School of Pharmacy

The Characteristics of HIV Patients Who are Long-Term Responders to Nevirapine.

Vijay Seshadri Tenneti

This thesis is presented for the degree of
Master of Pharmacy
of
Curtin University of Technology

September 2007

Declaration

This Thesis contains no material which has been accepted for the award of any other degree or diploma in any other University or institute.

To best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been given.

Signature: ……..

(Vijay Seshadri Tenneti)

Dated:

Abstract

Objective: To define Nevirapine long term responders (NLTRs) and identify the characteristics of patients who stay on the drug for long periods of time without adverse effects and maintain suppression of HIV replication without development of resistance.

Methodology: Demographic and clinical data was collected for all the patients >18 years old from Department of Clinical Immunology, Royal Perth Hospital, Western Australia. All the patients included in the study received Nevirapine as part of their first regimen of anti-retroviral therapy. Data collection and analysis were divided in three groups based on their length of stay on Nevirapine; short-term, medium and long-term responders. Patients who stayed on Nevirapine continuously for more than five years were considered long-term responders (60+ months). Patients who withdraw before six months of therapy due to the drug's side effects or any other reason are considered short-term responders (0-6 months). Patients whose length of stay on the drug is intermediate between these two groups are considered as medium responders (6-60 months). Investigation of the possible genetic influence on response to Nevirapine was made by analysis of genetic markers like HLA-B35+C4, HLA-DR1, and HLA-B14.

Results: We found no difference in the frequencies of HLA-DR1 or HLA- B14 in the three groups. However, we found that HLA-B35+HLA-C4 did not occur in any of the Nevirapine long term responders (NLTRs).This was not described in previous studies. Furthermore, when a survival analysis was performed, carriage of HLA-B35+HLA-C4 identified patients who were not NLTRs.

Conclusion: This study was carried out to determine whether or not factors like age, gender, baseline CD4+ count, baseline RNA, and HLA alleles would predict the length of stay on Nevirapine. Although the factors considered for the study have previously been associated with hyper-sensitivity reactions, they do not predict how long a patient would stay on the drug. However, it can be understood from the study that patients who had a combination of HLA alleles B35+HLA-C4 would not continue on Nevirapine use for a prolonged time.

Acknowledgment

It is my cherished privilege to acknowledge my debt and express my sincere gratitude to all those who have helped me accomplish this humble venture.

Words alone in no way shall amply articulate my deepest gratitude; valuation and indebtedness for my revered supervisors ***Prof. Michael Garlepp*** and ***Prof. Martyn French*** who have been a perpetual source of guidance and unflinching support. Their keen supervision, invaluable suggestions, illuminated vision and persistent encouragement have made this project possible today.

I am complaisantly beholden to ***Mrs. Leanne Haggart*** for her invaluable advice and kind cooperation extended to me during the period of the entire research. Her timely help and cooperation extended to materialize this project work.

I felicitously mark off the enlightening guidance and concrete suggestions rendered gladsomely by ***Mr. Satvinder Dhalival***, statistician, School of Public Health, Curtin University of Technology.

It behoves me to acknowledge the help of all faculty members and administrative staff of School of Pharmacy especially ***Prof. Bruce Sunderland***, Post graduate coordinator; School of Pharmacy, ***Mrs. Daphne D'Souza, Mrs. Charmaine D'Costa, Mrs. Jennifer Ramsay*** and ***Mr. John Hess*** for their active support.

I am deeply grateful to ***Mr. Steven Roberts,*** Lab Technician, Department of Clinical Immunology; Royal Perth Hospital for his manifold help.

I owe a grateful acknowledgement of gratitude to ***Ms Diana Blackwood,*** Senior Librarian, Library Information Services; Curtin University of Technology for providing me with valuable information about library literary sessions and assistance with Endnote.
Special word of thanks for ***Mr. Kailash Singh*** and ***Mr. Gautam Dalwadi,*** for providing me valuable help and sagacious guidance at all times.

Mere words cannot acknowledge my intense feelings of gratitude towards my treasured friends ***Yousuf, Rahul, Deepal, Vinitha, Deepika, Ashraf, Rafi, Dhanushka and Lydia*** for their supportive attitude and ever available help. I am indeed thankful to all my other friends in Perth for making this time memorable, with lots of sweet reminiscences to look back at.

Transcending all this, the unbounded affection, constant inspiration and blessings of my parents cannot be expressed by mere words. With a deep sense of reverence, I acknowledge my parents for their unconditional support and ever encouraging attitude. I owe a special word of thanks towards my younger sister ***Raji,*** for her cheerfulness and encouragement. My final debt is to all dear ones especially my grandmother ***Smt. Mahalakshmamma*** whose unruffled patience, silent encouragement to see me work has eventuated in this denouement.

I wish to dedicate this thesis to my parents who had always sacrificed their pleasures for my education and well being and made this possible for me and also to my supervisor ***Prof. Michael Garlepp*** who initiated this project and has given valuable insights and guidance on the directions of this project and on every decision I made throughout my master's and my life outside the realms of studies.

Above all, I bow my head before the Almighty God with whose grace and beatitude, I moved through this venture.

"MATHRU DEVO BHVA - Worship the Mother as God
PITHRU DEVO BHVA - Worship the Father as God
ACHARYA DEVO BHVA- Worship the preceptor as God"

Abbreviations

HIV	Human Immunodeficiency Virus
FIV	Feline Immunodeficiency Virus
SIV	Simian Immunodeficiency Virus
AIDS	Acquired Immuno Deficiency Syndrome
RPH	Royal Perth Hospital
WA	Western Australia
CNS	Central Nervous System
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitor
NtRTIs	Nucleotide Reverse Transcriptase Inhibitor
PIs	Protease Inhibitors
NVP	Nevirapine
NLTRs	Nevirapine Long Term Responders
DNA	De-oxy Ribo Nucleic Acid
cDNA	Complementary De-oxy Ribo Nucleic Acid
RT	Reverse Transcriptase
BBB	Blood-Brain Barrier
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

MTCT	Mother-To-Child-Transfer
dNTP	Deoxyribonucleotide triphosphate
AUC	Area under the curve
ARV	Anti-Retro viral
CSF	Cerebrospinal fluid
CYP450	Cytochrome P-450
CD	Cluster designation
IL-1A	Interleukin 1-Alpha
Gp	Glycoprotein
FDA	Food and Drug Administration
Th	Helper T cells
HAART	Highly Active Anti-Retroviral Therapy
RNA	Ribonucleic acid
m-RNA	Messenger Ribonucleic acid
MVB	Multivesicular bodies
HLA	Human Leukocyte Antigen
CCR	Chemokine Receptor
CTL	Cytotoxic T lymphocytes
INF-γ	Interferon Gamma
VL	Viral Load

ELISA	Enzyme-Linked ImmunoSorbent Assay
SI	Synctia-Inducing
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
GGT	Gamma Glutamyl Transferase

Table of contents

List of tables

List of figures

1 Introduction

1.1 Human Immunodeficiency Virus

Human Immunodeficiency virus (HIV) belongs to the lenti virus subclass of retroviruses. As with all viruses HIV cannot reproduce on its own and so must infect cells of a living organism in order to replicate[1]. HIV preferentially infects CD4 T- cells causing immune activation, which results in CD4 T-cell dysfunction and depletion. This effect on the immune system results in the development of an immunodeficiency syndrome known as Acquired Immuno Deficiency Syndrome (AIDS). This manifests as a persistent failure of immune cell function over time and intrusion by several different opportunistic agents, the result of which is loss of the ability of the body to fight infection and the subsequent acquisition of diseases. HIV was formerly defined as a non curable infection leading to death from one or more opportunistic infections. In recent times increasing arrays of effective antiretroviral drugs have become available.

1.1.1 HIV and AIDS in the World

Since AIDS was first recognised in the early 1980s, HIV/AIDS has emerged as one of the largest global threats to development and the economic and social stability of developing countries. HIV/AIDS is a global emergency. Already 20 million people, the equivalent of Australia's population, have died as a result of the pandemic and another 38 million currently live with the virus. Around 7.4 million people are living with HIV/AIDS in the Asia-pacific region alone. HIV/AIDS impacts on every level of society. It is one of the greatest threats to the economic and social development, stability, and security of developing countries[2].

The United Nations releases new estimates of the number of people living with HIV infection every year (Table 1-1 and Table 1-2). The number of people affected with the disease continues to rise in spite of the efforts to prevent worldwide HIV infection.

Table 1-1 HIV in World Population[3]

Number of people living with HIV in 2005	Total	40.3 million (36.7-45.3 million)
	Adults	38.8 million (34.5-42.6 million)
	Women	17.5 million (16.2-19.3 million)
	Children under 15 years	2.3 million (2.1-2.8 million)
People newly infected with HIV in 2005	Total	4.9 million (4.3-6.6 million)
	Adults	4.2 million (3.6-5.8 million)
	Children under 15 years	700 000 (630 000-820 000)
AIDS deaths in 2005	Total	3.1 million (2.8-3.6 million)
	Adults	2.6 million (2.3-2.9 million)
	Children under 15 years	570 000 (510 000- 670 000)

Table 1-2 Global Estimates for Adults and Children, End 2005[3]

Regional HIV and AIDS statistics and features, 2003 and 2005				
	Adults and children living with HIV	Adults and children newly infected with HIV	Adult prevalence (%)	Adults and child deaths due to AIDS
Sub-Saharan Africa				
2005	25.8 million	3.2 million	7.2 million	2.4 million
2003	24.9 million	3.0 million	7.3 million	2.1 million
North Africa and Middle East				
2005	510000	67000	0.2	58000
2003	500000	62000	0.2	55000
South and South-East Asia				
2005	7.4 million	990000	0.7	480000
2003	6.5 million	840000	0.6	390000
East Asia				
2005	870000	140000	0.1	41000
2003	690000	100000	0.1	22000
Oceania				
2005	74000	8200	0.5	3600
2003	63000	8900	0.4	2000
Latin America				
2005	1.8 million	200000	0.6	66000
2003	1.6 million	170000	0.6	59000
Caribbean				
2005	300000	30000	1.6	24000
2003	300000	29000	1.6	24000
Eastern Europe and Central Asia				
2005	1.6 million	270000	0.9	62000
2003	1.2 million	270000	0.7	36000
Western and Central Europe				
2005	720000	22000	0.3	12000
2003	700000	20000	0.3	12000
North America				
2005	1.2 million	43000	0.7	18000
2003	1.1 million	43000	0.7	18000
Total				
2005	40.3 million	4.9 million	1.1	3.1 million
2003	37.5 million	4.6 million	1.1	2.8 million

As per the WHO estimations, about 14000 new HIV infections occur worldwide every day and more than 95% of the infections are in the low and middle income counties.

Almost 2000 cases are children under 15 years of age and about 12000 are people aged 15-49 years of whom; almost 50% are women and 50% are 15-24 year olds. Barely about one in five HIV patients receive ARVs in the low- middle income countries[4]. Figure 1-1 gives an overview of use of ARVs in the world.

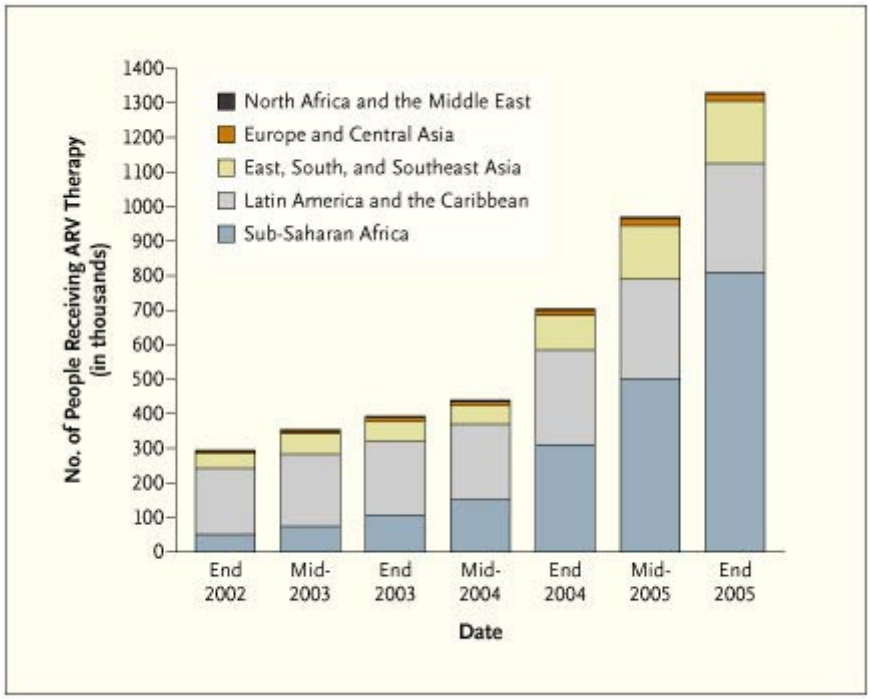


Figure 1-1 Use of ARV drugs in the World[4]

1.1.2 HIV in Australia

Table 1-3 HIV infection in Australia[3]

Estimated Population	20155000
Population Growth Rate	1.1%
Life Expectancy at birth	Male= 83 years Female= 78 years
Number of people living with HIV	16000 (9700-27000)
Adults aged 15-49 HIV prevalence rate	0.1% (<0.2%)
Adults 15 and over living with HIV	16000 (9600-27000)
Women aged 15 and over living with HIV	<1000 (<2000)
Death due to AIDS	<500(<1000)

Citizens from Australia accounted for major diagnosis for AIDS in the past decade. AIDS was diagnosed in 69% during 1996-2000 and 65.3% during 2001-2005 were among Australian born people. Figure 1-2 describes that there was no evidence of change

in rate of new acquired HIV infection in Australian Capital Territory, Western Australia, Tasmania and Northern Territory in the past ten years. However, there was an increase of 20% and 40% in New South Wales and Victoria respectively during the same period of time[5].

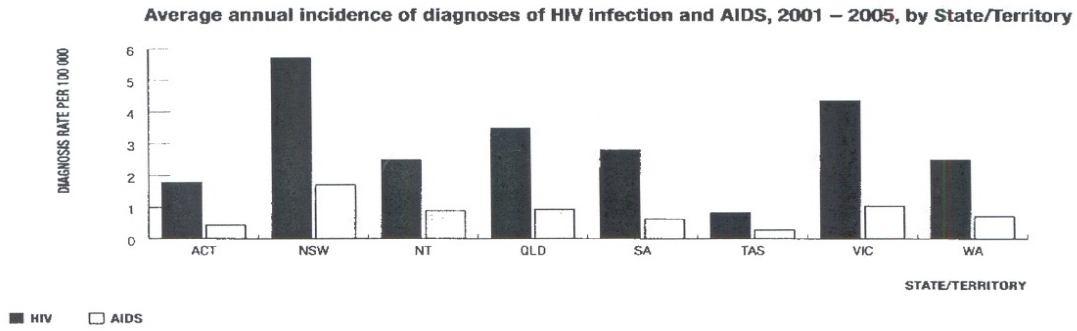


Figure 1-2 Average annual incidence of diagnosis of HIV infection and AIDS, 2001-2005, by State/Territory [5].

1.1.3 Background information for the present study

The development of antiretroviral therapy is the defining medical event in the management of patients with HIV infection that has occurred in the past two decades. Nevirapine a non-nucleoside reverse transcriptase inhibitor has shown confirmed efficacy in the treatment of HIV infection[6]. Licensed antiretroviral drugs have been developed rapidly in the last ten years doing miracles in the area of HIV/AIDS. It is estimated that Nevirapine therapy is an effective clinical and cost-effective option for HIV treatment compared to any other antiretroviral therapy[7] . The high efficacy rate of Nevirapine is due to its wide distribution (readily crosses placenta and BBB) and low lipid profile qualities (increases serum lipids less than other ARVs). But due to Nevirapine induced toxicities; mainly hypersensitivity reactions like liver toxicity, skin rashes, flu like symptoms and fever which occur within the first 4-6 weeks of start of NVP therapy ; and development of resistance much later if the advised treatment regimen is not adhered to, the long term use of the drug is under threat[8]. Nevirapine monotherapy suppresses HIV replication very effectively but resistance occurs rapidly and therefore, Nevirapine must be used in combination with other antiretroviral drugs[9]. Virological failure can occur due to: (a)

emergence of drug resistant virus (b) non adherence to therapy (c) interruptions to therapy[10, 11]. Despite good adherence some patients still experience virological failure, thus giving way to the thought that some other mechanisms are involved[12] . However, some patients also remain on Nevirapine therapy for long periods of time. Studies have already been done to confirm that there exists an association between HLA, IL-1A and immunodeficient patients[12]. A research done by Price et al confirmed that alleles carried at IL1A-889 or IL1A+4845 may predict the control of HIV replication in already immunodeficient patients responding to HAART. Some studies have supported that there exists an association between CCR5 and virological failure[13]. However, some studies oppose this theory stating that no such association occurs[14, 15].

This research aims to identify the characteristics of those patients who stayed on Nevirapine for long periods of time which may lead to the selection of people who might do well on the drug. This knowledge may allow a decision to be made whether Nevirapine should be administered to a patient based on their genetic profile and clinical characteristics, there by preventing unwanted complications of hepatitis or rash which might occur in patients treated with the drug. Hence in the present study genetic markers like HLA, IL-1A were used to investigate any association with response to Nevirapine therapy and HIV patients.

1.1.4 HIV Structure

Outside human cells HIV exists as uneven and globular particles; which are sometimes called virons. The surfaces of the particles are studded with numerous spikes (Figure 1-3). They measure approximately 0.4 microns in diameter and can be viewed using an electron microscope[16].

1.1.5 HIV a Retrovirus

Retroviruses have a double stranded RNA as their genetic material. They make a DNA copy of their RNA in order to replicate[17]. The DNA genes facilitate the process of

replication. An enzyme called reverse transcriptase facilitates this conversion. Once DNA is formed, it gets incorporated into human cells[18].

Lentivirus

Lentiviruses or slow viruses are a class of viruses which are characterized by existing dormant for long periods of time after the initial stages of infection. They take a long time between initial infection and appearance of symptoms. Other viruses belonging to this class are SIV (Simian Immunodeficiency Virus)[19] and FIV (Feline Immunodeficiency Virus)[20].

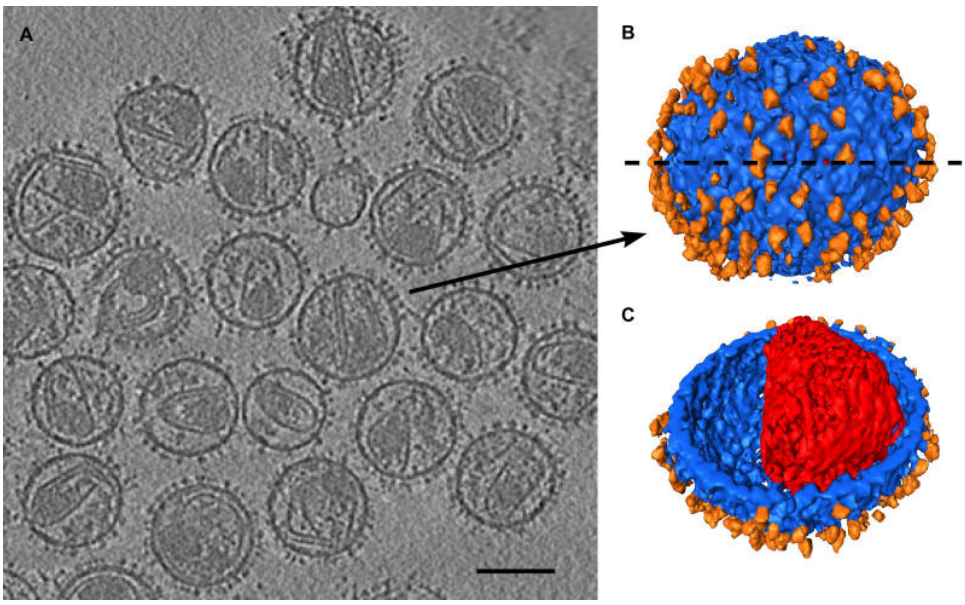


Figure 1-3 Three-Dimensional Reconstruction of HIV Virions from Cryo-Electron Tomography [16]

A) A slice through the xy plane of a reconstructed tomogram from the −6-µm defocus dataset. The spike complexes are clearly visible on the viral surface. Some of the cores appear disrupted, reflecting AT-2 treatment. Scale bar represents 100 nm.

