining these agents in order to achieve

more favorable pharmacokinetics for these drugs.

Long Term Metabolic Toxicities of Anti-retroviral Therapy.

Long Term Anti-retroviral Toxicities
Diabetes
Hypercholesterolemia
Lipodystrophy
Lipoatrophy
Lactic Acidosis

There has been a growing recognition of a cluster of metabolic toxicities of anti-retroviral therapy. Initially it was assumed that these were secondary to protease inhibitor usage, but it has now become evident that there are multiple, complex factors in the development of these toxicities including potential effects of HIV itself. Many of these abnormalites were observed prior to the development of triple anti-retroviral therapy but it is clear the incidence and prevalence of these toxicities have dramatically increased in the past 5 years. This leaves open the possibility that these toxicities were due to HIV itself but were observed less commonly because patients did not survive long enough to manifest them. There are also other co-morbid conditions that may contribute to these illnesses in patients with HIV-1 infection.

Glucose Intolerance

Up to 70% of patients initiated on protease have impaired glucose tolerance with initiation of protease inhibitors[6, 72, 73]. A much smaller proportion develops diabetes. High fasting glucoses can usually be controlled with oral hypoglycemic. Addition of insulin is occasionally required. Drugs such a metformin can be effective but are also associated with lactic acidosis, another side effect of anti-retroviral therapy. Secondary diabetes has not been associated with non-nucleoside reverse transcriptase inhibitors or with nucleoside analogues. Co-infection with hepatitis C has recently been implicated as a cause of diabetes but the association with HIV-1 related diabetes is unclear. It is also unclear how family history and obesity interplay in the decreased glucose tolerance observed with protease inhibitor usage.

Hyperlipidemia

Protease inhibitor therapy has been associated with increases in the total cholesterol, decreased high-density lipoprotein and increased triglycerides. The mechanism of action of increased cholesterol is unknown. There have also been case reports of accelerated athrogenesis and myocardial infarction in patients receiving protease inhibitors. Epidemiologic studies have shown an increase rate of coronary artery disease in HIV-1 infected patients treated with anti-retroviral medications when compared to aged matched, non –HIV infected controls. All protease inhibitors have been implicated with hyperlipidemia but not to the same degree. For example, ritonavir causes the largest increase in triglycerides, while amprenavir has the least effect on cholesterol. Discontinuation of protease inhibitors is associated with decreases in total cholesterol. Switching from a protease-based regimen to a triple nucleoside regimen has also been associated with approximately a 30-mg/dl decrease in cholesterol without a rebound in HIV-1 viral replication. It is difficult to solely implicate protease as the cause of this abnormality; the non-nucleoside reverse transcriptase inhibitor efavirenz is also associated with increases in cholesterol and switching from a protease-containing regimen to efavirenz does not result in a reduction of cholesterol. Anecdotal reports suggest that therapy with statins can reduce cholesterol levels but clinical trials demonstrating this have yet to be completed.

Lipodystrophy

Protease inhibitor therapy is associated with severe changes in body habitus[6, 74-78]. There is a loss of subcutaneous fat in face, arms and legs. There is accumulation of fat in the abdomen and the development of a buffalo hump. Women have increases in breast size. Patients with this syndrome do not have high cortisol levels. This maldistrabution of fat becomes evident after 6 months of therapy and

is most commonly seen in patients who have been treated with over 18 months of therapy. The true incidence of this syndrome is unknown but has been reported to occur in 10-80% of individuals. Historical databases have documented lipodystrophy prior to the use protease inhibitors but the number of affected patients was clearly much lower. Discontinuation of protease inhibitor therapy has not been associated with a reversal of symptoms. There are anecdotal reports of improvement of body habitus with anabolic steroids and with growth hormone.

