AN INVESTIGATION OF THE RATE OF CHANGE OF CD4 AND CD8 T LYMPHOCYTE COUNTS AND VIRAL LOADS IN HIV INFECTED PATIENTS ON IMMUNE BOOSTERS

BY

BRENDA THABISILE MKHIZE

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AUTHORS DECLARATION

This study represents the author's original work. It has not been submitted previously for any qualification to this or any other tertiary institution. Where use of work of others was made, such help has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Biomedical Technology, Faculty of Health Sciences, Durban University of Technology, Durban, South Africa; in the Global Clinical and Viral Laboratory, Merewent, Durban, South Africa under the supervision of Dr S Madurai (Global Clinical and Viral Laboratories) and Dr J K Adam (Department of Clinical Technology, Durban University of Technology, Durban).

SIGNED ……..
Brenda Thabisile Mkhize

DATE ……..

I, the undersigned approve the submission of this dissertation.

…….
Dr S Madurai, PhD

I, the undersigned approve the submission of this dissertation.

……
Dr J K Adam, PhD

DEDICATION

This thesis is dedicated to:

My late father, Wilson Themba Ndaba and my late sister, Zanele.

My mother, Elizabeth Ndaba, for her guidance and teaching me the value of perseverance and the power of prayer

My husband Sibusiso, my sons Kabelo and Mandla, my daughter Nokuzola for their continuous support

My sister, Ziningi, Thamba her husband and her family for their love, encouragement and support

My Lord, Jesus Christ, who sustained me in times of great difficulty. I lift my hands in total praise of Him.

ABSTRACT

In 2004, it was reported that KwaZulu-Natal had the greatest number of HIV infected people, approximately 1.8 million people, of whom an estimated 450 000 were in need of antiretroviral drug therapy based on their Cluster of Differentiation 4 (CD4) counts and clinical status. Studies on the success of antiretroviral drugs in improving the quality of life in HIV infected individuals have been extensively performed and published. However, there are no published data on the effect that immune boosters have in improving the quality of life in such persons.

Considering the side effects, toxicity, multi-drug regimens and drug resistance problems associated with antiretroviral therapy, alternative or supplementary therapies may play an important role in improving the quality of life in HIV infected people. Such therapy might help in situations where some patients who qualify for antiretroviral treatment are unable to access them because of several reasons such as long waiting lists, travelling costs, unwilling to take antiretroviral drugs, *etc*. Some patients have reservations in taking antiretroviral drugs. The stigma associated with the disease may be a major factor.

The aim of this study was to investigate the change in the immune status of HIV infected patients that were on the Inochi New Medicine immune booster, as well as, to assess the safety and efficacy of this immune booster in improving the patients' quality of life.

One hundred and thirty-one HIV infected patients with CD4 counts of less than or equal to 500 cells/mm^3, not on any anti-HIV therapy nor immune boosters were recruited from Ikhaya Lobomi Hospice and Care Centre and enrolled for the study.

Most participants that were successfully followed-up after 6 months on treatment, reported that they felt better after starting the medication, even though it was not always evidenced by an increased CD4 count or reduced viral load, as expected. This is in keeping with literature which indicates that CD4 counts do not always represent the clinical wellness of patients. In most participants, opportunistic infections present at baseline, disappeared after taking the Inochi New Medicine immune booster. Participants reported feeling well and were no longer feeling tired and sick. Most reported that their appetite had improved, diarrhoea had subsided and aching feet felt better. They also gained weight. The immune booster was observed to be safe to the majority of participants.

ACKNOWLEDGEMENTS

This study has opened my eyes into the extent of the devastation brought about by the HIV epidemic. There is never enough a person can do to help the infected and the affected.

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LIST OF ABREVIATIONS

AFSA	AIDS Foundation South Africa
AIDS	acquired immunodeficiency virus
ALT	alanine aminotransferase
APC	allophycocyanin
ARV	antiretroviral drug
AST	aspartate aminotransferase
BBC	British Broadcasting Corporation
BD	Becton Dickinson
^{0}C	degrees Celsius
CD3	cluster of differentiation - 3
CD4	cluster of differentiation - 4
CD8	cluster of differentiation – 8
CD16	cluster of differentiation – 16
CD38	cluster of differentiation - 38
CD45	cluster of differentiation – 45
CD56	cluster of differentiation - 56
$CD4^{+}$ T lymphocytes	cluster of differentiation – 4 positive T lymphocytes
CDC	Centres for Disease Control
cDNA	complementary deoxyribonucleic acid
CMV	cytomegalovirus
DNA	deoxyribonucleic acid

EDTA	ethylene- diamine tetra- acetic acid
FBC	full blood count
FITC	flourescin isothiocyanate
fl	femtolitre
Gp41	glycoprotein 41
Gp120	glycoprotein 120
Gp160	glycoprotein 160
HAART	highly active antiretroviral therapy
Hb	haemoglobin
HIV	human immunodeficiency virus
HIV/AIDS	HIV and AIDS
HTLV-3	human T-cell lymphotropic virus, type 3
IFN-γ	interferon- gamma
IRD	immune restoration disease
IRIS	immune reconstitution inflammatory syndrome
IRS	immune reconstitution syndrome
kDa	kiloDaltons
LAV	lymphadenopathy associated virus
LTRs	long terminal repeats
MCC	Medicines Control Council
MHC	major histocompatibility complex
ml	millilitre
mRNA	messenger- ribonucleic acid

NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide - reduced
NIAID	National Institute of Allergy and Infectious Diseases
NK cells	natural killer cells
NNRTI	non-nucleoside reverse transcriptase inhibitor
nm	nanometer
NRTI	nucleoside reverse transcriptase inhibitor
OAT	other alternate treatments
OD	optical density
PCP	*Pneumocystis carinii* pneumonia
PCR	polymerase chain reaction
PE	phycoerythrin
perCP	peridinin chlorophyll protein
PI	protease inhibitor
RNA	ribonucleic acid
RT	room temperature
RTIs	respiratory tract infections
SADC	Southern African Development Community
SIV	simian immunodeficiency virus
STIs	sexually transmitted diseases
SPSS	statistical package for social sciences
TB	tuberculosis
TBDOTS	tuberculosis directly observed therapy, short-course

T_c cells	T cytotoxic cells
TCR	T cell receptor
TCR/ CD3	T cell receptor/cluster of differentiation – 3 complex
T_H1	T helper cell- 1
T_H2	T helper cell- 2
TM	trademark
µl	microlitre
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNAIDS/WHO	Joint United Nations Programme on HIV/AIDS/ World Health Organisation
USA	United States of America
WHO	World Health Organisation

CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.1 INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is an unpleasant, fatal disease. It is presently an incurable disease. It results from an infection caused by the human immunodeficiency virus (HIV). It features a sudden onset of immunodeficiency, associated with life threatening opportunistic infections that may be harmless to non-immunocompromised persons. Harley (1999) pointed out that as soon as a person is diagnosed with HIV infection, they experience an emotional response to the diagnosis. They may deny, be angry, be depressed or accept the diagnosis. A similar emotional grieving experience is seen, as is in the case of persons diagnosed with a terminal disease. This disease becomes more stressful because they may not divulge their diagnosis due to the stigma associated with HIV infection. It has been identified that such stressful circumstances will propagate AIDS, as stress weakens the immune system (Giraldo, 2000; Irwin, 2001). AIDS renders the infected person vulnerable to opportunistic infections, malignancies and neurological disorders.

The opportunistic infections commonly associated with HIV/AIDS are *Pneumocystis carinii* pneumonia (PCP), cytomegaloviral (CMV) infection, mycobacterial infections, fungal infections such as candidiasis and *Cryptococcus neoformans* meningitis, malignant neoplasms such as Kaposi's sarcoma and malignant lymphoma (Klatt, 2005).

Early symptoms of HIV infection (within about 10 to 21 days) are a flu-like illness with sore joints, muscles and bones, fever and swollen lymph glands. The symptoms then disappear, in some people for even up to 20 years, the mean time being nine years. This period is called clinical latency where the person remains well until the symptoms of AIDS begin. In many cases, the person's immune system will fight off the invasion of HIV for many years before it succumbs and permits the AIDS to develop. The rate of progression from HIV to AIDS is increased in areas where adequate and proper health care to that opportunistic infection is not available. Poor nutrition also decreases the time to development of AIDS (Sanders, 2001). AIDS has become a global epidemic, a threat to human development since it has affected all regions of the world (UNAIDS, 2004b). It has affected people for more than 20 years without a cure. Therefore, any strategies that seem to be curbing this epidemic need to be explored.

1.1.1 The history of AIDS

It is believed that HIV was spread from monkeys to humans in Central Africa between the years 1926 and 1946 (Knight, 2000; Pakistan, 2001; WHO, 2000). Some believe that it was transmitted to humans by an experimental oral polio vaccine made from chimpanzee tissue cultures which was given to approximately a million people in Central Africa from 1958 to 1960. The vaccine may have been made with tissue contaminated with the simian immunodeficiency virus (SIV) (Devitt, 2000; Hooper, 2003). Grmek (1990) believed AIDS had been detected as far back as February 1952 in the United States of America (USA), where a twenty eight year old male patient was admitted to a hospital in Tennessee, USA, diagnosed with viral pneumonia that lasted

for two weeks. All his symptoms were suggestive of the pneumonia diagnosis with the exception of an elevated white cell count and a lowered total lymphocyte count. He later died of overwhelming sepsis caused by *Pseudomonas aeruginosa* (Grmek, 1990). His case was further discussed in 1982 where a retrospective diagnosis of *Pneumocystis carinii* pneumonia superimposed on a cytomegalovirus infection was made.

The first warning of the growing number of cases of pneumocystosis amongst the California and New York (USA) homosexual community was published in 1981 by Gottlieb *et al.*, Los Angeles physicians (Kanabus & Fredriksson, 2005; Morbidity and Mortality Weekly Report, 1981). The first official announcement of AIDS was published by the Centres for Disease Control (CDC), a federal epidemiology agency in Atlanta, USA, on the 5th of June 1981 (Grmek, 1990). This disease was found to be affecting homosexuals, as well as more males than females. The patients found to be affected were observed to have had an interaction with someone from New York City, USA, which led to the conclusion that this was an acquired infectious disease.

These patients started dying in 1981 due to an acquired immunodeficiency. It is suspected that they might have been suffering from this disease from 1978 (Grmek, 1990). The most common clinical presentation was thrush, *Pneumocystis carinii* pneumonia (PCP), toxoplasmosis and/or Kaposi's sarcoma (Grmek, 1990). The patients were exclusively homosexuals, predominantly white, some of them known to each other or were friends, mean age 39 years old. The disease was therefore known as a 'gay' disease.

This immunodeficiency disease was named as the acquired immunodeficiency syndrome (AIDS) in August 1982 (Grmek, 1990). It was thought that the name was suitable since the disease was acquired, not inherited: it resulted in a deficiency in the immune system, and it was a syndrome, with a number of manifestations rather than a single disease (Kanabus & Fredriksson, 2005). At that point in time it was known that there was a disease called AIDS but the causative organism was not yet known (Kanabus & Fredriksson, 2005). In 1982, patients that were heterosexual, some haemophiliac, women included, were identified in New York, USA (Grmek, 1990). This finding proved wrong the belief that AIDS was a 'gay' disease.

The African epidemic was not observed until 1983, when a Danish woman, who had worked as a surgeon in Central Africa, died of AIDS (Grmek, 1990). A number of middle class Zairian people, who were infected, went to Belgium for health care (Grmek, 1990). This might have caused the non-observance of this epidemic in Africa at that point. Most of these people were observed to have cryptococcal meningitis, while a few had Kaposi's sarcoma.

The belief was that AIDS affected white males that were homosexuals and blacks that were drug abusers (Grmek, 1990). It was suspected that the virus in Africa began to spread in Zaire, Rwanda, Chad and Uganda by the late 1970's through intravenous drug abuse (Grmek, 1990). In 1978, AIDS was observed in Haiti among the bisexual community who were black and poor. They were utilising unsterilized needles for their drug abuse habits (Grmek, 1990).

It was only in 1984 that the human immunodeficiency virus (HIV) was isolated as the causative agent of AIDS (Grmek, 1990). It was, however, called the lymphadenopathy associated virus (LAV) by the French, while the National Cancer Institute, USA, called it the human T-cell lymphotropic virus, type 3 (HTLV-3) (Grmek, 1990). It was only named as HIV in May 1986 by the International Committee on Taxonomy of Viruses (Kanabus & Fredriksson, 2005). Serologic screening tests for this virus were only available in 1985.

The disease in Africa has been mostly heterosexually spread (Grmek, 1990). The rapidity of its spread is probably partly due to the lack of health resources, poor general health because of starvation, long periods of social unrest, and economic disruption (Grmek, 1990). It was found to be affecting mostly young unmarried and middle-aged people (Barnett & Blaikie, 1992).

1.1.2 The prevalence of HIV/AIDS worldwide

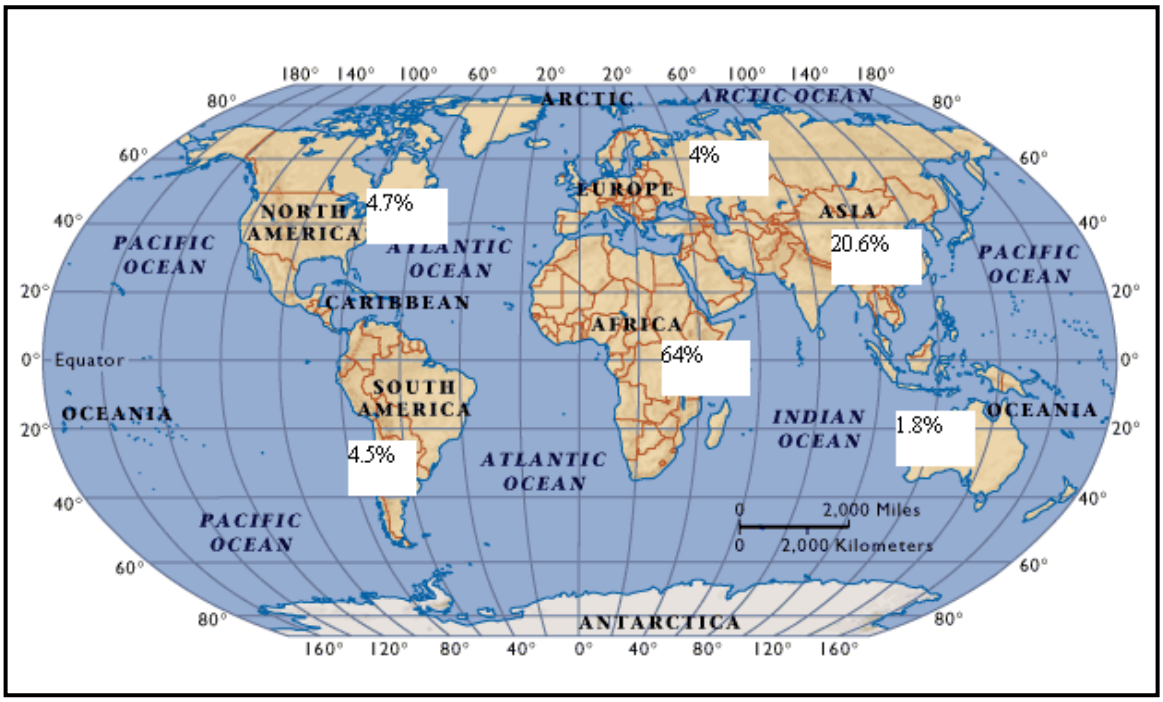

Figure 1.1: The map of the world showing global patterns of the spread of HIV, indicating the prevalence in the different continents (BBC News, 2005)

The global AIDS epidemic continues to expand despite the fact that effective prevention strategies exist (UNAIDS, 2004b). Figure 1.1 shows the prevalence of HIV in the different continents worldwide. Extensive spread of HIV occurred in the late 1970's in North America, South America and Australia, predominantly amongst the homosexual, bisexual and intravenous drug-using community (Barnett & Blaikie, 1992). The spread occurred in the Sub-Saharan region in the late 1970's through heterosexual and vertical transmission (Barnett & Blaikie, 1992). In Asia and North Africa, HIV was introduced in the 1980's by travellers and by imported contaminated blood and blood products (Barnett & Blaikie, 1992).