(B) Surface rendering of one of the virions. The membrane is represented in blue, the core in red and the spikes in orange.

(C) The same virus as in (B) viewed after removing half of the viral envelope along the dotted line to reveal the core.

HIV particles are encircled by two layers of coat made of fatty material called viral envelope or viral membrane. From this viral envelope project tiny spikes which are produced from proteins gp120 and gp41[16, 21]. These proteins are derived from viral pre-protein gp 160 splicing. The layer immediately underneath viral envelope is called the matrix and is made from protein p16 (HIV matrix protein). The viral core or capsid is made from protein p24 and is generally bullet shaped (as shown in figure 1-4). The viral core also has a protein called p9 (the HIV nucleocapsid protein) that is not covalently joined to the viral RNA[22]. The three enzymes required for HIV replication are found inside the capsid. They are reverse transcriptase, integrase and protease. HIV's genetic material, which consists of a pair of identical strands of RNA, is also held within the capsid.

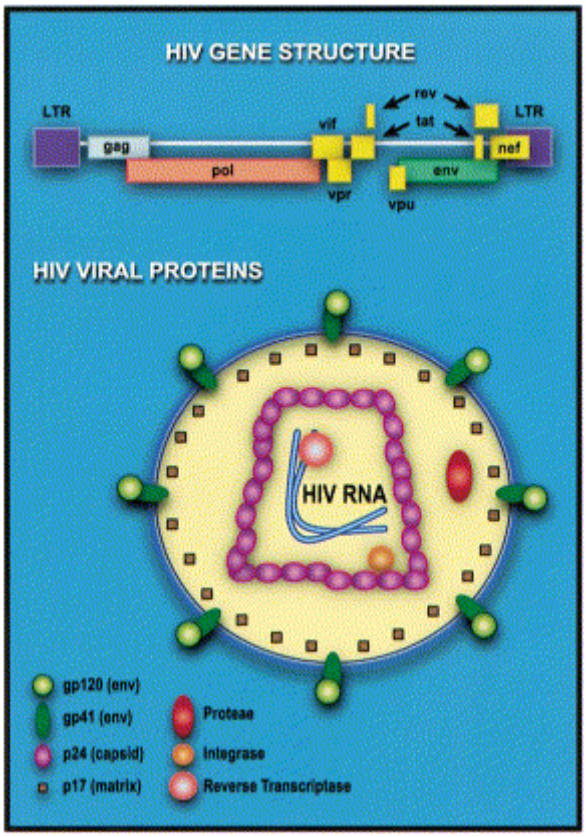


Figure 1-4 HIV viral proteins and gene structure[17]

1.1.6 Genetic Material

Most viruses and almost all organisms store their genetic material on long strands of DNA. Retroviruses store their genetic information on two strands of RNA. HIV's replication process is comparatively more complicated than other viruses due to the presence of RNA. HIV has nine genes viz; gag, pol, env (required to make structural proteins for new virus particles) tat, rev, nef, vif, vpr and vpu (code for proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease)[17]as described in Figure 1-4. A sequence called the long terminal repeat present at either end of each strand of RNA helps in controlling HIV replication (Figure 1-5).

Viral gene transcription is regulated by Tat and Rev and in due course useful for HIV replication. *In vitro* infection potential of HIV is increased by Vif gene as it associates with cytoskeletal elements[17]. Vpu gene plays its role by intervening in the assembly process whereas Vpr gene intervenes with the nuclear transport of viral genome. Nef is the gene with multiple functions including; down regulation of surface CD4 and MHC class I molecules, acceleration of clinical disease, modulation of signal transduction and ultimately favouring HIV entry into target cells by CD4 and chemokine dependant pathway[23]. A clear understanding can be obtained upon observing figure 1-5. An outline of HIV gene products is illustrated in the Table 1-4 below.

Table 1-4 HIV gene products[17]

Structural proteins	**Regulatory proteins**
Viral envelope proteins: gp 41 gp 120 Core proteins: p 24 (Capsid) p 16 (matrix) p 9(nucleocapsid)	Transcription regulators: Tat Rev Other proteins: Vif Vpu Vpr Nef

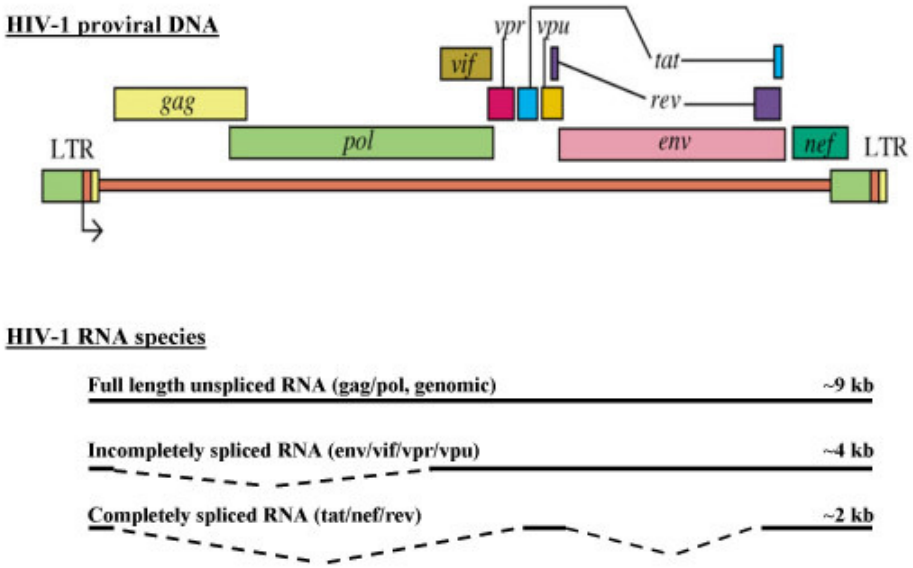


Figure 1-5 Schematic representation of HIV-1 and different RNA species

Gag: group specific antigen, Gag-Pol: group specific antigen-polymerase, Env: envelope, Tat; trans-activator of transcription, Rev: regulator of expression of virion proteins, Nef: negative effector, Vif: virion infectivity factor, Vpr: viral protein r, Vpu: viral protein u, LTR; long terminal repeat [1].

Life Cycle and mechanism of Replication

HIV replication can take place only inside human cells. The life cycle of HIV (Figure 1-7) can be explained through the following steps;

Step 1: HIV entry into human cells- The process of infection begins when a HIV particle comes in contact with a human cell having a surface molecule called cluster designation 4 (CD4)[24-26]. The cells which carry these molecules are called CD4 cells. Glycoprotein gp120 present in the viral envelope allows the virus to bind tightly to CD4 molecules. This binding causes a conformational change in the gp120 permitting it to bind to other molecules on the cell surface called the co-receptors. Later, the viral envelope fuses to the cell membrane, thus allowing the virus to enter the cell[27, 28]. Gp41 also present in the viral envelope plays a vital role in the fusion of virus to the cell (figure 1-7). Cell-to-cell spread of HIV also can occur through the CD4-mediated fusion of an infected cell with an uninfected cell.

Step 2: Reverse Transcription- The replication of HIV is possible only after its genetic RNA is converted to DNA. Enzyme reverse transcriptase present inside the capsid helps in the conversion of viral RNA to viral DNA. Upon entry the HIV capsid releases viral RNA and viral protein in the cytoplasm where the RNA undergoes the process of reverse transcription and results in the formation of complementary DNA (cDNA) by the viral reverse transcriptase enzyme[29]. The enzyme reverse transcriptase incorporates an erroneous nucleotide at every 1500-4000 bases; this results in the emergence of mutations. This phenomenon underlies the development of drug resistant strains in patients receiving anti-retroviral drugs[30].

Step 3: Integration- The viral DNA which is newly formed enters the nucleus of the human cell. Viral cDNA undergoes a process of integration randomly with the host cell genome. This reaction would be catalysed here by the enzyme viral integrase which helps in joining the viral DNA with host DNA. At this stage the viral DNA is called provirus.

Step 4: Transcription- The provirus can produce new viruses only when RNA copies are prepared that can be read by the host cell's protein-making machinery. These RNA copies are called messenger RNA (mRNA) and the process of production of mRNA is called transcription. Following integration, cellular transcription factors activate the viral

gene transcription thereby producing short and multiply sliced mRNA transcripts. These encode the regulatory proteins Tat, Rev and Nef. Tat acts by binding at the 5' end of viral DNA sequence and enhances the process of transcription by 1000 fold[31]. Rev links an RNA structure to the env gene region, thus causing the nuclear export of the incompletely spliced transcription units. HIV replication would get impaired significantly if the effects of Tat and Rev are inhibited[32, 33].

Step 5: Translation- HIV mRNA which is formed in the nucleus is now transferred to the cytoplasm. HIV proteins are critical to this process; for example, a protein encoded by the rev gene allows mRNA encoding HIV structural proteins to be transferred from the nucleus to the cytoplasm. Without the rev protein, structural proteins are not made. In the cytoplasm, the virus co-opts the cell's protein-making machinery including structures called ribosomes to make long chains of viral proteins and enzymes, using HIV mRNA as a template. This process is called translation. Pre-proteins pr55 and Gag-Pol are formed during translation. Gag-Pol gets cleaved to produce viral enzymes like protease, integrase and reverse transcriptase.

Step 6: Assembly and Budding- The mRNAs produce gp 160 which migrates to the cell membranes and is cleaved to form the regulatory proteins; VPr, Vpu, Vif and the envelope proteins gp 41 and gp 120. These HIV core proteins, enzymes and host membrane proteins are incorporated on the plasma membrane to form the viral envelope around the capsid which already contains the viral genomic RNA. The assembly process involves the utilisation of energy which is produced by unidentified cellular factors[34]. This immature viral particle buds off from the cell. At this stage of the viral life cycle, the core of the virus is immature and the virus is however not infectious. The viral enzyme protease cuts the long chains of proteins and enzymes that make up the immature viral core. Vpu induces CD4 degradation and causes the down-regulation of CD4 surface expression[35]. During the budding pathway, binding between the HIV Gag protein and molecules in the cell directs the accumulation of HIV components in special intracellular sacs, called multi-vesicular bodies (MVB), which normally function to carry proteins out of the cell. In this way, HIV actively hitch-hikes out of the cell in the MVB by seizing control over normal cell machinery and mechanisms.

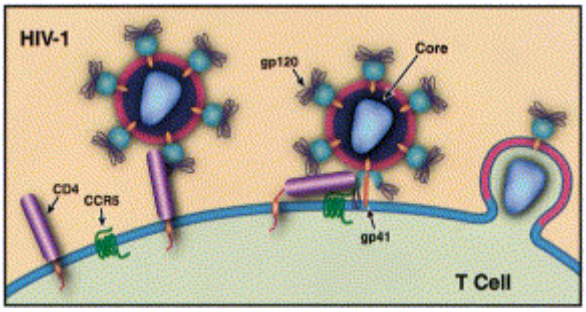


Figure 1-6 HIV entry through lymphocytes and monocytes by cognate recognition of viral glycoprotein 120[17]

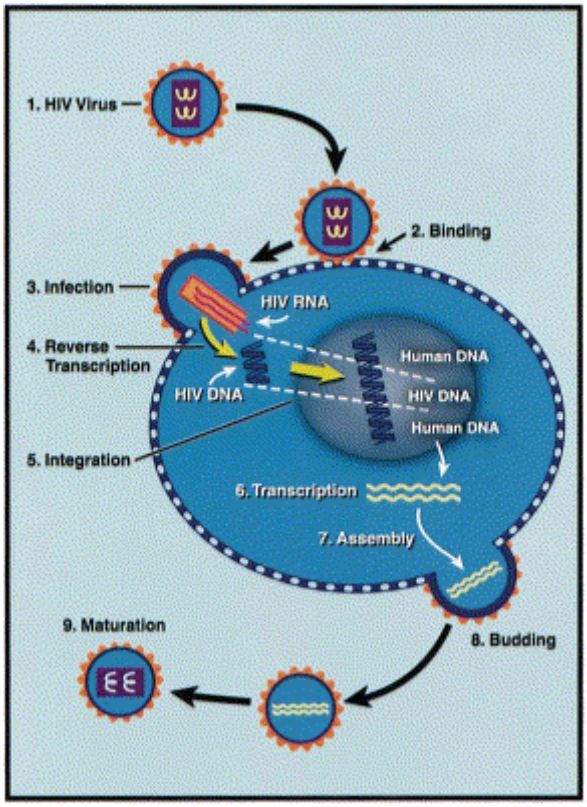


Figure 1-7 HIV replication with all the stages[17]

1.1.7 Pathogenesis of HIV disease

A broad perspective of the complexities of HIV disease has been achieved[36]. The clinical course of HIV infection comprises of the following stages: (a) Viral transmission (b) Primary HIV infection (c) Seroconversion stage d) Asymptomatic chronic infection (d) The symptomatic stage (e) AIDS defining illness (f) Advanced HIV (g) Death.

HIV enters the human body through blood, serum or vaginal secretions. HIV is spread most commonly during sexual intercourse with an infected partner, in case of adults. HIV also can be transmitted by contact with infected blood, most often by the sharing of needles or syringes contaminated with minute quantities of blood containing the virus[37]. Primary infection normally goes unnoticed when blood tests for HIV infection are performed. This may be due to lack of specificity and variable severity of clinical syndrome[38-40]. Mononucleosis like clinical syndrome may be associated with HIV primary infection[40]. 50%-70% of HIV infected patients experience a clinical syndrome of varying severity during primary infection. This can be established by retrospective analysis of patient clinical history[39]. A number of studies have confirmed that virologic and immunologic mechanisms together are associated with the pathogenic mechanisms which consecutively are associated with primary HIV infection[41-44]. During early stages of primary infection, cell-mediated and humoral mediated HIV –specific immune responses would be observed following the peak viremia[45-47]. In addition to these an array of cytotoxic T lymphocytes (CTLs) which are specific for HIV proteins and anti-HIV antibodies would also be detected. Once the virus enters the body, it infects a large number of CD4 cells and replicates rapidly. During this acute or primary phase of infection, the blood contains many viral particles that spread throughout the body, seeding various organs, mainly the gut lymphoid tissue and the lymph nodes.

During seroconversion stage the body fights the HIV antigens by producing antibodies to restore homeostasis. Flu-like symptoms appear in 70% of HIV infected people after 2-4 weeks of exposure to the virus. The human immune system fights back the virus with B-cell produced antibodies and Killer T cells (CD8+ T cells); thereby considerably reducing HIV levels. HIV looks for CD4 molecule with CCR5 and replicates rapidly. Once they find CCR5 they bind to these receptor sites on T-Helper lymphocytes and macrophages.

Macrophages especially in the genital area are rich in CCR5. HIV can be found in lymph after 2 days of infection and in blood after 5 days of infection. After 7 days tests detect the presence of HIV antigens. During early stages of primary infection, cell-mediated and humoral mediated HIV –specific immune responses would be observed following the peak viremia [41, 44, 47] .In addition to these, an assembly of CTLs which are specific for HIV proteins and anti-HIV antibodies would also be detected.

HIV- specific CTLs play a key role in the down regulation of viral replication. The vigorous immune response observed during early primary infection fails to eradicate the virus as the virus gets transformed from primary infection phase to chronic phase. During this chronic phase there would be a down regulation of HIV. However, viral replication would not be curtailed. The progression of HIV primary infection to AIDS would take eight – ten years[48, 49]. During this period of latency, HIV continuously and actively replicates at the lymphoid organs which serve as anatomic sites of replication[50-52]. This confirms the reality that clinical latency is not equivalent to disease latency. The virus may hide within the chromosomes of an infected cell and be shielded from surveillance by the immune system. Antiretroviral drugs attack only the replicating viruses and are not effective against latent reservoirs of the virus. There would be high turnover of CD4+ T cells during this stage where a large number of them are exhausted and restored daily. Latent phase is characterised by progressive loss of CD4+ T cells and persistent replication[50, 53]. Through the correlation of CD4+ T cells and development of clinical symptoms it can be understood that patients with a CD4+ T cell count > 500 cells/µl remain symptom free while patients with <500 cells/µl more frequently associated with appearance of constitutional symptoms. When the count falls below 200 cells/µl, there would be an increased vulnerability to opportunistic infections or neoplasms[54] . Thus, evaluation of the progression of HIV disease can be made based on the CD4+ T cell counts. This period of symptomatic stage of HIV infection is complicated by bacterial, fungal and viral infection like Pneumocystis jiroveci, Cytomegalovirus, Herpes Simplex virus, Mycobacteria, Influenza, Cryptococcus and Candida[55-59].

In the category of AIDS defining and/or advanced stage disease HIV infected individuals would have a CD4+T cell count of < 200 cells/µl. At this stage there would be equilibrium of viral load between peripheral blood and lymph nodes[60]. Profound immunosuppression is the characteristic of advanced stage disease where HIV-specific

cytotoxic activity would have been lost at this point[61]. In this stage, contrasting to the decreased number of CD4 T cells would be an increased number of cytokines such as INF-γ and IL-10[62] . Hence, this reflects that CD8+ T cells and monocytes/macrophages account for most of the mononuclear cells after the exhaustion of CD4+ T cells. Severe immunosuppression due to destruction of lymphoid tissue is the principal mechanism responsible for advanced/late stage HIV disease. Hence with complete depletion of immunity and acquiring life threatening opportunistic infections, the patient progressively sets off towards death[36]. Hence, upon observing the various stages of HIV pathogenesis it can be said that start of HIV therapy should be initiated during primary infection stage when a HIV-specific immune response is likely to be high.

1.2 HIV Testing

HIV testing is done in two phases. The first phase is diagnosis and monitoring viral load is the second phase. In diagnosis, HIV antibodies are detected. Monitoring of HIV infection is primarily done by Viral Load (VL) testing. This method is considered highly specific[63]. It looks for viral RNA and has a sensitivity of 50 copies of virus/ml blood. The commonly employed blood tests are ELISA (Enzyme Linked Immuno Sorbent Assay) and Western Blot which is considered gold standard and more specific than ELISA[64, 65]. These tests are used for the diagnosis of HIV antibodies. High titers of these antibodies would appear during the stage of seroconversion[38, 39] .

1.2.1 Following up of the Progression of HIV infection

Progression of HIV pathogenesis can be established by periodically monitoring CD4+ T-cells, viral load (VL), CD4/CD8 ratio and CD4%[36].