Lipoatrophy

Lipoatrophy is distinct from lipodystrophy, in that patients have wasting in the face arms and legs[5, 78-80]. This complication has been associated with nucleoside usage, particularly stavudine, and not with protease inhibitors. Patients with lipoatrophy do not have fat accumulations in the abdomen nor do they develop a buffalo hump. The loss of fat is thought to be due to mitochondria toxicity caused by interruption of mitochondrial DNA synthesis by the nucleoside analogues. As with lipodystrophy, discontinuation of nucleoside therapy has not been associated with a reversal of symptoms and there are anecdotal reports of improvement of body habitus with anabolic steroids and with growth hormone.

Lactic Acidosis

Lactic acidosis is a clinical syndrome characterized by fatigue, nausea, vomiting, diarrhea and high lactate levels in individuals who have no other obvious cause of lactemia[81-86]. Chronic lactic acidosis has been associated with all available nucleosides though stavudine has been most widely implicated. As with lipoatrophy, chronic lactic acidosis is thought to be caused by mitochondrial toxicity due to interruption of mitochondiral DNA synthesis by the nucleoside analogues. This causes increase in anaerobic glycolysis and the development of lactemia. The true incidence of chronic lactic acidosis is unknown but may be as low as one percent of patients. Substitution of stavudine by another nucleoside was associated with reversal of symptoms and normalization of lactate levels in a small cohort of patients.

Strategies to Improve Outcomes in Patients Treated with Anti-retroviral Therapy

Different strategies of treatment have been proposed to prolong the effectiveness of anti-retroviral therapy, preserve treatment options and reduce long-term complications of anti-retroviral medications. The concept of sequencing therapy is a based on the observation that regimens will eventually fail and thus the patient will have virus that is resistant to the failing combination of drugs. The initial regimen, then, should be one that has the least potential for cross-resistance with other agents. Subsequent regimens could be chosen to be fully active against the strain of HIV infecting the individual. This would result in a longer time to treatment failure and thus to an overall longer time of viral suppression.

Two main strategies have developed; a protease sparing regimen where individuals would be treated with a non-protease containing regimen initially and then receive a protease-containing regimen once the initial regimen fails. The protease first option would use a protease inhibitor as part of the initial regimen. Subjects who fail this regimen would then be treated with a second protease or combinations of protease inhibitor with a favorable resistance pattern. Nucleoside analogues or non-nucleoside reverse transcriptase inhibitors would be used in the second regimen to augment the potency of the other drugs. Currently, there is data to support both approaches though clinical trials designed to demonstrate the superiority of either approach have yet to be completed.

Protease Sparing Regimens

The triple nucleoside analogue containing regimen of zidovudine, lamivudine and abacavir is a commonly used protease-sparing regimen. This regimen has been shown to be comparable to a protease-containing regimen (zidovudine, lamivudine and abacavir) in

suppressing viral replication below 400 copies/ml in two large clinical trials[32]. This regimen has the benefit of simplicity. A combination tablet of zidovudine, lamivudine and abacavir is available, allowing for patients to take one pill, twice daily. In addition, this regimen does not increase serum lipid levels or fasting glucose. None of the long-term complications of anti-retroviral therapy have been described with this regimen. There are several drawbacks to this regimen. In one clinical trial, subjects treated with this triple nucleoside regimen were less likely to obtain a viral load below 50 copies/ml, suggesting that it may not be as potent as a protease containing regimen. In addition, subjects who fail this regimen may have significant cross-resistance to other nucleoside regimens. Thus the benefit of preserving protease and non-nucleoside sensitivity may be lost due to increase resistance to nucleoside analogues. Currently, there is little clinical data on the effectiveness of subsequent regimens in patients who have failed triple nucleoside therapy.

