ABSTRACT

An Examination of Non-nucleoside Reverse Transcriptase Inhibitors

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The Human Immunodeficiency Virus, or HIV, is the cause of AIDS, an immune syndrome that continues to have profound effects in the United States and around the world. There is currently no cure for AIDS, but there are several classes of medications that inhibit the reproduction of HIV, one of which are non-nucleoside reverse transcriptase inhibitors, or NNRTIs. This class of medications suppresses the enzyme reverse transcriptase, a HIV enzyme which converts viral RNA into human-compatible DNA. NNRTIs have become a cornerstone of the treatment of HIV, and continue to be used in treatment of patients along with a combination of other antiviral drugs. While there are several drawbacks to NNRTIs, they remain highly useful in patient care, and research continues in improving these medications and the lives of AIDS patients around the world.

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AN EXAMINATION OF NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

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By

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CHAPTER ONE

Introduction

The human immunodefiency virus, HIV, is the causative agent of AIDS, an autoimmune syndrome which remains prevalent in the United States and in many countries around the world. HIV has gained mass recognition and public awareness because of the deteriorating effects of the resulting disease AIDS, or Autoimmune Deficiency Syndrome, and HIV's characterization as a worldwide epidemic. The disease remains highly studied today, despite years of investigation, as researchers investigate a cure for the disease. While a cure has not yet been discovered, there are several dozen inhibitory medications for HIV, classified into four main categories. One of these highly researched categories is non-nucleoside reverse transcriptase inhibitors, a class of pharmaceutical medications which will be discussed in this paper. In order to understand the role of non-nucleoside reverse transcriptase inhibitors, otherwise known as NNRTIs, the structure of HIV as well as the worldwide magnitude of HIV is important to reference.

The Discovery of AIDS

AIDS is a relatively "new" disease, and the discovery of HIV is even more recent. Researchers speculate that HIV was likely in the human population from as early as the 1930s (Whiteside 2008). It is likely that the disease developed from virus transmission from other primates to humans, but there have been no definite conclusions as to how the

virus was initially transferred to or evolved in humans. The first recorded case of HIV infection was found in a blood sample taken from a patient in the Republic of Congo in 1959 (Grmek 1990).

The first noticeable discussions on AIDS began in the 1980s. The first move in the direction of the discovery of AIDS in the United States was an article by Michael Gottlieb in the New England Journal of Medicine in 1981 describing HIV-infected patients (Oldstone 1998). At the same time, Joel Weisman, a Los Angeles doctor, noticed a group of patients holding a unique cluster of rare diseases only found in immune-compromised adults (Grmeck 1990). This unique group of diseases are called opportunistic infections and typically include diseases such as *Pneumocystis carinii*, and Kaposi's sarcoma, a type of normally slow growing tumor (American 2005). While the cause of the syndrome was not known, the disease was thus named "Acquired Immunodeficiency Syndrome", or AIDS for short.

Through a series of investigations, in 1983, the virus causing AIDS was identified. A group of scientists, including Robert Gallo, Paul Ferino and Myron Essex, studied viruses that were suspected to have a role in in AIDS (Grmek 1990). At first HIV was thought to be several strains of the Human T-lymphotrophic virus or the Lymphadenopathy Associated virus, but by 1983, Gallo discovered that the virus was one uniform strain. This virus was isolated and named "human immunodeficiency virus", or HIV in 1984 (Arno 1992).

Human Immunodeficiency Virus

The Human Immunodeficiency Virus is of the family retroviridae, an infectious agent with a similar replication mechanism and structure to other common viruses, such

as influenza, chickenpox or hepatitis. Like all viruses, HIV contains a protein coat, called a capsid. The capsid acts like an envelope surrounding the rest of the virus, and keeping its genetic information within an outer coat. The capsid contains glycoproteins which act as recognition particles to recognize target cells. The inside of the virus contains a set of proteins called a "protein core" and genetic information via RNA. This is the virus's genetic material used to replicate, comparable to human DNA.

Viruses naturally destroy their host's cells by using glycoproteins to attach to the membrane of the host cell and entering into the cell. The virus will then insert viral DNA or RNA into the host cell. This insertion of viral genetic information causes the cell to produce more viral particles and stops the progression of the host cell's DNA translation and transcription. After a period of time, the virus replicates enough within the cell to burst or "lyse" the host cell. This not only destroys the host cell, but releases the replicated viruses to the rest of the body. Eventually the virus destroys enough cells to cause disruption to the balance of the body's functions.

On one hand, HIV is a typical virus as it follows the general flow of viral replication and lysis. On the other hand, HIV remains different from other viruses in that it remains dormant for a period of time without causing any symptoms. The virus replicates in macrophages for months or years, without rupturing the cell. After this phase, the virus invades CD4 positive T cells, white blood cells, and rapidly grows, rupturing these indispensable cells. Since white blood cells are essential in the body's natural immune response, HIV destroys the body's defenses against infection. (La Regina 2010). Thus other viruses and bacteria can easily invade the body. In this way, HIV itself does not cause death, but rather a *syndrome* of immune-deficiency. In an immune-

compromised system, minor diseases can become deadly since the body has no way to fight even common diseases. This is why HIV causes what is called an acquired immunodeficiency syndrome, or AIDS.

The Human Immunodeficiency Virus is known to be transferred from human to human primarily through blood transfusions, sharing needles with intravenous–drugs, sexual transmission, or mother to child transmission during the gestational period or birth. The virus is not very robust outside its primary environment, and consequently cannot be transferred through other mediums, like air, saliva, or skin contact.

The Autoimmune Deficiency Syndrome

Looking at the infection clinically, there are several stages of illness that an affected individual experiences. As noted earlier, the infection of HIV does not immediately cause AIDS, but rather slowly advances to the AIDS syndrome. The first stage, called the acute or primary infection, occurs within two to four weeks of exposure to the virus, and presents symptoms similar to that of influenza. After this brief symptomatic period, the individual moves in the second stage, or the asymptomatic HIV infection stage. During this stage, an infected individual experiences no symptoms while the virus starts to infect T-cells. The asymptomatic stage usually lasts years, sometimes ten or more, while the virus replicates slowly within the white blood cells without lysing the cell. The average length of the asymptomatic stage is eight years, although this depends mainly on the vulnerability of an individual's immune system (Whiteside 2008). Eventually, if not treated, the infection will progress to ARC, or AIDS Related Complex. ARC consists of symptoms such as fever, swollen glands and thrush. The fourth stage is called Acquired Immunodeficiency Syndrome, or AIDS, the most commonly known

period of HIV disease. By this time, the virus has dramatically reduced the immune system, and opportunistic infections are able to grow. White blood cells, specifically CD4-T cells, are at a very low count, and consequently, the infected individual may suffer from a variety of diseases, such as tuberculosis or Kaposi's sarcoma, a type of cancer.

Because of the severely detrimental symptoms of AIDS, HIV is highly tracked and generally well diagnosed. Currently, the prevalence of HIV differs among region and country, but is harder to measure in less developed countries. Moreover, since HIV does not directly create symptoms soon after transmission of the virus, some carriers of HIV may be undetected. However, using information from antenatal clinic surveys as well as other international organizations, estimations can be made on the prevalence of AIDS in the total population (Whiteside 2008). Generally worldwide, the prevalence of AIDS increased exponentially until the late 90s, but stabilized in the 2000s.

