COMBINATION ANTIRETROVIRAL THERAPY IN PRIMARY AND POST-PRIMARY HIV INFECTION

by

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ABSTRACT

Primary HIV infection is characterized by high levels of plasma viremia and a transient decrease in CD4+ T-lymphocytes count. The initial peak of viral load would subside on its own in a few weeks due to the development of the host's specific anti-HIV immune response. A virologic setpoint would then be established reflecting the balance between the basal level of viral replication and the host's response to it. Studies have shown that the level of this setpoint is predictive of long-term HIV disease outcome. By intervening with combination antiretroviral therapy during primary HIV infection, it is our hope that the virologic setpoint would be lowered even more resulting in a further delay of HIV disease progression. In this project, it was shown that double therapy with nucleoside analogs in patients with primary HIV infection could only induce transient viral suppression and partial immune reconstitution. The average reduction of viral load in the cohort from baseline to the virologic setpoint was about 1.3 log (95% reduction) in the presence of double therapy. CD4 cell counts were generally found to decline gradually once the virologic setpoint was established. This therapy was found to lack the anti-viral potency needed to treat patients with acute HIV infection most effectively. In contrast, the study of triple drug therapy in the same setting has shown that suppression of viral replication in the plasma (to less than 20 copies/mL) could be achieved in patients who had been through double therapy or had withdrawn from treatment. This antiviral effect of triple drug therapy was sustained (~100 weeks in one patient), with an associated increase in CD4 cell count. Therefore, as in established HIV infection, triple drug therapy is highly suggestive to induce long-term maximal viral suppression and immune reconstitution in patients with early (primary or post-primary) HIV infection. This may have relevant long-term clinical benefit, slowing down disease progression and prolonging the period of latency.
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CHAPTER I: INTRODUCTION

1.1 Classification of Retroviruses and HIV

Acquired immunodeficiency syndrome (AIDS) is now known to be caused by human immunodeficiency virus (HIV) infection. HIV is a retrovirus. As such, its genome consists of RNA, rather than DNA. There are six known genera of retroviruses: spumavirus, oncovirus, lentivirus, B-type retrovirus, C-type retrovirus and D-type retrovirus. The first three genera are human retroviruses, with HIV belonging to the lentivirus family. There are two different types of human immunodeficiency viruses, HIV-1 and HIV-2. HIV-1 is the most common cause of HIV infection throughout the world. HIV-2 appears to be less virulent than HIV-1 but is also associated with a smaller portion of AIDS cases in geographically distinct areas, such as West Africa (Clavel et al. 1987).

The genetic variability of HIV is high, due to the error-prone viral reverse transcriptase (Bebenek et al. 1989). To date, there have been 9 (designated A to I) different genetic subtypes (clades) of HIV-1 identified by phylogenetic analyses of the nucleotide sequences of a large number of HIV-1 isolates. These 9 clades form the major group (Group M) of HIV-1. Clade B is more prevalent in North America, Europe, Latin America, Japan, Australia and the Caribbean. Clades A and D predominate in Central and Eastern Africa while clade C is more prevalent in India and southern Africa. Clade E was identified in Thailand, clade F was described in Brazil, clade G was found in Russia and Gabon, and clade H was identified in Zaire and Cameroon. It is important to note that these data were generated from small descriptive studies. A large scale well-designed study is needed to accurately map out the geographical distribution of HIV-1 Group M subtypes and any variation that may occur over time. A second group (Group O) of HIV-1 has been identified recently in patients from Cameroon. HIV-1 group O isolates are the most genetically distinct of the identified subtypes (Dale et al. 1996) and their prevalence outside Africa is not yet known.
1.2 Structure of HIV

As seen by electron microscopy, the HIV-1 (hereafter referred to as "HIV") virion is an enveloped icosahedral structure. It has a cone shape core capsid covered with a lipid bilayer membrane. The diameter of a HIV virion is about 100 nm. The capsid contains 2 identical copies of positive sense RNA genome. The genomic size of HIV is about 9.8 kb. p24 is the main structural protein forming the capsid. A number of viral enzymes are pre-formed and present inside this capsid. These include the HIV reverse transcriptase, protease and integrase. The lipid bilayer is produced when the virion buds off from the cellular plasma membrane and is thus host cell derived. On this membrane, the major components are the gp120 external or surface protein and the gp41 transmembrane protein. These 2 proteins are critical for viral binding, fusion and entry into the host cells (Gelderblom et al. 1989). The structure of HIV-1 is shown in Figure 1.

Figure 1. The structure of a HIV-1 virion.
1.3 HIV Life Cycle

The gp120 surface glycoprotein mediates the binding between target cells and the virus itself. Binding occurs between the gp120 env protein and the cellular CD4 molecule (Willey et al. 1986). Virtually all cells bearing CD4 molecules on their membrane are susceptible to HIV infection. Following the binding of gp120 to CD4, the gp41 transmembrane viral protein is exposed and binds with the fusion receptor (CCR5 or CXCR4) on the target cell to mediate the fusion of viral envelope and cell membrane (Gallaher 1987).

After the fusion process has occurred, HIV viral RNA is released in the cytoplasm and rapidly reverse transcribed into a linear double-stranded complementary DNA (cDNA) by the viral reverse transcriptase (encoded for by viral pol gene). The reverse transcription process generates the long term repeat (LTR) regions at both ends of the proviral DNA which are necessary for integration. The reverse transcription of HIV is the one step in its life cycle that generates the great genetic variability of the virus. The HIV reverse transcriptase is highly error-prone due to the absence of 3'-5' exonuclease activity that would be required to replace mis-incorporated bases (Ji and Loeb 1992). This allows the virus an intrinsic property of evading the immune surveillance and developing resistance to antiretroviral agents. As such, given the kinetics of viral replication in vivo, all single mutations at each nucleotide position of the genome are produced everyday.

The proviral cDNA generated from reverse transcription is then transported into the nucleus and integrated permanently into the host genome by viral integrase (encoded for by viral pol gene) (Englund et al. 1995). The integrated proviral DNA can stay inactive in this integrated form for a long time or, if the cell is activated, begin a new cycle of replication immediately.

NF-κb is an inducible cellular cytoplasmic transcription factor. It is heteromeric and consists of protein subunits of 50kDa, 65kDa and 37kDa in size. The 37kDa protein is termed Iκb (Baeuerle and Baltimore 1989). Phosphorylation of Iκb by protein kinase C acts as an activation signal which causes the dissociation of the subunits (Shirakawa and Mizel 1989). p50/p65 are translocated into the nucleus where they are capable of binding to the NF-κb motifs of many T
cell genes and HIV LTR (Fujita et al. 1992). These subunits bind to the NF-κb enhancer element and activate HIV transcription. Thus, activation of CD4+ T-lymphocytes triggers cellular proliferation and renders the cell fully permissive for active HIV replication and transcription.

The activation mentioned above induces a relatively low basal level of HIV transcription. The mRNA produced are then transported out of the nucleus into the cytoplasm for processing and translation to occur. The mRNA are spliced and only regulatory viral proteins, such as Tat and Rev, are made. Tat and Rev are transported back into the nucleus where they interact with the integrated HIV genome. Tat binds to the trans-acting response (TAR) element located at the 5' end of the HIV transcript. This regulates HIV gene expression and enhances the otherwise ineffective process of elongation of viral mRNA transcripts (Peterlin et al. 1993). Rev binds to the Rev response element (RRE). As with the Tat-TAR interaction, Rev-RRE binding can also be stabilized by cellular factors. The binding of Rev to RRE allows the accumulation of unspliced viral mRNA (Malim et al. 1989). This allows a shift from the production of regulatory proteins to structural proteins from coding regions like gag, pol and env. Many viral mRNA are multicistronic in which more than one protein can be produced from a single mRNA species, a phenomenon which is mediated by ribosomal frame shifting and initiation site scan-through.