A regimen of two nucleoside analogues and the non-nucleoside reverse transcriptase inhibitor efavirenz can also be used instead of a protease-containing regimen.[34] The combination of zidovudine, lamivudine and efavirenz was superior in suppressing viral load below either 400 copies/ml or 50 copies/ml than a regimen containing indinavir in a large clinical trial. The combination of didanosine, stavudine and efavirenz had similar rates of patients with undetectable viral loads in a non-controlled trial. Pill counts are decreased in this regimen as with triple nucleoside regimens. Efavirenz is dosed once daily at bedtime because of the central nervous system side effects. When combined with newer formulations of didanosine and with lamivudine, there is potential for a once daily regimen. Efavirenz has been associated with increases in cholesterol but not with diabetes. In addition, body habitus changes have not been described with efavirenz containing regimens. Sensitivity to protease inhibitors in preserved and there is less potential for nucleoside resistance because fewer of these agents are used in the initial regimen. As in the case of triple nucleoside therapy, there is little data on the efficacy of regimens once an individual has failed an efavirenz-containing regimen.

Protease Containing Regimens as Initial Therapy

Despite the potential problems of protease inhibitors, there is still good rationale to use this drugs as part of the initial regimen. Protease inhibitors have proven potency and have the longest track record for efficacy. There is data that patients who fail protease inhibitors can be successfully treated with other protease inhibitors[87, 88]. Several studies have shown that approximately 60 percent of subjects who failed nelfinavir containing regimens have undetectable viral loads for up to a year when treated with regimens containing the combination of ritonavir and saquinavir. In trial of multiple protease inhibitor failures, the combination of lopinavir/ritonavir suppressed viral load to below 400 copies in 82% of patients. Numerous studies have demonstrated the use of a non-nucleoside reverse transcriptase inhibitor plus a protease inhibitor is associated with better virologic outcomes than regimens that do not contain non-nucleoside regimens in patients who failed prior protease inhibitor regimens. Thus the optimal regimen for protease inhibitor failures seems to be combinations of protease inhibitors plus a non-nucleoside reverse transcriptase inhibitor. This strategy requires that use non-nucleoside reverse transcriptase inhibitors be deferred until a patient has failed because resistance to these agents is broad and confers resistance across the class drugs.

Although there is good rationale for pursing either a protease first strategy or a protease sparing strategy, there is little clinical data demonstrating the superiority of either tactic. A major problem with either is the level of cross-resistance among nucleoside analogues. All anti-retroviral regimens are based upon a foundation of nucleosides. Every regimen

change because of virologic failure usually entails changing the nucleosides as well as the other agents. Although there are currently 6 of these agents available, cross-resistance and over lapping toxicities limit the number of usable nucleosides. This reduces the number of potential regimens to two or three per patient regardless of the initial choice of protease sparing or protease containing strategy.

Resistance Testing

Resistance testing has become a major element in the management of failures of anti-retroviral therapy. Currently there are two types of resistance tests available; genotypic testing and phenotypic testing. A third method call virtual phenotype is a hybrid of the two technologies. Use of resistance testing can identify the specific agents in a failing regimen to which the virus is resistant[41, 89]. It can also allow a clinician to new agents that are fully active against the isolated from a specific patient.

Genotypic resistance testing is a technique where the viral genome from individual patients is amplified through PCR and sequenced. The genome is then analyzed for the presence of specific mutation that has been associated for specific mutations to specific agents. For example, the T215F/Y substitution in the HIV-1 reverse transcriptase genome has been associated with resistance to zidovudine. Mutations in the protease genome can confer resistance to a particular agents; the D30N substitution is only associated with nelfinavir resistance and I50V is only associated with amprenavir resistance. Accumulation of numbers of mutations seems to be associated with increased rates of resistance. The primary mutation for stavudine resistance is rarely found, but accumulations of multiple mutations for zidovudine have been associated with resistance to stavudine. Similarly, there is no single mutation associated with resistance to lopinavir/ritonavir but accumulations of 6 or more mutations associated with resistance to other protease inhibitors leads to decreased sensitivity to this agent. Some mutations confer resistance across the class of agents. The Q151M complex and the S69SS insertion mutations confer resistance to all nucleosides. Similarly the K103N mutation in the same genome confers resistance to all non-nucleoside reverse transcriptase inhibitors. Protease inhibitors have mutations that confer cross-resistance to many of the drugs in the class. For example the L90M is associated with resistance to saquinavir, ritonavir, indinavir and nelfinavir. There is, however, no single mutation that confers resistance across the protease inhibitor class of drugs. Genotyping is easy to perform and readily available. Its major disadvantage is that is it an indirect measure of resistance and that interpretation of the results are complex.