Currently, AIDS is still prevalent within the United States, with an estimated population of 1.5 million infected individuals currently residing in North America (UNAIDS 2010). In other words, an estimated 0.5-0.9 % of all individuals in North America are infected with HIV as of 2007 (AIDS 2007). Despite an increase in research to prevent further transmission of AIDS, as well as advances in medicine, the number of individuals in North America who become newly infected every year has remained fairly stable since 2001 (Condon 2008). Consequently, AIDS has continued to be relevant in North American healthcare. In relation to the economy, the average cost of treating an individual with HIV/AIDS ranges from 20,000 to 24,700 dollars annually. Accounting for the estimated number of individuals in the United States with AIDS, this totals around

7 billion dollars annually (Hellinger and Fleishman 2000). In developed countries, such as the United States, this economic strain is distributed through public sources of funding, such as insurance. However, in undeveloped countries, there are not such easily obtained structures to help with the management of HIV/AIDS.

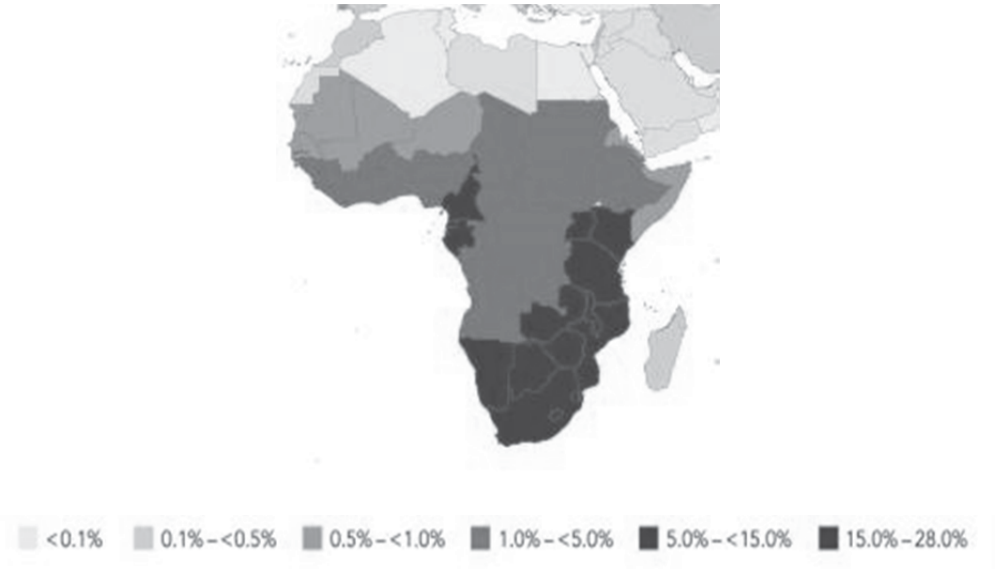

Figure 1
Map of the Prevalence of AIDS in Africa
(UNAIDS 2010)

Around the world, the continent of Africa, where the earliest HIV positive patient was discovered, continues to be the highest at risk for being infected by HIV. The virus is specifically most prevalent in the sub-Saharan regions of Africa, where an estimated 5-6% of the adult population is infected by HIV (UNAIDS 2010, Whiteside 2008). The impacts of HIV and AIDS are important for several reasons. In countries where there are a high percentage of infected populations, AIDS greatly increases mortality rates, reducing average lifespan. It also removes independent-working adults from working population and orphans children.

Among African countries, controlling for other factors, the presence of AIDS decreases the average life expectancy by 10 or more years. For example, in South Africa during 1997, the highest number of female deaths was among the age group of 60-64 years. In 2000, the highest number of female deaths peaked at both 30-34 and 60-64 (UNAIDS 2001). While not quite as drastic, a similar trend is shown among male death rates, with younger cohort mortality rate being almost as high as the oldest cohorts. Subsequently, South African children remain in poverty after losing parents to AIDS, and the decrease in working adults has slowed the economy of South Africa. . In countries where AIDS is widespread, even where birth rate is enough to increase a population, AIDS can create an overall negative growth rate. Such is the case of Botswana, where around one fourth of the population is infected with HIV. (Whiteside 2008, UNAIDS 2010). In this fashion, HIV impacts the individual as well as the greater population. Since AIDS can debilitate infected persons for years, the number of dependent adults increases in a population, requiring more adults to stay home as caregivers, lowering the general working force. In countries such as South Africa, HIV has had lasting effects on the entire nation because of the results of AIDS.

While Africa has the most adult prevalence rate by far, other continents and regions also have significant HIV-infected populations. In central and south America for example, HIV has risen over the past 10 years and has not seen the stabilization of numbers as in other regions. Central and South America have an adult prevalence rate of around 0.5% (UNAIDS 2010). Southeast Asia as well as North Asia have substantial HIV infected populations due to drug trafficking and international tourism (Marlink and Kotin 2004; UNAIDS 2010).

While AIDS treatment and mortality rates are still too high, education on HIV prevention has become widespread and treatment options for HIV are readily available (NIH). Within the last 10 years and the prevalence of HIV has been reduced around the globe, or at least stabilized in many regions (UNAIDS 2010).

Research for a Cure

Because of the steady increase of worldwide AIDS and its rapid detriment to individuals, media attention and public awareness of HIV increased in the early 1990s. AIDS was termed an epidemic, and health officials emphasized promoting education and research to prevent the spread of HIV; research on how to cure or prevent HIV began early after the isolation of the virus in 1983 (Stolley 2009). The search was intensified by the fact that HIV was one of the first discovered human lentiviruses, that is, a virus with a long incubation period and a slow progression.

The primary ambition of many viral researchers and AIDS drugs since the discovery of HIV has been to create a vaccine. While a method of complete immunization of HIV would be the ideal prevention and thereby treatment of HIV, at present, no such comprehensive immunization is in existence, although it has been a subject of investigation (La Regina 2010). The main reason why a vaccine for HIV has not yet been developed is because of the rapid replication and mutation cycle of the virus. Reverse transcriptase, the enzyme which replicates the virus's RNA, works quickly and with little precision. The result is a rapid mutation rate of about one mutation per viral replication cycle, with many versions of the virus even among different regions within an individual (Morse, 1993; Telesnitsky and Goff, 1997). Vaccines are created by copying a

virus's RNA and injecting an inactive version of the RNA into the body for natural antibodies to form. The human body builds up many different antibodies, each specific to one virus. Since HIV RNA mutates extremely quickly, a vaccine will only be able to work for a specific version of HIV, and not be able to prevent against the other strains of the virus. In this fashion, HIV is similar to familiar viruses such as the common cold since this virus maintains many versions and no effective vaccine is available. Additionally, HIV invades T-cells, white blood cells used in the creation of an antibody.

Despite these prominent complexities, research on HIV vaccines still continues in hopes of finding an effective vaccine and cure for AIDS. Many companies have proposed vaccines that have reached clinical trials, but none have reached approval. A more recent drug is the vaccine called AIDSVAX, which is the first AIDS vaccine to have reached Phase III clinical trials. However, the vaccine was found to not be effective in this last phase of trials (Stolley 2009, Shah 2006). Several other drugs have also been investigated under clinical trials, but all have resulted in the same conclusion.

Antiretroviral Medications

Despite the incapability of researchers to create an effective vaccine, other types of medications have been developed over the past 15 years. Further investigations revolved around the structure of HIV and the mechanisms of replication and transmission. Knowing the method of replication could then be targeted to inhibit the virus. Since the mechanisms for virus RNA replication differ in many aspects from

human DNA replication, these processes can theoretically be inhibited in the human body without undesirable effects to the patient.

The resulting medications are not cures, but rather suppressors of the human immunodeficiency virus replication process. They have been proved an effective treatment to delay the advancement of AIDS and create longer and healthier lives for AIDS patients.