Traditionally CD4 T- cells and VL are considered the best markers. The lower the CD4/CD8 ratio goes the worse the condition of the patient. CD4% gives an insight about the patient's immune system[66]. Higher the CD4%, the better it is for the patient. Non-HIV patients have CD4% > 50%, well established HIV patients have 20-35% and HIV immunodeficient patients have <15%.

1.2.2 HIV affects immune system

One of the proteins, named gp 120, recognizes a protein on helper T-cells named CD4, and associates with it. Gp 120 is a sugar-containing protein called a glycoprotein, which has a molecular weight of approximately 120,000. The CD4 protein is a normal part of a helper (both Th1 and Th2) T-cell membrane. The CD4 is a specific receptor for HIV. As a consequence of the interaction with CD4 on helper T-cells, HIV exclusively infects the cells necessary to activate both B-cell and cytotoxic T-cell immune responses. Without helper T-cells, the human body cannot create antibodies appropriately, nor can infected cells containing HIV be properly eliminated. As a result, the virus would become capable to multiply, kill the helper T-cell in which it survives, infect adjacent helper T-cells, and repeat the cycle. These cycles will carry on until there is a substantial loss of helper T-cells.

The interaction between the virus and the immune system for supremacy is continuous. Human body counters to this onslaught through production of more T-cells, some of which mature to become helper T-cells. The virus eventually infects these targets and eliminates them. More T-cells would be produced, which in due course get infected and killed by the virus. This interaction may continue for up to ten years before the body eventually succumbs due to an inability to produce T-cells any-longer. This loss of helper T-cells ultimately results in the complete inability of the body to protect against even the weakest of organisms (all kinds of bacteria and viruses other than HIV) which usually would not be very harmful to humans. Thus people with HIV become more susceptible to infections and may possibly get certain kinds of cancer that a healthy body would be able to fight off. Hence, infections which are rarely seen in people with normal immune systems are deadly to those with HIV. This acquired form of immunodeficiency is called, AIDS. Even though AIDS is always a result of HIV infection, not all people who become infected by HIV develop AIDS.

1.2.3 HIV and co-receptors

Chemokine receptors are the proteins present on the cell surface that bind to small peptides called chemokines[67, 68]. Based on the number and location of conserved cysteines the chemokines are classified intro three groups; C, CC and CXC. The role of chemokine receptor gene CCR5 has been of strong interest since the discovery of its role in the entry of HIV-1 into human CD4 +ve cells[69]. Chemokine receptors of major importance for entry of HIV into human cells are CCR5 and CXCR4[70-72]Conformational changes in viral envelope gets provoked by attachment of CD4-gp 120. This allows CD4-gp 120 complex to interact with either CCR5 or CXCR4; as shown in figure 1-8. On the basis of cell tropism HIV can be classified into 2 categories; M-Tropic (macrophage tropic) and T-Tropic (T-cell tropic). T tropic strains operate with the CXCR4 co-receptor; while M-tropic strains operate with the CCR5 co-receptor [73]. Based on the receptor action, T-tropic and M-tropic strains are called X4 and R5 viruses respectively[67].

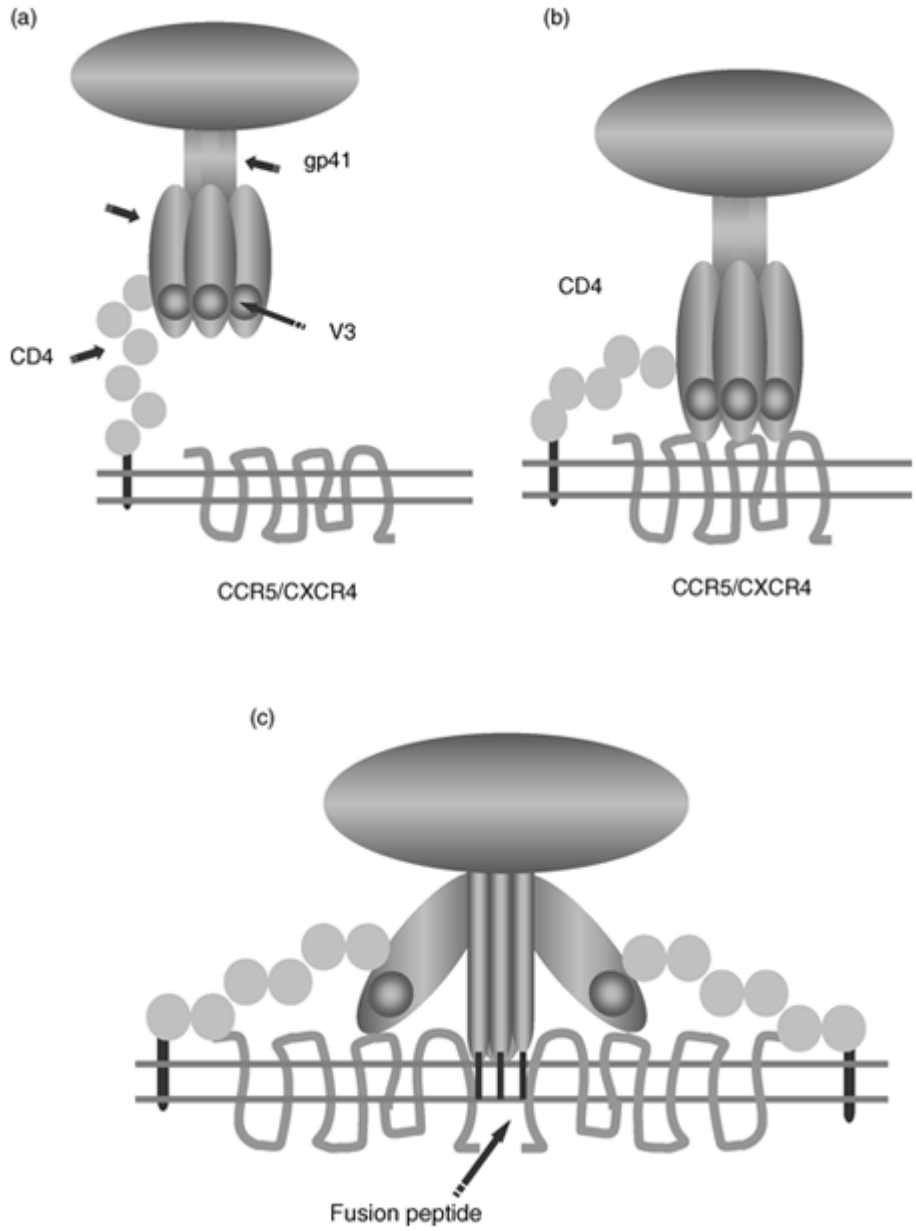


Figure 1-8 HIV and co-receptor entry[67]. The figure 1-8 illustrates a) CD4-gp 120 binding b) gp 120-coreceptor interaction c) viral and cell membrane fusion.

1.3 Treatment for HIV/AIDS

Drugs used in the treatment of infection by retroviruses are termed "antiretroviral drugs" (ARVs). Several classes of antiretroviral drugs have become available to treat infections by retroviruses since 1985. These different classes of ARVs operate at different stages in the life cycle of HIV. However, a revolution in the field of HIV medicine occurred in 1996 when "Highly Active Anti-retroviral Therapy" (HAART) was introduced[74]. This is a drug regimen involving a combination several ARVs, generally ≥ 3, to restrict viral replication and possible mutations[74, 75].

1.3.1 Highly Active Anti-retroviral Therapy (HAART)

Extensive research in the area of AIDS has resulted in the development of more than 20 anti retroviral drugs approved by FDA. Highly active antiretroviral therapy (HAART) has been responsible for a dramatic decrease in AIDS mortality since 1996. There has been a phenomenal drop in the HIV related mortality and morbidity since the introduction of HAART[74, 76] . Death rates and AIDS related illness have reduced drastically by 80% since HAART is introduced[77]. The higher the number of the virus inside the human body, higher the possibility of resistance to ARVs. Hence a combination therapy of ARVs suppresses HIV replication and protects against emergence of resistance. HAART substantially reduces viral replication and thus reduces the number of new viral copies formed and also the possibility of a superior mutation. HAART usually comprises of a combination of two NRTIs and one NNRTI or PI. Even though HAART has been quite useful in controlling HIV, extensive and prolonged exposure to three or more ARVs poses a major hurdle due to emergence of toxicities and adverse effects[76].

The aim of HAART is to control the levels of HIV RNA such that they remain at undetectable levels (50copies/ml). Initiation of HAART has totally transformed the clinical profile of HIV/AIDS; from a subacute lethal disease to a chronic ambulatory disease.

Introduction of HAART resulted in profound suppression of HIV replication and significant increase in CD-4 T cell counts[78]. There has been a remarkable decrease in the number of deaths due to AIDS (Figure 1-9), new AIDS cases over the past decade and an

increase in the number of people surviving with AIDS in Europe and North America. This can be attributed to the improvements in diagnosis and treatment. However, fairly few people from underprivileged countries have received this benefit.

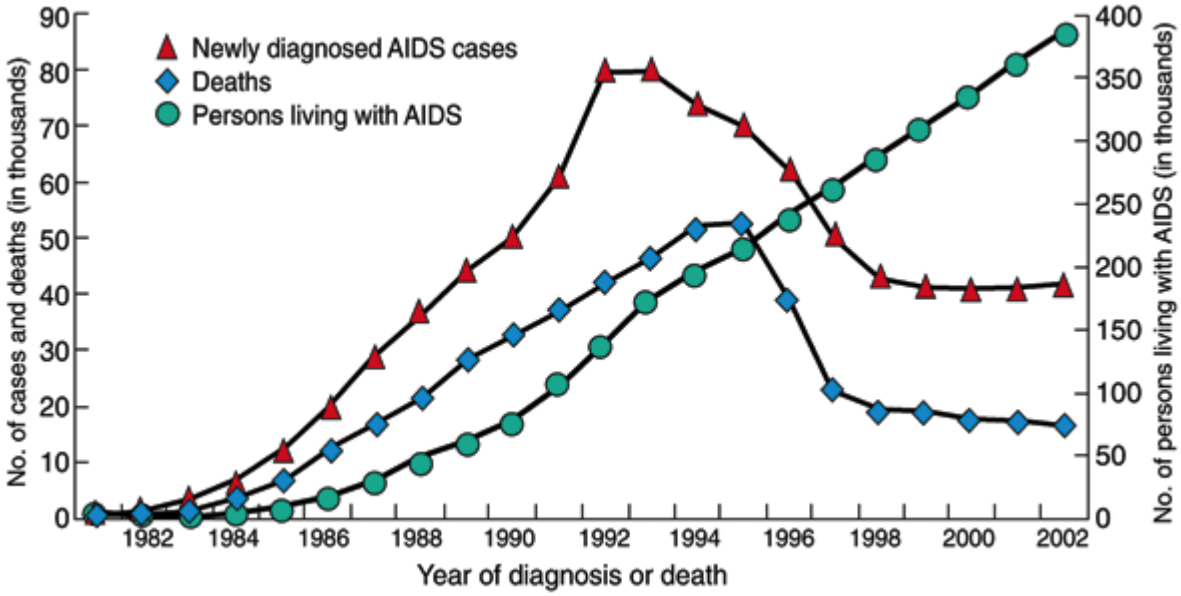


Figure 1-9 HIV and HAART in the United States (1983-2003) [77]

1.3.2 Advantages of HAART

HAART plays a vital role in the reduction in disease progression and in the reduction in AIDS diagnosis. Early commencement of HAART may cause reduction in occurrence of opportunistic infections which inturn may reduce the extent of hospitalisations[79, 80]. Reduction in AIDS related deaths can be minimised through HAART intervention[81].

1.3.3 Commencing ARVs

All AIDS cases are individualised. Commencement of ARVs depends upon the patient and the clinician. However HAART can be started for all patients with no symptoms but have a low CD4 count (<350 cells/ml) or have a high viral load (100,000 cells/ml)[82]. The reason behind this rationale is with time immune competence declines in untreated patients and further more sets of T cells would get exhausted. Secondly, there is always a

threat of emergence of quasi-species which increase with time on emergence of mutations which results in formation of syncytia-inducing (SI) form of virus. The SI form of the virus is undoubtedly a more aggressive form of HIV. Hence earlier commencement of ART might prevent the emergence of SI form of HIV and may possibly prevent rapid cell death.

Early commencement of ARVs is advantageous as it may cause earlier suppression of viral replication and prolongation of disease- free life .This aids to preserve immune function and also prevents drug related toxicities. However, for a simple understanding of when to commence ARV therapy Wood et al[80]; described as shown in figure 1-10.

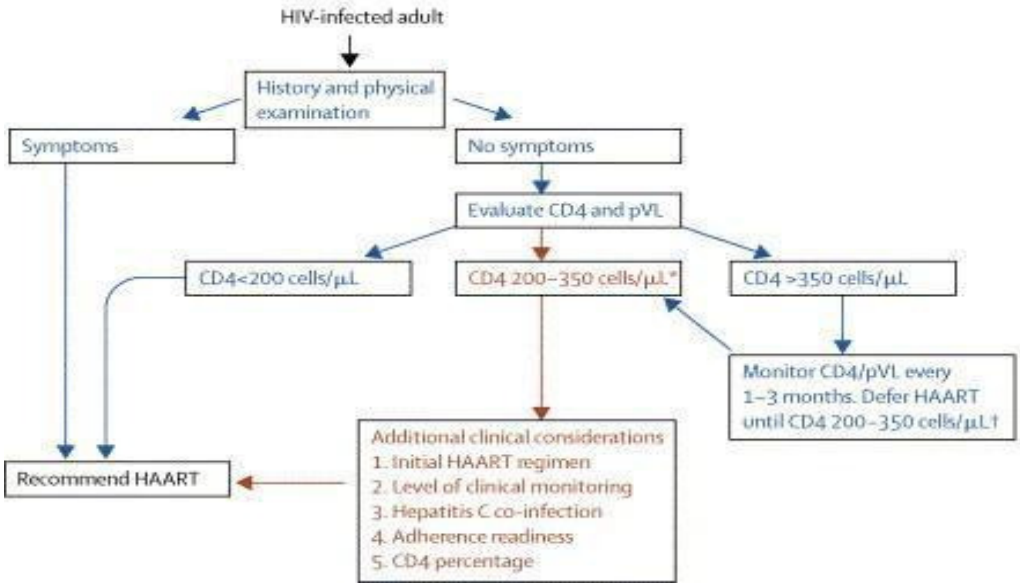


Figure 1-10 Commencement of ARVs; Figure 1-10 describes the guidelines regarding considerations for the optimal time to initiate HAART[80].

1.3.4 Disadvantages of early commencement of ARVs

Early commencement of ARVs may not always be most favourable as it may expose the patients to adverse effects early on and there would be high chances of emergence of resistance with suboptimal regimens[83, 84]. This may lead to limitation of future options of ARVs for the patient[85].

1.3.5 Adherence to HAART

HAART often comprises of a complex regimen[86]. With HAART adverse events are frequent. This may lead to dose interruptions, discontinuation of therapy and considerable decline in quality of life. Adverse events pose the main reason for compromise to adherence. HAART fails in approximately half of the patient population and non-adherence plays an important role to some extent[87]. Hence, adherence is recognized as an important determinant to successful ARV therapy. Virological failure of potent ARVs was mostly attributed to poor adherence. Several studies have strongly supported the association between adherence and adverse events of ARVs. Surveys of people receiving HAART have proved that adverse effects account for 10-15% or more of those patients who discontinue therapy and 30% of patients missed doses in their first three days of therapy. Adherence was considered a barrier to ARV treatment success[88]. Near perfect adherence is required for successful treatment outcomes in HIV.

Adherence necessitates a healthy and reliable trusting patient-physician relationship, wherein the patient should be made the active participant[89]. The physician should explain thoroughly to the patient before the first prescription of HAART. This should include knowledge of treatment and possible adverse effects. Involvement of family, friends, and support staff improves adherence. A patient's ability to identify treatment regimen and understanding the relationship between adherence and medication resistance also predict better adherence[90].

Table 1-5 Possible strategies to improve adherence to antiretroviral therapy [86]

Medication related:
Inform, anticipate and treat side effects
Avoid adverse drug interactions
Reduce dose frequency and number of pills if possible
Patient related:
Negotiate a treatment plan that the patient understands to which he/she commits
Take time to educate and explain goals of therapy and need for adherence
Recruit family and friends to treatment plan and need for adherence
Provide written schedule of medications, daily or weekly pill boxes, alarm clocks or other devices
Develop adherence support groups or add adherence to regular agenda of support groups
Physician related:
Establish trust
Serve as educator and source of information, ongoing support and monitoring
Monitor ongoing adherence and intensify management in periods of low adherence
Use health care team for difficult patients and patients with special needs, such as peer educators for injection drug users or adolescents
Consider impact of new diagnoses on adherence, particularly depression, and drug use
Health team related:
Use nurses, pharmacists, peer educators, volunteers, and drug counsellors to reinforce adherence
Provide training to support team related to adherence
Add adherence interventions to job descriptions of support team members

Factors that influence adherence:

The various possible factors which could aid in maintenance of adherence can be understood from figure1-11;

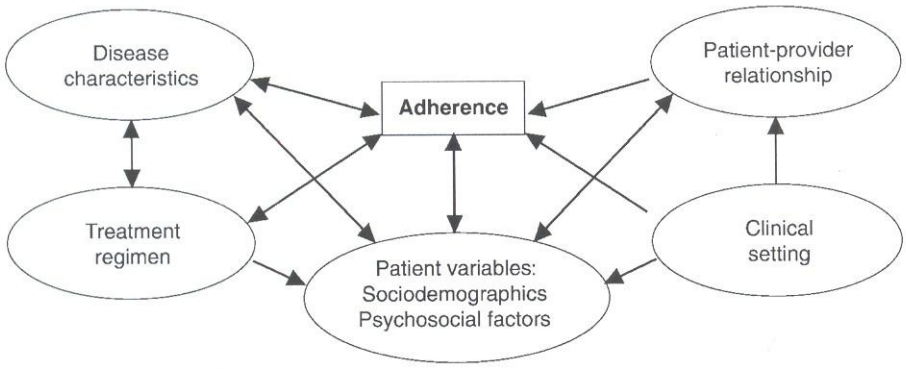


Figure 1-11 Factors which could aid in adherence[91]

1.4 HIV/AIDS Treatment

The following classes of drugs are included under HAART; 1) Nucleotide Reverse Transcriptase Inhibitors(NRTIs) (2) Nucletide Reverse Transcriptase Inhibitors (NtRTIs) (3) Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) (4) Protease Inhibitors (PIs) (5)Novel agents which includes; immune stimulators, vaccines, integrase inhibitors, antisense drugs and entry inhibitors like chemokine co-receptors antagonists, attachment inhibitors and entry inhibitors. Sites of action of the various classes of ARVs can be observed from figure1-12.