Phenotypic testing is a technique where the HIV-1 from a particular patient is grown in the presence of increasing of an anti-retroviral drug. Recent advances in molecular biology and robotics have allowed for the introduction of this method into clinical practice. In this particular method of phenotypic, the RT and PI portions of the HIV-1 genome are cloned an amplified by PCR. It is then integrated into standardized reporter virus that is grown in the presence increasing amounts of anti-retroviral drugs. An IC_{50} is calculated based on the concentration of drug required to inhibit viral replication by 50%. The $IC_{50\ is}$ compared to a similar result for a standardized, wild type virus and the results are reported out fold increase in IC_{50} over the wild type virus. Cut-offs based on the variability of the test are also provided. (e.g. >2.5 fold increase for the Phenosense assay).Values above the cut-off are considered resistant. As more clinical data is accumulated, phenotype cut-offs are being adjusted for each agent. For example, the cut-off for didanosine and stavudine is > 1.7 fold increase; the cut off for abacavir > 4.0 fold increase, and the cut-off for lopinavir/ritonavir is > 10 fold increase in IC_{50}. Phenotyping has an advantage over genotyping in that it is a direct measure of susceptibility and the results are easily interpretable.

Numerous studies have shown that resistance testing can improve clinical out-comes in patients who have failed anti-retroviral medications[90]. Typically, these are strategy trials, where resistance testing is provided to the primary physicians of one group of patients. The out come of this group of patients is compared to a control group in whom results of resistance testing was not provided. Subjects in whom resistance testing is provided had lower viral loads, longer time to treatment failure and require fewer medications. These results have been observed in studies that use either genotype and phenotype assays. Despite these results the optimal time to obtain a resistance test has not been delineated. While resistance to has been found in acute seroconverters, the prevalence of resistance has been too low to warrant routine testing of these individuals. Similarly, there prevalence of resistance in untreated patients with long standing HIV infection is very low. Patients who have failed initial therapy with anti-retroviral medication can clearly benefit from resistance testing while those who have failed multiple regimens may not because high levels of cross resistance across all classed of drugs. Currently, resistance testing is recommended for individuals who have failed an anti-retroviral regimen.

Future Directions

A major thrust in anti-retroviral drug development has been the simplification of regimens. The concept behind this effort is that patients will be more compliant if there are fewer pills to take on a less frequent basis and that the medications have less side effects. Fixed dosage combinations of anti-retroviral drugs are already available. Zidovudine and lamivudine are available in a single pill marketed as Combivir; zidovudine, lamivudine and abacavir are available in a single pill call Trizivir. Didanosine is now available as an enteric capsule that can be given once daily and does not have the gastrointestinal side effects associated with the previous formulation. A pro-drug of amprenavir is under development that will reduce the pill count associated with this medication. New agents that can be given once daily in each of the classes of drugs are also under development. It is hoped that in several year, there will be a real possibility of providing once daily regimens that have small pill burdens to most patients.

There is also an effort to develop new drugs that are active against virus that is resistant to currently available agents. The most promising of these are the fusions inhibitors. These are drugs that interfere with the HIV-1 GP41 interaction with co-receptors, blocking fusion of HIV-1 with the CD4 lymphocyte. Two prototypes of these agents, termed T-20 and T-1249, have been tested in humans. Therapy with these agents resulted in greater than 1.0 log decrease in HIV-1 viral replication in patients who had highly resistant virus. These fusions inhibitor are peptides and must be given parenterally. In early clinical trials, subcutaneous injection was equivalent to IV administration, leaving open the possibility for wide spread out patient usage. Future trials will focus on use of these agents in combinations with other medications and in patients will little or no resistant virus.