Table 1.1: Global prevalence of HIV in 1990 and in 2003, showing a continuous increase in the different continents (Kanabus & Fredriksson, 2005; UNAIDS, 2004b)

Area	Reported AIDS in 1990	Estimated HIV in 1990	Estimated HIV in 2003
Africa	77 043	>5 500 000	25 000 000
North America	156 658	1 000 000	1 000 000
South America	28 937	1 000 000	1 600 000
Asia	843	500 000	7 400 000
Europe	41 564	500 000	580 000
Oceania	2 334	30 000	32 000
TOTAL	307 379	<9 000 000	<37 000 000

By the end of 1990, over 307 000 AIDS cases worldwide had been officially reported to the World Health Organisation (WHO) (Kanabus & Fredriksson, 2005) (Table 1.1). However, the estimated number of infected people was 8 to 10 million, more than what had been reported (Kanabus & Fredriksson, 2005). This anomaly could be as a result of the stigma attached to the disease, the so-called "gay disease" or the "prostitutes' disease" that made people not to readily report on the AIDS cases.

As reported by UNAIDS (2004b), almost universally, mainstream society disapproves, and does not tolerate behaviour such as illicit drug use, sex between men, and prostitution, and that these have been associated with HIV/AIDS. Therefore, people infected with HIV will not readily disclose this information because of not wanting to be labelled and ostracized.

In 2003, almost 5 million people globally became newly infected with HIV. This was the greatest number in any one year since the beginning of the epidemic (UNAIDS, 2004a). The estimated HIV prevalence by 2003 was more than 37 million people globally (Table1.1). It was reported that by 2004, more than twenty million people around the world had died of AIDS-related diseases (Kanabus & Fredriksson, 2005). It was also reported that by 2004 almost forty million people globally were living with HIV, with 4.9 million people newly infected with HIV (Kanabus & Fredriksson, 2005). This data indicates that the epidemic had been continuing to grow and many people were and are still dying. This epidemic is devastating families, communities, continents and the whole world at large. It is seriously affecting the development of countries and widening the gap between poor and rich countries. Poor countries are becoming even poorer because of the diminishing human resources required in uplifting their economy.

1.1.3 Prevalence of AIDS in Africa

AIDS has reached endemic proportions worldwide, particularly in Sub-Saharan countries (Wilson & Blower, 2005). In Table 1.1 the high prevalence of AIDS in Africa compared to other countries is indicated, as far back as in 1990. The estimated HIV prevalence has been high compared to other continents probably because the African continent is still developing and lacks resources, such as proper and adequate health care facilities.

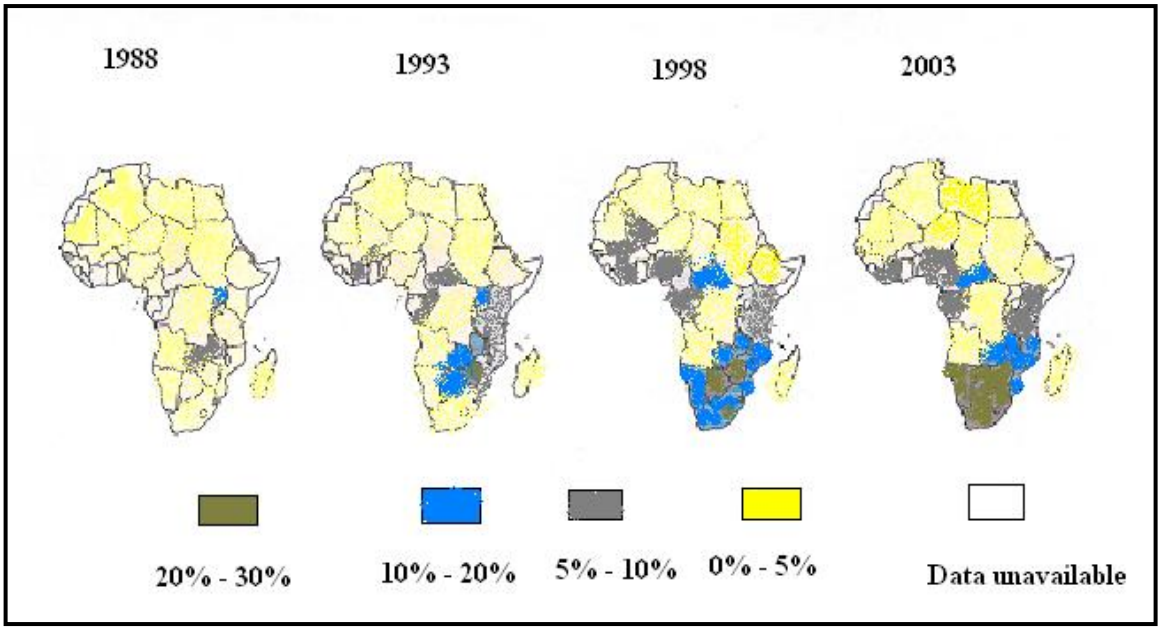

Figure 1.2: AIDS prevalence in Africa between 1988 to 2003, showing the extent at which HIV was spread in the different countries, particularly in the Sub-Saharan region (Adapted from UNAIDS, 2004b)

Figure 1.2 indicates the spread of HIV in African countries between 1988 and 2003. The most affected countries showing an increasing prevalence were in the Sub Saharan region, where the prevalence was 0 - 5% in 1988 and it increased to 20 - 30% in 2003 (Figure 1.2).

Sub-Saharan Africa is still the most affected part of the world, having more than 60% of all people living with HIV, approximately 25.4 million (UNAIDS, 2005) and 2.4 million people dead, because of AIDS (BBC News, 2005). The impact of this epidemic has been most severe in the poorest countries of the African continent (Kanabus & Fredriksson, 2005). In Botswana, 37.3% of the adult population were living with HIV in 2003, while in South Africa 21.5% were infected with HIV (UNAIDS, 2004b). In 2004, UNAIDS/WHO reported that South Africa had the largest number of people living with

HIV/AIDS in the world, an estimated 5.3 million people. Uganda had decreased its prevalence rates from 14% in the early 1990s to approximately 5% in 2004 because of strong prevention campaigns (UNAIDS/WHO, 2004).

It is suggested that the high HIV prevalence in Sub-Saharan Africa is due to situations such as poverty, poor women forced to be commercial sex workers, undiagnosed and untreated sexually transmitted infections (STIs) (Cohen, not dated). UNAIDS (2004c) reported that civil wars, sexual abuse and violence, particularly against females and young women, are also causes of very high HIV prevalence rates. Young women are coerced or forced during their first sexual encounters, which most of the time are without condoms (UNAIDS, 2004c). Infection is concentrated in the socially and economically productive groups aged between 15 and 45, with women infected more than men, with infection occurring at younger ages for girls (Cohen, not dated).

HIV infections need to be prevented and progression to AIDS delayed since the disease has a devastating and significant impact on health services, the labour force, and on the society at large.

1.1.4 HIV prevalence in South Africa

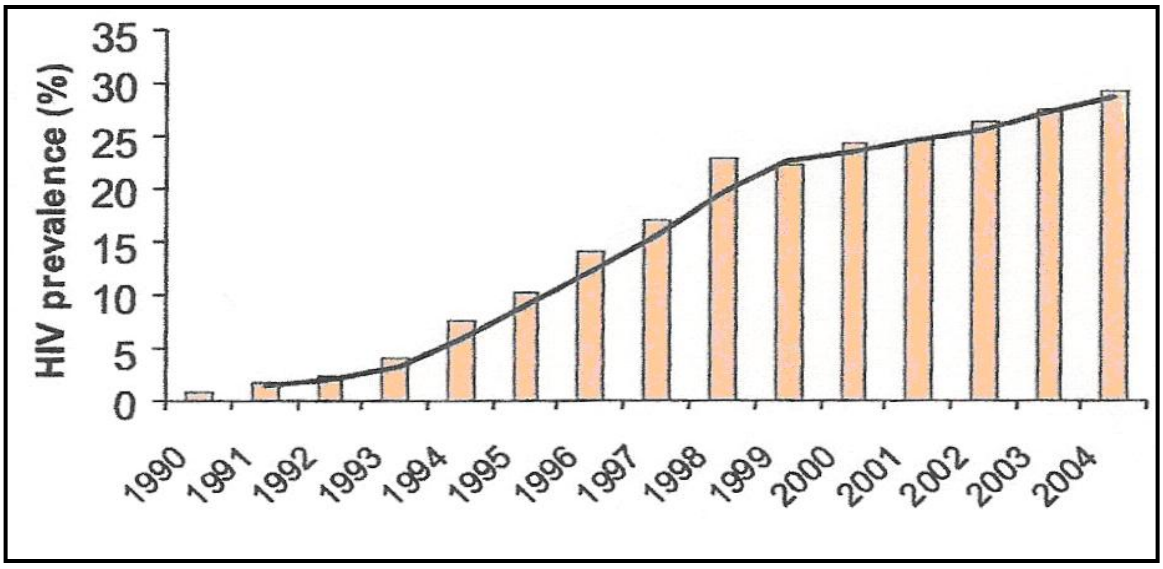

Figure 1.3: National HIV prevalence trends among antenatal clinic attendees in South Africa showing a steady increase between 1990 and 2004 (Department of Health, 2004a)

Figure 1.3 shows that the HIV prevalence in South Africa increased steadily but progressively between 1990 and 1998. Thereafter, there was no significantly sharp increase between 1999 and 2004. The rate of prevalence among pregnant women in 2004 was 29.5% compared to the 27.9% observed in 2003 (Department of Health, 2004a).

Table 1.2: HIV prevalence trends in antenatal clinic attendees in South Africa, between 1998 and 2004, showing how the different age groups were affected (Department of Health, 2004a)

AGE GROUP	1998	1999	2000	2001	2002	2003	2004
<20	21.0	16.5	16.1	15.4	14.8	15.8	16.1
20 – 24	26.1	25.6	29.1	28.4	29.1	30.3	30.8
25 – 29	26.9	26.4	30.6	31.4	34.5	35.4	38.5
30 – 34	19.1	21.7	23.3	25.6	29.5	30.9	34.4
35 – 39	13.4	16.2	15.8	19.3	19.8	23.4	24.5
40 – 44	10.5	12.0	10.2	} 9.8	17.2	15.8	17.5
45 – 49	10.2	7.5	13.1				

Table 1.2 indicates that the 25 to 29 years age group is the group that has always been affected the most (Department of Health, 2004a). This indicates that HIV infection is higher among women in their late twenties and lower among teenagers. It is also observed that there was a sharp increase in HIV prevalence in the 25 to 29 years age group and the 30 to 34 years age group between 2003 and 2004, from 35.4% to 38.5% and 30.9% to 34.4% respectively (Department of Health, 2004a). This age group is probably made up of married women and those that are co-habiting who might find it difficult to influence change of behaviour in their partners, such as using a condom when engaging in a sexual activity.

1.1.5 HIV prevalence in the province of KwaZulu-Natal

South Africa was said to have by the end of 2003, 5.3 million people infected with HIV disease in an estimated population of 40 million (UNAIDS, 2004a). This means that approximately 21.5% of the population was infected compared to the reported prevalence rate of 24.5% in 2000. The 21.5% prevalence rate in 2003 indicated stabilization in new infections. There seemed to be a cessation in the exponential growth of the epidemic in South Africa. In 2005, UNAIDS suggested that in 2004 an estimated 3.1 million people were newly infected, while 2.3 million died of AIDS. This is suggestive of a stabilization of the prevalence of HIV, but definitely not indicating a slowing down of the epidemic. Even though no actual figures were available, with only estimations and surveys done in pregnant women, it is encouraging to note that people seem to be becoming aware of the HIV/AIDS disease.

Table 1.3: Provincial HIV prevalence trends in antenatal clinic attendees in South Africa between 1998 and 2004, with comparisons of prevalence rates in the nine different provinces (Department of Health, 2004a)

PROVINCE	1998	1999	2000	2001	2002	2003	2004
KwaZulu-Natal (KZN)	32.5	32.5	36.2	33.5	36.5	37.5	40.7
Mpumalanga (MP)	30.0	27.3	29.7	29.2	28.6	32.6	30.8
Gauteng (GP)	22.5	23.9	29.4	29.8	31.6	29.6	33.1
Free State (FS)	22.8	27.9	27.9	30.1	28.8	30.1	29.5
North West (NW)	21.3	23.0	22.9	25.2	26.2	29.9	26.7
Eastern Cape (EC)	15.9	18.0	20.2	21.7	23.6	27.1	28.0
Limpopo (LP)	11.5	11.4	13.2	14.5	15.6	17.5	19.3
Northern Cape (NC)	9.9	10.1	11.2	15.9	15.1	16.7	17.6
Western Cape (WC)	5.2	7.1	8.7	8.6	12.4	13.1	15.4
National	**22.8**	**22.4**	**24.5**	**24.8**	**26.5**	**27.9**	**29.5**

The levels of HIV prevalence in South Africa differ between the provinces (Table 1.3). The province of KwaZulu-Natal was reflecting consistently high prevalence rates in the surveys that have been performed (Berry & Fredriksson, 2004; Department of Health, 2004a), *ie*. rates that were 10% higher than the national averages. There is also an

apparent increase in the prevalence rates each year, which calls for an emergency solution to this epidemic. Potential interventions employed that may assist in curbing this pandemic need to be investigated and explored. Therapies or interventions that appear to be beneficial in curbing this epidemic need to be ethically tried. In 2004, Cullinan reported that KwaZulu-Natal had the greatest number of HIV infected people, approximately 1.8 million people, of whom an estimated 450 000 were in need of antiretroviral drug therapy based on their Cluster of Differentiation (CD) 4 (CD4) counts and clinical status (Cullinan, 2004). Many reasons have been cited as a cause of the high HIV rates in KwaZulu-Natal. They include a high rate of sexual abuse; two large ports in Durban and Richards Bay where foreign visitors enter or leave and prostitution is high amongst truck drivers along major transport routes; low usage of condoms and high poverty (Erskine, 2005). Some religious denominations discourage the use of condoms, citing it as ungodly or satanic, therefore posing problems with curbing the currently only proven mechanisms of spreading the disease (Inanda HIV infected patient, personal communication).