Following viral transcription and translation, HIV proteins undergo modification and packaging through glycosylation, myristilation, phosphorylation and cleavage by the virally encoded protease. The protease cleaves the gag-pol polyprotein which is essential for the final viral assembly (Kaplan et al. 1994). The viral core is assembled from the HIV proteins, enzymes and genomic RNA at the plasma membrane of the cell. Budding of the progeny virion occurs through the host cell membrane, where the core acquires its external glycoprotein-incorporated lipid membrane envelope. The mean HIV generation time (from the virion binding to the release of progeny) is about 2.6 days (Ho et al. 1995).
Figure 2. The general overview of HIV life cycle.
Figure 3. Schematic representation of NF-κB (a), Tat (b) and Rev (c) activities in the HIV life cycle.
1.4 Epidemiology of HIV Infection

Cases of *Pneumocystis carinii* pneumonia and Kaposi's sarcoma in homosexual men were reported by physicians in the United States in 1981, leading to the formal description of the clinical syndrome now known as AIDS (Gottlieb et al. 1981). The first recognized cases of acute HIV infection were not described until 1984 (Feorino et al. 1984). Over the past decade, HIV infection has reached an epidemic level with approximately 8 million people in the world having AIDS and a further 22 million people being infected with HIV. The infection is not limited to homosexual males and intravenous drug users (IVDUs) as in the early 1980’s, but has now spread to all identified groups in society. No one has been spared. Quite sadly, the most vulnerable among us, those least able to fend for themselves are particularly prone to infection.

This is particularly true in Asia and Africa. The World Health Organization (WHO) estimates that by the year of 2000, 40 million people will be infected, worldwide (Chin 1991). This infection will have an immense impact on 21st century society.

1.5 Diagnosis of HIV Infection

The enzyme-linked immunosorbant assay (ELISA) is the most commonly used test to screen for the presence of antibodies to HIV because of its low cost, standardized procedure, and high reliability. HIV antigens are placed on beads or in wells after which the serum of a patient is added. If the patient has IgG antibodies against these HIV antigens, they would bind to the antigen-coated wells. In the presence of a substrate, a color reaction occurs, which is read as an optical density (OD). The more HIV antibodies present, the higher the OD. The sensitivity and specificity of the ELISA are both greater than 99 percent (Ranki et al. 1989). If the result is negative, no further tests are performed. If the result is positive, the test is repeated. If it is positive once again, Western blot analysis will be required to confirm the presence of anti-HIV antibodies, as this is the required confirmatory test before a final diagnosis can be made.
In this test, the major HIV antigens are separated by weight using gel electrophoresis and are then blotted onto paper. The patient's serum is added and if HIV antibodies are present, they adhere to the paper. Then enzyme-linked anti-IgG antibody is introduced which attaches to the Ab-Ag complex. When a substrate is added, a banding pattern appears that indicates the presence of antibodies to various HIV antigens (Phair and Wolinsky 1989).

Figure 4. Examples of different reactions by an HIV-1 Western Blot assay.

As seen in the Figure 4, a positive Western blot is based on reaction with specific bands representing three different gene products: p24 (gag), p31 (pol), and an env band (either gp41, gp120, or gp160). A Western blot strip is interpreted as negative only if it contains no viral bands. However, there would be specimens with band patterns that do not fit the criteria for either a positive or negative test result. These are interpreted as indeterminate. An indeterminate pattern may represent a non-specific reaction or a stage of disease prior to the development of a full antibody response (seroconversion). A second Western blot assay should be done (usually 2 weeks later) on indeterminate samples to confirm the test results, or to show evolution to a positive result in the case of seroconversion.
1.6 Overview of Primary HIV Infection

Acute/ Primary HIV infection is defined as the initial period of time in which an individual has contracted the HIV virus. The body is exposed to the virus and associated symptoms will likely be developed during this primary HIV infection period. Clinically, an individual would be classified as having an acute HIV infection if they can be categorized into the following scenarios:

1) a positive HIV test result at present time with a documented negative result in the past 6 months
2) a negative or indeterminate Western Blot confirmatory test at present time with a positive test for HIV viremia
3) a positive HIV test result at present time with no documented negative test in the past 6 months, but a definite exposure with symptoms of acute HIV infection during the past 6 months.

Most patients (50-80%) will experience mononucleosis-like symptoms while others will be asymptomatic. The most common symptoms include headache, fever, rash, pharyngitis, lymphoadenopathy, weight loss, nausea, and myalgia (Niu et al. 1993). The symptoms usually last for a few weeks before slowly subsiding on their own, without medical treatment.

Primary HIV infection is characterized by high levels of plasma viremia and a transient decrease in CD4+ T lymphocytes count in the circulation. This initial burst of viral replication following acute HIV infection would abate in a matter of weeks even without any external intervention. This is due to the development of an immune response to HIV in the host. This is associated with the development of antibodies against HIV antigens and a positive diagnostic test for HIV. Cytotoxic T-lymphocytes are also generated by the immune system to combat HIV infection. In the first six months of infection, a person may test “negative” for HIV despite an extremely high circulatory viral burden. The period of development of the immune response usually last from 1 to 6 months after the initial infection with HIV. Plasma viral load then decreases and reaches a “setpoint”. These events (and the subsequent development of symptoms, often after many years)
are shown in Figure 5. HIV disease progression depends largely on the virologic setpoint. This setpoint is a reflection of the delicate balance between active viral replication and immune response against HIV (Ho 1996). It is obvious that both virologic and immunologic factors play important roles in determining the subsequent clinical outcome of the disease.

![Generalized virologic and immunologic course of HIV disease](image)

**Figure 5.** Graphic representation of natural history of HIV disease (Sagg et al. 1996).

### 1.7 Viral Kinetics in Primary HIV Infection

HIV replication *in vivo* occurs at extremely high rates at all stages of HIV infection. Plasma viral load increases greatly and reaches a peak within the first 2 weeks of infection. Dissemination of virus to organs such as lymph nodes and the central nervous system (CNS) also occurs during this time. This leads to the establishment of viral reservoirs. By treating infected patients with appropriate antiviral drugs, Ho *et al* were able to gain information about the
kinetics of HIV replication. Administration of potent triple combination anti-HIV therapy was found to decrease patient plasma viral load exponentially. This rapid decay indicates that HIV replication is highly active (Ho et al. 1995) even during the phase of clinical latency. The estimated HIV production rate was found to be $10.3 \times 10^9$ virions per day. The life span of a free virion in plasma was estimated at about 7 hours with a half life of less than 6 hours. That is to say, half of the plasma HIV population in the circulation turns over every 6 hours (Perelson et al. 1997). With such a rapid turnover of virions, the generation of viral diversity and the increased opportunity for viral escape from therapeutic agents are inevitable. The duration of the HIV life cycle is about 2.6 days. This rapid generation of HIV accounts for more than 99% of the total body burden of HIV in infected patients (Ho et al. 1995). The remaining plasma virions are believed to be generated by a latent pool of cells such as non-activated CD4+ lymphocytes, tissue macrophages and other long-lived cell populations (Perelson et al. 1996). A general summary of viral replication and the cell populations involved is shown in Figure 6.

Figure 6. Schematic summary of the viral dynamics of HIV-1 infection in vivo, including tissue reservoirs. Shown in the centre is the free HIV virion population in the plasma (Perelson et al. 1996).
1.8 Cellular Tropism of HIV

HIV exhibits cellular tropism. HIV isolates that replicate well in monocytes and macrophages but not in transformed T cells are termed as M-tropic strains. Those that can infect transformed T cells but not monocytes or macrophages are termed T-tropic strains (Cheng-Mayer et al. 1988). There appears to be a selective transmission of a rather homogeneous and macrophage-tropic HIV population during acute HIV infection (Connor et al. 1993). The viral determinant of cellular tropism lies in the V3 loop of the gp120 subunit of the env protein (Cheng-Mayer et al. 1990), whereas the cellular determinant lies in the existence of certain co-receptors. The cellular co-receptor CXCR4 was reported to be essential for the entry of T-tropic HIV strains into target cells (Feng et al. 1996). It is a G-protein coupled receptor with 7 transmembrane helices. Its natural ligand was later found to be chemokine SDF-1. Antibodies against this receptor successfully block the entry of T-tropic strains but not M-tropic strains.

It was found that β-chemokines like RANTES, MIP-1α and MIP-1β block the entry of M-tropic strains into target cells. It was discovered that only the binding profile of the β-chemokine co-receptor CCR5 can explain the inhibitory effects of those three chemokines on M-tropic HIV strains entry. The binding of these chemokines to the CCR5 receptor prevents entry of M-tropic strains (Murphy 1996).