References

1. Fischl, M.A., et al., *The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial.* N Engl J Med, 1987. **317**(4): p. 185-91.
2. Hammer, S.M., et al., *A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team.* N Engl J Med, 1997. **337**(11): p. 725-33.
3. Deeks, S.G., et al., *HIV RNA and CD4 cell count response to protease inhibitor therapy in an urban AIDS clinic: response to both initial and salvage therapy.* Aids, 1999. **13**(6): p. F35-43.
4. Siliciano, J.D. and R.F. Siliciano, *Latency and viral persistence in HIV-1 infection.* J Clin Invest, 2000. **106**(7): p. 823-5.
5. Saint-Marc, T., et al., *A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy.* Aids, 1999. **13**(13): p. 1659-67.
6. Carr, A., et al., *A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors.* Aids, 1998. **12**(7): p. F51-8.
7. Fauci, A.S., et al., *Immunopathogenic mechanisms of HIV infection.* Ann Intern Med, 1996. **124**(7): p. 654-63.
8. Niu, M.T., D.S. Stein, and S.M. Schnittman, *Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment intervention in humans and animal retrovirus infections.* J Infect Dis, 1993. **168**(6): p. 1490-501.
9. Pantaleo, G., et al., *HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease.* Nature, 1993. **362**(6418): p. 355-8.
10. Autran, B., F. Hadida, and G. Haas, *Evolution and plasticity of CTL responses against HIV.* Curr Opin Immunol, 1996. **8**(4): p. 546-53.
11. Blankson, J.N., J.E. Gallant, and R.F. Siliciano, *Proliferative Responses to Human Immunodeficiency Virus Type 1 (HIV-1) Antigens in HIV-1-Infected Patients with Immune Reconstitution.* J Infect Dis, 2001. **183**(4): p. 657-661.
12. Ho, D.D., et al., *Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection.* Nature, 1995. **373**(6510): p. 123-6.
13. Perelson, A.S., et al., *HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time.* Science, 1996. **271**(5255): p. 1582-6.
14. Finzi, D., et al., *Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy.* Nat Med, 1999. **5**(5): p. 512-7.
15. Shen, A., et al., *Establishment of latent HIV-1 infection of resting CD4(+) T lymphocytes does not require inactivation of Vpr.* Virology, 2000. **278**(1): p. 227-33.
16. Siliciano, R.F., *Reservoirs for HIV-1.* Curr Infect Dis Rep, 1999. **1**(3): p. 298-304.
17. Vergis, E.N. and J.W. Mellors, *Natural history of HIV-1 infection.* Infect Dis Clin North Am, 2000. **14**(4): p. 809-25, v-vi.
18. Perelson, A.S., P. Essunger, and D.D. Ho, *Dynamics of HIV-1 and CD4+ lymphocytes in vivo.* Aids, 1997. **11**(Suppl A): p. S17-24.
19. McCune, J.M., et al., *Factors influencing T-cell turnover in HIV-1-seropositive patients.* J Clin Invest, 2000. **105**(5): p. R1-8.

20. Mellors, J.W., et al., *Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion.* Ann Intern Med, 1995. **122**(8): p. 573-9.

21. Mellors, J.W., et al., *Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.* Science, 1996. **272**(5265): p. 1167-70.

22. Mellors, J.W., et al., *Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection.* Ann Intern Med, 1997. **126**(12): p. 946-54.

23. O'Brien, W.A., et al., *Changes in plasma HIV RNA levels and CD4+ lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure. VA Cooperative Study Group on AIDS.* Ann Intern Med, 1997. **126**(12): p. 939-45.

24. O'Brien, W.A., et al., *Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS.* N Engl J Med, 1996. **334**(7): p. 426-31.

25. Katzenstein, D.A., et al., *The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. AIDS Clinical Trials Group Study 175 Virology Study Team.* N Engl J Med, 1996. **335**(15): p. 1091-8.