1.1.6 Worldwide distribution of subtypes of HIV

Two types of HI viruses namely HIV-1 and HIV-2 have been reported (Kandathil *et al*., 2005; Menting, 2001). HIV-1 is the predominant virus world-wide while HIV-2 appears to be endemic in West Africa (Mellors, 1999). HIV-1 and HIV-2 differ from one another by their pathogenicity as well as their nucleotide sequences (Rodenburg *et al*., 2001).

Viruses related to HIV have been found in many non-human primate species and have been named simian immunodeficiency virus (SIV) (Mellors, 1999). These primate viruses are lentiviruses, the subfamily of retroviruses.

Human immunodeficiency virus and SIV have been classified on the basis of their nucleotide and protein sequences. At present seven lineages have been identified (Foley, 2002; Stebbing & Moyle, 2003). The closest known relative to HIV-1 is SIV*cpz*, isolated from a chimpanzee (Huet *et al*., 1990). The overall genomic organisation of this virus is similar to HIV-1, but its sequence is more divergent from any HIV-1 reported thus far (Salemi *et al*., 2000). Simian immunodeficiency viruses have also been isolated from African green monkeys (SIV*agm*), sooty mangabeys (SIV*smn*), macaques (SIV*mac*) and mandrills (SIV*mnd*) (Fukasawa *et al*., 1988).

HIV-1 has evolved, differing from one geographical location to another. This variability at the genomic level is attributed to the high mismatch error rate of the HIV reverse transcriptase enzyme, coupled with the absence of proof-reading capacity, diploid genome, and rapid turnover of the virus *in vivo* (Kandathil *et al*., 2005). The strains of HIV-1 have been classified into 3 groups: namely group M (major group), the O group (outlier group), and the N group (new group), (Kalish *et al*., 2004) according to the genetic variation between members of the groups, namely a 30% amino acid sequence variation in the *gag* proteins, and 47% in the *env* proteins (Rodenburg *et al*., 2001). It is reported that all groups of HIV-1 are found in Africa, with group M viruses responsible for the global AIDS pandemic (Kandathil *et al*., 2005). More than 90% of HIV-1 infections globally are caused by the M strain of the HIV-1 group (Wainberg, 2004).

Group N and group O are fewer in numbers and are found mainly in Central Africa (Kalish *et al*., 2004; Kandathil *et al*., 2005; Rodenburg *et al*., 2001). It has been reported that there are nine genetically distinct HIV-1 M group subtypes or clades and 2 sub-subtypes identified according to the different sequences in their *env* and *gag* genes, with differences of approximately 25 to 35% for the *env* and 15% for the *gag* proteins (Kandathil *et al*., 2005; Wainberg, 2004). The subtypes are namely A, B, C, D, F, G, H, J and K (Kalish *et al*., 2004; Kandathil *et al*., 2005; Malim & Emerman, 2001). Subtypes A and F have subtypes known as sub-subtypes A1, A2 and F1, F2 respectively (Peeters, 2002; Stebbing & Moyle, 2003). These subtypes represent different lineages of HIV with some geographical associations (HIV Sequence Database, 2002). HIV-1 group N and group O have no defined subtypes as yet (Kandathil *et al*., 2005).

1.2 STRUCTURE AND PATHOGENESIS OF HIV

1.2.1 The structure of the human immunodeficiency virus

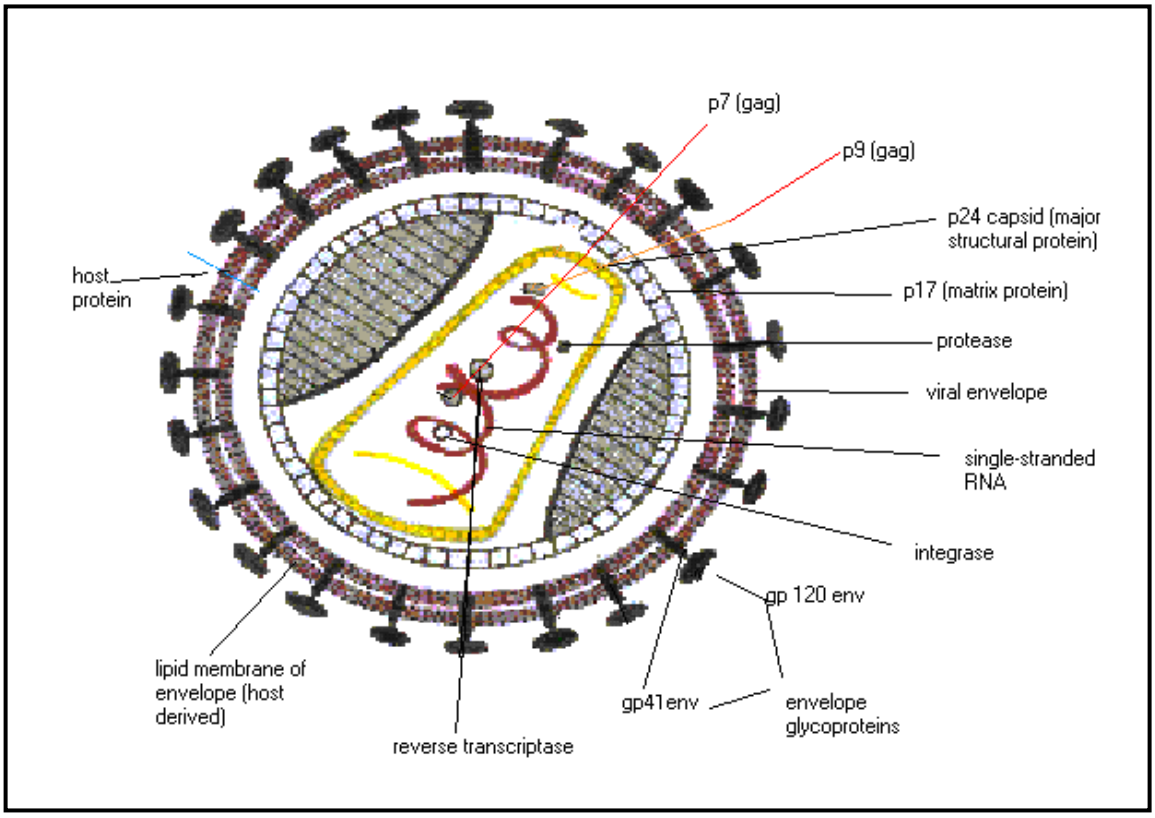

Figure 1.4: The structure of HIV showing the various viral proteins and enzymes enveloped with a polyhedral capsid (adapted from http://www.tulane.edu/)

According to the International Committee on Taxonomy of Viruses, HIV belongs to a family of ribonucleic acid (RNA) viruses, the Retroviridae family, the genus of Lentivirus (Menting, 2001). These are viruses which replicate in dividing cells (Menting, 2001) and can infect non-dividing cells such as macrophages (http://www.unifr.ch/biochem/DREYER/LENTIVIRU1.htm).

HIV is a round particle with a size of between 100 -120 nm in diameter and it is surrounded by a lipid bilayer. It has a half-life of less than 3 hours. It produces deoxyribonucleic acid (DNA) from single stranded RNA using its unique reverse transcriptase enzyme. It is enveloped with a polyhedral capsid. The outer envelope is acquired during virion budding and is studded with approximately 72 spikes formed by two major viral envelope glycoproteins (gp120 and gp41) inserted in the lipid bilayer. The central core consists of four viral proteins (p24, the major capsid protein; p17, a matrix protein; p9; p6 and p7; and three viral enzymes: reverse transcriptase, integrase and protease), all required for viral replication (Figure 1.4).

Table 1.4: Genes found in the HIV RNA genome that mediate replication and other regulatory functions in the life cycle of HIV (Mellors, 1999; NIAID, 2004a)

HIV gene	Protein product	Size	Function in life cycle of HIV	Localisation
Gag	Core protein precursor	p55	Viral core proteins: p24: major capsid protein p17: matrix protein p6 and p7: provides nucleocapsid	Virion
Env	Envelope proteins	gp120 gp41	gp120: major envelope protein, mediates virion binding to CD4cell surface receptor gp41: mediates fusion of viral envelope and cell membrane	Infected cell plasma membrane, virus envelope
Pol	Reverse transcriptase Integrase Protease	p51 p32 p15	Converts single-stranded viral RNA into viral DNA duplex. Integrates viral DNA duplex into host cell genome as provirus DNA Cleaves core precursor polyprotein into functional core proteins	Virion
Tat	Transactivator of transcription protein	p16/p14	Essential regulatory protein during viral transcription, upregulates *rev, nef* and *tat*	Infected cell nucleus, nucleolus
Rev	Regulator virion protein	p19	Regulatory protein, promotes export of viral mRNAs from nucleus into cytoplasm, downregulates *tat*	Infected cell nucleus, nucleolus
Vif	Virion infectivity factor	p23	Plays role in viral budding and infectivity of free virions	Infected cell cytoplasm
Vpu	Viral protein U	p16	Promotes release of budding virions from host cells	Infected cell membrane
Nef	Negative regulatory factor	p27– p25	Regulatory protein, important for virulence, downregulates CD4, negatively regulates *tat* and *nef*	Infected cell membrane, cytoplasm
Vpr	Viral protein R	P10 – 15	Regulatory protein, weak transcriptional activator	Virion
Tev/Tnv	Tripartite tat-env-rev protein	p28	Exhibits both *tat* and *rev* functions, regulatory protein	nucleolus/ nucleus
LTR	Long terminal repeats	Approx 730bp	Play essential role in replication, flanks the genome	Virion

The single-stranded HIV RNA genome is about 9.8 kilobases long (Hope & Trono, 2000; Oelrichs, not dated) and it is flanked by long terminal repeats (LTRs) at both its 5' and 3' ends. It contains three genes essential for retroviral replication, the *gag*, *env* and *pol* genes, and also at least six additional genes that mediate regulatory or other functions in the life cycle of HIV (Figure 1.4 and Table 1.4).

1.2.2 Pathogenesis of HIV

Transmission of the HIV disease is usually through infection with human blood, vaginal fluid, semen or breast milk, containing HIV (Roitt, 1997). Human immunodeficiency virus may enter and infect the human by direct inoculation in the blood stream or by close contact with mucosal surfaces (Lapenta *et al*., 1999). The following are the known routes of transmission:

- unprotected penetrative sexual intercourse homosexually or heterosexually;
- sharing of contaminated needles and syringes;
- occupational needlestick exposure in health care workers;
- blood transfusion of infected blood;
- infected mother to her baby either during pregnancy, at birth or through breastfeeding.

The chief target cells for HIV infection are human (the host) $CD4^+$ T lymphocytes (also called T helper cells) and macrophages (Kupfer *et al*., 1999). These cells are responsible for co-ordinating and activating the immune response. CD4 protein receptors are present on the surface of these host cells and act as high affinity receptors for the virus.

Human immunodeficiency virus uses the CD4 receptor with a co-receptor namely CCR5 or CXCR4 to enter the host cell and thus infect that cell (Coakley *et al*., 2005). During early stages of HIV infection, viral isolates tend to use CCR5, the β-chemokine receptor for viral entry, whilst later, when HIV progresses to AIDS, viral isolates tend to use CXCR4, an α-chemokine receptor (Farber & Berger, 2002; NIAID, 2004a). Iyengar *et al*. (1999) indicated that macrophage-tropic, non-syncytium- inducing HI viruses use CCR5 chemokine and T cell-tropic, syncytium-inducing HI viruses use CXCR4 as co-receptors for HIV infection after the initial binding to the CD4 receptor. This, therefore, means that HIV binding to susceptible cells through the CD4 receptor is not enough for its pathogenesis. There has to be co-receptor binding as well. Moyle *et al*. (2005) indicated that the mere presence of CD4 and either CCR5 and/or CXCR4 on cells designates those cells as potentially susceptible for HIV infection.

There are two types of T helper cells: T_H1 cells which promote cell mediated response, seen in primary HIV infection and T_H2 cells which promote the humoral response. These two subsets look the same and have the same T cell markers and receptors.

However, they secrete very different cytokines upon activation. T_H1 cells secrete great amounts of IL-2 and IFN-γ, which are vital in the cell-mediated immune response. T_H2 secrete IL-4, IL-5 and IL-6, providing help to B cells for antibody production, which forms part of the humoral immune response.

The CCR5- macrophage-tropic virus predominates in infecting the T_H1 CD4 cells during the initial stages of the infection, and later in the AIDS stage the CXCR4- T cell- tropic virus predominates in infecting the T_H2 CD4 cells, a phenomenon called viral tropism where a switch in virus population exists. Human immunodeficiency virus replication is efficiently supported in T_H2 CD4 cell clones than in T_H1 clones, suggesting preferential HIV replication in T cells producing T_H2-type cytokines (Becker, 2004).

1.2.3 The life cycle of the human immunodeficiency virus

The HIV envelope (*env*) is a polyprotein precursor, also called glycoprotein 160 (gp160). Gp160 is processed by cellular proteases to yield a non-covalent complex of an external glycoprotein, gp120, and a transmembrane glycoprotein, glycoprotein 41 (gp41) (Figure 1.5).

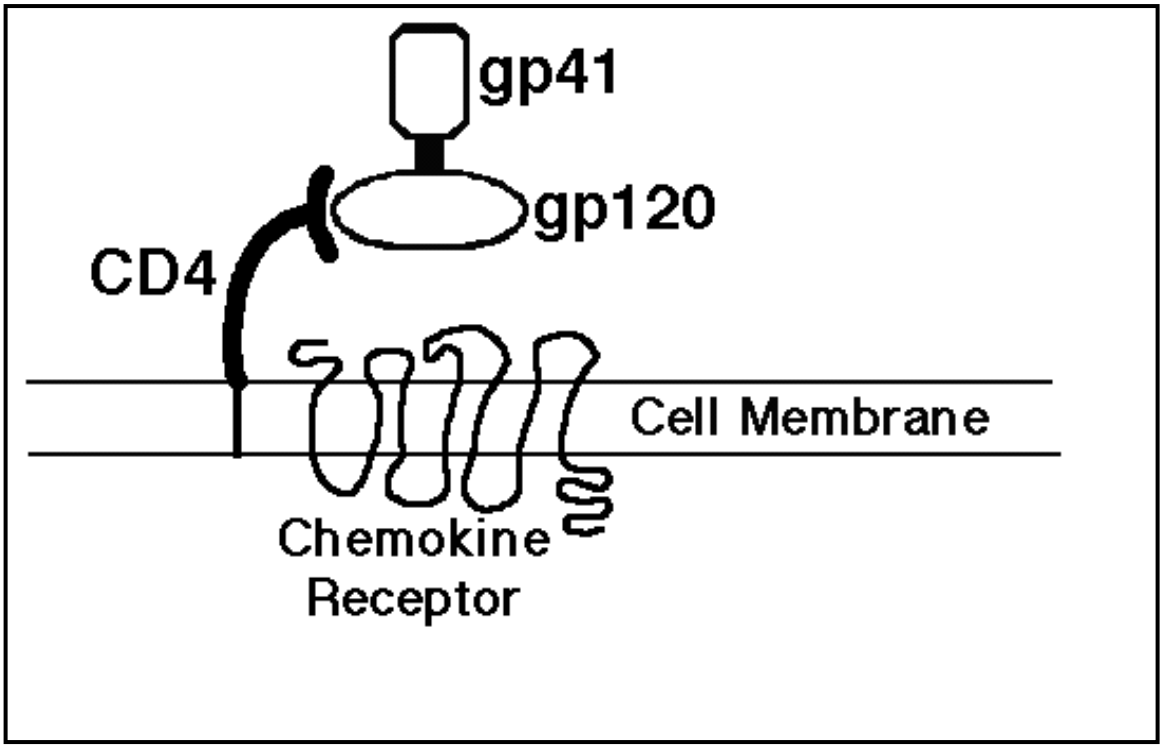

Figure 1.5: The interaction of HIV gp120 with the CD4 receptor. The chemokine coreceptor (CCR5 or CXCR4) in the cell membrane induces a conformational change in gp41 that allows fusion of virus with the cell membrane (Klatt, 2005)

The HIV gp120 binds tightly to human (host) cell surface CD4 molecules and initiates fusion of gp41 of the viral envelope with the cell membrane of the host cell. Following fusion of the virus with the host cell, HIV enters the cell. The genetic material of the virus, which is RNA, is then released and undergoes reverse transcription into DNA. HIV uses its reverse transcriptase enzyme to catalyze the conversion of the viral RNA into DNA. Then the viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the host cell, using the integrase enzyme to catalyze this process. Activation of host cells results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated into viral proteins. The new viral RNA forms genetic material of the next generation of viruses.