1.9 Role of Cytokines and Cellular Activation

HIV replication is more efficient in activated cells. The turnover is especially rapid in cells that are stimulated by exogenous factors such as an opportunistic pathogen. Exogenous stimuli lead to immune activation and the production of HIV-inducing cytokines which speed up viral replication. Examples of these HIV-inducing cytokines are TNF-α, IL-β and IL-6. Studies have found that PBMCs, plasma and lymphoid tissue isolated from HIV-infected patients have increased levels of these pro-inflammatory cytokines (Poli and Fauci 1995).
Cultures of PBMC supernatants have found that many cytokines up-regulate viral replication in HIV-infected cells. They include IL-1β, IL-2, IL-3, IL-6, IL-12, TNF-α, TNF-β, M-CSF and GM-CSF. In contrast, IFN-α and IFN-β suppress viral replication (Poli and Fauci 1995). The molecular basis for which these cytokines affect HIV replication is best understood by looking at TNF-α, which activates the cellular transcription factor NF-κB (Lenardo and Baltimore 1989), the mechanism of which we described above (Poli et al. 1990).

All of the stimulatory cytokines are present in particularly high concentration during acute HIV infection, thereby providing an additional stimulus to maintain viral replication at such a high level.

1.10 Immunological Features of Primary HIV Infection

The main target cell for HIV infection is the CD4+ T helper lymphocyte of the immune system. The destruction of these naive and memory T cells caused by initial exposure to HIV is an important factor in limiting the host response to the virus.

The down-regulation of the initial viremia is thought to be mediated by HIV-specific CD8-bearing cytotoxic T lymphocytes (CTLs) (Pantaleo et al. 1997). Specific Vβ expansions of CTL clones is the hallmark of the primary immune response to HIV (Pantaleo et al. 1994). Antigenic stimulation elicits an immune response in which CTLs of the Vβ clone that recognize the antigen would be activated. The high level of viral replication may be controlled with this massive activation of the CTL response. However, there is a rapid decay in the CTL activity believed to be caused by a phenomenon known as clonal exhaustion. Clonal exhaustion depends on the presence of high levels of viral antigens throughout the lymphoid system (Zinkernagel et al. 1996). These high levels of antigen provoke a rapid and vigorous immune response that is associated with the mobilization of all T cell clones that are able to recognize the viral epitope. Since the ultimate fate of all activated immune cells is to die, the consequence of this massive mobilization is the deletion of those clones that have received exhaustive stimulation (Cohen et al. 1992). As HIV infection is associated with overwhelming viral replication in lymphoid
tissues, it is no surprise that clonal exhaustion can take place. The HIV-specific T cell repertoire is therefore severely compromised even during primary infection (Pantaleo et al. 1994), a phenomenon that is further exacerbated by the relative absence of CD4+ T cell help. As a result, it will be more likely for the virus to evade a less effective immune system and facilitate its dissemination to various body compartments (Coffin 1995). It may thus be essential to control viral replication by other external interventions at the earliest possible time to prevent these events from occurring.

1.11 Antiretroviral Therapies

Currently, there are 3 different classes of anti-HIV agents available for use in clinical practice. They aim at interrupting different steps of the HIV viral life cycle. These classes of drugs include nucleoside analog reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Nucleoside analogs are competitive inhibitors of the HIV reverse transcriptase. Cellular phosphorylation of these analogs is required for their conversion into biologically active forms. These analogs have higher affinities for the active site of HIV reverse transcriptase than its natural substrates. The inhibition is therefore competitive. Once incorporated into the cDNA during the process of reverse transcription, they act as chain terminators (Cheng et al. 1987). Thus, viral DNA synthesis is halted. Included in this class of antiviral agents are zidovudine (ZDV), lamivudine (3TC), didanosine (ddl), zalcitabine (ddC) and stavudine (d4T).

Non-nucleoside reverse transcriptase inhibitors (NNRTI) are potent inhibitors of HIV-1 (but not HIV-2) reverse transcriptase. They target at a non-substrate binding site of the reverse transcriptase enzyme (Smerdon et al. 1994). They do not require cellular activation to be active. The binding of these inhibitors to the RT may induce changes to the active site of the enzyme, thus affecting its function (Rodgers et al. 1995). These NNRTI include nevirapine, efavirenz and delavirdine.
Protease inhibitors that target viral protein processing and virion assembly appear to be the most promising recent advance in antiretroviral therapy. By inhibiting the HIV-1 protease enzyme that cleaves the Gag-Pol fusion protein, virion assembly and budding of infectious virions would be inhibited (Katz and Skalka 1994). This radically decreases the amount of mature infectious virions being released from a target cell. Examples of protease inhibitors are saquinavir, indinavir, nelfinavir and ritonavir.

Figure 7. HIV replication cycle and potential sites for antiretroviral attack. Currently available agents inhibit reverse transcription (nucleoside analogues & non-nucleoside agents), assembly and release (protease inhibitors) (Hirsch et al. 1997).

In order to achieve the goals of reducing viral load and restoring immune function over a long period of time, combination therapy with multiple agents is required. Drug resistance is the most common cause for treatment failure. Monotherapy using any one of the antiretroviral agents mentioned above leads to the rapid development of high-grade drug resistance. Combination therapy provides two critical advantages over monotherapy. First of all, two or more drugs together may have additive or synergistic interactions with increased efficacy. Numerous clinical trials have demonstrated that combinations of 2 or 3 antiretroviral agents resulted in
greater and more sustained antiviral effects. Secondly, combination therapy may delay the emergence of drug-resistant viral strains (Watson and Wilburn 1992).

Now with our understanding of HIV viral dynamics, host immune function and antiretroviral drugs, it is reasonable to hit HIV infection early and hard with combination therapy in order to slow down immune deterioration, delay disease progression and prolong survival. As the virologic setpoint of plasma viral load after acute HIV infection is predictive of the long-term clinical outcome (Mellors et al. 1995). Combination therapy may be particularly beneficial if it is instituted before the setpoint is established. Thus, aggressive treatment at the time of acute/early HIV infection may lower the setpoint and improve the long-term clinical outcome.

In summary, it is important, therefore, to control viral replication in the early stage of the infection so as to minimize the total body viral burden. The immune response during primary HIV infection is especially crucial in determining this burden. Disease progression would be slow if the immune system is able to curtail viral replication earlier and more effectively. This relates to the fact that viral replication would be reduced more quickly and damage to the immune system induced by ongoing high level viral replication would also be minimized. With a lower viral burden, the dissemination of virus into different body compartments will be lessened, further easing the strain on the immune system. Given our ability to intervene with early, aggressive antiretroviral therapy, acute medical intervention while these events are occurring may allow viral load to decrease more rapidly to a lower setpoint. This may have relevant long-term clinical benefit, prolonging the period of latency. It has been suggested that if early antiretroviral therapy is to be considered, it should be limited to what is required to accomplish the stated goal of controlling viral replication while minimizing drug exposure early in disease. It is our objective, therefore, to evaluate the efficacy of double and triple drug therapy in patients with primary HIV infection. By monitoring their virologic and immunologic responses, it can be determined if the treatments are satisfactory in inducing prolonged viral suppression, the first step in our ultimate goal to reducing disease progression.
CHAPTER II: THE COMBINATION ANTIRETROVIRAL THERAPY STUDIES

2.1 Methods

2.1.1 Double Nucleoside Analog Therapy Study

Study Population

Patient Inclusion criteria:
1. Positive HIV test with a known negative over the past 6 months.
2. Current indeterminate HIV test result, evolving to a full positive test result in follow-up.
3. Current negative test result, with a positive test for direct viral detection (p24 or plasma viral load).
4. Positive test result with no history of a recent negative test result but a definite exposure to HIV in the past six months with a subsequent illness compatible with acute HIV infection.

Patient Exclusion criteria:
1. Contraindication for antiretroviral therapy or other conditions which, in the opinion of the physician, made therapy inappropriate.

In this observational pilot study, 12 patients presenting with acute HIV infection and documented seroconversion who fit our criteria were recruited at St. Paul’s Hospital in Vancouver, Canada. The diagnosis of HIV infection was made by standard ELISA and confirmatory Western blot assays. All participants provided written informed consent prior to the initiation of the study. Overall, 8/12 were male and 4/12 were female. The mean age of the study group was 38.1 (17-50) years old. The risk factors for HIV infection were intravenous drug use (6/12), heterosexuality (3/12), homosexuality (2/12) and homosexuality with IV drug use (1/12). These baseline characteristics are summarized in Table 1.
Table 1. Demographic characteristics and baseline characteristics of the 12 acute HIV infected patients enrolled in the double nucleoside analog therapy study.