26. Boyle, M.J., et al., *HIV seroconversion illness.* Med J Aust, 1993. **158**(1): p. 42-4.

27. Schacker, T., et al., *Clinical and epidemiologic features of primary HIV infection.* Ann Intern Med, 1996. **125**(4): p. 257-64.

28. Veugelers, P.J., et al., *Determinants of HIV disease progression among homosexual men registered in the Tricontinental Seroconverter Study.* Am J Epidemiol, 1994. **140**(8): p. 747-58.

29. Rodman, J.H., et al., *A systemic and cellular model for zidovudine plasma concentrations and intracellular phosphorylation in patients.* J Infect Dis, 1996. **174**(3): p. 490-9.

30. Spruance, S.L., et al., *Clinical efficacy of monotherapy with stavudine compared with zidovudine in HIV-infected, zidovudine-experienced patients. A randomized, double-blind, controlled trial. Bristol-Myers Squibb Stavudine/019 Study Group.* Ann Intern Med, 1997. **126**(5): p. 355-63.

31. Gulick, R.M., et al., *Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy.* N Engl J Med, 1997. **337**(11): p. 734-9.

32. Staszewski, S., et al., *A dose-ranging study to evaluate the safety and efficacy of abacavir alone or in combination with zidovudine and lamivudine in antiretroviral treatment-naive subjects.* Aids, 1998. **12**(16): p. F197-202.

33. Montaner, J.S., et al., *A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study.* Jama, 1998. **279**(12): p. 930-7.

34. Staszewski, S., et al., *Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team.* N Engl J Med, 1999. **341**(25): p. 1865-73.

35. Markowitz, M., et al., *A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection.* N Engl J Med, 1995. **333**(23): p. 1534-9.

36. Cameron, D.W., et al., *Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease. The Advanced*

HIV Disease Ritonavir Study Group. Lancet, 1998. **351**(9102): p. 543-9.

37. Cameron, D.W., et al., *Ritonavir and saquinavir combination therapy for the treatment of HIV infection.* Aids, 1999. **13**(2): p. 213-24.
38. Moore, R.D. and R.E. Chaisson, *Natural history of HIV infection in the era of combination antiretroviral therapy.* Aids, 1999. **13**(14): p. 1933-42.
39. Roca, B., C.J. Gomez, and A. Arnedo, *Adherence, side effects and efficacy of stavudine plus lamivudine plus nelfinavir in treatment-experienced HIV-infected patients.* J Infect, 2000. **41**(1): p. 50-4.
40. Durant, J., et al., *Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study.* Aids, 2000. **14**(10): p. 1333-9.
41. Cozzi Lepri, A., et al., *Resistance profiles in patients with viral rebound on potent antiretroviral therapy.* J Infect Dis, 2000. **181**(3): p. 1143-7.
42. Hertogs, K., et al., *Phenotypic and genotypic analysis of clinical HIV-1 isolates reveals extensive protease inhibitor cross-resistance: a survey of over 6000 samples.* Aids, 2000. **14**(9): p. 1203-10.
43. Mocroft, A., et al., *The use of and response to second-line protease inhibitor regimens: results from the EuroSIDA study.* Aids, 2001. **15**(2): p. 201-9.
44. Lucas, G.M., R.E. Chaisson, and R.D. Moore, *Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions.* Ann Intern Med, 1999. **131**(2): p. 81-7.
45. Ledergerber, B., et al., *Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study.* Lancet, 1999. **353**(9156): p. 863-8.
46. Carpenter, C.C., et al., *Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel.* Jama, 2000. **283**(3): p. 381-90.
47. Gulick, R.M., et al., *3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine.* Ann Intern Med, 2000. **133**(1): p. 35-9.
48. Eron, J.J., et al., *Efficacy, safety, and adherence with a twice-daily combination lamivudine/zidovudine tablet formulation, plus a protease inhibitor, in HIV infection.* Aids, 2000. **14**(6): p. 671-81.
49. Scevola, D., et al., *Reversal of cachexia in patients treated with potent antiretroviral therapy.* AIDS Read, 2000. **10**(6): p. 365-9, 371-5.
50. Rizzardi, G.P., et al., *Virological and immunological responses to HAART in asymptomatic therapy-naive HIV-1-infected subjects according to CD4 cell count.* Aids, 2000. **14**(15): p. 2257-63.
51. Autran, B., et al., *Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease.* Science, 1997. **277**(5322): p. 112-6.
52. Douek, D.C., et al., *Changes in thymic function with age and during the treatment of HIV infection.* Nature, 1998. **396**(6712): p. 690-5.
53. Palella, F.J., et al., *Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.* N Engl J Med, 1998. **338**(13): p. 853-60.
54. Mocroft, A., et al., *Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study*