The viral RNA and viral proteins assemble just inside the cell membrane of the host cell into a new virus. Amongst the viral proteins is HIV protease, which is required to process other HIV proteins into their functional forms. The protease enzyme is used for the assembly and maturation of fully infectious viral progeny. The HIV protease is responsible for cleaving gp160 into gp120 and gp41 (Heaphy, 2003).

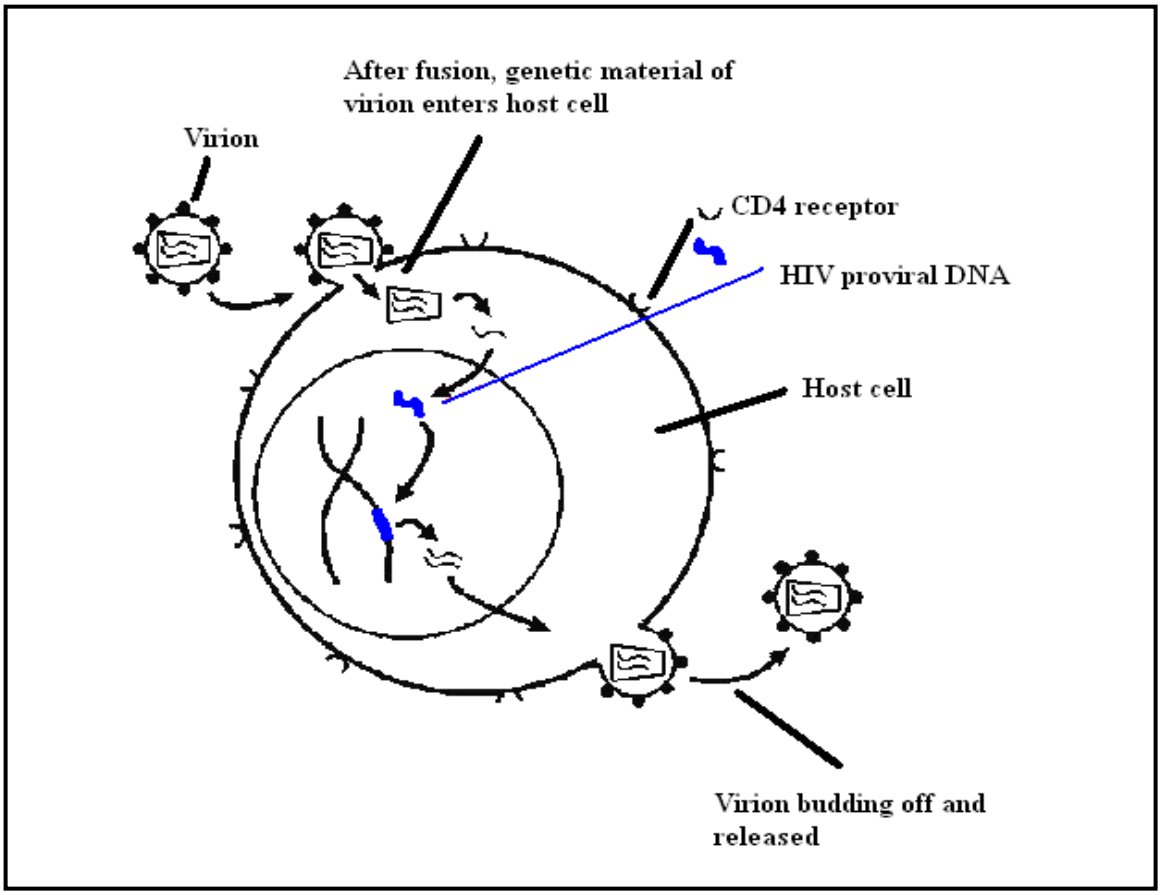

Figure 1.6: The life cycle of a human immunodeficiency virus showing how the reverse transcriptase enzyme makes an HIV proviral DNA that is incorporated into the host cells (Klatt, 2005)

Following assembly at the cell surface, the virus then buds forth from the cell and is released to infect other host cells (Gandhi *et al*., 1999) (Figure 1.6). Replication of HIV occurs *in vivo* primarily in activated CD4 T lymphocytes and mononuclear macrophages, which also express CD4 receptors. It can produce more than 10 billion viral particles per day (Hare, 2004).

1.2.4 Association of CD4 and HIV viral load in AIDS

Mellors (1999) suggested that the development of AIDS is primarily caused by continuous rounds of HIV infection and replication, resulting in virus-mediated and immune-mediated killing of CD4 T lymphocytes. Circulating CD4 T lymphocyte numbers fall progressively in HIV infection, resulting in profound depletion (Roitt, 1997). The infected person then becomes prone to life threatening infections caused by normally non-pathogenic agents, which is known as AIDS.

In early seroconversion, a high HIV viral load is experienced with little or no change in the CD4 count, showing that the person's immune system is still intact. The disease then reaches a stage of clinical latency where the person's viral load declines and the CD4 count stabilizes. This is when the immune system is still able to fight off the HIV. The last stage of the disease is AIDS, a stage in the clinical process when the viral load is at its peak, with levels reaching over one million viral copies per millilitre of plasma and the person's immune system collapses, with CD4 counts reaching levels of well below 200 cells/mm^3, even as low as zero (Malim & Emerman, 2001).

As CD4 counts decrease, CD8 counts increase, and CD3 total T lymphocyte counts remain constant (Kunkl, 1997). The AIDS stage is associated with increased susceptibility to opportunistic infections, because HIV infects the very cells that co-ordinate the immune response, leaving the body susceptible even to normally harmless micro-organisms. It is these opportunistic infections, rather than the HIV itself, that make HIV disease so deadly.

1.2.5 The development of a normal immune response with focus on the lymphocyte population

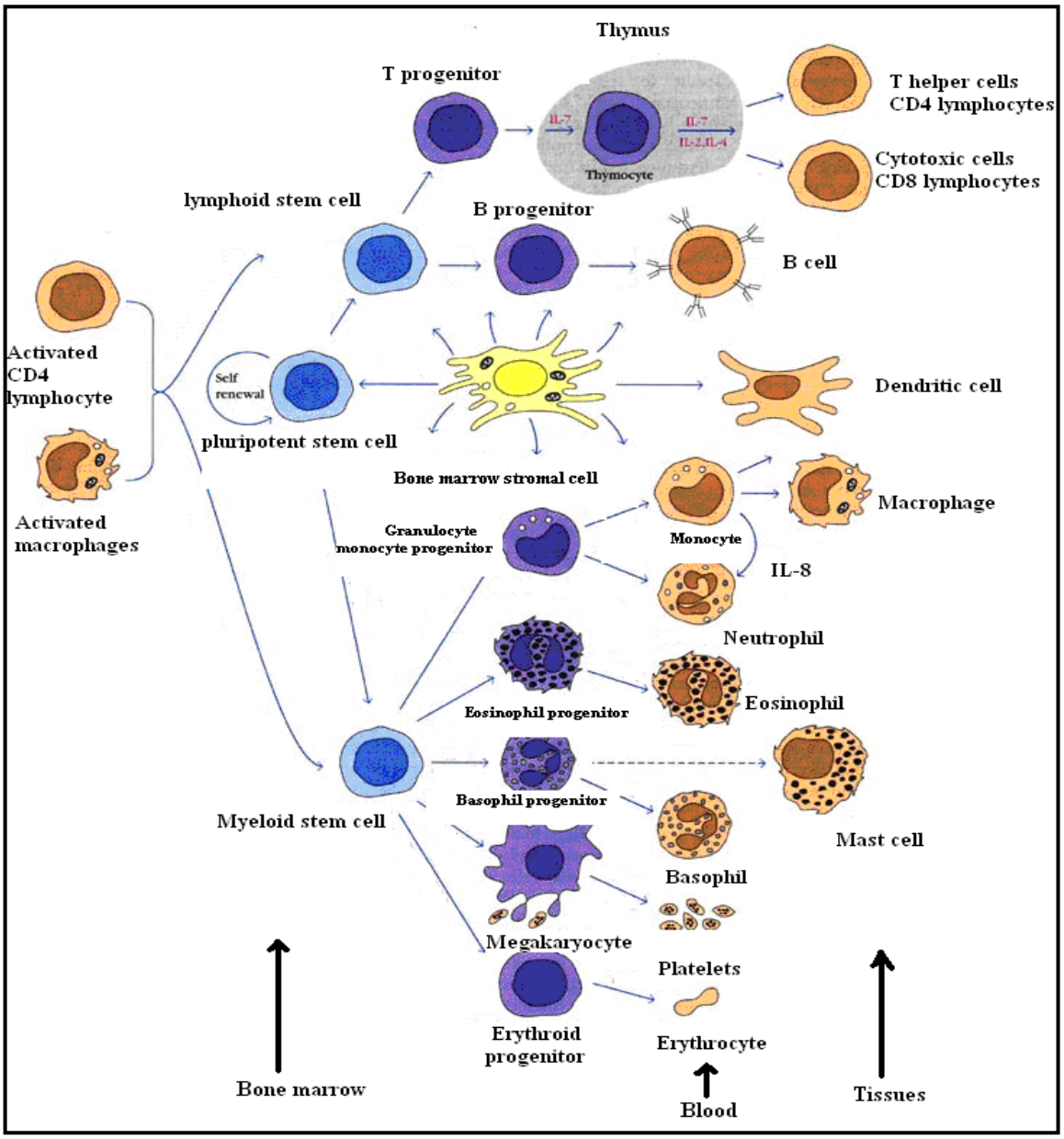

Figure 1.7: The normal development of blood cells in the bone marrow. The diagram shows that they develop from a common pluripotent stem cell, induced by activated immune cells and it also shows the normal lymphocyte development (Adapted from http://www.whfreeman.com)

The principal parts of the immune system are the bone marrow, the thymus, the lymphatic system and the spleen. The bone marrow is the active site of blood cell production. Blood cells are made from uncommitted/pluripotent stem cells into committed/unipotent stem cells. The pluripotent stem cells have the ability to differentiate into a variety of lineages. They may become committed to myeloid or lymphoid lineages. The myeloid lineage produces erythrocytes, platelets, neutrophils and monocytes. The lymphoid lineage produces T lymphocytes and B lymphocytes. As shown in Figure 1.7, marrow cells when differentiated and mature, are released into circulation for their respective functions, namely, monocytes and neutrophils as phagocytes; platelets for healing of injury and B and T lymphocytes for the immune system. Lymphocytes are produced in the bone marrow from a pluripotent stem cell and those destined to be T lymphocytes migrate to the thymus for differentiation and maturation known as thymic education. This is a process of 'teaching' lymphocytes to recognize self cells from non-self cells and therefore elicit an immune response against non-self cells for the purpose of protecting the body from invasion. B lymphocytes migrate to the bone marrow. The immune system also ensures that there are cells in tissues such as the mucosa and organs such as the spleen and lymph nodes, which play a safeguarding role against invading pathogens (Qasim *et al*., 2004). Mature B and T lymphocytes are stimulated by a foreign antigen *eg*. bacterial or viral, to divide and produce a line of descendent cells, also known as a clone of activated immune cells. Memory cells are produced as well and they home in tissues and are able to respond to any later invasion by the same antigen.

1.2.5.1 Cell-mediated and humoral immunity

Lymphocytes are principal mediators of the immune system. There are two major classes of lymphocytes: T lymphocytes and B lymphocytes (Hoffbrand & Pettit, 1995). T lymphocytes express a T cell receptor (TCR)/CD3 complex and B lymphocytes express a surface immunoglobulin on their cell membranes (Hoffbrand & Pettit, 1995). T lymphocytes are responsible for the cell mediated immune response whereas B lymphocytes are responsible for the humoral immune response. Cell-mediated immunity is mediated by T lymphocytes and it involves the production of antigen-specific cytotoxic T lymphocytes, activated macrophages, activated natural killer (NK) cells and various cytokines that influence the function of other effector cells in response to a foreign antigen. This arm of the immune system is active against target cells such as virus infected cells or tumour cells and cells with intracellular bacteria, which are not susceptible to antibodies. Humoral immunity is mediated by B lymphocytes and it involves the production of antibody molecules in response to a foreign antigen.

T lymphocytes produce cytokines which activate other immune cells. Activated immune cells become specially equipped to recognize a particular pathogen or antigen and respond to it. CD4 T lymphocytes, also known as T helper cells are active in inducing and helping the synthesis of immunoglobulins (antibodies) by B lymphocytes and also by activating CD8 T lymphocytes to multiply and differentiate. CD8 T lymphocytes have a cytolytic capability when activated and are able to lyse body cells displaying epitopes of foreign antigen on their surface. These lymphocytes are critical in the recognition and elimination of such altered self- cells, called target cells.

1.2.5.2 CD4 and CD8 T lymphocytes

T lymphocytes are further classified into CD4 T lymphocytes and CD8 T lymphocytes according to the receptors expressed on their cell surfaces. CD4 T lymphocytes function as T helper (T_H) cells and CD8 T lymphocytes function as T cytotoxic (T_c) cells in a normal immune reaction. The T cell receptor (TCR) and CD4 molecules on the surface of T helper cells are designed to recognize peptide epitopes bound on major histocompatibility complex II (MHC II) molecules on infected cells, whereas the TCR and CD8 molecules on the surface of T cytotoxic cells are designed to recognize peptide epitopes bound on MHC I molecules on infected cells (Figure 1.8). The TCR specifically helps the T lymphocyte recognize any foreign antigens on T lymphocytes. The TCR is associated with CD3 for signal transduction and both CD4 and CD8 T lymphocytes express CD3 (Hoffbrand & Pettit, 1995).