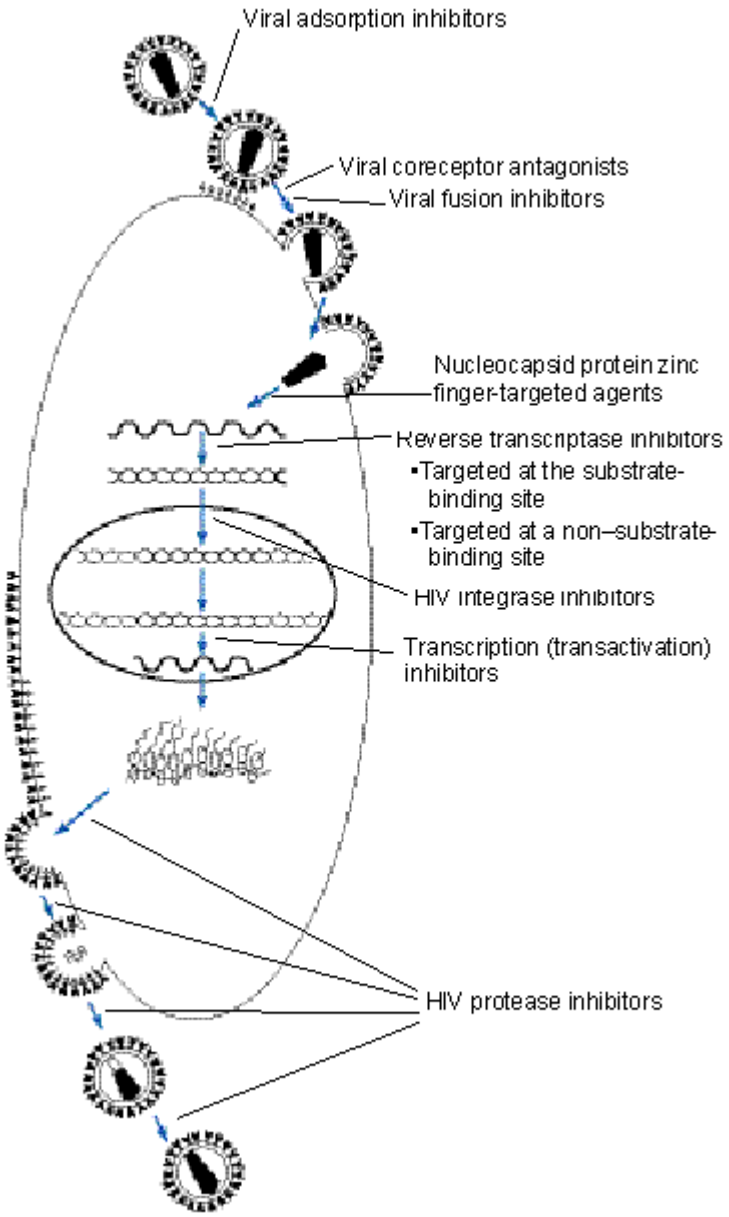


Figure 1-12 Sites of action of current and investigational antiretroviral agents for treatment of HIV infection[92].

1.4.1 Nucleoside Reverse Transcriptase Inhibitors

The following drugs are included in Nucleoside Reverse transcriptase Inhibitors;

Table 1-6 NRTI

Brand Name	Generic Names	Abbreviation
Combivir	Zidovudine + Lamivudine	AZT + 3TC
Emtriva	Emtricitabine	FTC
Epivir	Lamivudine	3TC
Epzicom/Kivexa	Abacavir + Lamivudine	ABC+ 3TC
Retrovir	Zidovudine	AZT or ZDV
Trizivir	Abacavir + Zidovudine + Lamivudine	ABC + AZT + 3TC
Truvada	Tenofovir + Emtricitabine	TDF + FTC
VIDEX	Didanosine	ddl
VIDEX EC	Didanosine: delayed-release capsules	ddl
Viread	Tenofovir DF	TDF
Zerit	Stavudine	d4T
Zerit XR	Stavudine: delayed-release	d4T
Ziagen	Abacavir	ABC

1.4.2 Non-Nucleoside reverse Transcriptase Inhibitors

The following drugs are included in Non Nucleoside Reverse Transcriptase inhibitors;

Table 1-7 NNRTI

Brand Name	Generic Names	Abbreviation
Rescriptor	Delavirdine	DLV
Sustiva	Efavirenz	EFV
Viramune	Nevirapine	NVP

1.4.3 Protease Inhibitors

The following drugs are included under Protease inhibitors;

Table 1-8 PIs

Brand Name	Generic Names	Abbreviation
Aptivus	Tipranavir	TPV
Crixivan	Indinavir	IDV
Invirase	Saquinavir (hard gel cap)	SQV (HGC)
Kaletra	Lopinavir/Ritonavir	LPV/r
Levixa	Fosamprenavir	FPV
Norvir	Ritonavir	RTV
Prezista	Darunavir	
Reyataz	Atazanavir	ATV
Viracept	Nelfinavir	NFV

1.4.4 Entry/Fusion Inhibitors

The following drugs are included under entry/fusion inhibitors;

Enfuvirtide(T20)

1.4.5 Fixed dose combinations of ARVs

Table 1-9 combinations of anti-retrovirals drugs

Brand Name	Generic Names	Abbreviation
Atripla	Efavirenz + Emtricitabine + Tenofovir)	EFV + FTC + TDF
Combivir	Zidovudine + Lamivudine	AZT + 3TC
Trizivir	Abacavir + Zidovudine + Lamivudine	ABC + AZT + 3TC
Truvada	Tenofovir + Emtricitabine	TDF + FTC

1.4.6 Dosage Forms of the available anti-retrovirals

Table 1-10 Dosage forms

Generic Name	Brand and Other names	Capsules	Tablets	Liquid Form	IV
Abacavir	Ziagen, ABC		✓	✓	
Abacavir,Lamivude	Epzicom			✓	
Abacavir,Lamivudine, Zidovudine	Trizivir			✓	
Didanosine	VIDEX ddl, VIDEX EC		Chewable enteric coated tablet	Buffered powder can be dissolved	
Emtricitabine	Emtriva, FTC, COVIRACIL	✓		✓	
Emtricitabine, Tenofovir DF	Truvada		Film coated tablet		
Lamivudine	Epivir, 3TC		Film coated tablet	Flavoured	
Lamivudine, Zidovudine	Combivir		✓		
Stavudine	Zerit, d4T	✓		✓	
Tenofovir DF	Viread, TDF		✓		
Zidovudine	Hivid, ddC	✓	✓	✓	✓
Delavirdine	Rescriptor, DLV	✓		Can be dissolved	
Efavirenz	Sustiva, EFV	✓	✓		
Nevirapine	Viramune,NVP		✓	✓	
Atazanavir	Reyataz, ATV	✓			
Fosamprenavir	Lexiva, FPV		✓		
Indinavir	Crixivan, IDV	✓			
Lopinavir/Ritonavir	Kaletra, LPV/r	✓		✓	
Nelfinavir	Viracept,NFV		✓	Oral Powder	
Ritonavir	Norvir,RTV	✓		✓	
Tipranavir	Aptivus,TPV	✓			
enfuvirtide	Fuzeon,T-20				Injection only

1.4.7 Anti-retroviral Agents approved by FDA

Mechanism of action of NNRTIs

NNRTIs reversibly inhibit HIV-1 reverse transcriptase reducing viral DNA synthesis.

Table 1-11 FDA approved NNRTIs

NNRTIs	Dosing Frequency	Daily Pill burden	Common side-effects
Nevirapine	Twice daily	2	Rash, elevated liver enzymes
Efavirenz	Once daily	1 or 3	Rash, Nervous system symptoms
Delavirdine	Three times daily	6	Rash

Mechanism of action of NRTIs

NRTIs are converted by cellular enzymes to active phosphorylated metabolites that inhibit viral reverse transcriptase and viral DNA synthesis, preventing HIV replication. The specific site of action differs for each drug; these differences are exploited in combination regimens. They act as false substrates for reverse transcriptase and thereby causing chain termination. The resulting viral DNA is incomplete and prevents HIV replication[86, 93]. NRTIs and NtRTIs are derivatives of naturally occurring nucleosides that make up DNA. Similar to all naturally occurring nucleosides NRTIs need to be tri-phosphorylated. The tri-phosphorylated form is an active form which competes with the natural deoxy-nucleotide tri-phosphates for incorporation into growing DNA chains and following incorporation into DNA, by DNA chain termination. As a result a full length copy of the DNA is not produced.

Table 1-12 FDA approved NRTIs

NRTIs	Dosing Frequency	Daily Pill burden	Common side-effects
Zidovudine	Twice daily	2	GI
Didanosine	Twice daily Once daily	2-4 1	Peripheral Neuropathy
Stavudine	Twice daily	2	Peripheral Neuropathy
Lamivudine	Twice daily Once daily	2 1	GI
Lamivudine/ Zidovudine	Twice daily	2	GI
Abacavir	Twice daily	2	GI, Allergic reaction
Abacavir/ Lamivudine	Once daily	1	GI, Allergic reaction
Abacavir/ Lamivudine/ Zidovudine	Twice daily	2	GI
Emtricitabine	Once daily	1	Headache, GI

Mechanism of action of NtRTIs

Tenofovir is metabolised to active Tenofovir diphosphate which inhibits viral polymerases and terminates the DNA chain after incorporation into viral DNA[93].

Table 1-13 FDA approved NtRTIs

NtRTIs	Dosing Frequency	Daily Pill burden	Common side-effects
Tenofovir	Once daily	1	GI
Tenofovir/Emtricitabine	Once daily	1	Headache, GI

Mechanism of action of Protease Inhibitors

Inhibit HIV-1 and HIV-2 proteases preventing maturation and replication[94, 95] .

Table 1-14 FDA approved PIs

PIs	Dosing Frequency	Daily Pill burden	Common side-effects
Saquinavir mesylate	Twice daily (with Ritonovir)	12	GI
Indinavir	Three times daily Twice daily(with ritonovir)	6 6-8	Nephrolithiasis, GI
Ritonovir	Twice daily	12	GI, dyslipidemia
Nelfinavir	Three times daily Twice daily	9 4-10	GI
Lopinavir/Ritonavir	Twice daily	6	GI
Atazanavir	Once daily Once daily(with ritonavor)	2 3	Benign hyperbilirubinemia
Fosamprenavir	Twice daily Twice daily(with ritonavir) Once daily (with ritonavir)	4 4 4	Rash

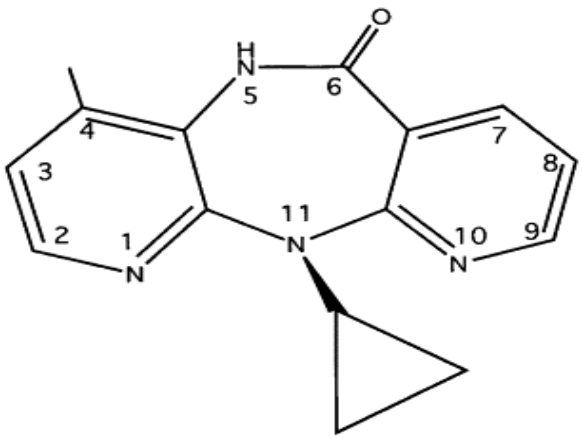
Mechanism of action of Fusion Inhibitors

They bind to viral glycoprotein subunit gp41 and by inhibiting its function, block viral fusion with the CD4 receptor of the host cell and thus viral entry to the cell[96].

Table 1-15 FDA approved Entry Inhibitors

Entry Inhibitors	**Dosing Frequency**	**Daily Pill burden**	**Common side-effects**
Enfuvirtide(T20)	Twice daily	2 SC injections	Injection site reactions

1.5 Nevirapine Pharmacology



Nevirapine

Figure 1-13 Nevirapine chemical structure

The chemical name of NVP is 11-cyclopropyl-5,11- dihydro-4 methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one[97].

NVP is a white to off- white crystalline powder with molecular weight of 266.3 and molecular formula $C_{15}H_{14}N_4O$. NVP is low- molecular weight compound. It is weakly basic with a pKa of 2.8 and highly lipophilic with partition coefficient of 83[98]. NVP is extremely soluble at pH < 3 but at neutral pH its aqueous solubility decreases to 0.1 mg/ml[98]. The absolute bioavailability is approximately 90%[99] . It is 50%-60% protein bound. NVP absorption is not affected by concomitant administration of food, didanosine or antacids. NVP concentration in human CSF is 45% of that found in plasma, which is almost equal to the plasma free fraction which is not bound to plasma protein. NVP was shown to cross the blood-brain barrier (BBB) more effectively than other anti-retroviral drugs like didanosine, stavudine, zidovudine, delavirdine, indinavir or saquinavir[100].It is proven that use of NVP can show beneficial effects in the elimination of HIV-1 induced dementia because of its ability to cross BBB and accumulate in CSF[101]. The volume of distribution is 1.21 ± 0.09 L/Kg. Metabolism of NVP is mediated by 3A4 family of isoenzymes of the CYP-450 system resulting into the formation of glucuronides ultimately. This is confirmed by *in vitro* studies with human liver microsomes.

Approximately 80% of the given dose of NVP is recovered from urine primarily as glucuronide conjugates of hydroxylated metabolites while 10% was found in faeces. Table 1-16 gives a brief idea about the pharmacokinetics of NVP.

Table 1-16 NVP Pharmacology[102]

Parameter	Dose
AUC µg h/ml	Adult: 55.95(51.49-69.74)
	Infant: 62.6 ± 15.7
C_{max} (µg/ml)	Adult: 5.86(5.52-7.22)
	Infant: 1.24 ± 0.34
t_{max} (h)	Adult: 2-4
	Infant: 14 ± 8.9

Pharmacokinetics of other common ARVs shown in Table 1-17.

Table 1-17 Pharmacology of other important antiretroviral drugs[103]

Drug	Volume of distribution L/Kg	Renal Clearance	Half life hours(h)	Protein Binding (%)	Bioavailability (%)
Zidovudine	1.6	400ml/min/70kg	1.1	25	60-70
Lamivudine	1.3	260ml/min	2-4	36	82
Didanosine	1	400ml/min	1.4	<5	25-43
Satavudine	0.5	240ml/min	1.6	Negligible	80
Delavirdine	Not Reported	Not available	2.8-7.3	99	Well absorbed
Saquinair	10	Not Reported	13	98	4
Ritonavir	0.5	<0.1 L/hr	3-5	99	60
Indinavir	2.25	Not Reported	1.8	60	Well absorbed
Nelfinavir	2-7	Not Reported	3.5-5	>99	30 without food & 43 with food

1.5.1 Adverse effects associated with Nevirapine

Nevirapine was approved by the FDA in 1996 and since then it has exhibited impressive results and gained an authoritative place in the treatment of HIV infection. NVP is usually a well tolerated ARV drug[86]. However, rash is the most common adverse effect that necessitates discontinuation if severe. Rash usually tends to occur during the first few weeks of therapy. The occurrence of rash was observed in 17% of the patient population as stated by Murphy et al[104]. Patients who experience rash during the first two weeks of therapy should be educated not to increase the dose of NVP. Rash due to NVP is generally mild- moderate in severity which typically appears on the face, trunk and extremities[105] . They are maculopapular, erythematous cutaneous eruptions with or without pruritis. However, rare and life-threatening skin reactions may also occur in patients using NVP [106]. The role of HLA molecules in the hypersensitivity reactions to NVP therapy can be attributed to HLA class I alleles HLA-Cw-08, HLA B-14 and HLA-DR1 where NVP or its metabolite when coupled with HLA antigens may be expressed on the cell surface and may bring about NVP hypersensitivity reactions or hepatotoxicities.

Management of NVP related rashes must be based on their severity. Mild-moderate rashes (Grade 1-2) are usually limiting and the rashes usually resolve without discontinuing NVP therapy. Use of oral anti-histamines or topical steroid creams may be sufficient to control grades 1-2 rashes. Rash along with gastrointestinal manifestations (characterised by diarrhoea), severe pruritis, vomiting, oral lesions and/or ulceration, muscle or joint pains mandates the discontinuation of NVP. NVP should not be rechallenged in these patients.

Hepatotoxocity is recognized to be a major safety concern with the use of NNRTIs[107]. Development of severe hepatotoxocity has been linked with the use of NVP [108]. Progression of liver disease would be accelerated in HIV-1 patients who are co-infected with HBV or HCV and hence is a concern as their condition would deteriorate further if they were placed on NNRTIs[109].

Hepatotoxicity associated with NVP was found with chronic dosing and not with a single dose[110]. However, there is a high risk (17%) of early hepatotoxicity with the use of NVP[111, 112] .

The common adverse effects associated with NNRTIs are listed in Table 1-18.

Table 1-18 NNRTI adverse effects[86]

NNRTI adverse effect	% of patients affected
Rash, total	17-24
Rash, severe	4-8
Stevens-Johnson syndrome	0.5
Fever	5-10
Elevated AST and ALT levels, and/or GGT	3-8
Nausea	7-11
Headache	7-10

1.5.2 Role of NVP in Mother-To-Child- Transfer of HIV

Mother-To-Child-Transfer (MTCT) is a very important source of HIV infection in children and breast-feeding may be a major risk in such cases and chances of acquiring HIV is doubled to the child[113, 114]. However, the HIVNET 012 (The HIV Network for Prevention Trials) established the safety and efficacy of NVP in the prevention of MTCT. In the HIVNET pregnant women received a single dose of oral NVP during labour and the new born received one dose of NVP (2mg/kg) within 72 hours of birth. The single dose of NVP was administered alongside the usual dose of Zidovudine to the mother. The results of HIVNET 012 have been substantiated by other studies[115-118].

1.5.3 Nevirapine Resistance

Studies performed prior to HIVNET 012 demonstrated the rapid emergence of NVP resistance upon monotherapy[119-121]. Although NVP has proved efficacious to prevent MTCT, further research revealed that there exists a high chance of getting NVP resistance due to the emergence of viral mutations even with a single dose of NVP. The Y181C mutation was most commonly observed in the infants from the HIVNET studies. However, K103N mutation was also observed in the study. The results were confirmed by PACTG 316 trial where 11% of the women were found to have NVP resistance[122]. A detailed explanation about NVP resistance was provided by Giovanni Maga et al[123]; stating that NVP resistance could occur due to combination of emergence of steric and thermodynamic barriers for drug binding and loss of stabilizing interactions[123].

1.5.4 Adverse reaction with NRTIs

NRTIs have been the foundation for HIV therapy since the availability of Zidovudine in 1986. Ever since, a range of NRTIs have been invented. All of them have the same mechanism of action but many adverse effects of each agent are quite unique. In order to elicit an effect on HIV, NRTIs first must be phosphorylated intracellularly by the host specific cell enzymes. But the major untoward effect with NRTIs is that they not only inhibit HIV reverse transcriptase but also cellular DNA polymerases; mitochondrial polymerase γ leading to mitochondrial toxicity. Most common adverse effects with NRTIs are lactic acidosis with severe hepatomegaly and steatosis. Relationship between

genetic markers and ARV drug hypersensitivity reactions with Abacavir have been identified in previous studies. The major treatment-limiting side effect associated with the use of Abacavir is an early onset multi-system drug hypersensitivity reaction which includes combination of rash, fever and gastrointestinal symptoms, occurring within 6 weeks of initiating treatment. Susceptibility to Abacavir hypersensitivity is strongly predicted by the presence of a specific HLA allele - HLA-B57 which characterizes the prevailing risk factor for the drug's hypersensitivity reactions. However the common adverse effects with NRTIs are listed in Table 1-19

Table 1-19 NRTI adverse effects[86]

NRTI, adverse effect	% of patients affected
Zidovudine	
Headache	12–18
Fatigue	2–7
Neutropenia	2–31
Nausea	4–26
Anaemia	1–7
Insomnia	4–5
Vomiting	3–8
Myalgia	5–8
Myopathy	6–18
Didanosine	
Pancreatitis	5–9
Diarrhoea	15–28
Peripheral neuropathy	2–20
Elevated AST and ALT levels	6–10
Stavudine	
Peripheral neuropathy, total	13–24
Gastrointestinal	4–6
Headache	3–5
Elevated AST and ALT levels	5–10
Lamivudine	
Nausea	4
Headache	8
Fatigue	4
Insomnia	4
Abacavir	
Nausea/vomiting	5–15
Fever	5–7
Dizziness	5–10
Insomnia	2–7
Rash	3–5
Diarrhoea	1–12
Hypersensitivity reaction	3–5

1.5.5 Adverse effects with PIs

To prevent emergence of resistance, PIs require accurate dosing, and adherence must be maintained. Since the risk of emergence of drug resistance is superior to the potential danger of adverse events, it is always advisable to maintain adherence. Hence it is not advised to reduce the dose of a PI drug in an attempt to reduce the adverse effects. Alongside other adverse effects of PIs, reports about syndrome of metabolic disorders have been identified in patients receiving PIs compared to those patients who are PI naive and the ones whose treatment is PI-sparing. The common adverse effects observed in PIs are listed in Table 1-20.