Since HIV is a retrovirus, it utilizes unique enzymes that are not found in humans. Retroviruses' common features can be inhibited to prevent the advancement of AIDS. HIV specifically utilizes four crucial enzymes in this lifecycle: reverse transcriptase (RT), Ribonuclease-H (RNAse-H) within the RT, integrase (IN), and protease (PR) (Alcaro 2011). Most FDA approved AIDS medications inhibit one of these enzymes, and they can be conveniently classified into categories by the type of enzyme it inhibits. These four enzymes have been primary targets in past and present research on therapies to inhibit viral growth.

At present, there are over two dozen antiretroviral (ARV) medications, each targeting a different step the process of virus replication (Adamson 2010). Antiviral drugs are a relatively new invention; in the early 1980s, very few effective treatments had been approved for viruses, and none were HIV applicable. The first HIV-specific drug approved by the FDA as a treatment of the retrovirus was AZT, or Retrovir, licensed in 1987 (De Bethune 2010). The drug was introduced within a three years after research into HIV treatment started, but clinically AZT prolonged patients' lives for only 6 to 18 months and clearly was a short-term solution (De Bethune 2010; Condon 2008). Because of HIV's rapid mutation rate, patients soon became intolerant to the medication.

Furthermore, the drug was cumbersome, as it required 12 pills a day taken at 4hr intervals, was quite expensive, and could produce dangerous side effects. The discovery of another inhibitor, Saquinair a protease inhibitor changed the protocol of HIV treatment. Clincians found that when both medications were taken together, the virus was much less likely to mutate around both inhibitors. Consequently, a therapy of drugs, called HAART, "highly active antiretroviral therapy", became more common starting in late 1990s (Waters 2007) This therapy constituted a combination of drugs taken at the same time, and proved more effective against mutation in HIV strands. Since its implementation, HAART remains the conventional treatment for AIDS patients. Because of HAART, there is not a specific single drug that is considered best, but rather a mixture of drugs that differ based on the patient's need. The many other types and categories of medications that have been developed under the umbrella of HAART medications will be discussed in further chapters.

Specifically, NNRTIs, non-nucleoside reverse transcriptase inhibitors started to be developed in 1990. By 1996 the first NNRTI treatment was introduced to HIV patients, a compound called Nevirapine, known commercially as Viramune. This compound allowed patients to receive the drugs in less rigorous regimens and with less risk involved than with AZT (Merluzzi et al. 1990; Waters 2007).

This first NNRTI was initially thought to be and was developed as a NRTI, but soon was discovered to have different properties than other NRTIs (Proudfoot et al. 1993). Nucleoside Reverse Transcriptase Inhibitors (NRTIs), not to be confused with NNRTIs, are artificial nucleosides or nucleotides that are analogs of natural deoxynucleotides. Both compounds inhibit the Reverse Transcriptase (RT) enzyme in

HIV. The RT enzyme in HIV is a type of DNA polymerase that transcribes single stranded RNA into single stranded DNA (Named “reverse” since DNA polymerase generally transcribes DNA into RNA). In the regular development of HIV, the virus would use its RT to produce viral DNA, which would then be incorporated into the host cell DNA. The host would then transcribe and translate the viral DNA into proteins which are usable by the human immunodeficiency virus. When a NRTI is incorporated into the DNA sequence, however, the host cell is no longer able to translate the HIV DNA sequence, even if it is incorporated into the cell. Consequently, the HIV DNA is terminated, and the replication of HIV is inhibited. NRTIs work by binding on the catalytic site of the RTs, thus being competitive inhibitors of the natural substrates, dNTPs.

On the other hand, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are composed of a diverse group of chemical structures. While NRTIs describe as few specific types of molecules, NNRTIs simply describe any other molecule which can be used to inhibit reverse transcriptase. NNRTIs are named as such because they are composed of structures other than analogs of dNTPs, therefore being “non nucleoside”. Because of the nature of NNRTIs, whereas NRTI’s must be converted metabolically by cell kinases, NNRTIs do not require conversion. NNRTIs are chemically diverse with over 50 classes of molecules (De Bethune 2010), but their universal feature is their ability to induce a conformational change in reverse transcriptase, which inhibits the catalytic activities of RT. They also generally share significant hydrophobicity, aromatic structure and small size (Parniak 2000). Being non-competitive inhibitors and uncompetitive

inhibitors, NNRTIs work even while the substrate (RNA) is in the RT enzyme. Another positive aspect of NNRTIs is their high specificity for HIV-1, which makes them more effective against HIV, but also less effective against other retroviruses.

CHAPTER TWO

The Function of Non-Nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside reverse transcriptase inhibitors were developed from the class of HIV inhibitors called Nucleoside reverse transcriptase inhibitors NRTIs, or the variant nucleotide reverse transcriptase inhibitors NtRTIs. The first NRTI, Zidovudine or AZT, was developed in the late 1980s. From this has stemmed a wide variety of inhibitors, not limited to RT inhibitors. Among these are protease inhibitors, ribonuclease inhibitors, and integrase inhibitors.

It should be noted here that almost all developed HIV drugs are targeted toward one type of HIV. There are two known types of HIV, HIV-1 and HIV-2. Both strains can lead to AIDS; however, HIV-2 is slower acting and a rarer form. HIV-1 is more common in North America and is more frequently targeted in developing treatment options. Since these viruses differ in the content of their genetic material, treatments are specific for one type of virus only. The treatments described subsequently are intended for HIV-1 only.

Essential Components of the Replication Process

The enzymes and proteins involved in HIV replication have features which make them specific to HIV-1 and different from eukaryotic proteins. These enzymes are essential to HIV replication. Reverse transcriptase, protease, and integrase are some of the enzymes which play a part in replicating HIV RNA. When a retrovirus enters a host cell, it uses the reverse transcriptase enzyme to generate DNA from the complementary single RNA that the virus brings. Once this DNA has been transcribed, the enzyme

integrase inserts the viral DNA into the host's existing DNA. Consequently, the host cell translates this viral DNA into viral proteins needed for the production of more viruses. At the end of this cycle, the viral enzyme protease cleaves the newly produced viral proteins from the host proteins, releasing them for future HIV molecules.

There are several proteins which are also vital to HIV replication and have proven important in AIDS research. Among the many proteins of HIV, proteins involved in the entry (fusion) of the virus into the T-cell have thus far been the most significant of the studied-proteins. One such HIV surface protein discovered is gp120. This protein interacts with T-cell membrane proteins, specifically CD4, CCR5 and CXCR4. Gp120 initially binds to the CD4 receptor on the T-cell, and induces a conformational change, exposing the gp120 subunit, gp41. Gp120 then binds to the cofactor CCR5 or CXCR4. Gp41 is exposed in the complex at this point, and approximates the T-cell and HIV membrane, starting the fusion process. Once the HIV and the T-cell have fused, the virus can enter the cell and begin the processes of replication, insertion and translation etc.

Inhibitors of HIV

The above enzymes and proteins have played a role in research of HIV treatment. Each enzyme or process has been inhibited by a class of anti-HIV drugs. Protease inhibitors (PIs) are a large class of HAART drugs. By inhibiting the protease enzyme, the virus is not able to package its proteins into further viral capsids for dispersion. PIs bind to the protease enzyme and prevent the protease from binding to viral proteins to begin proteolysis. There are several compounds discovered which act as inhibitors, such as ritonavir or darunavir. Protease inhibitors have been marketed since the mid 1990s starting with saquinavir mesylate (SQV) in 1995. There are currently eleven FDA

approved protease inhibitors, and they are part of the HAART regimen, along with NNRTIs.