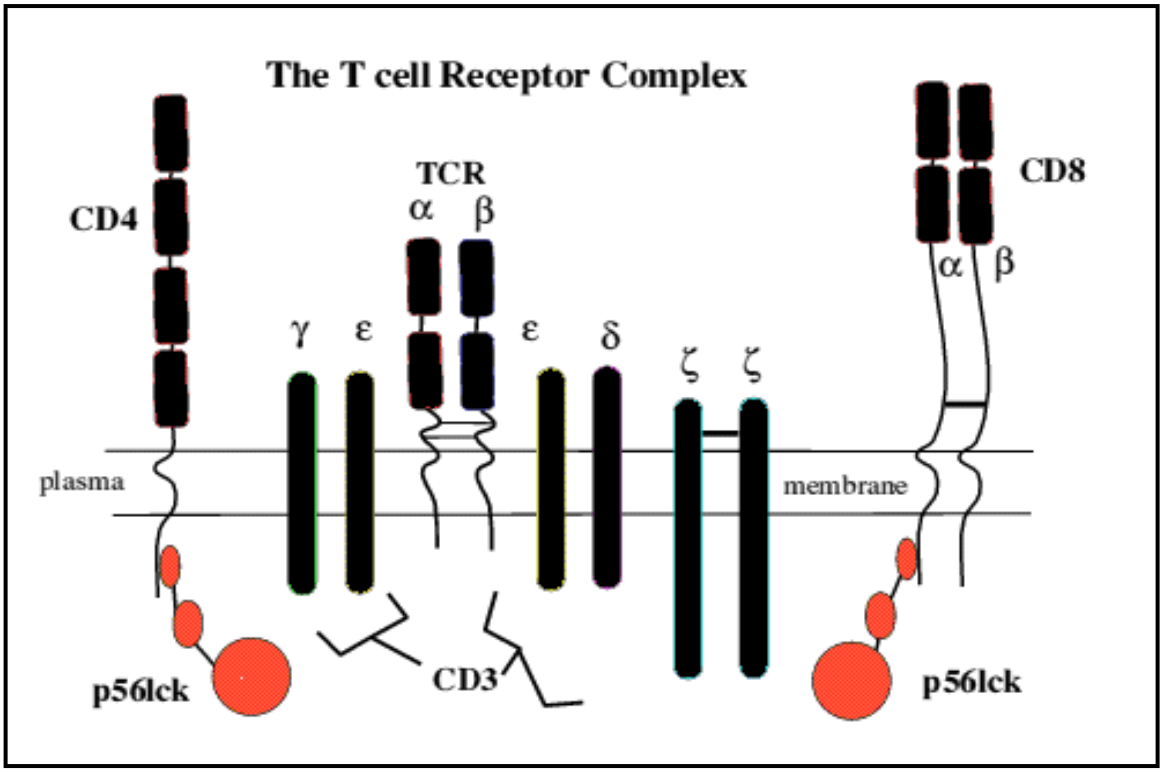

Figure 1.8: The structure of a T cell receptor (TCR) complex which includes the CD4 or CD8 molecules and CD3, a signal transduction module in association with p56lck, an intracellular tyrosine kinase for T cell signalling (Klatt, 2005)

CD4 is a member of the immunoglobulin superfamily. It consists of an extracellular region of 370 amino acids organized into four domains, D1 to D4; a hydrophobic membrane-spanning region of 25 amino acids and a highly charged cytoplasmic tail of 38 amino acids. It is the primary high-affinity cellular receptor for HIV on T lymphocytes (Devaux, 1998). The CD4 antigen is a single chain transmembrane glycoprotein with a molecular weight of 59 kDa (Figure 1.8). It binds to the non-polymorphic region of MHC class II molecules.

The CD8 antigen is a disulphide linked dimer (Figure 1.8). The molecular weight of each monomer α and β is approximately 32-34 kDa. CD8 binds to a non polymorphic domain of MHC class 1 molecules.

CD3 complexed with the T cell receptor (TCR/CD3 complex) is a multichain structure in charge of antigen recognition in T lymphocytes. CD3 is a member of the immunoglobulin family. The CD3 dimers transmit intercellular signals initiated through TCR:MHC/peptide-ligand engagement, resulting in recruitment and activation of tyrosine kinase activity and other downstream signalling events (Arnett, Harrison & Wiley, 2004). The TCR/CD3 complex contains exactly one TCR -α/β, one TCR –ε/γ, one TCR -ζ, one CD3 -ε/ϒ and one CD3 -ε/δ, all of which are required for its structural and functional integrity (Figure 1.8).

CD45, also known as the lymphocyte common antigen, is a receptor-linked protein tyrosine phosphatase that is expressed on all leukocytes, T lymphocytes included. The expression of CD45 is essential for the activation of T lymphocytes via the TCR, supporting signal transduction from the TCR in association with p561ck, an intracellular protein tyrosine kinase (Altin & Sloan, 1997). CD45 is a single chain transmembrane glycoprotein with two cytoplasmic phosphatase domains, of which the second domain is inactive. It weighs between 180–220 kDa, appearing in different isoforms. The extracellular domain is important in mediating interactions with other membrane surface proteins involved in T cell activation (Kung & Thomas, 1997).

In T cells CD45 associates with numerous molecules, both membrane-associated and intracellular; these include components of the TCR/CD3 complex and CD4/CD8 (Altin & Sloan, 1997). CD45 selectively regulates the activity of the tyrosine kinases, such as specific pools of antigen receptor associated p59fyn kinases and CD4 associated p56lck kinases in human T cells (Altin & Sloan, 1997; Kung & Thomas, 1997).

1.2.6 The immune system against the human immunodeficiency virus

Activated CD4 T lymphocytes proliferate and stimulate an immune response against the invading HIV. The activated CD4 T lymphocytes send a signal through a CD3 molecule which triggers the activation of CD8 T lymphocytes to produce cytokines that will be used to lyse HIV infected cells (Flamand *et al*., 2004; Kitchen *et al*., 2004). CD8 T lymphocytes secrete a pore-forming protein called perforin and serine proteases called granzymes to kill altered cells. Perforin molecules polymerize in the membrane of the infected cell, forming pores through which the granzyme B proteases enter the cytoplasm of the infected cell. A cascade of reactions are initiated that result in the fragmentation of the DNA of the target cell, and the altered cell (target cell) is killed by apoptosis (Haridas *et al*., 2003). Instead of attacking HIV directly, CD8 T lymphocytes inhibit virus spread by killing off other immune system host cells infected with HIV (Price *et al*., 2003; Roitt, 1997). The function of CD8 T lymphocytes is highly dependent on help from healthy and functioning CD4 T lymphocytes. This immune response is crippled in HIV disease since CD4 T lymphocytes are destroyed and thus CD8 T lymphocytes are not activated. A decrease in the production of CD8 T lymphocytes is associated with accelerated progression of HIV disease to AIDS (Kunkl, 1997). Jin *et al*.

(2002) reported that the magnitude, quality and specificity of an HIV specific CD8 T lymphocyte response may contribute to the control of HIV viraemia levels. The report also suggested that frequencies of HIV specific CD8 T lymphocyte responses are strong during earlier stages of the HIV infection when patients have high CD4 counts, whereas they are often low in later stages of infection particularly when patients' clinical situations deteriorate. Musey *et al*. (1999) cited that the primary immune response is characterized by a generalized lymphadenopathy and high absolute CD8 counts. The lymphadenopathy is indicative of migration and trapping of CD4 T lymphocytes in lymphoid tissues. Jin *et al*. (2002) cited that HIV infected long-term non-progressors have higher HIV specific CD8 T lymphocyte frequencies than those who progress to AIDS.

Chen *et al*. (2005) and Lineberger *et al*. (2002) suggested that the levels of CD4 and the co-receptor (CCR5 or CXCR4) affect the efficiency of viral entry and this may have consequences for the pathogenesis of HIV disease. Activated CD4 T lymphocytes express more CD4 receptors and co-receptors, therefore, making it easy for the HIV to gain entry into cells. This contributes and worsens immunodeficiency and accelerates the progression of the HIV disease to AIDS, which is characterized by a high viral load. Lederman (1998) indicated that a resting, inactivated CD4 T lymphocyte is not susceptible to HIV infection. During the course of the infection, there is gradual loss of CD4 lymphocytes; initially, there is an increase in the number of CD8 lymphocytes which falls during disease progression from HIV disease to AIDS (McBreen *et al*., 2001).

1.3 MANAGEMENT OF AIDS IN ASSOCIATION WITH LABORATORY PARAMETERS

Human immunodeficiency virus disease collapses the immune function. Therefore, it is imperative for the clinician and/or the nurse, as well as the patient infected with HIV, to monitor the progress of the disease so as to respond to the disease by starting anti-HIV treatment and/or prophylactic antibiotics as and when necessary. The typical pattern of HIV primary infection is characterized by high levels of virus in the blood, followed by a progressive loss of CD4 T lymphocytes, an elevation of CD8 T lymphocytes, CD3 T counts remaining constant and a progressive impairment of T lymphocyte function. Between the primary infection and the final progression to AIDS, there is a period of clinical latency characterized by a low but steady increase in viral loads (Kunkl, 1997) (Figure 1.9).

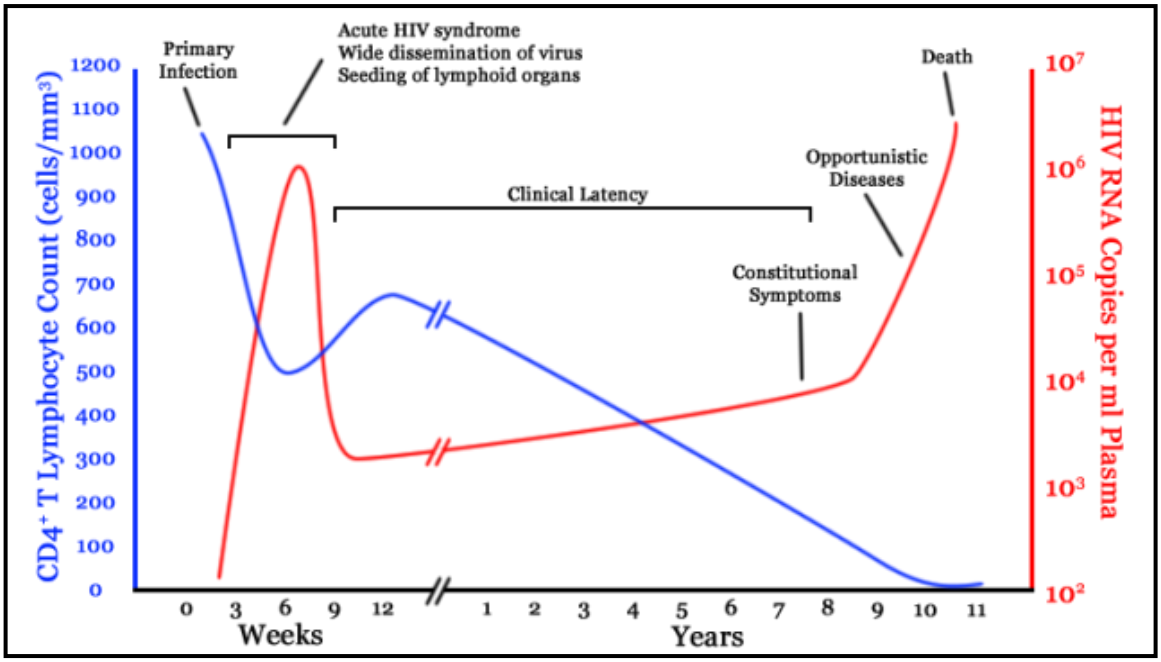

Figure 1.9: Typical pattern of HIV infection, showing high CD4 counts and low viral loads in primary infection and low CD4 counts and high viral loads leading to death in later stages of the infection. Shown also is the time it might take to develop opportunistic infections therefore manifesting symptoms (www.hivtraining.ucsd.edu/Powerpoint/HIV.Dx.Ugan.5.03.ppt)

The laboratory is essential in monitoring and managing HIV/AIDS as a chronic disease. It identifies the magnitude and nature of immune responses in HIV/AIDS patients. This is measured by running a series of immunological tests namely a CD4 count and CD4 percentage, a CD8 count and CD8 percentage, a CD4:CD8 ratio and an HIV RNA viral load test. A CD4 count and CD4 percentage is a measure of the level of immunodeficiency caused by HIV. The HIV RNA viral load is a relative measure of virus replication and cell killing, indicating the amount of virus in circulation.

Clinicians use these parameters as guidelines to make decisions on starting the administration of antiretroviral therapy (Baker, 1996; O'Brien & Goedert, 1998; Wilson *et al*., 2004), to ascertain response to anti-HIV treatment as well as to determine when to start prophylactic antibiotics. The efficacy of the antiretroviral drugs is assessed after a fixed time of therapy (*eg*.1 month, 3 months and 6 months later). In addition, CD4 and CD8 counts are used as prognostic markers to assess the degree of immune impairment or reconstitution in HIV seropositive individuals.

A normal finding in an HIV negative individual is a >1 ratio of CD4 T lymphocytes per CD8 T lymphocytes (HIV negative: CD4/CD8 ratio >1). When the HIV disease progresses to the AIDS stage, the CD4/CD8 ratio falls to <1 (HIV positive: CD4/CD8 <1). The dramatic fall in CD4 T lymphocytes will result in the decrease in the CD4: CD8 ratio. Keita-Perse *et al*. (1998) and Irwin (2001) suggested that when the CD4 count decreases, the CD8 count increases and *vice versa*. The clinical significance of CD4/CD8 ratio is that ratios indicating higher CD8 levels would indicate a lower infection and a higher resistance to HIV disease progression to AIDS (Kunkl, 1997). It follows then that improved ratios indicate decreasing likelihood of progression into full-blown AIDS (Irwin, 2001). High CD8 counts reflect a better capacity to attack HIV, therefore, avoiding the development of AIDS-related conditions (Kalams *et al*., 1999).