Table 1-20 PI adverse effects[86]

PI, adverse effect	% of patients affected
Saquinavir	
Diarrhoea	20
Nausea	11
Abdominal pain	9
Dyspepsia	8
Flatulence	8
Headache	5
Fatigue	5
Elevated AST and ALT levels	2-6
Indinavir	
Diarrhoea	5
Vomiting	4
Hyperbilirubinemia	10
Abdominal pain	9
Nausea	12
Headache	6
Ritonavir	
Nausea	23-26
Diarrhoea	13-21
Taste perversion	5-15
Elevated AST and ALT levels	5-6
Asthenia	9-14
Headache	5-6
Vomiting	13-15
Anorexia	6-8
Perioral dysesthesia	3-6
Hypertriglyceridemia	2-8
Hypercholesterolemia	2
Nelfinavir	
Diarrhoea	14-32
Nausea1	3-7

2 Methodology

2.1 Ethical approval

The study involved the collection and analysis of patient's recorded data, therefore ethical approval was obtained from Curtin University of Technology Human Research Ethics committee (Appendix). As this study involved the analysis of patient records, ethical issues arise in relation to confidentiality and release of data. A unique non-patient identifiable code (Unit Medical Record Number; UMRN) was allocated to each patient. The key to the code was held at all times by the Head of Department of Clinical Immunology, Royal Perth Hospital. Any coded data to leave the hospital was kept secure in accord with National Health and Medical Research Council guidelines and only group data will be released from the research. The data will be stored in the School of Pharmacy, Curtin University of Technology for a period of seven years in a locked cabinet.

2.2 Setting and Patient Population

The research was conducted at the Department of Clinical Immunology, Royal Perth Hospital (RPH), Western Australia. An extensive database held in the Department of Clinical Immunology was accessed for patient clinical records. The research was based on the review of the entire HIV database available at the Department of Clinical Immunology. HLA typing was done based on the data available from serological HLA typing and sequential typing. Various alleles of IL-1 alpha were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The researcher utilized only data from the hospital database and the experimental procedures required for the study included methods like PCR and RFLP were performed by the staff of the Department of Clinical Immunology, Royal Perth Hospital. Experimental procedure for IL-A genotyping was undertaken according to the method published by Price et.al[12].

2.2.1 Inclusion Criteria

The research included all the patients who received Nevirapine as part of the first regimen of anti-retroviral drugs. Patients who received any other anti retroviral drug prior to receiving NVP were not included in the study. All patients whose data was derived from the database were over 18 years of age and received NVP as their first drug of treatment. Adult HIV infected patients were considered for the research. RPH is the referral centre for patients with AIDS/HIV for the state of Western Australia.

2.2.2 Data Collection

The patient population was divided into three groups based on their length of stay on Nevirapine; short-term, medium and long-term responders. Patients who stayed on Nevirapine continuously for more than five years were considered long-term responders (60+ months or >60 months)). Patients who withdrew before six months of therapy due to the drug's side effects or for any other reason were considered short-term responders (0-6 months or <6 months). Patients whose length of stay on the drug is intermediate between these two groups are considered as medium responders (6-60 months). There were almost 500 cases of HIV in the hospital during the period of study. These cases were entered in Microsoft excel sheets and were randomized for selection. However, only patients to whom NVP was given as the first drug for treatment were taken into this study; hence the patient population in the study was only 79.

We considered several factors that might determine whether a patient on NVP would be a long term responder or a short term user of the therapy, based on the results of previously published studies[124].

Factors considered for the study were:

1. Age
2. Sex
3. Baseline CD4 T cell count
4. Baseline Viral load

5. History of IV drug use
6. Genetic factors previously associated with the development of nevirapine toxicity or long-term control of HIV replication. The genetic factors include;

    a) IL-1A genotype

    b) HLA genotype

The genetic markers which were examined as predictors of effective NVP long term use are;

1) IL-1A alleles, which have been associated with a good virological response to any antiretroviral therapy[12].
2) Class I and II HLA molecules, which have been associated with NVP hypersensitivity reactions[125].

2.2.3 Research Method

The research was based on the survey of the entire HIV database available at the Department of Clinical Immunology; Royal Perth Hospital. HLA typing was done based on the data available from serological HLA typing and sequential typing. Various alleles of IL-1A were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)[12].IL alleles were assessed as restriction fragment length polymorphism. Briefly, 150ng genomic DNA was placed in a 50 µl reaction mix containing 800µM dNTP, 2mM magnesium chloride, 800µM primers. DNA flanking IL was amplified with 1 U Taq platinum polymerase [96^0C 15 minutes (94^0C 1 min, 50^0C 1 min, 72^0C 2 min) x 45 cycles, 72^0C 5 min]. Amplicons were digested overnight with N*co*1 (37^0C), separated by electrophoresis on 15% polyacrylamide or 3% agarose gels and visualised using ethidium bromide. Plasma HIV RNA was assayed by Amplicor 1.0 (standard protocol, 400-750 000 copies/ml) (Roche Diagnostic Systems Inc., Branchburg, NJ, USA). Only data is used for this study. Techniques like PCR and RFLP were performed by the staff of the Department of Clinical Immunology at Royal Perth Hospital.

2.3 Hypothesis

Since the research was started with all the patients who had taken NVP as their first drug of therapy, the above set of factors were considered to investigate whether or not one of the factors would predict the stay of patients on NVP for long time. The factors considered for the search to predict the length of stay were; a) Gender b) Mode of infection c)Baseline CD4 d)Baseline RNA e)IL-1A genotype f)HLA type, particularly HLA- DR1 and HLA-B14, because these have been associated with toxicity in previous studies. We hypothesised that any of the above factors would predict the explanation for continuing NVP therapy or for discontinuing NVP therapy. If this hypothesis is proved correct, we can prevent the NVP induced toxicities to patients by recommending NVP only to those patients who are in concordance with the study results.

The data was taken from the RPH patient information sheet. The following information was employed for the present study.

Table 2-1 Group I Patient information sheet

UMRN	Sex	Age [a]	Code	ART date[b]	No.of months	Baseline CD4[c]	Baseline RNA[d]	IL-1A genotype	Continuing[e]	HLA B35+HLAC4	HLA DR1	HLA B14
Patient 1	M	53	1	28/01/1998	1	650	17608	1,1	No	No	No	No
Patient 2	M	45	5	24/06/2004	6	396	81283	1,1	No	No	No	No
Patient 3	M	38	12	15/03/1999	0	396	17099	1,1	No	No	No	No
Patient 4	F	45	2	10/12/1997	1	286	19415	1,1	No	No	No	No
Patient 5	M	40	1	14/10/1997	2	240	15543	1,1	No	No	No	No
Patient 6	M	49	3	19/08/1998	6	260	66089	1,1	No	Yes	No	No
Patient 7	F	37	2	15/11/2000	1	288	39300	1,1	No	No	No	No
Patient 8	F	58	2	9/07/1997	1	680	85114	1,1	No	No	Yes	1Yes
Patient 9	M	41	1	23/08/2005	1	576	660693	1,1	No	No	No	No
Patient 10	M	32	1	12/05/1998	1	360	33864	1,2	No	Yes	Yes	No
Patient 11	F	43	2	27/06/2001	2	405	115000	1,2	No	No	No	No
Patient 12	M	37	12	5/11/1998	4	300	155479	1,2	No	No	No	No
Patient 13	M	55	1	8/03/2000	3	180	75000	1,2	No	No	No	No
Patient 14	M	62	1	15/08/2000	1	280	161000	1,2	No	No	No	No
Patient 15	M	54	1	1/12/2004	1	240	100000	1,2	No	No	No	No
Patient 16	F	34	11	11/11/1998	0	616	219111	1,2	No	No	No	No
Patient 17	M	65	12	15/04/2003	0	1081	ND	2,2	No	No	No	No
Patient 18	M	52	1	4/03/1998	1	400	39101	2,2	No	No	No	No
Patient 19	F	31	5	19/01/2002	1	756	11200	NA	No	No	Yes	No
Patient 20	M	34	1	14/11/2000	0	350	719400	1,1	No	No	No	No

[a] time of study
[b] Date of commencement of ART
[c] CD4 count
[d] RNA level in
[e] No = NVP discontinued
Yes = NVP continued

Table 2-2 Group II Patient information sheet

UMRN	Sex	Age [a]	Code	ART date[b]	No.of months	Baseline CD4[c]	Baseline RNA[d]	IL-1A genotype	Continuing[e]	HLA B35+HLAC4	HLA DR1	HLA B14
Patient 21	M	43	1	25/08/1998	11	672	22754	1,1	No	No	No	No
Patient 22	M	45	1	23/06/1997	21	780	154882	1,1	No	No	No	No
Patient 23	M	52	1	14/11/1994	22	456	ND	1,1	No	No	Yes	No
Patient 24	M	32	3	30/07/2001	9	696	52000	1,1	No	No	No	No
Patient 25	M	32	11	1/04/1998	46	644	115343	1,1	No	No	No	No
Patient 26	M	43	1	22/12/1997	27	342	5536	1,1	No	Yes	No	No
Patient 27	M	56	1	15/10/2001	55	255	89300	1,1	Yes	No	No	No
Patient 28	M	53	3	7/02/1999	12	51	178178	1,1	No	Yes	No	No
Patient 29	M	40	1	15/02/1997	11	52	389045	1,1	No	No	No	No
Patient 30	M	52	1	14/12/1994	24	289	ND	1,1	No	No	No	No
Patient 31	M	44	1	8/04/1998	23	437	84812	1,1	No	Yes	No	No
Patient 32	M	33	1	21/07/1997	23	100	515355	1,1	No	Yes	No	No
Patient 33	M	43	1	15/10/1994	25	323	ND	1,1	No	No	No	No
Patient 34	M	44	1	9/03/1995	43	320	ND	1,1	No	No	No	No
Patient 35	M	42	1	20/07/2001	12	237	130000	1,2	No	No	No	No
Patient 36	F	65	2	15/09/2002	44	250	2130	1,2	Yes	No	No	No
Patient 37	M	44	11	16/01/2001	19	224	41800	1,2	No	No	Yes	No
Patient 38	M	44	1	7/11/1997	22	640	82823	1,2	No	Yes	No	No
Patient 39	M	55	2	15/11/2004	18	396	81283	1,2	Yes	Yes	No	No
Patient 40	M	31	1	11/12/2003	30	531	106000	1,2	Yes	Yes	No	No
Patient 41	F	39	2	21/12/2001	23	364	30700	1,2	No	No	No	No
Patient 42	M	71	1	7/12/1994	40	576	ND	1,2	No	No	Yes	No
Patient 43	M	64	1	15/08/2002	45	44	750000	1,2	Yes	No	No	No
Patient 44	M	54	1	11/01/1999	32	620	579225	1,2	No	No	No	No
Patient 45	M	34	1	24/07/2001	58	290	188000	1,2	Yes	Yes	No	No
Patient 46	F	28	11	5/09/1997	10	322	118779	2,2	No	No	No	No
Patient 47	F	77	2	15/10/2002	43	148	4900	NA	Yes	No	No	No
Patient 48	M	43	1	30/07/1997	10	462	30200	NA	No	No	No	No
Patient 49	M	56	1	5/05/2005	12	476	14125	1,1	Yes	ND	No	No
Patient 50	M	47	1	31/05/2005	11	475	100000	1,1	Yes	No	No	No
Patient 51	M	32	5	9/11/2004	18	84	100000	1,1	Yes	No	No	No
Patient 52	M	34	3	13/06/2005	11	486	93325	1,1	Yes	No	No	No
Patient 53	M	37	1	31/08/2005	9	122	100000	1,1	Yes	Yes	No	No
Patient 54	M	56	1	11/08/2005	9	60	263027	1,2	Yes	Yes	No	No
Patient 55	M	44	1	17/02/2005	15	266	50119	1,2	Yes	No	No	No

Table 2-3 Group III Patient information sheet

UMRN	Sex	Age [a]	Code	ART date[b]	No.of months	Baseline CD4[c]	Baseline RNA[d]	IL-1A genotype	Continuing[e]	HLA B35+HLAC4	HLA DR1	HLA B14
Patient 56	M	50	1	15/09/1997	66	195	104702	1,1	No	No	No	Yes
Patient 57	M	40	11	8/07/1999	60	408	12500	1,1	No	No	Yes	No
Patient 58	M	33	1	10/11/1997	60	440	82088	1,1	No	No	No	Yes
Patient 59	M	44	1	15/09/2000	68	549	218000	1,1	Yes	No	No	No
Patient 60	M	39	10	17/02/1997	111	338	31623	1,1	Yes	No	Yes	No
Patient 61	M	48	1	17/02/1999	87	391	410663	1,1	Yes	No	No	No
Patient 62	M	37	3	28/01/1998	83	60	28000	1,1	No	No	No	No
Patient 63	M	34	1	11/02/1998	99	675	5407	1,1	Yes	No	No	Yes
Patient 64	M	44	1	15/08/1997	105	234	117350	1,1	Yes	No	No	No
Patient 65	M	29	3	16/10/1998	91	456	64802	1,1	Yes	No	No	No
Patient 66	M	43	1	14/10/1998	91	357	4976	1,1	Yes	No	No	No
Patient 67	F	29	12	2/12/1997	85	285	462674	1,1	No	No	No	No
Patient 68	M	42	1	15/06/1999	83	420	9515	1,1	Yes	No	No	No
Patient 69	M	43	1	15/06/1997	85	324	208930	1,1	No	No	Yes	No
Patient 70	F	39	2	6/05/1998	77	348	17054	1,2	No	No	No	No
Patient 71	F	43	12	30/09/1998	76	528	21854	1,2	No	No	No	No
Patient 72	M	47	1	28/01/1998	71	ND	ND	1,2	No	No	No	No
Patient 73	M	47	1	26/08/1998	93	72	110200	1,2	Yes	No	No	No
Patient 74	M	37	1	10/12/1998	89	612	123479	1,2	Yes	No	No	Yes
Patient 75	M	61	1	14/05/1999	84	300	34443	1,2	Yes	No	No	No
Patient 76	M	46	3	11/12/1997	101	594	157692	2,2	Yes	No	No	No
Patient 77	M	39	1	6/06/1997	107	276	47863	2,2	Yes	No	No	No
Patient 78	F	29	2	14/10/1998	84	255	7669	NA	No	No	No	No
Patient 79	F	44	2	9/06/1997	101	341	30903	NA	Yes	No	No	No

Patient information sheet details

UMRNs of the patients who participated in the study are not provided here due to ethical constraints. However, each patient is represented by a patient number.

Data regarding HLA-A, HLA-B, HLA-C, HLA-DRB1 and IL-1A were derived from the HIV database for all the patients included in the study. Control caucasian HLA A and B frequencies were obtained from the database of bone marrow donors held at RPH (n = 879). Control HLA C and DR frequencies were obtained from the Busselton population study details of which were held at RPH (n = 200).

The patient information sheet also allowed the collection of data regarding mode of acquisition of virus and is represented by a code as described below.

Code	=	Mode of transfer of HIV
1	=	Male to male sexual contact
2	=	Male to female sexual contact
3	=	Female to male sexual contact
4	=	Female to female sexual contact
5	=	Sharing of contaminated needles and/or syringes
6	=	Transplacentally or congenitally
7	=	Through breast milk
8	=	Recipient of blood or blood products or tissue transplant or semen
9	=	Needle stick injury or similar acute exposure to material capable of HIV transmission
10	=	Male to male sexual contact and sharing of contaminated needles

11 = Male to female sexual contact and sharing of contaminated needles

12 = Unknown

2.4 Statistical analysis

Statistical analysis of the data was performed using SPSS version 14 for windows. Chi-square ($\chi 2$) analysis was used to observe the any significant relationship between categorical variables gender and continuity with NVP. A Two –sample T-test was used to determine any significant difference between continuous variable; age and categorical variable; continuity of NVP in the two groups (Yes and No groups). For continuity of NVP use and for baseline RNA levels, Non-parametric Mann-Whitney test was performed. Similar to baseline RNA, baseline CD4 in the three groups was compared using Non-parametric Mann-Whitney test. P value less than 0.05 was considered significant. Association between IL-1A and continuity with NVP was analysed using Chi-squared test.

2.4.1 Survival Analysis

To determine whether or not all the patients involved in the study truly continued on NVP a survival analysis was performed. Data collected contains several patients from groups I, II and III do not reveal the continuity on NVP due to lack of relevant data. Hence to verify whether or not the patients received NVP and to eliminate confusion a survival analysis was performed to explain the actual time the patients received the drug and taking HLA-B35+HLA-C4 also as criteria. A Kaplan Meier survival analysis was performed since it estimates the survival function from life-time data. In this analysis a Yes/No preference was made use of, demonstrating that those patients with HLA-B35+HLA-C4 and those without the same combination. A Yes-censored and No-censored criterion was used to determine the continuity of patient's use of NVP, declaring that those who continue NVP were Yes-censored and those patients who did not continue were No-censored.

Survival time refers to the collection of procedures performed statistically to analyse data in which the outcome variable of interest is time until an event occurs. Here in our analysis we considered continuity with NVP as the outcome variable. While performing survival analysis for time on NVP we refer NVP continuity as survival time. Censoring was performed to eliminate the survival analysis data since we don't know the survival time exactly and some patients may still continue with NVP or in remission. Survivor functions represent the probability of survival of a person or group longer than some specified time. The median survival times for group I (without HLA-B35+HLA-CW4) and group II (with HLA-B35+HLA-CW4) were 91 months and 30 months respectively. From this median survival times we can interpret that that group I tend to continue on NVP therapy compared to group II. The Log Rank Test can be employed to compare the difference between two survival curves. A p- value < 0.05 based on the Log Rank test indicates a significant difference between the two curves.

3 Results

3.1 Patient group comparison

A total of 79 patients were included in the study. The frequency distribution of the length of time that patients stayed on NVP therapy is shown in figure 3.1.