Similarly, integrase inhibitors work by binding to the integrase enzyme and preventing viral DNA from being inserted into the host cell genome. There is currently one integrase inhibitor in use, raltegravir. In addition to these enzyme inhibitors, fusion and entry proteins have been targeted in viral inhibition. This has resulted in two inhibitors, the entry inhibitor maraviroc and the fusion inhibitor enfuvirtide.

Clearly, there is a large variety of inhibitors in existence today, with over FDA approved 30 drugs currently available for treatment of HIV. However, the majority of HIV inhibitors fall under the category of reverse-transcriptase-inhibitors, with 19 NNRTIs or N(t)RTIs.

The Reverse Transcriptase Enzyme

The structure and role of reverse transcriptase have been heavily studied to generate viable transcriptase inhibitors (De Bethune 2010; La Regina 2010). Several RT structures have been studied and "reconstructed" in a three-dimensional form in order to better understand the enzyme's process (Esnouf et al. 1995). The HIV-1 reverse transcriptase enzyme is an asymmetric heterodimer, made of a p66 subunit consisting of 560 amino acids, and a p51 subunit of 440 amino acids. Both dimers are coded by the same gene, called the Pol gene (De Bethune 2010). Consequently, they are the same except that the p66 includes an extra domain of the RNaseH.

While both units are essential to transcription, the p51 is rather compact and is thought to play more of a structural role, while the p66 retains an open position and contains the catalytic domain (Abbink 2000). The last 120 amino acids of the p66 subunit

compromise RNAseH, the enzyme needed to degrade RNA after its transcription. The p66 unit of RT is often compared to a right hand with different portions representing the thumb, palm and fingers (Alcaro 2011). The catalytic domain is in the center of these three structures, near the "palm". The whole transcription complex is formed when ribonucleic acid passes along the palm, with the thumb and finger section folding down on the RNA.

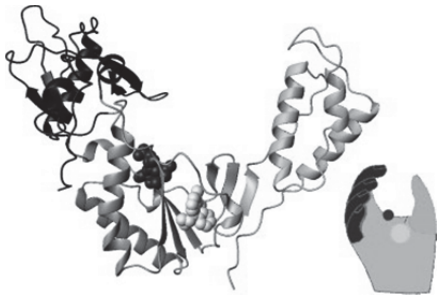

Figure 2
Structure of the Reverse Transcriptase Enzyme
(De Bethune, 2010)

The virus contains a small amount of RNA already inside its protein coat. When a retrovirus such as HIV enters a cell, it uses its RT enzyme to convert the viral RNA into proviral DNA, which then is inserted into the host. The method in which HIV RT transcribes is complex and a multistep process. In the first step, a specific site on the viral RNA, called the primer binding site (PBS), binds to a special t-RNA primer. This tRNA is called $tRNA^{Lys3}$ (Abbink 2000). The RT recognizes this RNA-RNA complex and starts the production of DNA on the 3' end using the viral RNA as a template. This new DNA strand is termed the –DNA. In a several step process, the –DNA is fully transcribed, and the RNA bound to the DNA is degraded. Then, a four step process RT catalyzes the production of another DNA strand, this time with the –DNA as a template. The new

DNA is termed +DNA, and these two strands then form a double stranded helix which will be incorporated in the host cell DNA.

Inhibitors of Reverse Transcriptase

Reverse transcriptase inhibitor drugs work on this complex by binding to a hydrophobic site at a few amino acids (10 angstroms) away from the catalytic binding site (Tucker 2008). The NNRTI then induces a conformational change in the enzyme which reduces catalytic activity. Since NNRTIs are noncompetitive or uncompetitive, they can bind to the p66 complex even while it transcribing, making NNRTIs a unique and effective method of inhibiting reverse transcriptase. NRTIs, or nucleoside reverse transcriptase inhibitors, the other class of RT inhibitors, work by combining an analog nucleoside into the newly processed DNA. This prevents the viral DNA from being translated and transcribed into viral proteins. There are eleven NRTIs that are currently used in the treatment of HIV infection. Among these is the first antiviral medication discovered, Azidothymidine, in 1987.

In research of novel inhibitors, a variety of compounds considered potential NNRTIs are assessed according to their potency for further trials and eventual marketability. NNRTIs have an advantage over other inhibitors in that they are naturally minimally toxic (Das et al. 2004). While many compounds may initially appear to be an effective NNRTI in vitro, the results in vivo often differ. There are requirements that researches seek in anti-viral drugs. The compound must have antiviral activity against wild-type and mutant viruses, high oral bioavailability, minimal side effects, and a relative ease of synthesis (Janssen et al. 2005). While there are a plethora of possible

NNRTIs, many have high-risk side effects or have differing responses in-vivo. Nonetheless, six drugs have been successfully approved to treat patients and are currently available.

The Progression of Drug Discovery

The first three NNRTIs discovered were approved for treatment of HIV-1 infection within a period of two years. These drugs are Nevirapine, Delavirdine, and Efavirenz. Nevirapine, NVP (brand name Viramune), was the first NNRTI approved in 1996 by the FDA. The drug, BI-RG-587, is a derivative of dipyridodiazepinones (Merluzzi et al. 1990). Produced by Boehringer Ingelheim, Nevirapine was used in combination with NRTIs to improve virus inhibition. Nevirapine was found to be highly effective even when taken orally, an improvement from NRTIs (Parniak 2000). In vivo and vitro, HIV was able to mutate and resist the drug within a month (La Regina 2010). A new extended-release version of Nevirapine was recently approved in 2011, allowing for simpler regimes. Nevirapine remains in use yet today in HAART treatments.

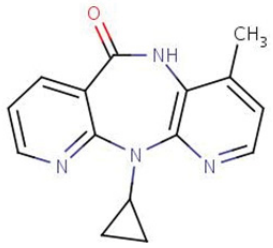

Figure 3
Chemical Structure of Nevirapine
(The US Department of Health and Human Services 2013)

Delavirdine, called Rescriptor by brand name, was discovered in 1993 and approved in 1997 by the FDA. Researchers in the Upjohn Laboratories and Mount Sinai

Medical Center discovered the potency of this compound against HIV RT(Romero 1993). Derived from the antiviral candidate atevirdine mesylate, Delaviridine contains some important modifications to improve antiviral potency from its precursors. Notably, it has an indole moiety, alterations in the central linker and the substitution of aminopyridine (Romero et al. 1993).

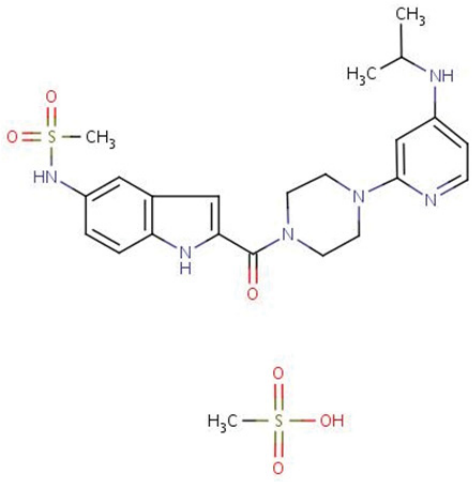

Figure 4
Chemical Structure of Delavirdine
(The US Department of Health and Human Services 2013)

Delavirdine falls under the category of bis(heteroaryl)-piperazine (BHAP) derivatives. The drug has a potency similar to Nevirapine, but additionally, researchers were interested to discover that the compound also heightened sensitivity of the reverse transcriptase to Nevirapine in resistant strains of RT (Dueweke 1993). Interestingly, while a feature of its metabolism in vivo causes it to lose efficacy, it also seems to raise levels of protease inhibitors (Parniak 2000). When used with conjunction with PIs, Delavirdine can heavily improve overall treatments. As with Neverapine, however, Delavirdine can easily lose potency when mutations arise in HIV nucleic acid like other BHAP compounds(La Regina 2009).