An infection of CD8 T lymphocytes by HIV provides an additional reservoir for the virus and may also contribute to the immunodeficiency seen in the HIV disease progressing to AIDS. In HIV infected individuals at the AIDS stage, the cytotoxic capability of CD8 T lymphocytes is ineffective at eliminating the viral infection since

these cells are killed and those still surviving are not activated since CD4 T lymphocytes are killed as well. Laboratory test results of a CD4 count and a HIV RNA viral load indicate the stage of the disease, thus providing guidelines for treatment initiation. The laboratory data must be used in conjunction with the clinical presentation of the patient. The following clinical guidelines are used to treat HIV infected patients:

a) The patient is in the WHO stage 4 of HIV disease (clinical AIDS), irrespective of CD4 count
b) The patient is in the WHO stages 1, 2 or 3 of HIV disease, with CD4 count of < 200 cells/mm^3
c) WHO stages 2 or 3 of HIV disease lymphocyte count of $< 1.2 \text{ X}10^9/l$
d) WHO stage 4 of HIV disease irrespective of the total lymphocyte count

Table 1.5: World Health Organization (WHO) guidelines for clinical staging of the HIV/AIDS disease, showing symptoms presenting in each stage (WHO, 2005)

WHO clinical staging	Symptoms presenting
Clinical stage 1	Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2	Moderate and unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media) Herpes zoster, recurrent oral ulcerations, papular pruritic eruptions, angular cheilitis, seborrhoeic dermatitis, fungal fingernail infections
Clinical stage 3	Unexplained chronic diarrhoea for longer than one month, unexplained persistent fever for longer than one month, severe weight loss (>10% of presumed or measured body weight), oral candidiasis, oral hairy leukoplakia, pulmonary TB diagnosed in last two years, severe presumed bacterial infections, acute necrotizing ulcerative stomatitis, gingivitis or perodontitis, unexplained anaemia and or neutropaenia and or thrombocytopaenia for more than one month
Clinical stage 4	HIV wasting disease, Pneumocystis pneumonia, recurrent severe or radiological bacterial pneumonia, chronic herpes simplex infection, oesophageal candidiasis, extrapulmonary TB, Kaposi's sarcoma, central nervous system toxoplasmosis, HIV encephalopathy, extrapulmonary cryptococcosis including meningitis, disseminated non-tuberculous mycobacteria infection, progressive multifocal leukoencephalopathy, candida of trachea, bronchi or lungs, cryptosporidiosis, isosporiasis, visceral herpes simplex infection, cytomegalovirus infection, any disseminated mycosis, recurrent non-typhoidal salmonella septicaemia, lymphoma (cerebral or B cell non-Hodgkin), invasive cervical carcinoma, visceral leishmaniasis

The World Health Organisation (WHO) has revised the system of staging the HIV/AIDS disease developed in 1990, which guides the disease progression (WHO, 2005) (Table 1.5). Sanders (2001) suggested that there usually is no going back when one has progressed from one stage to the next. According to the Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa (Department of Health, 2003), as well as the National Antiretroviral Treatment Guidelines (Department of Health, 2004b), currently in South Africa, the criteria for starting antiretroviral treatment is indicated as when the CD4 count is less than 200 cells/mm^3, irrespective of the WHO stage, or when in WHO stage 4 disease, irrespective of the CD4 count.

1.3.1 CD4 count in HIV infected people

A CD4 count is used for staging HIV disease, for monitoring progression of the HIV disease to AIDS, for predicting the risk of complications and debilitating infections as well as for monitoring the extent of effectiveness of anti-HIV treatment (van Leth *et al*., 2004). A study performed by Hogg *et al*. (2001), noted that a CD4 count was the only independent predictor of mortality. A CD4 count in healthy people is about 600 to 1500 cells/mm^3. CD4 lymphocyte counts are often slightly higher in HIV infected women compared to HIV infected men; however, showing no therapeutic advantage (Carter, 2003). CD4 lymphocytes in untreated HIV infected people decrease by about 30 to 100 lymphocytes per year (Highleyman, 2003). Increases in CD4 counts seen in people on effective anti-HIV treatment indicate that the treatment is successful in slowing down HIV replication in cells.

CD4 counts in this study were measured by comparing baseline results, namely from samples taken from HIV infected individuals on their first visit (before immune booster medication was taken), with follow-up results, (from samples taken after 6 months on immune booster medication).

Many factors affect CD4 counts, *viz*. age, gender, ethnic groups (Bussmann *et al*., 2004), the time of day sampling is performed, stress levels and social isolation, the menstrual cycle, acute infections (Highleyman, 2003), over-exercising and pregnancy (Irwin, 2001). There is evidence that CD4 counts are lower in the morning and higher in the evening (Irwin, 2001). Irwin (2001) reported that diurnal variation is less seen in lower CD4 counts *ie*. in levels below 100 cells/mm^3 (Guarner *et al*., 1997) . Since CD4 counts vary, it is, therefore, suggested that a CD4 percentage be used in conjunction with absolute CD4 counts to monitor disease progression (Guarner *et al*., 1997). CD4 percentages demonstrate smaller variations than an absolute count, which may demonstrate wider variations on repeated measurements (Dayama *et al*., 2003; Kunkl 1997). The CD4 percentage is the percentage of CD4 lymphocytes in the total number of lymphocytes. Guarner *et al*. (1997) and Hare (2004) suggested that to avoid suspecting treatment failure when there is a drop in the absolute number of CD4 T lymphocytes during therapy, due to normal variations and not due to the progression of HIV disease, a CD4 percentage must be used. A CD4 percentage in healthy people is between 30% and 60%.

1.3.2 CD8 count in HIV infected people

A CD8 count in healthy people is about 250 to 1300 cells/mm^3. HIV infected people may have more CD8 lymphocytes than CD4 lymphocytes (Highleyman, 2003). Early in the HIV infection the CD8 counts are higher, and this is related to the excessive immune activation of CD8 T lymphocytes (Hare, 2004). Later in HIV infection, a persistently higher CD8 count may indicate a reduced risk of developing AIDS within the next few years (Harrer *et al*., 1996). Valdez *et al*. (2002) in agreement, stated that HIV-infected individuals who maintain control of HIV infection have high frequencies of HIV-specific CD8 effectors in comparison with individuals with progressive disease. Kunkl (1997) indicated that when AIDS develops, almost all T lymphocytes in circulation become CD8 positive T lymphocytes. Natural killer (NK) subsets, which are CD16 positive-CD56 positive, possessing the most potent cytotoxic activity, become reduced in numbers (Kunkl, 1997). The presence of the increased activation marker CD38 in CD8 lymphocytes is predictive of disease progression (Kunkl, 1997). CD38 is reported to be a marker of residual HIV replication, with its expression declining steadily in individuals with undetectable viraemia on HAART (Benito *et al*., 2005). The CD8 percent is considered as more a useful indicator of immune function than an absolute CD8 count, since it demonstrates smaller variations than an absolute count, which may demonstrate wider variations on repeated measurements (Dayama *et al*., 2003; Kunkl 1997).

1.3.3 CD3 count in HIV/ AIDS

Expression of the CD3 surface marker is specific for T lymphocytes, so this count will measure all the subtypes of T lymphocytes present in blood. When AIDS develops, CD3 counts decrease in numbers (Kunkl, 1997). A decrease in a CD3 positive T lymphocyte count has been identified as a predictive factor of AIDS development in both HIV infected adults and infants (Chinen *et al*., 2001). The CD3 count provides a useful quality control check in the laboratory as it indicates the sum of CD4 T lymphocytes and CD8 T lymphocytes, which should be within 10% of the CD3 value (Kunkl, 1997).

1.3.4 CD45 count in HIV/ AIDS

The CD45 antigen is present on all human cells of haematopoietic origin, except erythroid cells, platelets and their precursor cells. It is expressed at a higher density on lymphocytes. CD4 lymphocytes can be divided into naïve/resting cells, known as CD45RA cells and memory/activated cells, known as CD45RO cells. CD45 is expressed in at least 5 isoforms, depending on the activation status of the cell. The different isoforms arise from variable splicing of exons 4, 5 and 6, near the amino-terminus of the extracellular domain, which encode A, B and C determinants respectively. The CD45 antibody recognizes a common structure of the different isoforms, therefore reporting all isoforms as a CD45 count (Sewell *et al*., 1999).

A study performed by Ullum *et al*. (1997), reported a loss of CD45RO cells early in HIV infection followed by increased loss of CD45RA cells in later stages of the infection. They reported that the loss of $CD4^+$ CD45RA cells is important in the pathogenesis of a terminal HIV infection.

1.3.5 HIV RNA viral load in HIV/ AIDS

Baker (1996) suggested that the extent of viraemia which is measured by the HIV RNA in blood is the best marker of HIV disease progression, better than a CD4 count. It can indicate when to stop using an ineffective treatment and when to switch to another regimen. The viral load test is beneficial in discordant cases, where both the CD4 count and the viral load test are increased (Farzadegan *et al*., 1998). A patient eligible for antiretroviral treatment might not be included in the programme if only the CD4 count is used. In such cases when a CD4 count is greater than 200 cells/mm^3 and the viral load is greater than 10 000 copies/ml, antiretroviral therapy must be considered (Farzadegan *et al*., 1998; USA Department of Health and Human Services, 2005). Gupta & Gupta (2004) cited that the viral load usually ranges between 100 and 10 000 000 HIV copies/ml in untreated individuals, though it may be lower in those on treatment. Hare (2004) suggested that more than 10 billion viral particles can be produced per day in untreated individuals.

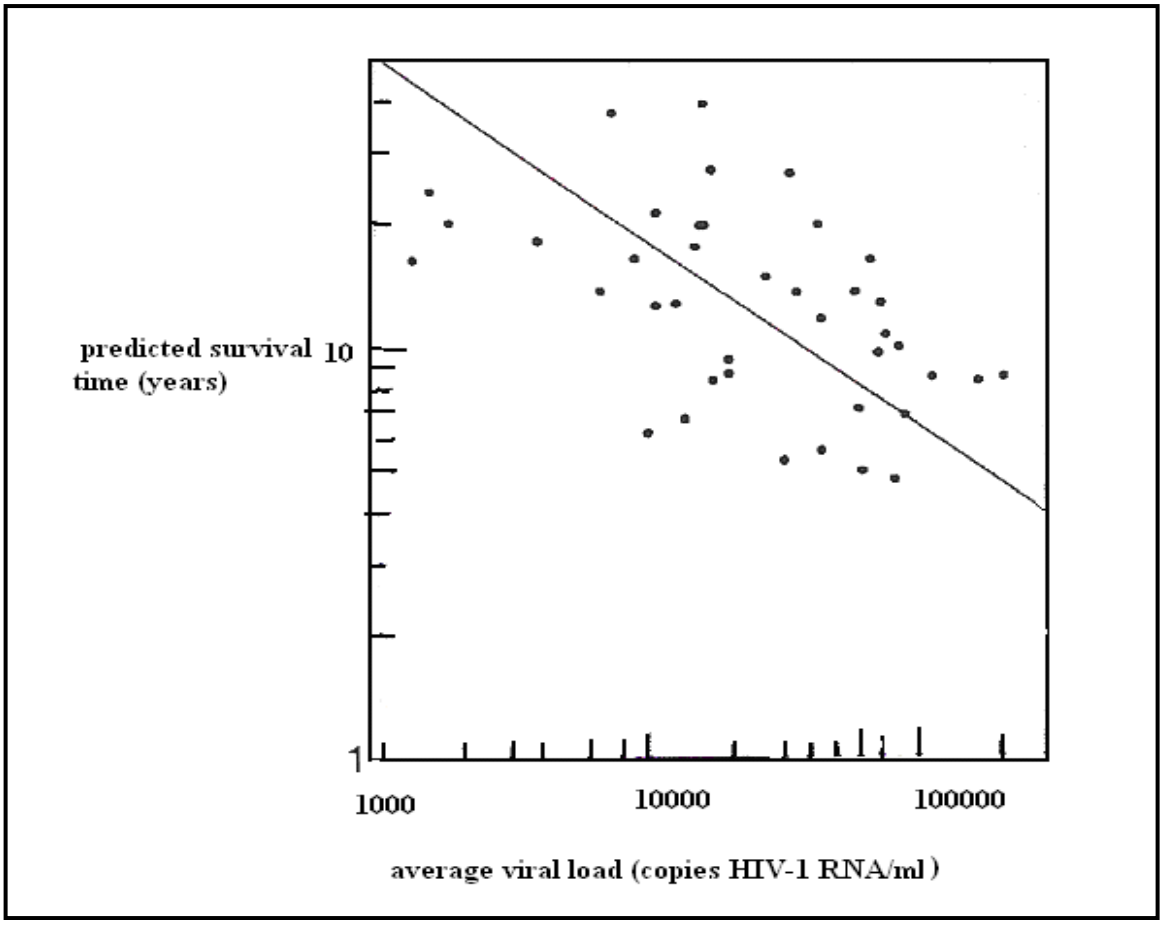

Figure 1.10: The relationship between viral load and survival time, showing that if viral load increases, disease progression will increase as well (Arnaout *et al.*, 1999)

The lower the viral load, the longer the time HIV disease takes to progress to AIDS and the longer the survival time for the patient (NIAID, 2004a) (Figure 1.10). Hare (2004) in agreement, suggested that higher viral loads predict more rapid progression to AIDS and death. Viral load is said to correlate with lymphocyte destruction (Arnaout *et al.*, 1999; Hare, 2004). There appears in some people, events of transient episodes of low level HIV viraemia in otherwise a well suppressed viral load, a phenomenon known as a 'blip' (Huff, 2004). It has been suggested that blips are due to the release of virions from reservoirs on protected sites in the body where replication of the virus continues at a low level. This is not of any concern because the viral load reverts to undetectable levels.

CD4 counts and viral loads are used to monitor the course of the disease over time, to ascertain the response to anti-HIV treatment as well as to determine when to start prophylactic antibiotics. Persistently detectable viraemia and high baseline levels carry a poor prognosis; risk of progression to AIDS is low at HIV RNA copy numbers < 10 000/ ml (Gupta & Gupta, 2004). Viral load is expressed in two ways *ie*. in the number of copies per ml and in logarithm of the number of copies, to the base 10 [$\log_{10}$]. The logarithmic transformation of the absolute number of copies is the preferred unit of measurement since it is more stable than the absolute number of copies (Hare, 2004; http://cfenet.ubc.ca/guide/page/sectc/consc2.html).

Hare (2004) indicated that a change in viral load of >0.5 log copies/ml, which is approximately a 3-fold change, may be considered to represent a true biological event, whereas a change of <0.5 log copies/ml may be due to random variability. Diurnal variation in stable HIV viral loads is said to be approximately 0.4 log copies/ml.

1.4 ANTIRETROVIRAL THERAPY

Azidothymidine (AZT) was discovered in September 1986 to be able to slow down the attack of the AIDS virus. It was first synthesized in 1964 as a possible anti-cancer drug, but it proved ineffective (Kanabus & Fredriksson, 2005). Antiretroviral therapy was introduced in 1996 (WHO, 2003a) and AZT had been approved in March 1987 by the U.S. Food and Drug Administration (FDA) as the first antiretroviral drug to be used as a treatment for AIDS (Kanabus & Fredriksson, 2005).

Antiretroviral drugs are given to patients for the rest of their lives as part of HIV clinical care, for treating HIV infection and thus making AIDS a manageable chronic illness. The aim of treatment is to reduce the amount of viral particles to very low levels namely below 50 copies/ml of blood so that the immune system can be reconstituted and be able to fight opportunistic infections. The indicators of successful therapy are the decrease in the HIV RNA viral load to undetectable levels in plasma, increase in CD4 counts, a decline in opportunistic infections as well as progressive weight gain (Demeter *et al*., 2001).

Untreated people have relatively high levels of HIV antigens in circulation, prompting the immune system to produce antibodies and other immune responses against the viral antigen. However, these are insufficient and ineffective in eradicating or suppressing the virus. Hunt *et al*. (2003) indicated that untreated HIV infected patients have high levels of T lymphocyte activation, which results in rapid proliferation and apoptosis of those cells as well as enhanced replication of HIV in infected cells. There is enhanced CD4 T lymphocyte susceptibility to HIV infection when these cells are activated. Activated cells express more CD4 molecules which HIV uses to gain entry into cells. Musey *et al*. (1999) indicated that in untreated patients, progressive T lymphocyte dysfunction is generally irreversible. Musey *et al*. (1999) further suggested that maintenance of T helper cell responses have an impact in controlling viral replication. Therefore, any form of intervention that yields immune reconstitution will help in controlling viral replication.