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
</tr>
<tr>
<td>Male</td>
<td>8/12  (66.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>4/12  (33.3%)</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>38.1</td>
</tr>
<tr>
<td>Risk Factors for HIV infection</td>
<td></td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>6/12  (50%)</td>
</tr>
<tr>
<td>Heterosexuality</td>
<td>3/12  (25%)</td>
</tr>
<tr>
<td>Homosexuality</td>
<td>2/12  (16.6%)</td>
</tr>
<tr>
<td>Homosexuality with IV drug use</td>
<td>1/12  (8.3%)</td>
</tr>
</tbody>
</table>

Drug Treatments

All 12 study patients were naïve to antiretroviral treatment prior to the enrollment in this study. All patients received the combination of zidovudine (ZDV) and lamivudine (3TC). All treatments were initiated within 2 weeks of the patients’ first clinic visits. Over time, therapy may have been changed in some patients based on tolerance and/or clinical or virologic response criteria.
2.1.2 Triple Drug Therapy Study

Study Population

Patient Inclusion criteria:
1. Positive HIV test with a known negative over the past 6 months.
2. Current indeterminate HIV test result, evolving to a full positive test result in follow-up.
3. Current negative test result, with a positive test for direct viral detection (p24 or plasma viral load).
4. Positive test result with no history of a recent negative test result but a definite exposure to HIV in the past six months with a subsequent illness compatible with acute HIV infection.

Patient Exclusion criteria:
1. Contraindication for antiretroviral therapy or other conditions which, in the opinion of the physician, made therapy inappropriate.

In this study, three patients presenting with acute HIV infection and documented seroconversion who fit our criteria were recruited at St. Paul’s Hospital in Vancouver, Canada. The diagnosis of HIV infection was made by standard ELISA & confirmatory Western blot assays. All subjects provided informed consent prior to inclusion in the study. All three patients were male and their baseline characteristics are summarized in Table 2.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Risk Factor</th>
<th>Initial Therapy</th>
<th>Subsequent Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient M</td>
<td>M</td>
<td>24</td>
<td>IVDU</td>
<td>ZDV/3TC</td>
</tr>
<tr>
<td>Patient N</td>
<td>M</td>
<td>49</td>
<td>Homosexuality</td>
<td>ZDV/ddI</td>
</tr>
<tr>
<td>Patient O</td>
<td>M</td>
<td>32</td>
<td>Homosexuality</td>
<td>ZDV/3TC</td>
</tr>
</tbody>
</table>

Table 2. Demographic characteristics and baseline data of the 3 patients enrolled in the triple drug therapy study.
Drug Treatment

All patients were initially treated with double nucleoside analog therapy, as shown above. This was eventually replaced by triple drug therapy in these patients due to the significant rebound of their plasma viral load or suboptimal virologic results. As shown, 2/3 patients (Patient M and N) received 2 nucleoside analogs with a protease inhibitor while the other study subject (Patient O) received a nucleoside analog, a protease inhibitor and a non-nucleoside reverse transcriptase inhibitor. The nucleoside analog used in this study were ZDV, 3TC and d4T. The protease inhibitors used were Indinavir (IDV) and Saquinavir (SQV). The non-nucleoside reverse transcriptase inhibitor used was Nevirapine (NVP).
Sample Processing

Study samples in both studies were collected at the time of clinic visits at St. Paul’s Hospital. Plasma samples were isolated from whole blood within 2 hours of their collection by centrifugation. The plasma samples were separated into 0.5ml aliquots and immediately stored at -70 °C until the virologic assays were performed. The CD4+ lymphocytes count was measured using flow cytometry, performed in the clinical laboratory at St. Paul’s Hospital.

Plasma HIV RNA Assay

Plasma viral load was quantitated using the Roche HIV Amplicor Monitor assay (Mississauga, ON) (Sagg et al. 1996). Target RNA sequences in the clinical samples are quantified in reference to both the internal and external standards. The assay has a higher limit of quantitation of 750,000 HIV RNA copies/mL plasma and a lower limit of 500 copies/mL plasma. In cases of viral load measurement below 500 copies, the Roche Molecular Systems Prototype Ultra Direct assay (Alameda, CA) was used. This assay has a lower limit of quantitation of 20 copies/mL plasma. All assays were preformed and interpreted according to the manufacturer’s instructions.

Genotypic assays

Genotyping of HIV strains was performed in most study samples to detect mutations associated with drug resistance to nucleoside analogues. An extensive number of studies have shown that drug resistance to reverse transcriptase and protease inhibitors is caused by specific mutations in the reverse transcriptase and protease genes (Mellors et al. 1996). Currently, there are two main genotypic approaches to the detection of drug resistance mutations: one is based on DNA sequencing technology and the other on probe hybridization. AZT resistance (conferred by point mutation of the HIV genome at codon 215) and 3TC resistance (conferred by point mutation at codon 184) were tested prior to therapy using DNA sequencing on theses study samples.
2.2 Results

2.2.1 Double Nucleoside Analog Therapy Study

Individual viral load and group median results for the 12 patients enrolled in this study (Patient A to L) at baseline, month 1, month 3 and month 6 are presented in Table 3.

Table 3. Individual viral load and group median results of Patient A to L at various time-points of the study. Plasma viral load is presented as \( \log_{10} \) HIV RNA (copies/mL). Data not available at a certain time-point is denoted as N/A.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Baseline</th>
<th>Month 1</th>
<th>Month 3</th>
<th>Month 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.95</td>
<td>2.70</td>
<td>3.02</td>
<td>2.52</td>
</tr>
<tr>
<td>B</td>
<td>3.56</td>
<td>1.30</td>
<td>2.90</td>
<td>2.03</td>
</tr>
<tr>
<td>C</td>
<td>3.15</td>
<td>1.30</td>
<td>1.64</td>
<td>2.04</td>
</tr>
<tr>
<td>D</td>
<td>5.00</td>
<td>2.78</td>
<td>4.89</td>
<td>4.55</td>
</tr>
<tr>
<td>E</td>
<td>6.32</td>
<td>5.43</td>
<td>5.06</td>
<td>N/A</td>
</tr>
<tr>
<td>F</td>
<td>4.74</td>
<td>2.85</td>
<td>2.20</td>
<td>2.76</td>
</tr>
<tr>
<td>G</td>
<td>5.22</td>
<td>3.73</td>
<td>3.98</td>
<td>3.88</td>
</tr>
<tr>
<td>H</td>
<td>2.28</td>
<td>1.30</td>
<td>2.98</td>
<td>1.36</td>
</tr>
<tr>
<td>I</td>
<td>5.02</td>
<td>2.69</td>
<td>1.51</td>
<td>4.04</td>
</tr>
<tr>
<td>J</td>
<td>5.22</td>
<td>5.12</td>
<td>4.52</td>
<td>N/A</td>
</tr>
<tr>
<td>K</td>
<td>4.76</td>
<td>5.53</td>
<td>5.13</td>
<td>5.05</td>
</tr>
<tr>
<td>L</td>
<td>4.07</td>
<td>2.19</td>
<td>2.42</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Median 4.86 2.78 3.00 2.76
CD4 cell count data at the corresponding time-points are shown in Table 4.

Table 4. Individual CD4 cell counts and group median of Patient A to L at various time-points of the study. CD4 cell count is presented as cells/μL. Data not available at a certain time-point is denoted as N/A.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Baseline</th>
<th>Month 1</th>
<th>Month 3</th>
<th>Month 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>870</td>
<td>620</td>
<td>500</td>
<td>470</td>
</tr>
<tr>
<td>B</td>
<td>380</td>
<td>890</td>
<td>820</td>
<td>895</td>
</tr>
<tr>
<td>C</td>
<td>710</td>
<td>880</td>
<td>909</td>
<td>940</td>
</tr>
<tr>
<td>D</td>
<td>410</td>
<td>540</td>
<td>390</td>
<td>420</td>
</tr>
<tr>
<td>E</td>
<td>530</td>
<td>610</td>
<td>750</td>
<td>N/A</td>
</tr>
<tr>
<td>F</td>
<td>640</td>
<td>620</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>G</td>
<td>400</td>
<td>540</td>
<td>560</td>
<td>580</td>
</tr>
<tr>
<td>H</td>
<td>540</td>
<td>770</td>
<td>910</td>
<td>850</td>
</tr>
<tr>
<td>I</td>
<td>340</td>
<td>290</td>
<td>340</td>
<td>N/A</td>
</tr>
<tr>
<td>J</td>
<td>970</td>
<td>920</td>
<td>860</td>
<td>N/A</td>
</tr>
<tr>
<td>K</td>
<td>N/A</td>
<td>560</td>
<td>570</td>
<td>540</td>
</tr>
<tr>
<td>L</td>
<td>360</td>
<td>710</td>
<td>630</td>
<td>N/A</td>
</tr>
<tr>
<td>Median</td>
<td>530</td>
<td>620</td>
<td>615</td>
<td>560</td>
</tr>
</tbody>
</table>