Group. Lancet, 1998. **352**(9142): p. 1725-30.

55. Weverling, G.J., et al., *Discontinuation of Pneumocystis carinii pneumonia prophylaxis after start of highly active antiretroviral therapy in HIV-1 infection. EuroSIDA Study Group.* Lancet, 1999. **353**(9161): p. 1293-8.

56. Moore, R.D. and R.E. Chaisson, *Costs to Medicaid of advancing immunosuppression in an urban HIV-infected patient population in Maryland.* J Acquir Immune Defic Syndr Hum Retrovirol, 1997. **14**(3): p. 223-31.

57. Keiser, P., et al., *Protease inhibitor-based therapy is associated with decreased HIV- related health care costs in men treated at a Veterans Administration hospital.* J Acquir Immune Defic Syndr Hum Retrovirol, 1999. **20**(1): p. 28-33.

58. Gebo, K.A., et al., *Costs of HIV medical care in the era of highly active antiretroviral therapy.* Aids, 1999. **13**(8): p. 963-9.

59. Deeks, S.G., *Failure of HIV-1 protease inhibitors to fully suppress viral replication. Implications for salvage therapy.* Adv Exp Med Biol, 1999. **458**: p. 175-82.

60. Deeks, S.G., *Determinants of virological response to antiretroviral therapy: implications for long-term strategies.* Clin Infect Dis, 2000. **30 Suppl 2**: p. S177-84.

61. Miller, V., et al., *The impact of protease inhibitor-containing highly active antiretroviral therapy on progression of HIV disease and its relationship to CD4 and viral load.* Aids, 2000. **14**(14): p. 2129-36.

62. Mocroft, A., et al., *Virological failure among patients on HAART from across Europe: results from the EuroSIDA study.* Antivir Ther, 2000. **5**(2): p. 107-12.

63. Staszewski, S., et al., *Rebound of HIV-1 viral load after suppression to very low levels.* Aids, 1998. **12**(17): p. 2360.

64. Staszewski, S., et al., *Virological response to protease inhibitor therapy in an HIV clinic cohort.* Aids, 1999. **13**(3): p. 367-73.

65. Bangsberg, D.R., et al., *Adherence to protease inhibitors, HIV-1 viral load, and development of drug resistance in an indigent population.* Aids, 2000. **14**(4): p. 357-66.

66. Chesney, M.A., et al., *Adherence: a necessity for successful HIV combination therapy.* Aids, 1999. **13**(Suppl A): p. S271-8.

67. Giron-Gonzalez, J.A., et al., *Effect of patient adherence to antiretroviral therapy on CD4+ cell count, HIV-1 RNA, and serum concentrations of tumor necrosis factor and its soluble receptors.* Eur J Clin Microbiol Infect Dis, 2000. **19**(11): p. 852-8.

68. Cheever, L.W. and A.W. Wu, *Medication Adherence Among HIV-Infected Patients: Understanding the Complex Behavior of Patients Taking This Complex Therapy.* Curr Infect Dis Rep, 1999. **1**(4): p. 401-407.