Human immunodeficiency virus relies upon many host cell proteins and mechanisms to complete its life cycle. The goal of antiretroviral therapy is to inhibit HIV replication. Antiretroviral drugs interfere with production of reverse transcriptase and protease, which are enzymes the virus must use in order to reproduce within the host cell (Hepp report, 2003). Currently available antiretroviral agents inhibit the viral enzymes required by HIV for intracellular viral replication: reverse transcriptase, which is essential for completion of the early stages of HIV replication; protease, which is required for the assembly and maturation of fully infectious viral progeny. Integrase is required for the integration of HIV-1 DNA into the chromosome of the infected cell (Di Santo *et al*., 2003).

Table 1.6: Classes of antiretroviral drugs, single or in combination, used for viral control with their physiological and adverse effects (side effects) (Bartlett, 2005; HEPP report 2003; NIAID, 2004a; NIAID, 2004b)

DRUG	PHYSIOLOGICAL EFFECTS	EXAMPLE OF DRUG	COMMON ADVERSE EFFECTS
Nucleoside Reverse Transcriptase Inhibitors (NRTI)	Inhibit viral RNA-dependent DNA polymerase (reverse transcriptase) and are incorporated into viral DNA. They are chain terminating.	Zidovudine (AZT) Lamivudine (3TC) Stavudine (d4T) Didanosine (ddI) Zalcitabine (ddC) Tenofivir (TDF) Abacavir (ABC) Emtricitabine (FTC) Combivir (CBV) [AZT +3TC] Epzicom [ABC + 3TC] Truvada [TDF + FTC] Trizivir [AZT + 3TC + FTC]	Bone marrow suppression Myopathy, neuropathy Mitochondrial toxicity Lipodystrophy Pancreatitis Anaemia, leukopaenia Nausea,vomiting, diarrhoea Hypersensitivity
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)	Not incorporated into viral DNA. Inhibit HIV replication by binding non-competitively to reverse transcriptase.	Nevirapine (NVP) Delavirdine (DLV) Efavirenz (EFV)	Hepatoxicity Rash, dizziness Hyperlipidaemia
Protease Inhibitor (PI)	Competitively inhibit the protease. Prevent the maturation of virions capable of infecting other cells.	Saquinavir (SQV) Ritonavir (RTV) Indinavir (IDV) Amprenavir (APV) Nelfinavir (NFV) Fosamprenavir (FPV) Atazanavir (ATV) Lopinavir/Ritonavir (LPV/r)	Hyperglycaemia Hyperlipidaemia, Lipodystrophy, Osteopaenia, Osteoporosis Hepatitis Rash
Fusion/ Integrase Inhibitor	Prevents virus from attaching to a CD4 cell by changing shape of the outer covering of the virus.	Enfuvirtide (T-20)	Injection site reaction: swelling and hardening, pain, itching at the spot

The antiretroviral therapy regimen used is a triple combination of synergistic antiretroviral agents, which includes a nucleoside reverse transcriptase inhibitor which inhibits the reverse transcriptase, a non-nucleoside reverse transcriptase inhibitor which inhibits the reverse transcriptase and a protease inhibitor which inhibits protease (Garcia *et al*., 1999). The newest class of antiretroviral drugs includes fusion and attachment inhibitors, called integrase inhibitors which stop HIV before it gains entrance to human CD4 T cells (Berger, 2004; www.aidsinfonet.org, 2005) (Table 1.6).

Havens (2003) reported that combination therapy with 3 or more antiretroviral medications, known as highly active antiretroviral therapy (HAART), is recommended to attain durable suppression of HIV replication and prevent or reverse HIV related symptoms or immune system dysfunction. Havens (2003) also suggested that these combinations must be reviewed, and carefully thought out, for better management of AIDS in the event of drug resistance, when the drugs need to be changed. King (1998) further indicated that disease progression in people with low CD4 counts is associated with the number of antiretroviral drugs taken, namely single drug or triple drug regimens. According to a WHO report (2003a), triple-drug combination antiretroviral therapy is given in many African countries including South Africa, in areas where such resources are available. Treatment is started when CD4 counts fall to levels of 200 cells/mm^3 or less, and/or when clinical symptoms of AIDS are showing (Cullinan, 2003), and/or HIV RNA levels exceeding 100 000 copies/ml, irrespective of the CD4 count (Chesebro & Everett, 1998; USA Department of Health and Human Services, 2005).

AIDS can be reversed by antiretroviral therapy (Garcia *et al*., 2004; Loemba *et al*., 2002; Temesgen *et al*., 2004) and viral loads are expected to decrease when CD4 counts increase (Jamil *et al*., 2002), whilst CD8 counts remain constant and CD8 responses become stronger (Gray *et al*., 1998; King, 1998). Rises in CD4 counts and lowered viral loads have been said to indicate good prognosis and therefore increased lifespan for HIV infected individuals (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for SA, 2003; Smith *et al*., 2003). Reports cite a significant reduction in HIV-associated mortality and morbidity in people taking these drugs (Hunt *et al*., 2003; Sterling, 2003). Loemba *et al*. (2002) showed that antiretroviral therapy during acute stages of HIV infection may be beneficial for regeneration of both CD4 and CD8 lymphocytes and restoration of immune functions. A study performed by Gray *et al*. (1998), indicated a persistent increase in absolute numbers of both naïve and memory CD4 cells over 6 months along with an increase in CD8 memory cells in patients on HAART. Contradictory to this report, Hunt *et al*. (2003) indicated that most HIV infected people often do not have normal CD4 counts, even after suppression of plasma HIV RNA levels (viral load), as would be expected. He indicated that CD4 counts may not increase even a year after therapy. Therefore, although most patients experience CD4 gains while receiving antiretroviral therapy, CD4 counts may not increase in some patients on such therapy even a year after treatment. In contradiction, Spritzler *et al*. (2003), reported that in some cases of incomplete viral load suppression, there is sustained increase in CD4 T cells. Price *et al*. (2003) also suggested that CD4 T lymphocytes remain stable or continue to increase despite ongoing viral replication. Jamil *et al*. (2002) concurred with the view of Loemba *et al*. (2002), by suggesting that immune reconstitution may occur among people on antiretroviral treatment, but he

further suggested that they may not achieve undetectable viral loads. This means that CD4 T lymphocytes may increase in the presence of high viral loads. This type of discordant response is most common among heavily pre-treated individuals, who are unable to suppress HIV but still experience improved health due to CD4 T lymphocyte recovery (Loemba *et al*., 2002). Havens (2003) indicated that while there might be ongoing viral growth, as long as CD4 counts remain high, people feel well and have no illnesses or symptoms associated with AIDS.

The conventional and expected pattern when a person is on antiretroviral therapy is a dramatic increase in CD4 T lymphocyte counts in the first few months of antiretroviral treatment, followed by a gradual increase during the following months (King, 1998), and a decrease in viral loads (Schlossberg, 2001). Bartlett (2005) suggested that an expected mean increase of CD4 counts is approximately150 cells/mm^3 in the first year on HAART. He indicated that treatment failure will be when there is a failure to increase the CD4 count by 25 to 50 cells during the first year of therapy.

People who have a significant CD4 T lymphocyte rise in the absence of reduced viral load are less likely to develop an AIDS-related illness. The absence of a CD4 T lymphocyte rise, even in people with viral suppression, is associated with disease progression.

1.4.1 The antiretroviral programme in South Africa

The planned antiretroviral treatment rollout in South Africa was only effected in March 2004 in very few sites within provinces of South Africa (Mohapeloa, 2004), such as KwaZulu-Natal, Gauteng and Western Cape. Currently, these antiretroviral treatment programmes do not cater for all people from rural settings. The Africa Focus Bulletin (2004) reported that not more than 10 000 people were receiving antiretroviral treatment at public health facilities in South Africa by August 2004 in spite of the government's target of providing this treatment to 53 000 people by 31 March 2005. The Aids Foundation of South Africa (AFSA) (2005) reported that by January 2005 only about 29 000 people were on antiretroviral treatment at more than 113 public sector facilities in South Africa. The KwaZulu-Natal provincial government had indicated that it would increase the number of people on antiretroviral treatment to 30 000 by March 2006 (PlusNews, 2005). This might not have catered for all the people who qualified for the treatment, and who might not even have been able to wait for the said date. AFSA (2005) reported that by January 2005, only 8467 people in KwaZulu-Natal were on antiretroviral treatment. Barron (2003) indicated that an antiretroviral therapy programme requires basic infrastructure such as water, sanitation, electricity, communication, consultation rooms, transport, sufficient drugs supply and laboratory support for it to be successful. It is well known that in many areas in South Africa, particularly in the rural areas such infrastructural facilities are non-existent. There is a shortage of health care workers, such as doctors, pharmacists, dieticians, laboratory personnel *etc*. to successfully render an adequate antiretroviral monitory service (Berry, 2004).

Table 1.7: Antiretroviral regimens used in South Africa and the laboratory parameters that should be used in routine monitoring during treatment (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa, 2003)

Regimen	Drugs	Laboratory test used to monitor	Frequency of testing
1a First-line	Stavudine + Lamivudine + Efavirenz	CD4 count Viral load ALT	Staging + 6-monthly Baseline + 6-monthly Symptomatic
1b alternate first-line	Stavudine + Lamivudine + Nevaripine	CD4 count Viral load ALT	Staging + 6-monthly Baseline + 6-monthly Baseline, week 2, 4 and 8, thereafter 6 monthly
2 second-line	Zidovudine + Didanosine + Lopinavir/Ritonavir	CD4 count FBC Fasting cholesterol, fasting glucose	Staging + 6-monthly Baseline + 1,3,6 monthly Baseline, 6 months and thereafter yearly

Antiretroviral therapy is available in different types of regimens in South Africa (Table 1.7). When there is development of resistance or any other factor that indicates that the first-line regimen has to be changed, then consideration has to be made to change to a different type of regimen (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa, 2003).

1.5 SIDE EFFECTS OF ANTIRETROVIRAL DRUGS AND DEVELOPMENT OF DRUG RESISTANT STRAINS

There is no doubt that antiretroviral drugs can control HIV and make AIDS a chronic manageable condition, as reported in many studies (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for SA, 2003). However, it has been indicated in the Afruka Products Company report (2004), that long term use of antiretroviral therapy also brings unsightly, painful and unpleasant side effects including the "evil triplets" of diarrheoa, nausea and vomiting. Other side effects of antiretroviral drugs include bone marrow suppression, liver toxicity, rash, kidney dysfunction, eye disease, lipodystrophy, peripheral neuropathy, myopathy, anaemia and leukopaenia (Table 1.6). The majority of these toxicities are not life threatening but can affect the quality of life and impact on the patients' willingness to adhere to the regimens. It is also reported that the immune reconstitution seen in antiretroviral therapy can trigger an inflammatory reaction soon after treatment is started, called immune reconstitution syndrome (IRS), immune restoration disease (IRD) or immune reconstitution inflammatory syndrome (IRIS) (Gardner, 2005). These are believed to result from the restored immune function responding to previously unrecognized antigenic stimuli (Gardner, 2005). The majority of IRS is reported to occur within two to six months after antiretroviral treatment in patients with lower CD4 counts. IRS may be severe or life threatening, featuring opportunistic infections, malignancies or inflammatory disorders.

These side effects can lead to patients being non-compliant to the strict antiretroviral drug regimens. These drugs have to be taken every day and at the same time or at least

90% of the time, for the drug to be effective. Non-compliance to the drug regimens is a major barrier in the complete success of antiretroviral treatment because of the toxicity problems posed by the antiretroviral drugs (Irwin, 2001; Foli *et al*., 2004; Kuritzkes, 2004). Drug non-compliance, inability to tolerate the drug regimen because of side effects and inadequate diet, contribute to the development of antiretroviral drug resistant strains that are not treatable. Drug resistance is the major limiting factor in the effective therapeutic management of HIV with antiretroviral drugs (Kuritzkes, 2004; Loemba *et al*., 2002). This results in antiretroviral drugs losing efficacy over time because of the resistance of HIV to such drugs. Antiretroviral drug resistant strains are a result of genotypic variants carrying mutations in the viral protease and reverse transcriptase and this confers resistance to antiretroviral compounds (Brindeiro *et al*., 2002). Carlos *et al*. (1999) indicated that these mutations alter the composition of the most immunogenic regions of the envelope glycoprotein (gp120), and this can affect the immune recognition and response to these altered strains.

Adherence is an important determinant of both the degree and duration of virologic suppression (New Jersey Department of Health & Senior Services, 2001). Moodley *et al*. (2003) stated that in developing countries, adherence to the drug regimen cannot always be ensured because of multiple doses that have to be taken, the price of drugs and other practical issues: *eg*. the fear of stigmatization. These issues also include improper taking of the drug regimen because of lack of patient education in taking these drugs; domestic abuse or violence and discrimination resulting in depression as well as active alcohol or drug abuse which affects the effectiveness of the antiretroviral drugs.

As reported by Cullinan (2002), the South African government has always been reluctant to provide antiretroviral therapy because of its toxicity, prohibitive costs and the complexity of antiretroviral drug management, since it can lead to drug resistant strains because of non-adherence.

Some antiretroviral drugs have basic food requirements arising from the effect food has on drug absorption through the gastrointestinal tract (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for SA, 2003). Some drugs have to be taken every 8 hours on an empty stomach *eg*. indinavir, others require refrigeration, while others require at least one litre of water a day to avoid kidney stones (Family Health International, 2004). In rural settings, where owning a watch, a refrigerator or accessing clean water is a luxury, taking antiretroviral drugs is difficult, the inabilities or drawbacks may create room for drug non-compliance . People from rural areas may not be able to access these drugs because of issues such as transport problems; not being able to come to urban hospitals to access these drugs and long waiting lists.

1.6 PROHIBITED USE OF ANTIRETROVIRAL DRUGS

When a patient is discovered to be suffering from tuberculosis (TB) and already on antiretroviral treatment, they should change the antiretroviral regimen to the one compatible with rifampicin, a commonly administered anti-TB drug, to avoid unacceptable drug interactions between antiretroviral drugs and rifampicin (WHO, 2005). The use of antiretroviral drugs in combination with anti-TB drugs is complicated

by overlapping toxicity profiles and by drug-drug interactions (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for SA, 2003). In addition, most of the time, patients have to go to different clinics to access antiretroviral drugs and anti-TB drugs. This adds to non-compliance, where patients end up not collecting their TB medication and therefore not completing their anti-TB treatment regimen (Mavata, personal communication). It is also often necessary for patients with CD4 counts less than 200 cells/mm^3 to take prophylactic antibiotics for *Pneumocystis carinii* pneumonia (PCP) as well (Benson *et al*., 2004). This might increase the pill burden and result in non-compliance to the antiretroviral regimen.

Efavirenz is not recommended for use in women who could become pregnant because of its potential teratogenic effect on foetuses in the first semester (WHO, 2005). Some antiretroviral drugs, such as Efavirenz and Nevaripine, can lower blood concentrations of oral contraceptives (WHO, 2005).