The baseline HIV RNA levels of this cohort ranged from 191 copies/mL plasma (2.28 log) to 2,068,973 copies/mL plasma (6.32 log). The mean baseline viral load was calculated to be 235,671 copies/mL plasma (5.37 log). Patients' initial CD4 count ranged from 340 cells/μL to 970 cells/μL. There was no evidence of primary drug resistance to ZDV or 3TC, although 3TC resistance was not evaluated in 5/12 samples. The mean baseline CD4 count was calculated to be 559 cells/μL. These data are summarized in Table 5.
Table 5. Virologic and immunologic baseline results of the 12 acute HIV infected patients enrolled in the double nucleoside analog therapy study.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Plasma Viral Load</td>
<td></td>
<td>5.37 ± 1.09 log</td>
</tr>
<tr>
<td>Mean CD4 T-Lymphocytes Count</td>
<td></td>
<td>559 ± 215 cells/µL</td>
</tr>
<tr>
<td>Primary ZDV resistance</td>
<td></td>
<td>0/12</td>
</tr>
<tr>
<td>Primary 3TC resistance</td>
<td></td>
<td>0/7 (5/12 not tested)</td>
</tr>
</tbody>
</table>

Figure 8 shows the changes in mean plasma viral load and CD4 T-lymphocytes count of the study cohort. Mean plasma viral load was shown to decrease from the baseline value. It reached a nadir of 3.7 log_{10} copies/mL at week 24 (2.76 if the median is considered). However, viral suppression was not maximal as the mean plasma viral load never reached the limit of quantitation of our assay (20 HIV RNA copies/mL plasma, 1.30 log) during the study. No patients were maximally suppressed at week 24, although 4 had values below the limit of quantitation of the standard assay (500 copies/mL). The virologic setpoint appears to have been established as early as the first month of therapy, with a decrease in plasma viral load of almost 2 log_{10} (99%) on average, an effect that is substantially more marked than could be expected in patients with chronic infection treated with this double combination therapy.

Mean CD4 T-lymphocytes count was found to increase after the initiation of double antiviral therapy. As shown in Figure 8, it rose from 559 cells/µL at baseline to a maximum of 776 cells/µL at week 8. No further gain was made over the period of observation. If anything, a gradual decline in mean and median CD4 cell count was observed from weeks 4-8 to week 24.
Plasma Viral Load and CD4 Cell Counts in Patients with Primary HIV Infection Receiving Double Nucleoside Analog Therapy

Figure 8. The changes in mean plasma viral load and mean CD4 T-lymphocytes count (±SD) of patients with primary HIV infection treated with double nucleoside analog therapy to 24 weeks, number of patients evaluated at each time point is shown at the bottom of the graph.

Statistical Analyses

Statistical analyses of the study cohort were done to compare the mean plasma viral load and CD4 cell count before and after treatment. Data collected at baseline were designated as before treatment and those collected at the end of the study (week 24) were designated as after treatment. The null hypotheses (H₀) claim that mean plasma viral load and CD4 cell count are the same before and after drug treatment. The alternative hypotheses (H₁) claim that mean plasma viral load decreases after treatment and CD4 count increases after treatment.
For mean plasma viral load, the baseline value of the cohort is $5.37 \log_{10}$ with a standard deviation of 1.09 and a sample size of 12. The value at week 24 is $4.07 \log_{10}$ with a standard deviation of 1.2 and a sample size of 9. The test statistic (z statistic) is calculated to be 2.55. Since this is an upper-tailed test, the p-value is the area under the z curve and to the right of 2.55. P-value is found to be 0.0054, which is smaller than the significance level of 5%. Therefore, $H_0$ is rejected ($p<0.05$) and the decrease in mean plasma viral load with the presence of double drug therapy is statistically significant.

For CD4 T-lymphocytes count, the baseline value of the cohort is 559 with a standard deviation of 215 and a sample size of 11. The value at week 24 is 673 with a standard deviation of 247 and a sample size of 8. The test statistic (z statistic) is calculated to be $-1.05$. Since this is an lower-tailed test, the p-value is the area under the z curve and to the left of $-1.05$. P-value is found to be 0.1469, which is larger than the significance level of 5%. Therefore, $H_0$ cannot be rejected ($p<0.05$). The increase in CD4 cell count with the presence of double drug therapy in this study does not suggest a statistical significance.

Among the 12 patients enrolled in this double nucleoside analog study, 2 (Patients A and B) have been selected to illustrate particular therapeutic effects for a more extended period of time, up to 69 weeks of observation. (See Figures 9 and 10)
2.2.1.1 Patient A

Patient A was a 41 year-old female IVDU who presented with documented acute HIV infection. The viral isolate at baseline showed no primary resistance to either ZDV or 3TC. Her treatment started with the combination of ZDV/3TC that was later modified to d4T/3TC at week 8 due to side effects. She has been on this combination of nucleoside analogs since that time and the changes in viral load and CD4 cell count are presented in Figure 9.

Figure 9. The changes in plasma viral load and CD4 T-lymphocytes count of Patient A. Therapeutic regimens are shown at the bottom of the graph.

Once treatment was initiated, plasma viral load decreased from 4.95 at baseline to 2.52 log_{10} copies/mL at week 4. This represents an elimination of >99% of the virus in the plasma. However, there was a rebound to 3.3 log by week 6. Minor fluctuations of plasma viral load were observed for the next 17 weeks (week 6 to week 23). At week 29, plasma viral load has significantly increased to 4.4 log_{10} copies/mL. At this time, 3TC resistance was identified from the viral isolates obtained from the plasma. Interestingly, despite this finding, some level of virologic suppression has been maintained to week 69 and the patient is reluctant to agree to the change in therapy recommended by her physician.

CD4 T-lymphocytes count of Patient A did not return to the baseline level until week 29. Thereafter, they gradually increased to a level 140 cells/µL above the baseline value by week 58.
2.2.1.2 Patient B

Patient B was a 20 year-old heterosexual male who presented with acute HIV infection. The viral isolate at baseline showed no resistance to ZDV. Primary 3TC resistance was not tested. He was treated with the combination of ZDV/3TC. At week 19, his treatment was modified to d4T/ddI/Indinavir triple therapy. Six weeks later, he was placed onto d4T/3TC double therapy (due to ddI and Indinavir toxicity) which he has continued to the present time (Figure 10).

Figure 10. The changes in plasma viral load and CD4 T-lymphocytes count of Patient B. Therapeutic regimens shown at the bottom of the graph.

Once ZDV/3TC treatment was initiated, plasma viral load was maximally suppressed within 3 weeks. However, this level of suppression was not sustained, with a progressive rebound between weeks 5 and 19. At that time, triple drug was initiated, with excellent virologic suppression once again. After week 25, a return to double therapy (d4T/3TC) was followed by a loss of virologic containment. Plasma viral load continued to rise and finally exceeded the baseline value by approximately 1.0 log_{10} copies/mL at the end of week 69. The CD4 T-lymphocytes count was found to increase significantly following the initiation of treatment, by 510 cells/µL in 3 weeks. As plasma viral load rebounded, CD4 cell count decreased, but has now been relatively stable over 69 weeks.
2.2.2 Triple Drug Therapy Study

The results generated from this part of the study are presented as three individual case reports. Patients M, N and O initially received double drug therapy during the acute/early phase of their illness. As their disease progressed into the post-primary infection stage (more than 6 months after the infection), their treatments were switched to a triple combination therapy regimen as described below.

Genotypic analysis of HIV viral strains were done in 2/3 cases with no evidence of primary zidovudine or lamivudine resistance. The baseline HIV RNA levels and CD4 counts, prior to the initiation of any treatment, are presented in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Patient M</th>
<th>Patient N</th>
<th>Patient O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Viral Load (log_{10} copies/mL)</td>
<td>4.87</td>
<td>4.96</td>
<td>5.26</td>
</tr>
<tr>
<td>CD4 Counts (cells/μL)</td>
<td>680</td>
<td>380</td>
<td>270</td>
</tr>
<tr>
<td>Primary ZDV resistance</td>
<td>sensitive</td>
<td>N/A</td>
<td>sensitive</td>
</tr>
<tr>
<td>Primary 3TC resistance</td>
<td>sensitive</td>
<td>N/A</td>
<td>sensitive</td>
</tr>
</tbody>
</table>
2.2.2.1 Patient M

Patient M was a 24 year-old male IVDU who presented with acute HIV infection. The viral isolate at baseline showed no resistance to ZDV or 3TC. He was initially treated with the combination of ZDV/3TC. At week 24, his treatment was modified to d4T/3TC/Indinavir triple therapy due to virologic failure of the initial regimen. Viral load and CD4 cell count results to week 46 are shown in Figure 11.