Efavirenz was approved a year after delavirdine as another potent NNRTI. Also known as L-743,726 (DMP-266) or commercially as Sustiva, Efavirenz is a benzoxazinone, a noncompetitive inhibitor, with excellent potency against HIV-1 wild-type and various mutant reverse transcriptases (Young et al. 1995). This drug came from a new class of NNRTIs, the 1,4-dihydro-2H-3,1-benzoxazin-2-ones.

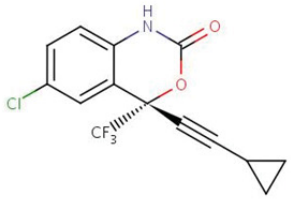

Figure 5
Chemical Structure of Efavirenz
(The US Department of Health and Human Services 2013)

Efavirenz was found to have excellent bioavailability. However the drug is less potent when taken orally and can produces moderate side effects especially to the CNS, frequently headaches or insomnia, or maculopapular rash which diminish the number of patients who can receive the medication (Adkins and Noble 1998). HIV is less likely to mutate and become resistant to Efavirenz, and so the drug is useful for patients who have HIV strands which are already resistant to Nevirapine and Delavirdine. Still, on occasion HIV can produce a single mutation with confers high resistance to Efavirenz. Like other anti-viral drugs, Efaverinz shows synergistic inhibition when paired with other anti-viral medications, such as protease inhibitors (Adkins and Noble 1998).

After the approval of Efavirenz in 1998, no new NNRTIs were approved until ten years later, in 2008. Etravirine, or TMC125-R165335, was discovered in 2004, as a possible NNRTI candidate. The drug was developed from imidoyl thiourea (ITU),

diaryltriazines (DATA) and diarylpyrimidine (DAPY) analogs, with Etravirine being a DAPY analogue. (Chemical formula of 4-[[6-amino-5-bromo-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy]-3,5-dimethylbenzonitrile). DAPY derivatives have a natural advantage in that they can bind in two confirmations of the RT, they have torsional flexibility, and they are compact and can easily reorient within the NNRTI-binding-pocket (Das et al. 2004). This makes them adaptable inhibitors against mutant RTs, and flexible enough to be slightly effective against HIV-2 as well as effective against HI-1 (Das et al. 2004).

Figure 6
Chemical Structure of Etravirine
(The US Department of Health and Human Services 2013)

Etravirine is also generally more potent than its precursors Nevirapine and Delavirdine. (Andries et al. 2004). In many ways, Etravirine showed similar properties as Efavirenz. In initial in-vitro research, the drug also showed lower susceptibility to mutant RTs, needing several mutations of HIV to decrease RT susceptibility to Etravirine. This discovery was especially significant because certain mutations in the RT enzyme could render all NNRTIs ineffective, otherwise known as cross resistance. Etravirine seemed to hold potency in mutant-resistant reverse transcriptase resistant. (Schiller & Youssef-Bessler 2009; Andries et al. 2004).

Rilpivirine was approved as a NNRTI in 2011, since being researched since 2004. Rilpivirine is also known as R278474 and commercially as Edurant. Diaryltriazines (DATA) and Diarylpyrimidines (DAPY) were studied in an effort to develop a potent NNRTI, since these compounds have high potency in T cell cultures. The result was the finding of Rilpivirine, from the DAPY class of anti-HIV compounds with the E-isomer of 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]]amino]-2-pyrimidinyl]amino-benzonitrile (Janssen et al. 2005; Herrewege et al. 2004). Rilpivirine, like its predecessors, was chosen because of its high affectivity in vitro to inhibit against mutant RTs as well as wild-type RTs The antiviral and immunosuppressive activity ranged at 0.2-12 EC_{50} nM in the first study of the compound (Herrewege et al. 2004).

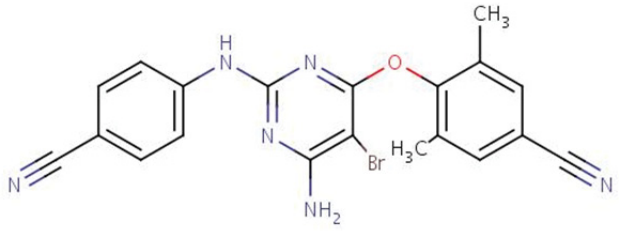

Figure 7
The Chemical Structure of Rilpivirine
(The US Department of Health and Human Services 2013)

Many of these NNRTI and NRTI compounds are helpful and have advanced medical treatment of patients infected with HIV. Unfortunately, as mentioned, they were found to lose potency when HIV mutations bypassed the inhibition of reverse transcriptase. These drugs had shown the most resistance to HIV mutation, however and are more able to inhibit HIV than many other possible options.

The constant mutation of HIV allows it to escape many treatment options by mutating to a drug-resistant strain. The HIV replication cycle has three factors which stimulate mutation: very high replication rate, a transcriptase lacking exonuclease proof-reading ability, and a viral population that is highly heterogeneous. In short, an affected individual is likely to hold many versions of HIV at any given time. Furthermore, patients who contained a strand of drug-resistant HIV could pass it to others. It is estimated that around one-fourth of HIV infected individuals are treatment-naïve with resistance to medication: these individuals have never received a HIV drug but have a strand which is resistant to one or more medications (Stolley 2009). Among the entire spectrum of HIV inhibitors, more development was needed in order to treat HIV extensively. As mentioned earlier, the advancing of HAART has greatly reduced the number of cases where HIV is able to mutate and replicate. Nevertheless, continued study remains to develop drugs which potencies remain unaffected by HIV mutations.

Since the early 1990s researchers have worked to develop NNRTIs that function despite RT mutation. A common strategy is the improvement of current NNRTIs by adding moieties or substitutions. These NNRTIs are called second-generation NNRTIs. The second- generation drugs worked slightly better than the previous generation, and in conjunction with other drugs, they prove more effective. Some of the drugs discussed above are among this class of drugs, for example efavirenz.

As HIV mutates in resistance to treatments, more and more classes of NNRTIs are being developed to inhibit HIV, up to the fourth generation. NNRTIs have become a foundation in HIV-1 treatment because of their effectiveness over other ARVs (De

Bethune 2010). However, while their treatment is useful, it is still often necessary to combine different categories of ARVs in order to keep the virus at bay. These treatments work to prevent the spread of the virus much longer than past treatments, heightening the quality of life for AIDS patients.

CHAPTER THREE

The Efficacy of Non-nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside reverse transcriptase inhibitor based regimens have demonstrated virologic potency and durability over the fifteen years of NNRTIs. Still, the theoretical potency and efficacy of non-nucleoside reverse transcriptase inhibitors are important only if they also produce desired results clinically in patients. Many anti-retroviral medications are expensive and find little usefulness in underdeveloped countries. As a whole, however, NNRTIs are comparatively inexpensive than other ART costs, ranging from 300-600 dollar monthly (Stolley & Glass 2009). Some NNRTIs are ineffective in certain populations because of the prevalence of NNRT-resistant viral strains in HAART-naïve patients. Consequently, the functional role of NNRTIs for HIV infected individuals in developed or undeveloped countries often vary. Also, like any sort of medication, side effects and drug interactions must be considered. These factors play a role in the determination of NNRTIs utility and preferential use among HIV-1 positive individuals.

Combination Drug Therapy

Because of the rapid mutation rate of HIV, any single anti-HIV drug must be used in conjunction with others in order to reduce the likelihood of producing mutant HIV-inhibitor resistant strains. NNRTIs are just one weapon in the arsenal of anti-HIV drugs to deter HIV growth an individual patient. Generally, one NNRTI is used in combination with one or two other antiviral drugs in HAART, (Highly Active Antiviral Retrovirus Therapy).