69. Stevens, V.M., J.L. Neel, and D.L. Baker, *Psychosis and nonadherence in an HIV-seropositive patient.* AIDS Read, 2000. **10**(10): p. 596-601.

70. Moatti, J.P., et al., *Adherence to HAART in French HIV-infected injecting drug users: the contribution of buprenorphine drug maintenance treatment. The Manif 2000 study group.* Aids, 2000. **14**(2): p. 151-5.

71. Murri, R., et al., *Patient-reported nonadherence to HAART is related to protease inhibitor levels.* J Acquir Immune Defic Syndr, 2000. **24**(2): p. 123-8.

72. Chronister, C.L. and A.S. Gurwood, *Type 2 diabetes in association with HIV-1 protease inhibitors in HIV-*

infected patients. J Am Optom Assoc, 1998. **69**(11): p. 695-8.

73. Yarasheski, K.E., et al., *Insulin resistance in HIV protease inhibitor-associated diabetes.* J Acquir Immune Defic Syndr, 1999. **21**(3): p. 209-16.

74. Calista, D. and A. Boschini, *Cutaneous side effects induced by indinavir.* Eur J Dermatol, 2000. **10**(4): p. 292-6.

75. Carr, A., et al., *Abnormal fat distribution and use of protease inhibitors.* Lancet, 1998. **351**(9117): p. 1736.

76. Carr, A., et al., *Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor- associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study.* Lancet, 1999. **353**(9170): p. 2093-9.

77. Carr, A., *HIV protease inhibitor-related lipodystrophy syndrome.* Clin Infect Dis, 2000. **30 Suppl 2**: p. S135-42.

78. Polo, R., et al., *Lipoatrophy, fat accumulation, and mixed syndrome in protease inhibitor- naive HIV-infected patients.* J Acquir Immune Defic Syndr, 2000. **25**(3): p. 284-6.

79. Saint-Marc, T. and J.L. Touraine, *The effects of discontinuing stavudine therapy on clinical and metabolic abnormalities in patients suffering from lipodystrophy.* Aids, 1999. **13**(15): p. 2188-9.

80. Strobel, M., P. Muller, and P. Claudel, *Complete reversibility of severe nucleoside-induced lipodystrophy.* Aids, 1999. **13**(18): p. 2606-7.

81. Gerard, Y., et al., *Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy.* Aids, 2000. **14**(17): p. 2723-30.

82. Tantisiriwat, W., et al., *Elevated Lactate Levels in Hospitalized Persons with HIV Infection.* AIDS Res Hum Retroviruses, 2001. **17**(3): p. 195-201.

83. Chariot, P., et al., *Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion.* J Hepatol, 1999. **30**(1): p. 156-60.

84. Khouri, S. and H. Cushing, *Lactic acidosis secondary to nucleoside analogue antiretroviral therapy.* AIDS Read, 2000. **10**(10): p. 602-6.

85. Lenzo, N.P., B.A. Garas, and M.A. French, *Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report.* Aids, 1997. **11**(10): p. 1294-6.

86. Miller, K.D., et al., *Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases.* Ann Intern Med, 2000. **133**(3): p. 192-6.

87. Deeks, S.G., et al., *Novel four-drug salvage treatment regimens after failure of a human immunodeficiency virus type 1 protease inhibitor-containing regimen: antiviral activity and correlation of baseline phenotypic drug susceptibility with virologic outcome.* J Infect Dis, 1999. **179**(6): p. 1375-81.

88. Tebas, P., et al., *Virologic responses to a ritonavir--saquinavir-containing regimen in patients who had previously failed nelfinavir.* Aids, 1999. **13**(2): p. F23-8.

89. Havlir, D.V., et al., *Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens.* Jama, 2000. **283**(2): p. 229-34.

90. Clevenbergh, P., et al., *Persisting long-term benefit of genotype-guided treatment for HIV- infected patients failing HAART. The Viradapt Study: week 48 follow-up.* Antivir Ther, 2000. **5**(1): p. 65-70.