---

Figure 11. The changes in plasma viral load and CD4 T-lymphocytes count of Patient M

Therapeutic regimens are shown at the bottom of the graph.
Antiretroviral therapy was not initiated in this patient until the second week of follow-up. His plasma viral load at week 2 had stayed at the same level as at baseline (4.87 \( \log_{10} \) copies/mL). Once double drug therapy was initiated, plasma viral load was reduced by almost 2 \( \log_{10} \) in the next 2 weeks. Viral load continued to fall until it reached a nadir of 1.83 \( \log_{10} \) copies/mL at week 12, but had rebounded significantly by week 24, to within 1.5 \( \log_{10} \) copies/mL of baseline.

Due to sub-maximal virologic suppression, the combination of two nucleoside analog (d4T and 3TC) with a protease inhibitor (Indinavir) was administered. The plasma viral load fell immediately to below the limit of quantitation of our most sensitive assay. Maximal suppression has now been maintained over 16 weeks.

The increase in CD4 T-lymphocytes count paralleled the reduction in plasma viral load. At week 12, when the plasma viral load reached its initial nadir, a peak increase in the CD4 cell count was measured. The subsequent rebound in viral load was accompanied by a CD4 cell count decline. With the initiation of triple combination therapy, the CD4 cell count rose again to 928 cells/\( \mu \)L at week 35.
2.2.2.2 Patient N

Patient N was a 49 year-old homosexual male who presented with acute HIV infection. Primary resistance to ZDV and 3TC was not measured in this patient’s viral isolates. He was initially treated with the combination of ZDV/ddI. At week 17, his double nucleoside analog therapy was terminated at his request. Treatment was re-initiated again at week 60. The triple drug combination of ZDV, 3TC and Saquinavir was administered, as part of an ongoing clinical trial. Therapy has been continued to the present time (week 156), without interruption. The changes in viral load and CD4 count over time are presented in Figure 12.

---

**Figure 12.** The changes in plasma viral load and CD4 T-lymphocytes count of Patient N. Therapeutic regimens are shown at the bottom of the graph.
The plasma viral load of Patient N decreased to below the limit of quantitation (<20 copies/mL) following the initiation of double combination therapy with ZDV/ddI. Maximal viral suppression was maintained for 14 weeks (week 7 to 21), even with the termination of treatment at week 17. Viral load slowly rebounded after week 21 and it plateaued at approximately 3.0 log_{10} copies/mL off treatment. This represents a very impressive response to double combination therapy.

When triple drug therapy was started at week 60, plasma viral load dropped immediately from the plateau level to below the limit of quantitation. Maximal viral suppression was once again achieved, and has been maintained for over 100 weeks.

As plasma viral load decreased following the initiation of the antiviral treatment, the CD4 cell count slowly increased. Even after the termination of double drug therapy, the CD4 count continued to rise until it reached a maximum of 920 cells/μL at week 26. As viral load rebounded off treatment, CD4 count gradually decreased to the baseline value by week 58. With the start of triple drug therapy, the CD4 count has gradually recovered to 820 cells/μL at week 156.
2.2.2.3 Patient O

Patient O was a 32 year-old homosexual male who presented with acute HIV infection. The viral isolate at baseline showed no resistance to ZDV or 3TC. He was initially treated with the combination of ZDV/3TC. At week 11, his treatment was modified to d4T/3TC due to intolerance. The triple drug combination of 3TC, Indinavir and Nervirapine was initiated at week 25 due to suboptimal virologic results achieved while on the d4T/3TC combination. Viral load and CD4 cell count results to week 64 over time are shown in Figure 13.

Figure 13. The changes in plasma viral load and CD4 T-lymphocytes count of Patient O. Therapeutic regimens are shown at the bottom of the graph.
Plasma viral load had decreased by 1.67 log_{10} copies/mL by week 4 on ZDV/3TC, but never reached the limit of detection. The replacement of ZDV by d4T had little effect. A virologic setpoint of 4.0 log_{10} copies/mL was established by week 8. This represents a decrease of 1.1 log from the baseline. With the start of triple drug therapy at week 25, plasma viral load slowly decreased to below the limit of quantitation. This viral suppression had been sustained since week 51.

The CD4 T-lymphocytes count changes paralleled the changes in plasma viral load. Sustained values of 400 cells/μL or higher have been measured while the patient has had maximal virologic suppression while receiving triple combination therapy.
CHAPTER III: DISCUSSIONS

The goal of antiretroviral therapy is to limit or delay HIV disease progression. With our understanding of viral dynamics and the properties of antiviral drugs, combination therapy is the most effective way to achieve that goal. This is supported by various clinical trials in which significant reduction in disease progression and mortality were observed in advanced HIV patients undergoing combination therapy (Henry et al. 1997; Hirsch et al. 1997). However, the benefit of subjecting patients to aggressive antiviral treatment at the earliest stage of the disease is still controversial. HIV reverse transcriptase is very error-prone (Mansky and Temin 1995). Given this mutation rate and the dynamic nature of HIV replication, Coffin et al argued that, on average, every mutation at every position of the HIV genome would occur numerous times each day (Coffin 1995). Viral diversity increases the likelihood of viral escape from therapeutic agents. Therefore, the failure of monotherapy in treating primary HIV infection is inevitable. Effective antiretroviral therapy must therefore contain a combination of potent antiretroviral agents which exerts maximal viral suppression of viral replication. Studies have shown that viral population during acute HIV infection is relatively homogeneous, less diverse and small. The viral population in acute infection is usually composed of only one principal strain that is monocytotropic (Zhu et al. 1993). It would therefore be easier to prevent viral diversity and dissemination with early antiviral treatment. A logical rationale for early aggressive treatment against primary HIV infection can clearly be defined. Our objective in this project was, therefore, to evaluate the efficacy of aggressive antiviral treatment using double and triple drug combinations in patients with primary HIV infection.

The fact of defining our study inclusion criteria as listed in previous section makes for a uniform population, although there would still be some heterogeneity. There are some that feel only those patients with indeterminate or negative tests are the "true" representatives of acute HIV infection. We have some of those patients in our study, but it is clearly not our whole population. The group is too small to break it down in this manner and still generate a statistically meaningful result. We are also excluding those who refuse therapy. Unless we have a reason to think that they are inherently different form those who accept therapy, this does not create a systematic bias.
Drug resistance is an inevitable result of the selection pressure of antiretroviral therapy. The dominance of mutant strains in a viral population is the major cause of treatment failure. A related issue may be the pre-existence of drug resistance in the initial viral inoculum, due to extensive use of anti-retroviral therapy in the community. If an antiviral treatment is able to suppress viral replication to a very low level, it will become highly unlikely that the required genetic mutation can occur. Even if the mutation does occur, the resistance developed would also be delayed and its relevance reduced. Genotypic analysis of viral strains were done on these patients at baseline. In this study cohort, no patient was found to have primary resistance to ZDV or 3TC, suggesting that its prevalence in the general HIV-infected population is still quite low. Physicians can therefore be more confident that the antivirals that they initially prescribe will be effective in most of their patients.

The combination of AZT/3TC is chosen to be the initial treatment of choice in our double nucleoside analog therapy study. It has been shown to be remarkably effective in suppressing HIV-1 replication for prolonged periods of time despite the rapid emergence of high level 3TC resistance in treated patients. There has been several mechanisms suggested to explain this. This particular 2-drug combination has the advantage of strong synergistic interaction against HIV. Mutation at codon 184, which causes 3TC-resistance, suppresses AZT resistance leading to prolonged antiviral activity due to the AZT component of the combination regimen. 3TC-induced mutation induces phenotypic reversion to ZDV sensitivity in ZDV-resistant strains (Larder et al. 1995). Mutation at codon 184 also leads to a 7-fold increase in fidelity of the reverse transcriptase enzyme which could potentially lead to decreased generation of viral quasispecies and inability of the virus to escape both drug and immune pressure. As genotypic analysis revealed no primary resistance to either of these drugs among our study cohort, the efficacy of this combination is expected to be quite effective.