The regimens (or groups of drugs) that are recommended for patients in the United States differ depending on whether the patient is therapy-naïve or not. Treatments also vary depending on the patient's CD4 cell count, (measuring the progression of the disease), at the time of diagnosis. In certain cases, clinicians can prescribe a variety of regimens; in other cases, such as in the case of nursing mothers, only a few regimens that can be considered.

The HHS panel on Antiretroviral Guidelines includes one "preferred" NNRTI-based regimen for antiretroviral therapy (ART) naïve patients. The preferred regimens score high in "optimal and durable efficacy, favorable tolerability and toxicity profile, and ease of use." (HHS Panel 2012; Thompson 2012) Current guidelines for caregivers list the preferred regimens, and treatment-naïve patients are likely to receive a preferred regimen unless exceptions such as viral-resistance are present. There are currently three preferred regimens, with the combination of ART drugs Efavirenz (EFV), Tenofovir disoproxil fumarate (TDF), and Emtricitabine (FTC) is a preferred NNRTI regimen (HHS Panel 2012).

Efavirenz is a well-studied NNRTI, while Tenofovir disoproxil and Emtricitabine are NRTIs that are commonly used as a backbone to HAART regimens. The combination can be taken separately through individual pills or together in a single tablet (brand name Atripa) with the same bioequivalence (Mathias et al. 2007). Single-tablet regimens are fairly new and improve the quality of life for the HIV- infected individual. As a whole, this combination of ARTs shows many benefits and the first treatment given to 80-90% of newly diagnosed HIV patients (Edelman et al. 2012). Mainly, the regimen is highly preferred because of the tested efficacy in treatment naïve patients and the synergistic

anti-HIV activity in vitro (Sax et al. 2012; Feng et al. 2009; DeJesus et al. 2009). Versus the multi-pill regimen, the single-tablet regimen has shown more cost-effectiveness and lower healthcare costs, as much as 20% cost benefit at six months of treatment (Beck et al. 2012; Colombo 2013; Blasco et al. 2012). Studies have shown better adherence and quality of life correlated with the single-tablet regimen (Colombo 2013). Furthermore, patients on this regimen are less likely to have to change treatment plans because of toxicity, adherence or treatment failure; the regimen displays high safety and low risk (Jarrin 2013; Campbell et al. 2012). In contrast to another common preferred regimen, with the protease inhibitor atazanavir/ritonavir replacing the NNRTI, EFV, the EFV/TDF/FTC regimen was more likely to achieve virologic suppression in treatment naïve patients (Taniquichi et al. 2013, Hodder 2010). On the other hand, other studies have found EVG/FTC/TDF, an INSTI-based regimen to have more rapid efficacy (Cohen et al. 2011).

Alternatively, a patient may be recommended one of three other common regimens, although not quite as advantageous as the preferred regimen. Efavirenz (EFV), abacavir (ABC), and lamivudine (3TC) in one regimen, are associated more virologic failure than in the preferred regimen (Sax 2011). The second regimen is similar: rilpivirine along with abacavir and lamivudine. Rilpivirine, tenofovir disoproxil fumarate, and emtricitabine are combined in the third last alternative regimen.

Among all NNRTIs, Delavirdine and Etravirine are noticeably not recommended in any first-line treatment. Delavirine has shown high anti-viral activity in triple-drug therapy (Tran 2001). But, Delavirdine also shows a large interpatient variability, and the non-linear pharmacokinetics are significantly altered by concurrent food intake (Smith

2005; Tran et al. 2001). Delavirdine also requires high dosing frequency and is more likely than other NNRTIs to produce a rash (Carr & Cooper 2000). Etravirine shows high barrier to mutation, patient safety, and is promising for future regimen combinations, but research in larger clinical trials is still pending (Montaner 2008; Schiller 2009; Lazzarin et al. 2007).

Advantages and Disadvantages of Therapies

Even within the 'preferred' category of regimens, side effects are invariable. These are not uniform for all patients, and often the side-effects will subside after a period of time. The most commonly used NNRTI, Efavirenz, may cause adverse CNS effects in 40-50% of patients (Clifford 2005). Symptoms such as abnormal dreams, dizziness, headache and depression are often associated with EFV use (Haas 2004; Clifford 2005). These EFV-induced symptoms usually decline in patients within four weeks. On the other hand, those treated with a PI-based ART which also leads to neuropsychiatric disturbances, do not decline over time (Hawkins et al. 2005).

It is still contested as to whether EFV is safe for use in pregnant women, Generally, EFV is not begun on women who are pregnant or planning to become pregnant soon after EFV treatment t (Nirogi et al. 2012). Efavirenz is still highly studied in pregnant women to access the effects of the pharmokinetics and risks of birth associated with Efavirenz. The drug has been linked to high fetal and amniotic fluid exposure levels (Nirogi et al. 2012). In non-human primates studies, EFV at drug exposure levels similar to humans cause major congenital anomalies in the central nervous system of nonhuman primates, and first trimester use of efavirenz has been linked to reports of congenital neural tube defects (Cressey 2012; Pillay et al. 2012;

Cadman 1998) . There is no conclusive evidence that these damages transfer to human, however. There are a number of current studies with findings of no increased risk of unfavorable pregnancy in humans (Cressey et al. 2012; Ekouevi 2011; Bera et al. 2010).

Rilpivirine (RPV) is similar to efavirenz (EFV) in many ways, with high efficacy, tolerability, and in some studies, safety even greater than EFV , making RPV a very probable preferred regimen in the future (Thompson 2012; Cohen et al. 2012; Cohen & Andrade-Villanueva 2011; Wilkin et al. 2012). As a drug, RPV is formulated as an individual tablet and as a fixed-dose combination tablet as RPV/TDF/FTC with bioequivalence. However, virologic failure, or lack of efficacy, occurs more frequently in treatment-naïve patients with a high HIV count receiving RPV than patients receiving EFV (Cohen et al. 2013; Cohen & Andrade-Villanueva 2011) . Also, subjects with virologic failure on RPV were more likely to have genotypic resistance to other NNRTIs, and TDF or 3TC/FTC genotypic resistance, which makes further treatment more difficult (Rimsky et al. 2012). RPV has similar side effects to EFV, such as rash and central nervous system effects, but side effects are less common in RPV than EFV (Sharma & Saravolatz 2013). There are a number of limiting factors to Rilpivirine, such as beginning the therapy before a patient reaches higher baseline viral loads, and t

he risk of coadministration of other drugs (Sharma & Saravolatz 2013). The bioavailability of RPV is significantly reduced in acid-lowering agents, such as antacids, so RPV cannot be coadministered with antacids and H2-receptor antagonists, or proton pump inhibitors (James et al. 2012).

Nevirapine (NVP) is the third NNRTI that is recommended as an initial NNRTI. NVP is only recommended as an alternative, or substitute, to other combinations of

regimens. While NVP is not the first choice of treatment, there are a number of reasons which makes NVP more beneficial to a treat a HIV-infected individual than EVP. These extra-factors could include pregnancy, other drug interactions, a resistant-viral strain, toxicity, or side effects of other NNRTIs . NVP is well-tolerated in most studies with fair efficacy with low risk for side effects and combination drug interactions (Harris et al. 1998). Unlike RPV, NVP can be administered to patients with a high baseline viral load, or with high numbers of viral strains (Van Leth 2005). NVP is also tolerable among pregnant women and is not linked to birth defects as is EFV (Mirochnick 1998). Because of its low-risk even during pregnancy, NVP is often used to prevent mother-to-child transmission of HIV-1 (Eshleman et al. 2001).