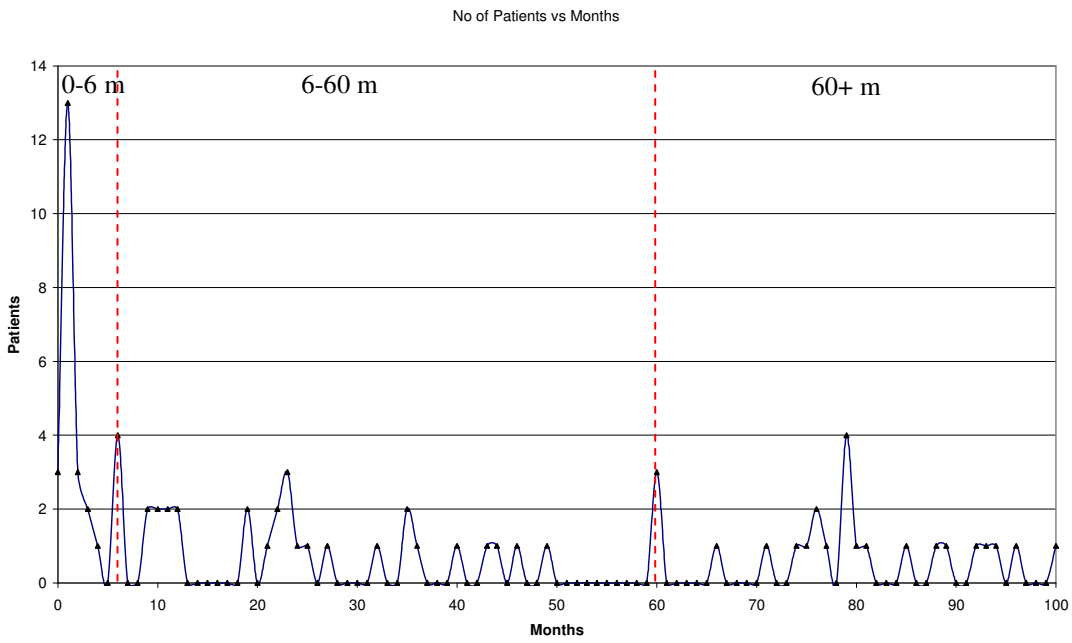


Figure 3-1 Frequency distribution of patients on Nevirapine according to duration of therapy

3.1.1 Definition of patient groups

From the above depiction of the data it was clear that a group of patients were discontinued from NVP therapy relatively early after commencement of treatment. Based on the clinical experience of one of the supervisors of this project (Professor Martyn French) the cut off point for this early withdrawal was arbitrarily taken as 6 months (Group I). The relevant data for this group are shown in Table 2-1. The remaining patients were then further divided into groups according to duration of therapy. Group II consisted of patients whose therapy had continued on the drug for between 6 months and 5 years (data shown in Table 2-2). Group III consisted of those whose treatment had persisted for longer than 5 years. It should be noted that some patients in each group were still taking NVP at the time of the study. These data are depicted in

Table 2-3. When conducting the survival analysis (section 3.1.9) whether patients were still on the drug was taken into consideration. Upon categorizing the population into these three groups, analysis of their characteristics was performed. In this analysis the factors considered were; sex, age, baseline CD4, baseline RNA, HLA and IL-1A genotype.

3.1.2 Effect of age on duration of NVP therapy

The effect of age of commencement on therapy on the ability to remain on long-term NVP was analysed in two ways. In the first those currently on NVP were compared with those who had ceased NVP. There was no significant difference in age between the two groups, ($p = 0.317$). Two –sample t-test was used.

Secondly, patients in three groups developed according to duration of NVP therapy were compared according to their age at the commencement of NVP therapy. When the patient group who had remained on NVP for longer than 5 years were compared with the two groups who had discontinued therapy, there appeared to be no significant difference (Table 3-1). Hence, this indicates that age does not have an effect on the length of stay on NVP.

Table 3-1 Mean age of patient in each group

Group	Number	Mean	P value
I	20	45.25	NS
II	35	45.97	NS
III	24	41.12	NS

NS= Not Significant

3.1.3 Effect of gender

There was no significant relationship between gender and whether or not the subject was continuing NVP use ($p = 0.165$). Chi-squared test was used. On comparing effect of gender to

duration of time on NVP, no significant association was observed (Table 3-2). Thus, results show that there existed no relation between gender and continuity of NVP use.

Table 3-2 Effect of gender on duration of NVP therapy

Group	Male	Female	P value
I	14	6	NS
II	31	4	NS
III	19	5	NS

NS= Not Significant

3.1.4 Effect of CD4 count

Baseline CD4 was not significantly different between those continuing NVP at the time of study and those who had discontinued treatment (p=0.288). Non-parametric Mann-Whitney test was used since the data was skewed.

When baseline CD4 counts of all the patients included in the study was compared with length of stay on NVP, no significant difference was observed (p=0.288). Individual CD4 counts of patients can be sighted from Table 3-3 and figure 3-2 represents the CD4 count vs. number of months in the study group. Non-parametric Mann-Whitney test was used since the data was skewed. The mean baseline CD4 values for groups I, II, III did not yield any significance differences. The average baseline CD4 was 437, 356.9, 352.4 respectively for groups I, II and III.

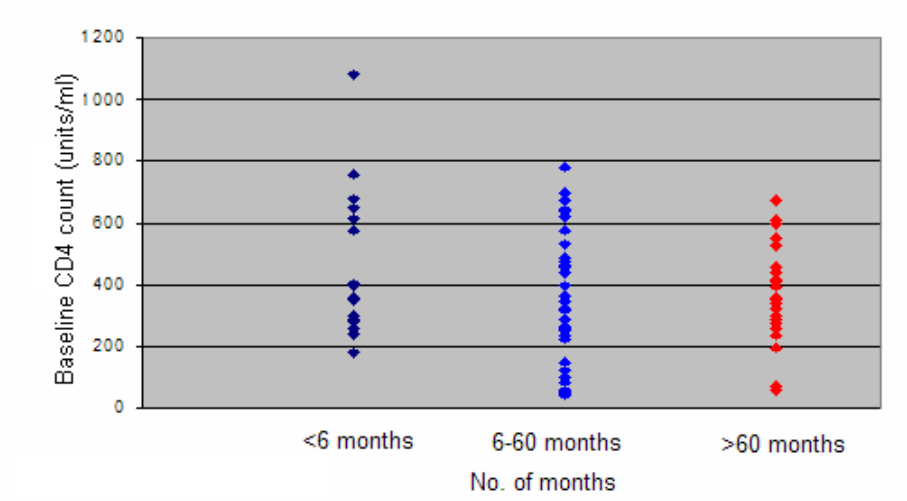


Figure 3-2 Baseline CD4 distribution

Table 3-3 Baseline CD4 distribution

CD4 Count	< 6 months	6-60 months	> 60 months
	180	44	60
	240	51	72
	240	52	195
	260	60	234
	280	84	255
	286	100	276
	288	122	285
	300	148	300
	350	224	324
	360	237	338
	396	250	341
	396	255	348
	400	266	357
	405	289	391
	576	290	408
	616	320	420
	650	322	440
	680	323	456
	756	342	528
	1081	364	549
		396	594
		437	612
		456	675
		462	
		475	
		476	
		486	
		531	
		576	
		620	
		640	
		644	
		672	
		696	
		780	

3.1.5 Effect of baseline RNA

Baseline RNA was not significantly different between those 2 groups (Yes, No) of continuing NVP use and those who had discontinued (p=0.872). Non-parametric Mann-Whitney test was used since the data was skewed. Comparing baseline RNA and continuity of NVP did not result any significantly different results (p=0.872). Table 3-4 represents the individual RNA values of the patients in the study and figure 3-3 represents Baseline RNA vs. number of months on therapy. Non-parametric Mann-Whitney test was used since the data was skewed. The mean baseline RNA values in the three groups did not yield any significant difference. The average baseline RNA values for groups <6m, 6-60m and >60m therapy were 138489.21, 149121.36 and 100538.56 respectively.

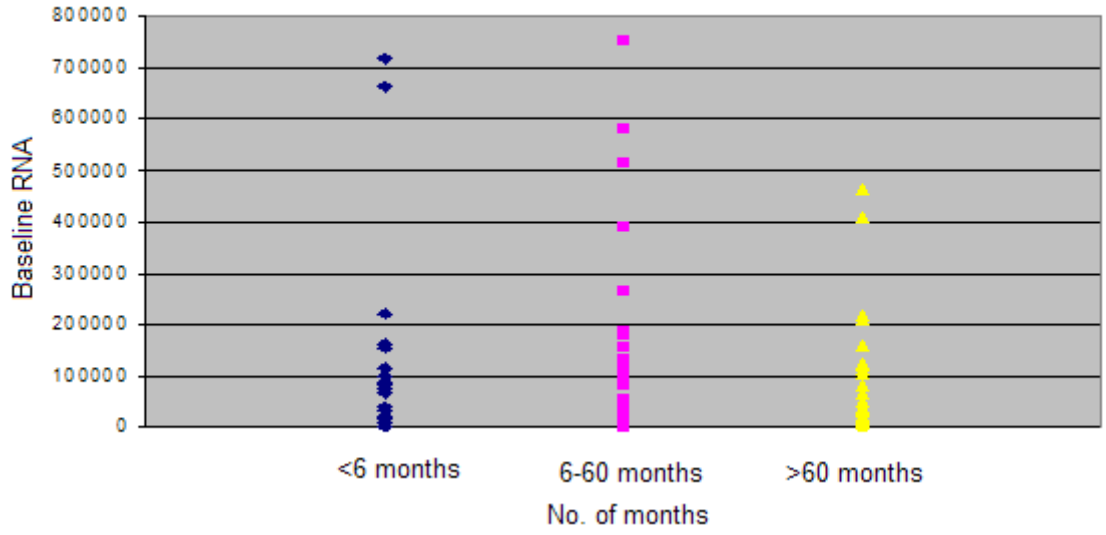


Figure 3-3 Baseline RNA distribution

Table 3-4 Baseline RNA distribution

HIV RNA	< 6 months	6-60 months	> 60 months
	17608	22754	104702
	81283	154882	12500
	17099	ND	82088
	19415	52000	218000
	15543	115343	31623
	66089	5536	410663
	39300	89300	28000
	85114	178178	5407
	660693	389045	117350
	33864	ND	64802
	115000	84812	4976
	155479	515355	462674
	75000	ND	9515
	161000	ND	208930
	100000	130000	17054
	219111	2130	21854
	ND	41800	ND
	39101	82823	110200
	11200	81283	123479
	719400	106000	34443
		30700	157692
		ND	47863
		750000	7669
		579225	30903
		188000	
		118779	
		4900	
		30200	
		14125	
		100000	
		100000	
		93325	
		100000	
		263027	
		50119	

3.1.6 Effect of IL-1A

There was no significant association between IL-1A genotype and continuing NVP use (p=0.931). Chi-squared test was used. When a Chi-squared ($\chi 2$) test was employed to identify any association between IL-1A and continuity of NVP, the result was found to be non significant (p= 0.931). Figure 3-4 represents number of patients vs. IL-1A and Table 3-5 represents the distribution of the IL-1A in the three groups.

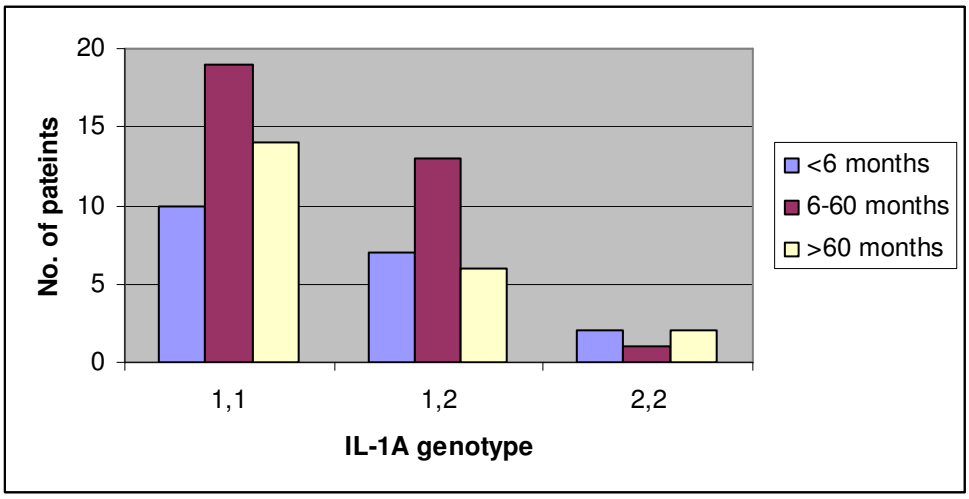


Figure 3-4 IL-1A genotype distribution

Table 3-5 IL-1A genotype distribution

IL-1A genotype	< 6 months	6-60 months	> 60 months
1,1	10	19	14
1,2	7	13	6
2,2	2	1	2

3.1.7 Effect of IV drug use

Upon observing the data collection sheet it is evident that only one patient from group I, three patients from group II and two patients from group III acquired HIV from mutual exchange of IV injections. However, taking the total population into consideration, the percentage of IV drug use was not significant.

3.1.8 Effect of HLA

The frequency of HLA alleles in different time groups can be observed from the following tables.

Table 3-6 HLA- A allele distribution

HLA A gene allele	0-6 months (n=22)	6-60 months (n=29)	60+ months (n=22)	Control Frequency
A1	27%	45%	18%	34%
A2	32%	34%	55%	47%
A3	18%	17%	27%	22%
A11	18%	24%	18%	12%
A23	5%	7%	0%	5%
A9	0%	3%	0%	NA
A24	9%	21%	18%	22%
A10	0%	0%	5%	NA
A25	0%	3%	5%	2%
A26	5%	0%	0%	5%
A29	14%	3%	5%	7%
A30	9%	7%	5%	5%
A31	0%	3%	5%	8%
A32	27%	3%	9%	5%
A33	5%	0%	0%	3%
A34	5%	3%	0%	2%
A36	5%	0%	0%	0%
A68	5%	3%	0%	6%
A69	0%	0%	0%	0%
A74	5%	0%	0%	0%

NA- not available

When a Chi squared analysis was performed comparing each individual HLA-A allele with the respective control frequency, the only significant association was for HLA-A 32 in 0-6 months group (27%) when compared against Control frequency (5%) ($p \leq 0.001$).

Table 3-7 HLA-B allele distribution

HLA B gene allele	<6 months (n=20)	6-60 months (n=29)	>60 months (n=22)	Control Frequency
B5	9%	0%	0%	NA
B7	4%	24%	35%	21%
B8	18%	7%	13%	25%
B13	9%	10%	9%	6%
B14	9%	0%	17%	6%
B15	0%	14%	9%	16%
B16	0%	3%	0%	NA
B17	0%	10%	0%	NA
B18	5%	10%	9%	7%
B27	9%	3%	13%	6%
B35	18%	27%	9%	13%
B37	0%	14%	0%	3%
B38	0%	3%	4%	3%
B39	5%	0%	4%	3%
B40	9%	24%	9%	19%
B41	0%	3%	9%	1%
B42	5%	0%	0%	0%
B44	14%	14%	17%	26%
B45	0%	3%	0%	1%
B49	5%	3%	17%	3%
B50	5%	0%	0%	3%
B51	14%	14%	4%	9%
B52	9%	0%	4%	1%
B53	0%	3%	0%	1%
B56	9%	0%	0%	3%
B57	5%	10%	13%	7%
B58	5%	0%	0%	3%
B35+CW4	18%	24%	0%	NA

NA- not available

When a Chi squared analysis was performed for all the HLA-B gene alleles comparing their frequencies in the subgroups of patients with the respective control frequencies, most of alleles were found not to be significantly different in frequency. However the frequencies of HLA-B14 in the 60+ group ($p < 0.05$), HLA-B 37 in 6-60 months group ($p < 0.01$), HLA-B41 in 60+

group ($p < 0.01$), HLA-B49 in 60+ group ($p < 0.001$) and HLA-B52 in the less than 6 months group ($p < 0.01$) were all significantly increased when compared with the respective control frequencies.

Table 3-8 HLA-C allele distribution

HLA C gene allele	<6 months (n=18)	6-60 months (n=26)	>60 months (n=22)	Control Frequency
Cw1	10%	8%	22%	3%
Cw2	5%	11%	4%	8%
Cw3	5%	31%	4%	32%
Cw4	19%	38%	9%	17%
Cw5	5%	4%	13%	18%
Cw6	24%	31%	13%	17%
Cw7	67%	35%	57%	52%
Cw8	10%	4%	17%	7%
Cw12	10%	4%	17%	10%
Cw14	10%	0%	0%	1%
Cw15	10%	11%	4%	6%
Cw16	10%	4%	4%	12%
Cw17	5%	8%	9%	2%

The frequencies of HLA-CW3 in the <6 months ($p < 0.025$) and 60+ patient groups ($p < 0.01$) were significantly different from the frequency in controls. Interestingly the frequency of HLA CW3 in the 6-60 month patient group was identical to control. By contrast the frequency of HLA –Cw4 in the 6-60 months group was significantly increased when compared to the control frequency ($p < 0.01$). HLA-CW17 in the 60+ group was also significantly increased when compared with its control frequency ($p < 0.025$).

Table 3-9 HLA-DRB1 allele distribution

HLA DRB1 gene allele	<6 months (n=17)	6-60 months (n=28)	>60 months (n=21)	Control Frequency
DR1	14%	14%	13%	22%
DR6	5%	3%	0%	NA
DR15	36%	21%	39%	21%
DR16	5%	3%	0%	3%
DR3	27%	18%	17%	23%
DR4	9%	28%	26%	34%
DR11	14%	18%	13%	17%
DR12	0%	3%	9%	5%
DR13	9%	10%	26%	22%
DR14	9%	21%	0%	4%
DR7	41%	21%	30%	30%
DR8	14%	7%	4%	5%
DR9	0%	7%	0%	5%
DR10	5%	3%	0%	1%

NA- not available

There existed no significant relation between most *HLA-DRB1* alleles and their respective control frequencies. The frequency of HLA-DR14 in the 6-60 months group was found to be significantly different from its control frequency when Chi-squared analysis was performed ($p < 0.001$).

In contrast to earlier published data (Littera et al.) we found no association between the presence of HLA-DR1 or HLA-B14 and early withdrawal from NVP therapy (Tables 3.6 to 3.9).

Since the 35.1 ancestral haplotype has previously been associated with AIDS progression the frequencies of the combination of HLA-B35 and HLA-CW4 (part of the 35.1 ancestral haplotype) in the patient groups and controls were compared with each other to determine any possible influence on the longevity of NVP therapy. The combination HLA-B35+HLA-CW4 did not occur in any of the NVP long term responders (NLTRs). This association between HLA-B35+HLA-CW4 and NLTRs was not described in previous studies. Furthermore, when a survival analysis was performed carriage of HLA-B35+HLA-CW4 identified patients who were not NLTRs. The median survival time for patients with HLA-B35+HLA-CW4 was 30 months compared to 91 months with those without HLA B35+HLA-CW4. The survival

distributions were significantly different (p= 0.002). Survival analysis here means the time analysed till the cessation of NVP use.

3.1.9 Survival Analysis

A survival analysis of patients with HLA B35+CW4 compared with those not bearing this combination of HLA antigens is shown in figure 3-2. The data was depicted in two ways. In the first all patients were included, even those whose duration of NVP therapy was shorter than 60 months but were still on the drug (non-censored). The second analysis made allowance for those patients who were still on NVP therapy but whose duration of therapy was less than 60 months (censored). The significant effect of HLAB35+CW4 on continuation of NVP therapy was clear no matter which analysis is considered.