As mentioned during introduction, the initial burst of viremia following acute HIV infection would abate in a matter of weeks even without any external intervention. This is due to the development of host immune response to HIV. That anticipated decline in viral load is part of the natural course of HIV infection. Our studies were limited by the sample size and thus did not provide a control group to assess the amount of viral load reduction by treatment in comparison
to the anticipated decline. However, since the objective of our studies is to evaluate the efficacy of double and triple drug therapy and determine if these treatments are satisfactory in inducing prolonged and maximal viral suppression, the absolute amount of viral suppression induced by each therapy would not be crucially necessary to our findings. Even if control groups did exist in our studies, the absolute amount of reduction indicated would be limited by the small sample size.

In our double drug therapy study, all patients were treated initially with the combination of ZDV and 3TC. The mean plasma viral load of all treated patients was found to decrease shortly after the initiation of drug therapy as shown in Figure 8. It was reduced by $1.3 \log_{10}$ copies/mL (median $2.0 \log_{10}$ copies/mL) from the baseline by the time a virologic setpoint was established at week 20 to 24. This degree of viral load reduction is considered biologically significant. It reflects a significant inhibition of viral replication in the plasma. This also suggests that 99% of the baseline plasma viral burden was eliminated with the presence of double nucleoside analog therapy. The combination of ZDV and 3TC thus was effective in preventing further viral replications. The viral reverse transcriptase is very possibly inhibited by the nucleoside analog combination. Based on our analysis, the amount of viral load reduction was found to be statistically significant after drug treatment. However, viral suppression was not maximal as the concentration of virus was still quantifiable by our most sensitive HIV assay. Even with 1-5% of the baseline viral load left, it is expected that the establishment of viral reservoirs in the body would still proceed and eventually lead to the failure to contain the high level of viral replication (Henry et al. 1997).

Host immune function was restored slowly after the initiation of double combination therapy. The rapid decrease of plasma viral load on treatment was followed by a gradual increase in CD4 counts. The effect of antiviral therapy on immune reconstitution seemed to be delayed. Elimination of plasma virions lowered the probability of CD4 cells being infected. Reduction of viral burden also facilitated a more effective CTL killing of infected CD4 cells. Thus, the immune system secured an opportunity to replenish itself. Immune reconstitution requires the differentiation of stem cells in the bone marrow. This process takes time, which explains the delay of CD4 rebound upon reduction of viral load. Nonetheless, the mean CD4 count of the
cohort has increased by more than 200 cells/μL by week 8 as shown in Figure 8. However, as the virologic setpoint (with some ongoing viral replication in the plasma) was established in most patients, immune reconstitution was “stalled”. Indeed, CD4 cell count began to decline from the maximum value (Figures 8). By month 6, the median increase in CD4 count was only 30.0 cells/μL compared to the baseline value (Table 4). The final increase in CD4 count, when compared to the baseline figure, was not found to be statistically significant.

In fact, only 8/12 patients (66.7%) had an increase in their CD4 lymphocytes count, with 2/12 patients (16.7%) showing no change, 2/12 patients (16.7%) a continued decline despite a partial virologic response to therapy. This heterogeneity could be due to different genetic backgrounds, as well as age or risk behaviors. It is interesting that the 2/12 patients who had declining CD4 counts were both IVDU’s and over 40 years of age.

Establishment of a virologic setpoint indicates the balance between viral replication and the host immune response has been reached. On double therapy, viral replication was not fully suppressed. As long as this is the case, CD4 counts will gradually decline. Thus, it is critically important to suppress viral replication as much as possible to allow for the host immune reconstruction to occur. Double drug therapy does not allow us able to achieve that goal.

To illustrate the virologic and immunologic response in patients receiving double drug therapy on an individual basis, the data of patients A and B were selected for presentation. As in the case of the cohort study, plasma viral loads of both patients A and B were found to be significantly decreased shortly after the initiation of double nucleoside analog treatment. However, maximal suppression was not achieved in either case, with subsequent early viral rebound. This result can be explained by the inadequate potency of double nucleoside analog therapy and the associated rapid selection of drug-resistant strains. In Patient A, a virologic setpoint was established by week 44 as shown in Figure 9. This setpoint was established at a level 1.3 log below the baseline (consistent with our cohort data) with a delayed CD4 cell count increase.

In Patient B, a virologic setpoint was established during the period of ZDV/3TC therapy, but this was only 0.5 log below the baseline level. There was a period of 6 weeks in which Patient B
received triple drug therapy when the viral load was shown to fall below the limit of quantitation of our assay. Even after the return to double nucleoside analog treatment, the viral load of Patient B rebounded only slightly for 7 weeks. Nevertheless, plasma HIV RNA levels then increased, eventually becoming higher than the baseline value (Figure 10). This indicated the failure of double nucleoside analog therapy in this patient. Nevertheless, Patient B responded well immunologically. His CD4 count was increased by almost 600 cells/µL within 15 weeks of treatment. The lymphocytes count was maintained at a high level for the remainder of the follow-up (69 weeks). The complexity of the immunologic response to antiviral treatment is again appreciated here. For a patient who ultimately did not respond well virologically under double nucleoside analog therapy, his immune function did seem to be relatively well maintained. Polyclonal Vβ T cell expansion against HIV might have had taken place during primary HIV infection in this patient. Thus despite the failure to suppress viral replication, there was still an adequate immune response to prevent CD4 counts from declining. Immune replenishment in this 20-year-old patient may also be more rapid and longer-term follow-up will help us determine if it will be maintained with an incomplete virologic response.

The relationship between antiviral treatment and immune response could be analogous to a sink of water with an open tap and an open drain. Immune replenishment of CD4 cells by the bone marrow would be like new water coming out of a tap. The level of water in the sink represents the number of existing CD4 cells in the body. Massive destruction of CD4 cells by viral replication would be like water being drained away by the opening at the bottom of the sink. Antiretroviral treatment reduces viral replication, which is analogous to closing down the drain, sometimes only partially. The CD4 count may now decline slower than before, cease to decline, or even rebound. It all depends on the flow of the tap, the size of the opening and the size of the plug which varies among individuals. If immune replenishment is fast and the treatment is effective in a patient, this patient will be more likely to have a rebound in CD4 cell count, thus an immunologic benefit from antiviral therapy. The size of the opening might have effectively closed down by treatment in Patient A, but the flow of the tap might be slow. Thus Patient A had decreasing CD4 count despite a reduction of viral load. In Patient B, the plug might not be effective in closing down the drain, but the tap might be wide open. Thus CD4 count could be
maintained at a high level. This underscores the truly individual nature of responses to antiretroviral therapy.

From our cohort study and the individual analyses of patients A and B, it is quite clear that double nucleoside analog was only able to suppress HIV viral replication partially. Maximal viral suppression was not achieved. Immune function was partially restored (in most cases) following the initial reduction of viral load. However, as a detectable virologic setpoint was established, the CD4 count slowly declined, albeit in a heterogeneous manner across the cohort. The current goal of anti-HIV treatment in acute/early HIV infection is to exert maximal antiretroviral pressure using a combination of potent drugs when the viral population is most homogenous and less diverse. According to our findings, the combination of two nucleoside analogs was not able to exert maximal antiviral pressure. The residual viremia may be due to inadequate drug penetration into certain tissues, drug resistance, persistence of a long-lived virus-producing population and a gradual activation of the latent pool of cells (Ho et al. 1995).

The analysis of Patient B gave us an insight toward the potential added benefit of triple drug therapy in acute HIV infection. This leads us to the more in-depth investigation of Patient M, N and O who were treated with triple drug therapy after being on double therapy as their initial regimen. It was of interest to us to determine how long maximal viral suppression could be sustained using triple combination in the post-primary infection setting after the failure of a two drug combination.