Despite the positive effects and tolerability of NVP, the drug is also linked to hepatotoxicity, sometimes severe (Sanne et al. 2005). The hepatic events generally occur within the first few weeks of treatment. The effects of the hepatotoxicity depend on several factors of the individual. The genotype of the treated individual, specifically with mutations of p-glycoprotein are associated with increased or decreased risk (Haas et al. 2006). Patients with hepatitis B or C, often common with HIV infection, are at higher risk for developing toxicity (Bonnet 2002). Higher CD4cell counts, or low body-mass-index have high hepatic side-effect risks. In some studies, the hepatotoxicity associated with NVP has resulted in liver failure (Sanne et al. 2005). Consequently, HIV-infected individuals treated with NPV must be closely monitored for hepatic events. Elevated serum transaminase levels, skin rash, fever or flu-like symptoms are other possible side-effects of NPV.

The Prevention of Mother-to-Child Transmission

Non-nucleoside reverse transcriptase inhibitors play a role in effectively preventing mother-to-child transmission HIV-1. This was eluded to in Nevirapine's role as a prophylaxis. HAART regimens for pregnant mothers are important not only in preventing disease transmission during birth, but also relating to breast-feeding, concerning birth-complications, fetal/infant antiretroviral drug exposure, or birth-defects.

In the absence of treatment, mother-to-child transmission of HIV rates at 20-45% (Olagunju et al. 2012). Transmission can occur during pregnancy, labor, delivery or breastfeeding. However, with antiretroviral drugs, the transmission can reduce to 2%. NNRTIs play a role in preventing transmission and preventing HIV viral load increase during pregnancy. Nevirapine, NNRTI, is fairly commonly used during pregnancy, while other drugs are less advantageous for use in pregnant women. As mentioned earlier, the NNRTI efavirenz tends to be associated with birth-defects (Bera 2010). The NNRTIs Etravirine and Rilpivirine are not established in well-controlled studies of pregnant women (Buckoreelall 2012). Since Delavirdine is not a preferred treatment option for all patients, it is generally not used in treatments for pregnant women either.

Pregnant women in developed countries are likely to continue the use of the HAART regimen throughout pregnancy, which can significantly lower the risk of transmission (Painstil & Andiman 2007). The NRTI Zidovudine regimen, (PACTG 076 regimen) and other HAART wide-spread use can achieve transmission rates as low as 1% (Cooper et al. 2002). Current transmission rates in the United States and developed countries are the result of non-adherence or missed opportunity to receive public health education or treatment (Painstil & Andiman 2007).

In developed countries, the consistent use of HAART, caesarean section and the directed avoidance of breastfeeding has lowered mother-to-child transmission to less than 2%, but these interventions are not possible or available in many underdeveloped countries (UNAIDS 2006).

Nevirapine is prevalent in transmission prevention and as HAART regiments for HIV-1 infected pregnant women. Since the late 1990s, a single-dose of Nevirapine to the mother during labor and the infant soon after birth has been used to prevent HIV-mother-to-child transmission (James 1997). This is typically called single-dose Nevirapine, or sd-NVP. Nevirapine was found to reduce risk of transmission even more than the NRTI Zidovudine, a drug which is also commonly used to prevent vertical transmission (Guay et al. 1999; Jackson et al. 2003). As opposed to a multi-drug regimen during birth, NVP is also highly cost effective in underdeveloped countries where women do not have access to consistent HAART treatment before pregnancy (Marseille 1999). Because of the single-dose treatment strategy, however, women are likely to develop resistant strains of HIV after birth. A study on women who received a single dose of NVP to prevent mother-to-child transmission during birth found that NVP resistant HIV-1 was found in both the women and the child, but that the resistant HIV strands faded over time (Eshleman , Mracna et al. 2001).

Women and infants in undeveloped countries may also receive NVP over longer-periods of time, such as several weeks before pregnancy or after birth for the infant. A recent study showed the effectiveness of this strategy. Women received HAART consistently while the infant received daily NVP for 6 weeks to inhibit any viral growth if

HIV was transmitted and found higher effectivity than just HAART or single dose NVP (Binagwaho 2013).

Transmission of HIV during breastfeeding is a major public health challenge, especially in underdeveloped countries. Risk of transmission during breastfeeding depends on a variety of factors: viral, clinical and genetic factors (Shetty & Maldonado 2013). In developed countries, breastfeeding can be avoided to reduce the risk of HIV transmission. In underdeveloped countries, lack of resources, reduced adherence, or expense of milk-formula increases the likelihood of breastfeeding and the risk of transmission (Pool et al. 2001). Often breastfeeding and retroviral therapy is more feasible than a complete avoidance of breastfeeding. This increases the importance of the NNRTI Nevirapine in reducing transmission in underdeveloped countries (Dabis 2005). The safety and efficacy of Nevirapine for even infant use make it very useful in these regions. The continued use of nevirapine through 12 months or even extended six-weeks has been shown to reduce mortality rates of infants and reduced risk of transmission in underdeveloped countries (Omer 2011).

Because of the possible complications and number of combinations of drugs, HAART is a complex pharmacological regimen for many patients (Johnson et al. 2000). Individuals may be take pills multiple times a day under special conditions; with or without food, separate from other medications, or other specifications that must be strictly adhered to in order to maintain proper treatment. The treatment variables can be vast and complicated, making treatment demanding or challenging for patients in many respects.

CHAPTER FOUR

Medication Adherence, Mutations and Advances in Non-nucleoside Reverse Transcriptase Inhibitors

Adherence

The complexity of HAART often produces variability in a patient's adherence, or consistency in carrying-out the patient-specific HAART regimen. A study in 1998 began groundbreaking research into the complexities of nonadherence, noting the extent to which a majority of patients on antiviral medications were likely to remain adherent to the therapy less than 80% of the time (Eldred et al. 1998). This began the process of researching and understanding how to improve adherence, including the development of simpler drug options. Because of the international-wide effort to stem HIV infections and the newfound importance of adherence in treatment results, a broad set of governmental agencies have sought to improve the general adherence of HIV-positive individuals (Ka'opua & Linsk 2007). Non-adherence shifts clinical-study results and compromises the effectiveness of the therapy (Garcia de Olalla et al. 2002). The optimal regimen adherence that a patient must maintain to receive the benefits of the treatment is 95% (Paterson et al. 200). Adherence below this percentage, increases chances of the development of treatment resistant HIV strands and ART failure (Chesney, 2003). In fact, research points to medication adherence being the strongest predictor of viral suppression (Bangsberg et al. 2000; Paterson et al. 2000). Consequently, NNRTIs efficacy are limited by the adherence of the treated individual.

Besides the obvious difficulties of taking the specified medications under the specified conditions, other difficulties such as treatment-side effects or lifestyle accommodations must be dealt with in order to maintain a regimen for an indefinite period of time. A number of social factors affect adherence, such as income, stable housing, and health care. Demographic characteristics, like race and gender, social support or health beliefs are other possible variables in adherence (Ka'opua & Linsk 2007).