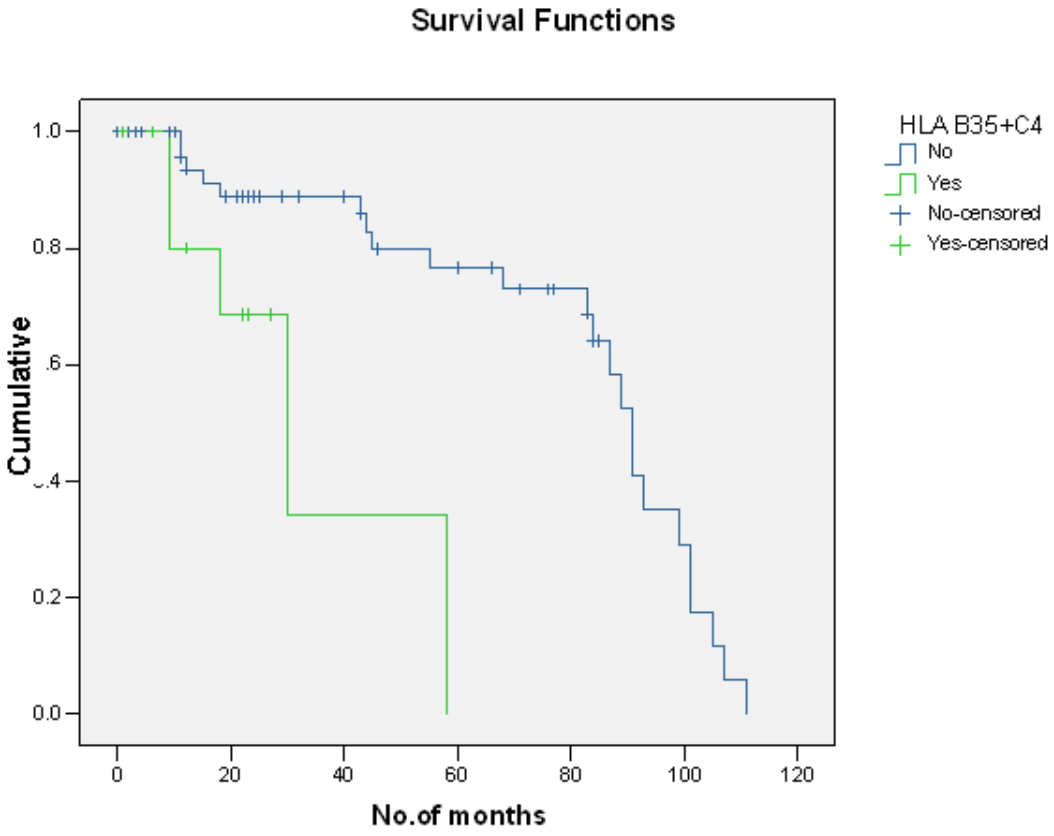


Figure 3-5 HLA- B35+HLAC4 Survival analysis

The median survival time for those with HLAB35+C45 is 30 months compared to those without: 91 months**.**

Table 3-10 Overall comparisons of survival curve

Test	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	9.987	1	0.002

The survival distributions are significantly different (p=0.002), thus indicating that subjects without HLA-B35+HLA-CW4 combination used NVP for a significantly longer duration compared to those without the combination of genotype. However, study on a larger cohort and potentially including treatment-experienced patients may divulge more reasons for NVP discontinuation.

3.1.10 Interpretation of survival curve

The graph shows that the survivor function for group with HLA-B35+HLA-CW4 consistently lies above that of the group that lacks the allele combination. However, the two groups were somewhat closer in the first few months of follow-up, but thereafter are quite spread apart. This widening gap suggests that the people who discontinued from NVP therapy had HLA-B35+HLA-CW4 combination and those without the allele combination continued therapy.

4 Discussion

This study examined the characteristics of long term responders of Nevirapine therapy considering different factors such as age, gender, baseline CD4 T cell count, baseline RNA levels, IL-1A genotype, and various HLA alleles. The data for the study was obtained from the Department of Immunology, Royal Perth Hospital, Western Australia. Royal Perth Hospital is the state regional centre for HIV/AIDS diagnosis and treatment. The present study investigated whether or not all the above factors predicted the long term response to NVP therapy. The main objectives of the study were to define Nevirapine long term responders and identify the characteristics of patients who stay on the drug for long periods of time without adverse effects and maintain suppression of HIV replication without development of resistance.

4.1 Significance of the study

NVP is a very potent drug and was the first NNRTI available for the treatment of HIV infection. It is an extensively used ARV drug and is a crucial component of HAART[126, 127]. NVP can be used in treatment simplification strategies and also involved in the treatment of NVP naïve patients[128-130] . Although the clinical results of Nevirapine therapy are promising there is still a concern about the high drop-out rate of patients from the therapy. Given the seriousness of this complication it is important to determine the most effective means of prevention of drop-out rates. Upon examining the patients genetic nature by evaluating the results from gene marker reports we may be able to predict whether the patient would adhere to the therapy or drop-out from the therapy.

4.2 Incidence of Adherence to Nevirapine based on gender

A research by Bersoff-Matcha et al[124], demonstrated that females had 7-fold increased risk of severe rash and were 3.5 times more likely to discontinue NVP therapy compared to men. Some people are intolerant to the adverse effects of the drug amongst which rash and liver toxicity are the major ones[6]. Nevirapine has an excellent safety profile and its penetration into CNS provides an option for patients with HIV associated dementia. But the severe adverse affects of NVP like hepatic toxicity, fever, oral lesions and ulcers and the most common treatment-limiting adverse effect rash restricted the universal use of the drug. The study by

Bersoff-Matcha et al started with the approach that female gender is more prone to acquire rash compared to males. The participants included in the study were ≥13 years of age who received ARV regimen with NVP as a component. The results in the study stated that severe rash occurred in 3.4% of the population. The risk of severe rash was 1.1% in men and 9.5% in females. The rate of discontinuation NVP was more among females (13.7%) compared to males (3%) due to occurrence of rash.

However, in this research at RPH, when we tried to observe any association between gender and continuity with NVP using Chi-squared test, a P value of 0.165 was obtained showing that there was no significant relationship between gender and continuity with NVP.

4.3 Incidence of adherence to Nevirapine based on CD4+T cell count and baseline RNA levels

A study performed by Sanne et al claimed that female patients with CD4+ T cell count > 250 cells/mm^3 and male patients with CD4+T cell count > 400 cells/mm^3 are more susceptible to get liver toxicity and are likely to discontinue NVP therapy[110]. The study recruited HIV-1 infected non-pregnant women and men in South Africa with plasma RNA levels >5000 copies/ml and CD4+ T cells/$mm^{3,}$ all of whom were ARV therapy naïve. The patient population who met the inclusion criteria was 468 who were enrolled between August 1999 and February 2000. 385 patients (82%) were administered NVP as part of their ARV regimen. 17% of the patients who received NVP acquired hepatotoxicity , 12.8% in men and 20.1% in women. The onset of hepatotoxic events started within 12 weeks of the start of NVP therapy in 80% of the patients who received NVP. In accord with other studies the study by Sanne et al confirmed that women with CD4+T cell count >250 cells/mm^3 and men with CD4+ T cell count >400 cells/mm^3 hepatotoxic events compared to those with lesser CD4 counts. The reason for this was attributed to low BMI (<18.5) for females due to low nutritional status.

In contrast to the previous studies, in our study none of the factors either, age, sex, baseline CD4+ T cell count, baseline viral RNA count seemed to influence long term use of NVP. Results from the study indicated that there was no significant difference between patient demographics between each of the three groups.

A study by Martin et al[131] attempted to establish a relation between NVP hypersensitivity and presence of HLA DR1 and CD4 status. Before start of their study, they predicted that there could be an association between HLA markers in class II region of the MHC and low CD4+ T cell counts and occurrence of NVP hypersensitivity. The study was started with the belief that MHC class II limits the CD4+ T cell mediated immune responses directed against drug specific antigens which inturn might be responsible for drug hypersensitivity. A total 235 patients who received NVP for more than 6 weeks without symptoms or who developed NVP induced reactions were included in the study. HLA typing was performed at HLA-DR, HLA-DQ, HLA-A, HLA-B and HLA-C loci at enrolment. Upon analysis it was revealed that threat of hepatic toxicity reactions due to NVP was most significantly associated with interaction between presence of HLA-DR1 and high CD4+ T cell count. The rate of occurrence of NVP toxicity was similar in those with low CD4+ T cell count and those with high CD4+ T cell count but who lacked HLA-DR1. Conversely, presence of HLA-DR1 and highCD4+ T cell count was associated with higher risk of NVP toxicity.

On comparing the results of the above study with our research, there was no such relation between HLA-DR1 and CD4+ T cell count and NVP toxicity which inturn reflects in adherence to NVP therapy.

A study by Littera [132] stated that a combination of HLA-B14 and HLA-C8 may determine the susceptibility to NVP hypersensitivity. They predicted that NVP toxicity is not either due to NVP dose or due to an association with cytochrome P450 enzyme. NVP toxicity could be due to a hypersensitive reaction in which NVP or its glucuronide metabolites act as specific antigens which elicit a T-cell mediated immune response in genetically susceptible individuals. Based on these principles they hypothesised that hypersensitivity reaction due to NVP may be HLA associated. A total of 49 HIV-1 positive patients were enrolled for the study. Before start of therapy all the patients were subjected to tests to determine the levels of liver enzymes, screening for hepatitis C virus antibodies and hepatitis B virus surface antigens. Levels of CD4+ T cell and CD8+ T Cells were also determined. The results demonstrated that 13 of the 49 patients developed hypersensitivity to NVP within 3-60 days of start of NVP therapy. HLA typing showed that six of the thirteen patients (48%) had HLA-B14, HLA-C8 antigens. The results proved the hypothesis acceptable where patients with HLA-B14 and HLA-C8

combination would develop hypersensitivity with NVP therapy. Thus the authors confirmed the role of HLA molecules in NVP hypersensitivity.

Upon comparing our study results with the study mentioned above, no correlation between HLA-B14 and HLA-C8 combination and continuity with NVP was demonstrated.

Immunologists at RPH headed by Patricia Price[12] performed study that tried to investigate whether polymorphisms in cytokine genes affected the control of HIV RNA for people receiving HAART over five years. They tried to throw light on the association between virological failure and polymorphisms in genes encoding pro-inflammatory cytokines. All the patients included in the study were recent cases who were detected HIV positive with low CD4+ T cell counts of <100cells/µl. A total of 81 patients met with the inclusion criteria and were placed on ARVs using a combination of a protease inhibitor, NRTI and NNRTI. IL1A-889 and IL1A+4985 alleles were assessed using restriction fragment length polymorphism (RFLP). The results state that carriage of allele 2 at position -889 in the IL1A gene could predict the control of viraemia for patients who are immunodeficient (low CD4+ T cell count) and responding to HAART. The same results were observed for polymorphisms at IL1A+4845 and thus in concordance with the predictions of IL1A-889. In conclusion they declared that alleles IL1A-889 or IL1A+4845 may expect to control of HIV replication in patients with a low CD4+ T cell who respond commendably with HAART.

When a comparison was made with the above study with our research, IL1A (1,1), (1,2) and (2,2) ; no significant relation between IL1A and continuity with NVP were observed (p= 0.931).

Upon performing a survival analysis for length of time on NVP and using presence or absence of HLA-B35+HLA-CW4 alleles as criteria, we did find that NLTRs never had the HLA-B35+HLA-C4 antigen combination. Thus, this finding suggests that carriage of these genes is a risk factor for toxicity or early development of NVP resistance. These two HLA antigens form part of the 35.1 and 35.2 ancestral haplotypes which have previously been associated with rapid progression to AIDS in patients infected with HIV (Flores- Villaneuva et al, 2003)[133]. Both HLA B35 and CW4 were present in some patients in the NLTR group so that it is unlikely that these alleles by themselves confer susceptibility to adverse reaction to NVP. Interestingly, neither DR11 nor DR1, which are present on the 35.1 and 35.2 haplotype, were not associated

with early withdrawal of NVP in this study. Whether the association of the 35.1 and 35.2 ancestral hapotypes with disease progression is relevant to the observation in this thesis is unclear, although the presence of this haplotype, or at least genes within that haplotype, may contribute to more severe disease by affecting both disease progression and continuity of treatment with one potentially valuable anti-retroviral drug

We stated in the hypothesis that the factors considered for the study may direct us towards preventing NVP induced toxicities to patients. In accordance with the hypothesis the study proclaims that those patients with a combination of HLA-B35+HLA-CW4 should not be administered NVP, though more information needs to be obtained as to why patients discontinued the therapy since complete information not available at the time of data collection. However, for those patients without the HLA-B35+HLA-CW4 allele can be recommended NVP therapy, for such patients NVP can be advised as part of the first regimen for HAART or NVP can be introduced if any of the previously used ARV drugs proved ineffective.

A number of other HLA-A, HLA-B and HLA-C antigens were increased in frequency in certain subgroups of patients compared to other subgroups and controls. Although, these analyses were not subject to Bonferroni correction, so that some of the weaker associations may have been demonstrated by chance alone, these observations might suggest that other ancestral haplotypes or at least HLA antigens could be implicated in the response to NVP. Clarification of this issue will require more detailed study of a larger group of patients and to explore reasons to ceasing the drug.

5 Conclusion and Future Studies

In conclusion it can be stated that various demographic and genetic factors considered for determination of NVP long-term response could not foretell the reasons for the high drop-out rate from NVP therapy. However, this study found that HIV patients with HLA-B35+HLA-CW4 antigen were prone to discontinue NVP. Further studies need to be undertaken to determine the reasons for this.

A comparison of these study results with studies in the same setup with a larger population would consolidate the results of this study. Further analyses of the frequency of HLA-B35+HLA-CW4 antigen in patients who developed hypersensitivity reaction to NVP (skin rashes and liver toxicity) is warranted. It would be helpful if studies involving other combinations of HLA antigens are studied for the betterment in the advancement of HIV medicine and care to HIV patients.

6 References

1. Nielsen MH, Pedersen FS, Kjems J. Molecular strategies to inhibit HIV-1 replication. Retrovirology. 2005; 2:10.

2. Australian Agency for International Development. In: Meeting the challenge:Australia's international HIV/AIDS strategy. 2004. Canberra: Australian Agency for International Development.

3. UNAIDS. Report on the global AIDS epidemic 2006. Switzerland; 2006.

4. Merson MH. The HIV-AIDS pandemic at 25--the global response. N Engl J Med. 2006; 354(23):2414-7.

5. National Centre in HIV Epidemiology and Clinical Research. HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia Annual Surveillance Report 2006.

6. Dieterich DT, Robinson PA, Love J, et al. Drug-induced liver injury associated with the use of nonnucleoside reverse-transcriptase inhibitors. Clin Infect Dis. 2004; 38 Suppl 2:S80-9.

7. Stringer JS, Sinkala M, Rouse DJ, et al. Effect of nevirapine toxicity on choice of perinatal HIV prevention strategies. Am J Public Health. 2002; 92(3):365-6.

8. Taburet AM, Paci-Bonaventure S, Peytavin G, et al. Once-daily administration of antiretrovirals: pharmacokinetics of emerging therapies. Clin Pharmacokinet. 2003; 42(14):1179-91.

9. Perinatal zidovudine plus nevirapine results in similar rates of mother-to-child transmission of HIV compared with nevirapine alone. Evid-Based Healthcare and Pub health. 2005; 9(1):32-33.

10. Phillips AN, Miller V, Sabin C, et al. Durability of HIV-1 viral suppression over 3.3 years with multi-drug antiretroviral therapy in previously drug-naive individuals. Aids. 2001; 15(18):2379-84.

11. Chen RY, Westfall AO, Mugavero MJ, et al. Duration of highly active antiretroviral therapy regimens. Clin Infect Dis. 2003; 37(5):714-22.

12. Price P, James I, Fernandez S, et al. Alleles of the gene encoding IL-1alpha may predict control of plasma viraemia in HIV-1 patients on highly active antiretroviral therapy. Aids. 2004; 18(11):1495-501.

13. O'Brien TR, McDermott DH, Ioannidis JP, et al. Effect of chemokine receptor gene polymorphisms on the response to potent antiretroviral therapy. Aids. 2000; 14(7):821-6.

14. Brumme ZL, Dong WW, Chan KJ, et al. Influence of polymorphisms within the CX3CR1 and MDR-1 genes on initial antiretroviral therapy response. Aids. 2003; 17(2):201-8.

15. Bratt G, Karlsson A, Leandersson AC, et al. Treatment history and baseline viral load, but not viral tropism or CCR-5 genotype, influence prolonged antiviral efficacy of highly active antiretroviral treatment. Aids. 1998; 12(16):2193-202.

16. Zanetti G, Briggs JA, Grunewald K, et al. Cryo-electron tomographic structure of an immunodeficiency virus envelope complex in situ. PLoS Pathog. 2006; 2(8):e83.

17. Chinen J, Shearer WT. Molecular virology and immunology of HIV infection. J Allergy Clin Immunol. 2002; 110(2):189-198.

18. Farnet CM, Bushman FD. HIV cDNA integration: molecular biology and inhibitor development. Aids. 1996; 10 Suppl A:S3-11.

19. Serres PF. Molecular mimicry between the trimeric ectodomain of the transmembrane protein of immunosuppressive lentiviruses (HIV-SIV-FIV) and interleukin 2. C R Acad Sci III. 2000; 323(11):1019-29.

20. De Rozieres S, Swan CH, Sheeter DA, et al. Assessment of FIV-C infection of cats as a function of treatment with the protease inhibitor, TL-3. Retrovirology. 2004; 1:38.

21. Freed EO, Martin MA. The role of human immunodeficiency virus type 1 envelope glycoproteins in virus infection. J Biol Chem. 1995; 270(41):23883-6.

22. Luciw PA. Human immunodefeciency virus and its replication Philadelphia: Lippincott-Raven; 1996.

23. Schaeffer E, Geleziunas R, Greene WC. Human immunodeficiency virus type 1 Nef functions at the level of virus entry by enhancing cytoplasmic delivery of virions. J Virol. 2001; 75(6):2993-3000.

24. Littman DR. Chemokine Receptors: Keys to AIDS Pathogenesis? . Cell. 1998; 93(5):677-680.

25. Dalgleish AG, Beverley PCL, Clapham PR, et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature. 1984; 312(5996):763-767.

26. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature. 1984; 312(5996):767-768.

27. Cladera J, Martin I, O'Shea P. The fusion domain of HIV gp41 interacts specifically with heparan sulfate on the T-lymphocyte cell surface. EMBO J. 2001; 20(1-2):19-26.

28. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science. 1998; 280(5371):1884-8.

29. Cen S, Khorchid A, Javanbakht H, et al. Incorporation of lysyl-tRNA synthetase into human immunodeficiency virus type 1. J Virol. 2001; 75(11):5043-8.

30. Tantillo C, Ding J, Jacobo-Molina A, et al. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. J Mol Biol. 1994; 243(3):369-87.

31. Parada CA, Roeder RG. Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. Nature. 1996; 384(6607):375-8.

32. Gracia JA, Gaynor B. Regulatory mechanisms involved in the control of HIV-1 expression. Aids. 1994; 8:S13-S17.

33. Emerman M, Malim MH. HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science. 1998; 280(5371):1880-4.

34. Tritel M, Resh MD. The late stage of human immunodeficiency virus type 1 assembly is an energy-dependent process. J Virol. 2001; 75(12):5473-81.

35. Schubert U, Anton LC, Bacik I, et al. CD4 glycoprotein degradation induced by human immunodeficiency virus type 1 Vpu protein requires the function of proteasomes and the ubiquitin-conjugating pathway. J Virol. 1998; 72(3):2280-8.

36. Pantaleo G, Fauci AS. Immunopathogenesis of HIV infection. Annu Rev Microbiol. 1996; 50:825-54.

37. Booth CL, Garcia-Diaz AM, Youle MS, et al. Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection. J Antimicrob Chemother. 2007; 59(3):517-24.

38. Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med. 1991; 324(14):954-60.

39. Daar ES, Moudgil T, Meyer RD, et al. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med. 1991; 324(14):961-4.

40. Tindall B, Cooper DA. Primary HIV infection: host responses and intervention strategies. Aids. 1991; 5(1):1-14.

41. Borrow P Lh, Hahn Bh, et al CTL activity associated with control of viremia in primary HIV -1 infection J Virol. 1994; 68:6103-6110.

42. Chakrabarti L, Isola P, Cumont MC, et al. Early stages of simian immunodeficiency virus infection in lymph nodes. Evidence for high viral load and successive populations of target cells. Am J Pathol. 1994; 144(6):1226-37.

43. Graziosi C, Pantaleo G, Butini L, et al. Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. Proc Natl Acad Sci U S A. 1993; 90(14):6405-9.

44. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol. 1994; 68(7):4650-5.

45. Pantaleo G, Demarest JF, Soudeyns H, et al. Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV. Nature. 1994; 370(6489):463-7.

46. Moore JP, Cao Y, Ho DD, et al. Development of the anti-gp120 antibody response during seroconversion to human immunodeficiency virus type 1. J Virol. 1994; 68(8):5142-55.

47. Reimann KA, Tenner-Racz K, Racz P, et al. Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Virol. 1994; 68(4):2362-70.

48. Buchbinder SP, Katz MH, Hessol NA, et al. Long-term HIV-1 infection without immunologic progression. Aids. 1994; 8(8):1123-8.

49. Lifson AR, Buchbinder SP, Sheppard HW, et al. Long-term human immunodeficiency virus infection in asymptomatic homosexual and bisexual men with normal CD4+ lymphocyte counts: immunologic and virologic characteristics. J Infect Dis. 1991; 163(5):959-65.

50. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993; 362(6418):355-8.

51. Pantaleo G, Graziosi C, Butini L, et al. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc Natl Acad Sci U S A. 1991; 88(21):9838-42.

52. Piatak M, Jr., Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science. 1993; 259(5102):1749-54.

53. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature. 1993; 362(6418):359-62.

54. Fauci AS, Lane HC. The acquired immunodeficiency syndrome (AIDS): an update. Int Arch Allergy Appl Immunol. 1985; 77(1-2):81-8.

55. Denis M, Ghadirian E. Mycobacterium avium infection in HIV-1-infected subjects increases monokine secretion and is associated with enhanced viral load and diminished immune response to viral antigens. Clin Exp Immunol. 1994; 97(1):76-82.

56. Heng MC, Heng SY, Allen SG. Co-infection and synergy of human immunodeficiency virus-1 and herpes simplex virus-1. Lancet. 1994; 343(8892):255-8.

57. Ho DD. HIV-1 viraemia and influenza. Lancet. 1992; 339(8808):1549.

58. Israel-Biet D, Cadranel J, Even P. Human immunodeficiency virus production by alveolar lymphocytes is increased during Pneumocystis carinii pneumonia. Am Rev Respir Dis. 1993; 148(5):1308-12.

59. Zhang Y, Nakata K, Weiden M, et al. Mycobacterium tuberculosis enhances human immunodeficiency virus-1 replication by transcriptional activation at the long terminal repeat. J Clin Invest. 1995; 95(5):2324-31.

60. Pantaleo G, Graziosi C, Demarest JF, et al. Role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection. Immunol Rev. 1994; 140:105-30.

61. Haynes BF, Pantaleo G, Fauci AS. Toward an understanding of the correlates of protective immunity to HIV infection. Science. 1996; 271(5247):324-8.

62. Graziosi C, Pantaleo G, Gantt KR, et al. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. Science. 1994; 265(5169):248-52.

63. Scosyrev E. An overview of the human immunodeficiency virus featuring laboratory testing for drug resistance. Clin Lab Sci. 2006; 19(4):231-45; quiz 246-9.

64. Lujan-Zilbermann J, Rodriguez CA, Emmanuel PJ. Pediatric HIV infection: diagnostic laboratory methods. Fetal Pediatr Pathol. 2006; 25(5):249-60.

65. Taye B, Woldeamanuel Y, Kebede E. Diagnostic detection of human immunodeficincy virus type-1 antibodies in urine, Jimma Hospital, south west Ethiopa. Ethiop Med J. 2006; 44(4):363-8.

66. Dunn DT, Gibb DM, Duong T, et al. Predictive value of absolute CD4 cell count for disease progression in untreated HIV-1-infected children. Aids. 2006; 20(9):1289-94.

67. Salem AH, Batzer MA. Distribution of the HIV resistance CCR5-Delta32 allele among Egyptians and Syrians. Mutat Res. 2007; 616(1-2):175-80.

68. Murphy PM. Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine Growth Factor Rev. 1996; 7(1):47-64.

69. Libert F, Cochaux P, Beckman G, et al. The deltaccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. Hum Mol Genet. 1998; 7(3):399-406.

70. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 1996; 272(5263):872-7.

71. Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature. 1996; 381(6584):667-73.

72. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature. 1996; 381(6584):661-6.

73. Picker LJ. Immunopathogenesis of acute AIDS virus infection. Curr Opin Immunol. 2006; 18(4):399-405.

74. Montaner JS, Cote HC, Harris M, et al. Nucleoside-related mitochondrial toxicity among HIV-infected patients receiving antiretroviral therapy: insights from the evaluation of

venous lactic acid and peripheral blood mitochondrial DNA. Clin Infect Dis. 2004; 38 Suppl 2:S73-9.

75. Weller IV, Williams IG. ABC of AIDS. Antiretroviral drugs. BMJ. 2001; 322(7299):1410-2.

76. Powderly WG. Long-term exposure to lifelong therapies. J Acquir Immune Defic Syndr. 2002; 29 Suppl 1:S28-40.

77. Fauci AS, Touchette NA, Folkers GK. Emerging infectious diseases: a 10-year perspective from the National Institute of Allergy and Infectious Diseases. Emerg Infect Dis. 2005; 11(4):519-25.

78. Dai Y, Qiu ZF, Li TS, et al. Clinical outcomes and immune reconstitution in 103 advanced AIDS patients undergoing 12-month highly active antiretroviral therapy. Chin Med J (Engl). 2006; 119(20):1677-82.

79. Gazzard B, Bernard AJ, Boffito M, et al. British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy (2006). HIV Med. 2006; 7(8):487-503.

80. Wood E, Hogg RS, Harrigan PR, et al. When to initiate antiretroviral therapy in HIV-1-infected adults: a review for clinicians and patients. The Lancet Infectious Diseases. 2005; 5(7):407-414.

81. Volberding P. When and where to start: guidelines for the initiation of antiretroviral therapy. AIDS Read. 2000; 10(3):150-5; discussion 171-4.

82. Holmberg SD, Palella FJJ, Lichtenstein KA, et al. The case for earlier treatment of HIV infection. Clin Infect Dis. 2004; 39(11):1699-704.

83. Colonno RJ, Thiry A, Limoli K, et al. Activities of atazanavir (BMS-232632) against a large panel of human immunodeficiency virus type 1 clinical isolates resistant to one or more approved protease inhibitors. Antimicrob Agents Chemother. 2003; 47(4):1324-33.

84. Wegner SA, Brodine SK, Mascola JR, et al. Prevalence of genotypic and phenotypic resistance to anti-retroviral drugs in a cohort of therapy-naive HIV-1 infected US military personnel. Aids. 2000; 14(8):1009-15.

85. Gallant JE. Initial therapy of HIV infection. J Clin Virol. 2002; 25(3):317-33.

86. Max B, Sherer R. Management of the adverse effects of antiretroviral therapy and medication adherence. Clin Infect Dis. 2000; 30 Suppl 2:S96-116.

87. Valdez H, Lederman MM, Woolley I, et al. Human immunodeficiency virus 1 protease inhibitors in clinical practice: predictors of virological outcome. Arch Intern Med. 1999; 159(15):1771-6.

88. Ickovics JR, Meade CS. Adherence to HAART among patients with HIV: breakthroughs and barriers. AIDS Care. 2002; 14(3):309-18.

89. Gross R, Bilker WB, Friedman HM, et al. Provider inaccuracy in assessing adherence and outcomes with newly initiated antiretroviral therapy. Aids. 2002; 16(13):1835-7.

90. Bangsberg DR, Hecht FM, Charlebois ED, et al. Adherence to protease inhibitors, HIV-1 viral load, and development of drug resistance in an indigent population. Aids. 2000; 14(4):357-66.

91. Ickovics JR, Meisler AW. Adherence in AIDS clinical trials: A framework for clinical research and clinical care. J Clin Epidemiol. 1997; 50(4):385-391.

92. Piliero P. Developments in HIV treatments: strategies for success. AIDS Read. 2004; 14(12):655-65.

93. Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. Clin Ther. 2000; 22(6):685-708.

94. Wlodawer A, Vondrasek J. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. Annu Rev Biophys Biomol Struct. 1998; 27:249-84.

95. Flexner C. HIV-protease inhibitors. N Engl J Med. 1998; 338(18):1281-92.

96. Dietrich U. HIV-1 Entry Inhibitors. AIDS Rev. 2001; 3:89-97.

97. De Clercq E. HIV-1-specific RT inhibitors: highly selective inhibitors of human immunodeficiency virus type 1 that are specifically targeted at the viral reverse transcriptase. Med Res Rev. 1993; 13(3):229-58.

98. Cheeseman SH, Hattox SE, McLaughlin MM, et al. Pharmacokinetics of nevirapine: initial single-rising-dose study in humans. Antimicrob Agents Chemother. 1993; 37(2):178-82.

99. Lamson MJ, Sabo JP, MacGregor TR, et al. Single dose pharmacokinetics and bioavailability of nevirapine in healthy volunteers. Biopharm Drug Dispos. 1999; 20(6):285-91.

100. Susan L. Glynn MY. In vitro blood-brain barrier permeability of nevirapine compared to other HIV antiretroviral agents. J Pharm Sci. 1998; 87(3):306-310.

101. Gibbs JE, Gaffen Z, Thomas SA. Nevirapine uptake into the central nervous system of the Guinea pig: an in situ brain perfusion study. J Pharmacol Exp Ther. 2006; 317(2):746-51.

102. van Heeswijk RP, Veldkamp AI, Mulder JW, et al. The steady-state pharmacokinetics of nevirapine during once daily and twice daily dosing in HIV-1-infected individuals. Aids. 2000; 14(8):F77-82.

103. Warren JB. Chemotherapeutic agents for human immunodeficiency virus infection: mechanism of action, pharmacokinetics, metabolism, and adverse reactions. Clin Ther. 1998; 20(1):2-25.

104. Murphy RL, Montaner J. Nevirapine: a review of its development, pharmacological profile and potential for clinical use. Exp opin Invest Drugs. 1996; 5:1183-99.

105. Ingelheim B. Viramune Product Information. Ridgefield,CT, USA: Boehringer Ingelheim Pharmaceuticals,Inc.; 2005.

106. Wetterwald E, Le Cleach L, Michel C, et al. Nevirapine-induced overlap stevens-johnson syndrome/toxic epidermal necrolysis. Br J Dermatol. 1999; 140(5):980-2.

107. Palmon R, Koo BC, Shoultz DA, et al. Lack of hepatotoxicity associated with nonnucleoside reverse transcriptase inhibitors. J Acquir Immune Defic Syndr. 2002; 29(4):340-5.

108. Sulkowski MS, Thomas DL, Mehta SH, et al. Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections. Hepatology. 2002; 35(1):182-9.

109. Sulkowski MS, Thomas DL, Chaisson RE, et al. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. Jama. 2000; 283(1):74-80.

110. Sanne I, Mommeja-Marin H, Hinkle J, et al. Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. J Infect Dis. 2005; 191(6):825-9.

111. Wit FW, Weverling GJ, Weel J, et al. Incidence and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. J Infect Dis. 2002; 186(1):23-31.

112. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003; 349(5):474-85.

113. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet. 1999; 354(9181):795-802.

114. Jackson JB, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. Lancet. 2003; 362(9387):859-68.

115. Moodley D, Moodley J, Coovadia H, et al. A multicenter randomized controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early

postpartum mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis. 2003; 187(5):725-35.

116. Taha TE, Kumwenda NI, Gibbons A, et al. Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: NVAZ randomised clinical trial. Lancet. 2003; 362(9391):1171-7.

117. Lallemant M, Jourdain G, Le Coeur S, et al. Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. N Engl J Med. 2004; 351(3):217-28.

118. Dorenbaum A, Cunningham CK, Gelber RD, et al. Two-dose intrapartum/newborn nevirapine and standard antiretroviral therapy to reduce perinatal HIV transmission: a randomized trial. Jama. 2002; 288(2):189-98.

119. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol. 1994; 68(3):1660-6.

120. Havlir DV, Eastman S, Gamst A, et al. Nevirapine-resistant human immunodeficiency virus: kinetics of replication and estimated prevalence in untreated patients. J Virol. 1996; 70(11):7894-9.

121. Wei X GS, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature. 1995; 373:117-122.

122. Cunningham C. Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections In: Genotypic resistance analysis in women participating in PACTG 316 with HIV-1 RNA >400 copies/ml. 2002. Seattle, Washington.

123. Maga G, Amacker M, Ruel N, et al. Resistance to nevirapine of HIV-1 reverse transcriptase mutants: loss of stabilizing interactions and thermodynamic or steric barriers are induced by different single amino acid substitutions. J Mol Biol. 1997; 274(5):738-47.

124. Bersoff-Matcha SJ, Miller WC, Aberg JA, et al. Sex differences in nevirapine rash. Clin Infect Dis. 2001; 32(1):124-9.

125. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet. 2002; 359(9308):727-32.

126. Kumarasamy N, Vallabhaneni S, Cecelia AJ, et al. Reasons for modification of generic highly active antiretroviral therapeutic regimens among patients in southern India. J Acquir Immune Defic Syndr. 2006; 41(1):53-8.

127. Idigbe EO, Adewole TA, Eisen G, et al. Management of HIV-1 infection with a combination of nevirapine, stavudine, and lamivudine: a preliminary report on the Nigerian antiretroviral program. J Acquir Immune Defic Syndr. 2005; 40(1):65-9.

128. Maggiolo F, Arici C, Airoldi M, et al. Reasons for discontinuation of nevirapine-containing HAART: results from an unselected population of a large clinical cohort. J Antimicrob Chemother. 2007; 59(3):569-72.

129. Montaner JS, Reiss P, Cooper D, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. Jama. 1998; 279(12):930-7.

130. Negredo E, Cruz L, Paredes R, et al. Virological, immunological, and clinical impact of switching from protease inhibitors to nevirapine or to efavirenz in patients with human immunodeficiency virus infection and long-lasting viral suppression. Clin Infect Dis. 2002; 34(4):504-10.

131. Martin AM, Nolan D, James I, et al. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1*0101 and abrogated by low CD4 T-cell counts. Aids. 2005; 19(1):97-9.

132. Littera R, Carcassi C, Masala A, et al. HLA-dependent hypersensitivity to nevirapine in Sardinian HIV patients. Aids. 2006; 20(12):1621-6.

133. Flores-Villanueva PO, Hendel H, Caillat-Zucman S, et al. Associations of MHC ancestral haplotypes with resistance/susceptibility to AIDS disease development. J Immunol. 2003; 170(4):1925-9.

7 Appendix

Presentation

To determine the characteristics of HIV patients who are Nevirapine Long-term responders

Vijay Tenneti[1], Prof. Michael Garlepp[1], Dr. Martyn A French[2] and Steven Roberts[2]

[1] School of Pharmacy, Curtin University of Technology, WA;

[2] PathWest Immunology, Royal Perth Hospital and School of Surgery and Pathology, University of Western Australia;

This study was presented at the following conference:

Australian Pharmaceutical Science Association, Adelaide, Australia, 2-5th Dec 2006.

Objective: The objective of the research is to define Nevirapine long term responders (NLTRs) and identify the characteristics of patients who stay on the drug for long periods of time without adverse effects and maintain suppression of HIV replication without development of resistance.

Methodology: The patient population was divided into three groups based on their length of stay on Nevirapine; short-term, medium and long-term responders. Patients who stayed on Nevirapine continuously for more than five years were considered long-term responders. Patients who withdraw before six months of therapy due to the drug's side effects or any other reason are considered short-term responders. Patients whose length of stay on the drug is intermediate between these two groups are considered as medium responders. There were almost 500 cases of HIV in the hospital during the period of study. But patients to whom NVP was given as the first drug for treatment were taken into the study; hence the patient population in the study was only 79. Investigation of the possible genetic influence on response to Nevirapine was made by use of genetic markers like HLA-B35+C4, HLA-DR1, and HLA-B14.

Results: We found no difference in the frequencies of HLA-DR1 or HLA- B14 in the three groups. However, we found that HLA-B35+C4 did not occur in any of the NVP long term responders (NLTRs), which was not described in previous studies. Furthurmore, when a survival analysis was performed, carriage of HLA-B35+C4 identified patients who were not NLTRs. The median survival time for patients with HLA B35+C4 was 30 months compared to 91 months with those without HLA B35+C4. The survival distributions were significantly different ($p= 0.002$).

Conclusions: Although, the factors are considered for the study are associated with hypersensitivity reactions, they do not predict how long a patient would stay on the drug. There was no significant relationship between gender, age, duration of Nevarapine use and whether or not the patient was continuing Nevarapine.

Dieterich DT RP, Love j, Stern JO. Drug-induced liver injury associated with the use of non-nucleoside reverse-transcriptase inhibitors. *Clin Infect Dis* 2004; 38:S80-S9.

Price P JI, Fernandez S, French MA. Alleles of genes encoding IL-1alpha may predict control of plasma viraemia in HIV-1 patients on highly active antiretroviral therapy. *AIDS* 2004; 18(11):1495-501.

Annalise M. Martin DNea. Predisposition to navirapine hypersensitivity associated with HLA-DRB1*0101 and aborgated by low CD4 t-cell counts. *AIDS* 2005; 19:93-9.

Curtin University of Technology Human Research Ethics Committee Approval Letter

minute

Curtin
University of Technology

Division of Heath Sciences

School of Pharmacy
GPO Box U1987
Perth WA 6845
Telephone +61 8 9266 7528
Facsimile +61 8 9266 2769
Email pharmacy@curtin.edu.au
Web www.curtin.edu.au

To	Mr Vijay Tenneti
From	Mrs Jennifer Ramsay Ethics Committee Secretary
Subject	Protocol Approval **PH-08-2006**
Date	15 June 2006
Copy	Professor Michael Garlepp

Thank you for your "Form C Application for Approval of Research with Minimal Risk (Ethical Requirements)" for the project titled **"TO DETERMINE THE CHARACTERISTICS OF HIV PATIENTS WHO ARE NEVIRAPINE LONG-TERM RESPONDERS"**. On behalf of the Human Research Ethics Committee I am authorised to inform you that the project is approved.

Approval of this project is for a period of twelve months from 24 February 2006 to 24 February 2007.

If at any time during the twelve months changes/amendments occur, or if a serious or unexpected adverse event occurs, please advise me immediately. The approval number for your project is **PH-08-2006**. *Please quote this number in any future correspondence.*

Mrs Jennifer H. Ramsay
Committee Secretary
Human Research Ethics Committee

Please Note: The following standard statement must be included in the information sheet to participants:
This study has been approved by the Curtin University Human Research Ethics Committee. If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784.