Patient M, N and O were seroconverters with primary HIV infection when they first enrolled in the study. They were initially treated with a combination of two nucleoside analogs. Patient M and Patient O follow the same virologic pattern of patients treated with double nucleoside analogs. Their viral load was reduced significantly (2 log or more) during the first few weeks of treatment. However, suppression was incomplete. Viral rebound occurred shortly after the viral load reached its nadir and a virologic setpoint was established at a level about 1 to 1.5 log below the baseline value. With the failure of double drug therapy in Patient M and O, triple combination therapy was initiated in the post-primary infection stage. Patient M received two nucleoside reverse transcriptase inhibitors (NRTI) with a protease inhibitor while Patient O received a NRTI, a protease inhibitor and a non-nucleoside reverse transcriptase inhibitor.
(NNRTI). Their plasma viral loads fell below the limit of detection from the previous setpoint after triple drug therapy was initiated as shown in Figures 11 and 13. The reductions were more than 100 fold (2 log) in both cases, indicating the clearance of virions, loss of productively infected cells and prevention of new cell infection. Maximal viral suppression was maintained for more than 15 weeks in both cases indicating the effect of triple therapy was not transient. As for the immunologic responses of Patients M and O, a decrease in plasma viral load was associated with an increase in CD4 counts. This suggested maximal and sustained viral suppression prevents the ongoing destruction of CD4 lymphocytes and allows replenishment of the immune system.

The virologic and immunologic benefits associated with triple drug therapy were even more apparent in Patient N, our most extensively followed patient in the study cohort. The plasma viral load of Patient N was quickly reduced to the limit of detection under double therapy as shown in Figure 12. This optimal virologic result was not observed in any another patient receiving 2-drug combinations in our studies. It could be due to the suitability of ZDV/ ddI treatment in this particular patient, his excellent compliance to the regimen or a number of other undefined host factors. The maximal viral suppression was maintained for more than 10 weeks, during which Patient N was on double therapy. However, as the patient was off treatment, rapid viral rebound occurred, indicating the persistence of viral stores in tissue reservoirs. The viral load increased by almost 2 log within 10 weeks of being off treatment. A virologic setpoint was established at a level 1.9 log lower than the baseline. This was a slightly greater difference than those observed in other patients receiving double therapy. This was probably due to the better virologic response to treatment experienced by Patient N in which maximal viral suppression was initially achieved. As triple drug therapy was initiated, a rapid virologic response was observed and has been sustained over 100 weeks. Immunologically, the patient responded well after the initiation of double therapy. CD4 counts gradually increased as the viral load fell to below the limit of detection. When the patient was taken off treatment, viral rebound occurred and a setpoint was established. As with previous observations, CD4 count ceased to increase, but rather, declined gradually. It was not until the initiation of triple drug therapy that the CD4 count rebounded. As maximal viral suppression was sustained over nearly 100 weeks, the CD4 has continued to increase, consistent with immune restoration. This provides solid evidence that
triple drug therapy has the potential to induce prolonged maximal viral suppression and immune reconstitution in patients with post-primary HIV infection. This finding may have long term benefits to patients who have failed to respond optimally to monotherapy or double therapy. It also provides a strong argument for aggressive antiviral therapy in the early stage of the disease. If patients were treated with triple drug combination at the time of initial diagnosis, it may be possible to lower the virologic setpoint to below the limit of detection, thus halting immune deterioration, prolonging clinical latency and delaying disease progression, perhaps indefinitely.

One may consider withdrawing treatment in patients who respond optimally to double or triple drug therapy. Studies have shown that patients being treated with combination therapy for as long as 30 months show no signs of drug-resistant strains of HIV. However, they do still harbor replication-competent HIV in some of their latent cell populations such as resting memory T cells (Finzi et al. 1997). These virions are believed to be in the long-lived cells that have evaded the effects of antiretrovirals and immune surveillance. Upon stimulation, infectious HIV can be cultured from these latent cells. Analysis of these virions have shown that they are not resistant to antivirals (Finzi et al. 1997). These non-activated infected cells serve as a reservoir capable of maintaining HIV infection even in the setting of optimal antiretroviral treatment. Thus this observation suggests that it would be premature to withdraw therapy even in patients with long-term maximal virologic suppression. According to the findings of our studies, it may be the ideal choice to treat patients during seroconversion with triple drug therapy and maintain the treatment for as long as possible. For patients experiencing viral rebound due to treatment failure of double nucleoside analog therapy, it is shown here that maximal viral suppression can be achieved by switching treatment to the 3-drug regimen, at least in the limited number of patients we have studied.
Despite advances in our understanding of primary HIV infection, there are still many areas which require further investigation. For example, it is still unclear why M-topic HIV strains predominate during the transmission process. It is also uncertain why some patients can generate polyclonal CD8+ T cell expansion (which controls the initial burst of HIV replication more effectively) while others cannot. In other ways, the primary immune response is also qualitatively different among individuals and the complete impact of acute intervention with antiretroviral therapy on this process is still unknown. More studies should be done to understand and identify the factors that allow some individuals to mount a more efficient immune response against HIV.

More extensive studies should be conducted to determine the duration of maximal viral suppression achieved by triple drug therapies in the setting of primary HIV infection and to see if viral rebound occurs over time. Genotypic analyses should be performed in patients with viral rebound to determine if resistant strains have been selected, or if the loss of virologic containment relates to other issues such as pharmacokinetics, drug levels or compliance. The sensitivity of newer assays has allowed the accurate quantitation of viral load down to very low levels. It has also allowed the detection of very early viral rebound, giving us additional insight into these issues.

The viral dynamics in tissue compartments other than the peripheral circulation must be understood in order to establish if complete eradication of HIV from the body will be possible. Although the virus produced by latent cells in these compartments account for less than 1% of the total body viral load, it could still serve as a reservoir for viral rebound when therapy is withdrawn. Methods of accurately quantifying viral load in different compartments must be developed in order to assess the real efficacy of an antiviral therapy. Future research should be targeted towards designing treatments which prevent de novo HIV replication and allow these compartments to “burn out” (Perelson et al. 1996). Better understanding of the viral dynamics in these latent pools may provide valuable insights towards HIV pathogenesis and therapeutic approaches.
CHAPTER V: CONCLUSION

In our studies of antiretroviral therapy in patients with acute HIV infection, it was shown that double therapy with nucleoside analogs could only induce transient viral suppression and partial immune reconstitution. The average reduction of plasma viral load in the cohort by the time a virologic setpoint was established was a median of $2.0 \log_{10}$ copies/mL below the baseline level. This finding is consistent with recent reports in patients with more chronic HIV infection where viral rebound and insufficient viral suppression occur when double therapy is given. Immune function was restored slowly in most patients after the initiation of treatment. However, CD4 counts were generally found to decline gradually once the virologic setpoint (representing sub-maximal suppression) was established. The combination of two nucleoside analogs may have inadequate antiviral potency to achieve the required suppression of viral replication in the blood and in tissues. Incomplete viral suppression may allow viral breakthrough to occur due to drug resistance or other factors. This residual viral replication leads to progressive immune disease. Our results suggest that the aggressive approach to antiretroviral therapy in established disease (with three or more agents given simultaneously) is also suggestive in patients with acute HIV infection.

Our limited study of triple drug therapy has shown that this therapeutic approach has the potential to lower the viral load to below the limit of detection in patients who previously either failed on double therapy or withdrew from treatment. The reduction of viral load from the previous setpoint to the detection limit was more than $2 \log$ in all three cases we have studied. The antiviral effect was sustained (up to 100 weeks in Patient N). Immune restoration was apparent for as long as maximal viral suppression was maintained as evidenced by rising CD4 counts. Thus triple drug therapy may be able to induce long term maximal viral suppression and immune reconstitution in patients with early HIV infection, even after the initial failure of double nucleoside analog therapy. It is apparent that a larger study of triple drug therapy in a primary infection setting is required to consolidate our findings about its efficacy. A control group of patients receiving no treatment in future studies will also help us assess treatment efficacy versus the anticipated viral load decline (as part of the natural course of disease) upon acute HIV infection. Nonetheless, this project has allowed us to look at the potential effectiveness of triple
drug therapy and justify the necessity of more researches on multi-drug therapy in a primary and post-primary HIV infection environment.

As mentioned by John Coffin (Coffin 1995), HIV disease progression would be analogous to a train heading towards a cliff. The speed of the train would represent the level of viral replication while the distance between the train and the cliff would represent the CD4+ T lymphocytes count. The cliff represents the onset of AIDS and the progression to death. The higher the rate of viral replication during primary HIV infection, the sooner the onset of AIDS. However, if the immune status is well preserved, the onset of AIDS can be delayed. This project has provided us with an insight that triple drug therapy could be very effective, even at the earliest stages of HIV infection, in slowing down the train as much as we can. The next step will be to look at any residual viral replication in other body compartments that may have evaded the host immune response as well as the antiretroviral drugs being administered. It is not until we understand the viral dynamics in those compartments and derive strategies to eliminate the virions they contain that we will have accomplished our ultimate goal of stopping the train.
CHAPTER VI: REFERENCES