Because of the obvious benefits of adherence as well as the complexity of regimen adherence for patients, recent advances in pharmacology have improved the ease of medication regimens by combining pills and adding slower release tablets. One pill, once a day has been shown to improve adherence in multiple studies (Airoldi et al. 2010; Hodder et al. 2010). The EFV/TDF/FTC single-pill regimen shows higher adherence and persistence, especially for marginalized groups (Taneja et al. 2012; Bangsberg et al. 2010) The introduction of two NNRTIs that can be taken once a day has improved the clinical application of Nevirapine and Rilpivirine. The extended-release formula of Nevirapine was developed recently with the aim of simplifying ART and improving adherence (Yone & Kengne 2012). The extended release has shown similar efficacy and tolerance to the immediate release (Ena et al. 2012). Similarly, the introduction of Rilpivirine, combined with emtricitabine-tenofovir in a single pill, has given this potent NNRTI regimen simplicity (Wainberg 2013). The Tenofovir-emtricitabine-efavirenz regimen can be recommended in limited-resource countries, such as India and Senegal in fixed-dose combinations (Pujari 2008; Landman et al. 2009).

Notable Reverse Transcriptase Mutations

NNRTIs have proven to be useful and effective in Highly Active Antiretroviral Therapy. However, if not combined into a regimen of drugs, NNRTIs are likely to lose efficacy as HIV mutates. Even within a regimen of drugs, there is a possibility of the reverse transcriptase enzyme mutating to confer resistance to NNRTIs, especially if the treatment regimen is not properly adhered to, as discussed in chapter 3. In the advent of the mutation to a resistant-strain of HIV, resistance may spread among a population and render NNRTIs ineffective.

In order to map mutations and discover improved HAART drugs, researches have studied the common mutations that confer resistance to current drugs, including NNRTIs . The development of second, third, and fourth generation NNRTIs, has in part resulted from the discovery of mutations and the consequent selection for drugs that are able to tolerate the most common mutations. The discovery and study of mutation is also important in predicting virologic response in treatment-naïve or treatment-experienced patients. These indicators can be helpful to individualize treatment options and to prevent virological failure clinically. For example, a study on HIV RT mutations studied the impact of mutation on viral load (Shulman et al. 2000). The results showed that with patients on EFV or ADV based therapy; common resistance- mutations could predict a high viral load and low HAART response (Shulman et al. 2000).

There are several well-documented mutations in reverse transcriptase (RT). These mutations generally disrupt the contact of the NNRTI to the binding pocket of RT, or

disrupt the interactions between the two entities. A third possible disruption is to the overall confirmation and size of the binding pocket of the RT. Some mutations create resistance to one NNRTI, while others will cause cross resistance, depending on the similarities of the NNRTIs and the extent of the mutation.

Efavirenz, an older NNRTI, is known to be highly susceptible to mutations that cause resistance. Mutations interfering with Efavirenz were especially frequent in RTs with K103N, V108I and P225H substitutions (Bacheler et al. 2000). The result of these mutations is efavirenz therapy failure in vivo, whether in high or low frequency (Geretti et al. 2009).

The K103N mutation is the most frequent mutation causing NNRTI failure. This mutation has been studied extensively, and directed the discovery of etravirine. The mutation creates cross-resistance in the older NNRTIs, including efavirenz and nevirapine (Lecossier et al. 2005). This mutation is notable especially in single-dose nevirapine treatments, where mothers or infants receive only one dose of nevirapine and are not necessarily followed by HAART or combined with antiretroviral treatments. Documentation of this typical mutation is numerous, both in developed countries or underdeveloped countries, especially south or Sub-Saharan Africa, where single-dose nevirapine therapy is common (Flys et al. 2006; Jackson et al. 2000). In some cases the K103N mutation remains long-term in both women and infants after single-dose nevirapin, one to two years after treatment (Flys et al. 2005; Coovadia et al. 2009). This is significant because the K103N refers cross-resistant, making those individuals susceptible to regimen failure (viral load increase) if given a NNRTI based regiment of HAART. The extent of persistence is still being studied since other research has shown a

significant decrease in the K103N mutation after 6 weeks following single-dose nevirapine (Loubser et al. 2006).

Another common but perhaps less significant mutation is G190A. This mutation affects efavirenz, nevirapine, greatly reducing the efficacy of both NNRTIs (Uhlmann et al. 2004). The mutation causes a change in the inhibitor-binding pocket of RT in HIV (Fan et al. 1996). G190A, however, is not fully advantageous for the HIV. The mutation not only confers resistance to NNRTIs, but also reduces activity in the HIV enzyme RNase H and DNA synthesis, decreasing replication efficiency (Wang et al. 2006).

The newer NNRTIs, etravirine and rilpivirine, have shown better efficacy n vitro, notably, even in the presence of mutations that create inefficacy for other NNRTIs. Notably, Etravirenz is not susceptible to the very common K103N mutation, a mutation which tends to cause cross-resistance among all NNRTIs (Varghese et al. 2009). The efficacy of Etravirenz is also unchanged by the mutation G190A (Xu et al. 2009).Rilpivirine is a little more susceptible than etravirenz to become ineffective to reverse transcriptsae mutations. Some common RT mutations that cause RPV resistance are M184I, E138K, and M184V (Rimsky et al. 2012).

Novel Non-Nucleoside Reverse Transcriptase Inhibitors

Throughout the development of NNRTIs , many discoveries of possible inhibitors have been lost on inefficacy in vivo, high-risk factors, or other clinical results that end in abandonment of the possible NNRTI. These are either a certain group of compounds or a specific molecule. For example, The first-generation NNRTIs tivirapine, a TIBo derivative, and loviride, a spirocyclopropyl derivative were initially found to be active against HIV-1 but were later found ineffective under clinical development, in one

instance (Witvrouw et al. 1999). A very large variety and group of NNRTI candidates have been examined for use and have not been selected as a viable candidate. However, these discoveries have led to study and discovery of second-generation NNRTIs. The second-generation NNRTIs efavirenz, etravirine and rilpivirine have advantages that past NNRTIs do not have, either in tolerability, efficacy, or ease of adherence.

There are currently several ongoing studies on novel NNRTIs that are candidates for ART. Lersivirine is an investigational drug that is undergoing phase II clinical trials but is not yet approved for use by the FDA. The drug is a 3-cyanophenoxpyrazole derived compound that was noted in 2009 to be very promising in vitro, safety and pharmacokinetics (Mowbray 2009). In vivo, it also seems to have high efficacy as well as tolerability (Fatkenheuer 2009). Lersivirine (UK-453,061) also shows promising use in NNRTI-resistant mutants. The new candidate drug binds the RT enzyme in a different interaction than previous NNRTIs, making mutations unproblematic for Lersivirine. The drug is also able to inhibit over 60% of viruses bearing key RT mutations, and has excellent sensitivity (Corbau et al. 2010). Lersivirine also seems to create fewer severe side effects than efavirenz when tested in phase II trials, with common side-effects being headache or nausea (Vernazza et al. 2011).

There are some studies investigating the use of 4,6-diarylpyrimidines and diarylbenzenes as NNRTIs, as reported with similar or improved activity to nevirapine (Ribone et al. 2012). A very recent discovery is the 1,2,4 triazole derivates, which are a series of compounds, have shown three possible NNRTIs with antiviral activity (Lie et al. 2013).

Further Development of Non-Nucleoside Reverse Transcriptase Inhibitors

Non nucleoside reverse transcriptase inhibitors have proven effective and clinically relevant. Efavirenz and nevirapine, as older first-generation or second generation NNRTIs, are still frequently prescribed and are widely used in the inhibition of HIV and in the prevention of vertical transmission. Newer drugs, like etravirine and rilpivirine have shown promising effects in the treatment against HIV-1, as well as high resistance to mutated strains of HIV-1. The better efficacy or tolerability in either drug have allowed patients to receive better, more efficient care. While NNRTIs are very useful today, there is still active research on improved future NNRTIs to lead the way to better treatment options and better HAART opportunities. The continued investigations into the generation of novel NNRTIs have opened the door for new possible NNRTIs as researchers and care-givers continue to improve the fight against HIV.

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