Clinical disease progression is a marker of treatment failure, and this necessitates that the regimen the patient is on be changed to a different regimen (Table 1.7). As stated in the WHO HIV/AIDS antiretroviral newsletter (2000), virological treatment failure can be defined as a failure to achieve undetectable viral load, or at least a 2 log decline in viral load from baseline after a reasonable period of time, typically one to two months. Immunological failure is defined as a declining CD4 count over time. Antiretroviral success is measured by the increase in CD4 counts, clinical improvements, opportunistic infections declining as well as weight gain of 10% of original body weight (WHO, 2001).

1.7 ALTERNATIVE THERAPIES AGAINST HIV/AIDS

Considering the side effects, toxicity, multi-drug regimens and drug resistance problems associated with antiretroviral therapy, alternative or supplementary therapies may play an important role in improving the quality of life in HIV infected people. Tshibangu *et al*. (2004) suggested that alternate herbal medicine can be used as a supplementary or alternative option in managing HIV/AIDS. However, it is imperative that clinically tried and proven products be used. World Health Organization (2001) indicated that even though further research needs to be done on traditional medicines, preliminary results on the evaluation of herbal preparations used for the management of HIV/AIDS in many African countries have shown encouraging results in improving the quality of life and clinical conditions in patients treated with such preparations (WHO, 2001). Such therapy might help in situations where some patients who qualify for antiretroviral treatment and yet are unable to access them because of several reasons such as waiting lists, travelling costs, unwilling to take antiretroviral drugs, *etc*. (Mavata, personal communication). Some patients have reservations in taking antiretroviral drugs; the stigma associated with the disease may be a major factor.

Case *et al*. (2005) reported that some ill people attend both public and/or private clinics and at the same time seek cures from traditional healers and non-prescribed medications from pharmacies. They further suggested that the socioeconomic status of people leads to their choosing which health facility to use: namely, less well educated and poor people are more likely to consult traditional healers whereas the educated lot will consult western health practitioners.

1.8 THE ROLE OF ALTERNATE HEALTH PRACTITIONERS IN THE TREATMENT OF HIV/AIDS

World Health Organization described traditional medicine as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines to treat, diagnose and prevent illnesses as well as maintain the well-being of people (WHO, 2003). In KwaZulu-Natal, there are two main subgroups of traditional healers, namely the izinyanga and izangoma (Giarelli & Jacobs, 2003). An inyanga is a herbalist who works mostly with indigenous plants whereas an isangoma is believed to have the power to communicate with ancestral spirits to bring about divination and healing (Giarelli & Jacobs, 2003). Traditional healers are said to treat patients' spiritual, psychological and physical well-being together (Richter, 2003). They often have high credibility and deep respect among the population they serve and are able to influence behaviours (Richter, 2003). They are important agents concerned with the provision of ancient knowledge to the society that believes in them. Colvin *et al*. (2001) reported of a study they undertook in a rural district of Hlabisa in KwaZulu-Natal, where traditional healers, staff from the local health clinic, community health workers and lay people such as shopkeepers were made supervisors of the community-based tuberculosis directly observed therapy, short-course (TB DOTS) programme. Patients had to choose their treatment supervisor. It is reported that patients that chose to be treated by traditional healers were satisfied because of their easy access and short waiting times (Colvin *et al*., 2001).

In Zulu tradition, health is closely associated with religious belief and therefore traditional healers are considered to be religious specialists (http://www.kzn.org.za/kzn/129.xml).

Adherents of the Shembe faith fuse Christian beliefs with Zulu cultural traditions. Many African people still celebrate tradition as an affirmation of their self-worth. It has been estimated that 80% of South Africans use herbal medicine on a regular basis for their primary health care, purely due to historical and cultural reasons (Smart, 2005; WHO, 2003), even though some of them also use western medicines (AFSA, 2005). In the majority of cases traditional healers are the first health care providers to be consulted with in rural areas where the people rely on traditional herbal medicine (Colvin *et al*., 2001; WHO, 2000). The South African government indicated that traditional health practitioners should support the national response to HIV/AIDS developed by the government, by being involved in the provision of comprehensive care developed for the HIV/AIDS care and treatment programme (Comprehensive HIV and AIDS Care, Management and Treatment Plan, 2003). This will provide a holistic health care approach to people that believe in this arm of the health system.

Traditional health practitioners were consulted by HIV infected people when antiretroviral drugs were not yet available. Some bogus traditional healers have misused the trust of people consulting them, due to administration of false remedies. This has caused mistrust between traditional healers or sangomas and western medical practitioners (AFSA, 2005). False claims have been identified. Likewise, traditional healers are reported not to be enthusiastic about collaborating with western clinicians because they fear giving away their hard-learned knowledge on herbal medicines

(Tshibangu *et al*., 2004). This trust has to be built by regulating the practice of the traditional medicine arm of the health system and regulating their medications through the Medicines Control Council (MCC) as done in other western forms of health care management systems. Protection of their indigenous knowledge systems must be ensured.

In Sub-Saharan Africa, the ratio of traditional healers to the population is approximately 1:500 as opposed to a ratio of 1:40 000 of medical doctors to the population (Richter, 2003). It is estimated that more than 200 000 traditional health practitioners are active throughout South Africa, and they are more deeply rooted within the local communities (Comprehensive HIV and AIDS Care, Management and Treatment Plan, 2003). An estimated 97% of HIV infected people consult them first before going to hospitals or clinics if their ailments are not cured (Comprehensive HIV and AIDS Care, Management and Treatment Plan, 2003).

A report by Tshibangu *et al*. (2004), suggested that a combination of a variety of herbs and their sub-components produces a significant effect. However, the majority of traditional herbal medicines used have not been subjected to thorough clinical trials to evaluate their safety and efficacy (Mills *et al*., 2005b; Tshibangu *et al*., 2004). Tshibangu *et al*. (2004) further suggested that incorrectly combined herbs and their toxic impurities may result in kidney or liver failure and even death. The African potato (*Hypoxis hemerocallidea*) and Sutherlandia (*Sutherlandia frutescens*) are herbs that are currently used in South Africa traditionally to treat a variety of maladies as well as to improve overall health in HIV/AIDS patients (Tai *et al*., 2004). Tai *et al*. (2004) stated

that *Sutherlandia frutescens* contains L-canavanine, a potent non-protein amino acid, which is an arginine antagonist and has antibacterial, antifungal, anticancer and antiviral activity. Mills *et al*. (2005a) also stated that *Hypoxis hemerocallidea* contains a sterol called hypoxoside, which once in the human gut readily converts to rooperol, a biologically active compound that balances the immune system. Laifer (2000) agreed with Mills *et al*. (2005a), that the African potato does not possess antiviral properties but he stated that it demonstrates significant immunomodulatory effects.

Laifer (2000) further suggested that if combined with widespread cheaper antiretroviral drugs, the African potato could offer an affordable and effective treatment regimen. Loemba *et al*. (2002), in agreement, indicated that immune based therapies may provide important and less expensive adjuncts to the management of HIV infections when used with antiretroviral drugs. However, in contradiction Mills *et al*., (2005b), suggested that co-administration of *Hypoxis hemerocallidea* and *Sutherlandia frutescens* with antiretroviral drugs may result in the early inhibition of antiretroviral drug metabolism and its transport and may put patients at risk of treatment failure, viral resistance and/or drug toxicity.

Any efficacy observed in herbal immune boosters will be beneficial to those individuals that are reluctant to start the antiretroviral regimen and will assist individuals that are not on the antiretroviral drug programme due to access problems or not being eligible to be on the programme as per their CD4 counts.

1.9 THE SOUTH AFRICAN GOVERNMENT'S SUPPORT OF TRADITIONAL MEDICINE

The current minister of health, Dr Tshabalala-Msimang has promoted the use of African traditional medicine and an immune boosting diet with antiretroviral drugs in the treatment of HIV and AIDS in South Africa (Mills *et al*., 2005a). Two African herbal medicines have been recommended by the South African Ministry of Health to be used as immune boosters, namely the African potato (*Hypoxis hemerocallidea*) and *Sutherlandia frutescens* (Clayden, 2005; Singh, 2004). The Afruka Company Report (2004) supported the use of medicinal herbs in whole plant forms in combination with nutritional supplements to boost the immune system so that pathogens can be destroyed and nutritional shortages due to reduction of the immune modulators, be supplemented. The report stated that immune boosters attempt to encourage the body's ability to fight HIV and strengthen the immune system against HIV's attack, preventing the development of resistant strains. The report also stipulated that herbal immune booster products are well tolerated by patients and pose no side effects, as they are made from natural products.

At a Southern African Development Community (SADC) ministerial sub-committee meeting on traditional medicine held in Harare, Zimbabwe, on the 16th September 2005, it was noted that SADC ministers supported the view of elevating traditional medicine to a higher level of recognition and they committed to formulating a policy framework for traditional medicine (Department of Health, 2005). It is stated that traditional medicine is affordable, accessible and effective, but it is not yet recognized as part of a formal

health system, it is still marginalized (Department of Health, 2005). It was noted from Dr Tshabala-Msimang's address at the launch of the Western Cape's National Reference Centre for African Traditional Medicines held on the 15th February 2004, that she supported locally produced traditional medicinal products as long as they are of proven quality and efficacy because she said they may provide an affordable alternative to some of the expensive imported synthetic drugs (Ministry of Health, 2004). BBC News (2004) reported that the Traditional Health Practitioner's Bill was adopted by the South African government in September 2004, and this bill was to ensure that traditional healers are licensed before they are allowed to work by being registered, and that their medicines are checked for efficacy, safety and thereafter made available to the public.

1.10 THE IMPACT OF NUTRITION ON HIV/AIDS

It is known that poor nutrition increases the progression of HIV to AIDS. Opportunistic infections and their associated symptoms limit food intake which contributes to malnutrition. Diarrhoea and vomiting worsen the AIDS state, resulting in wasting and accelerated disease progression (Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for SA, 2003). This is also supported by the view expressed in the SADC meeting on nutrition and AIDS held in Johannesburg in November 2002, that the use of food, immune boosting supplements and herbal remedies can reverse the effects of AIDS and can prevent the progression of HIV to AIDS (Giraldo, 2002).

Eating a balanced diet based on a variety of foods helps strengthen the immune system and maintain body weight to its optimum level. HIV infection poses a tremendous stress on the immune system. Rebuilding the immune system begins with support of the digestive system. A balanced diet should include proteins, fruits and vegetables, breads and grains and dairy products. Proteins are required to form antibodies and repair body damage. Fruits and vegetables provide a variety of vitamins and minerals. Bread and grains are sources of carbohydrates for energy. Dairy products provide calcium, proteins, vitamins and fat. Fats and sugars add calories which can result in weight gain if there is weight loss due to the wasting effect of AIDS. However, fats and sugars if consumed in increased amounts can aggravate symptoms of some opportunistic infections such as candidiasis (Kline, 2004; San Francisco Aids Foundation, 1999).

In the WHO Consultation on Nutrition and HIV/AIDS in Africa conference held in Durban on the 10th to the 13th April 2005, Dr Tshabalala-Msimang reported on the government's realisation that good nutrition is a critical component of a comprehensive response to diseases. She reported that patients on the antiretroviral programme in public health facilities are provided with a supplementary meal and multivitamin syrup or tablets as part of the Comprehensive Plan, to increase macro and micronutrient intake and maintain body weight. It was reported that only 153 000 people on antiretroviral treatment benefited from this government nutrition programme by the end of February 2005. The government's Comprehensive HIV and AIDS Care, Management and Treatment Plan (2003) states that nutritional therapy improves the functioning of the immune system and the body's ability to fight infection and therefore delay progression from HIV infection to the development of the AIDS defining conditions.

This should benefit HIV infected individuals that are not yet medically or clinically eligible for antiretroviral treatment or are on a waiting list to be in the antiretroviral therapy programme.

1.11 THE PRESENT INVESTIGATION

Studies on the success of antiretroviral drugs in improving the quality of life in HIV infected individuals have been extensively performed and published (Hunt *et al*., 2003; Jamil *et al*., 2002; King, 1998; Sterling, 2003). The exact rate of change in viral load, CD4 and CD8 counts in patients on antiretroviral treatment has been reported (Demeter *et al*., 2001; Keita-Perse *et al*., 1998; Smith *et al*., 2003; Sterling, 2003). However, there is no published data available yet on immune boosters improving the quality of life in such persons.

The study for this thesis was undertaken at the Ikhaya Lobomi Hospice and Care Centre in Botha's Hill in Durban, KwaZulu-Natal, South Africa (appendix 1). This study investigated the rate of change of CD4 counts and CD4 percentages, CD8 counts and CD8 percentages, CD3 counts and HIV RNA viral loads in patients on the Inochi New Medicine immune booster, a herbal product. As indicated by Kuritzkes (2004), that antiretroviral drugs have side effects and are toxic, any efficacy indicated by this herbal medication in slowing down the progression of HIV disease to AIDS will benefit those patients that are not on antiretroviral therapy programme. It will also benefit those not able to access antiretroviral drugs because of problems with cost or the fact that their clinical stage does not warrant antiretroviral therapy as yet.

Table 1.8: The Inochi New Medicine immune boosting products administered to HIV/AIDS patients at the Ikhaya Lobomi Hospice and Care Centre, showing their physiological effects (Mavata, unpublished)

PRODUCT	CONSTITUENTS	PHYSIOLOGIC EFFECTS
Inochi Clear the Heat tablets Use and indication: Reduces heat and inflammation and has antiviral properties.	Dandelion (*Taraxacum mongolicum)* root powder	*Taraxacum mongolicum* detoxifies virus toxins, eliminates heat.
	Aloe ferox	*Aloe ferox* regulates sugar metabolism and reduces heat, promotes healing.
	Scullcap herb *(Scutellaria baicalensis)* powder	*Scutellaria baicalensis* is antispasmodic, antioxidant, repairs damaged DNA.
	Turmeric *(Curcuma* sp) powder	Tumeric is a natural antibiotic, antioxidant, anti-inflammatory and anti-carcinogenic.
	Sutherlandia (*Sutherlandia microphyllia)*	Sutherlandia is an immune booster, an anti-inflammatory.
	Sarsaparilla (*Sarsaparilla officinalis)* root	*Sarsaparilla officinalis* root purifies blood of toxins, dispels infection and inflammation.
Inochi Nourish the Body tablets Use and indication: Immune booster	Astragalus (*Astragalus membranaceus)* powder	Astragulus is a tonic, strengthens immune function
	Dong quai (*Angelica sinensis*) root	The dong quai root activates blood circulation.
	Sutherlandia (*Sutherlandia microphyllia)*	Sutherlandia is an immune booster, an anti-inflammatory.
	African Potato *(Hypoxis hemerocallidea)* powder	*Hypoxis hemerocallidea* is an antioxidant and an immune booster.
	Marshmallow (*Althea officinalis)* root powder	*Althea officinalis* is a tonic and an anti-inflammatory, expectorant.
	Liquorice (*Glycyrrhizae uralensis)* root powder	*Glycyrrhizae uralensis* increases vital energy, a detoxifier, an expectorant.
	Ashwaganda (*Withania somnifera)*	*Withania somnifera* is a tonic, rejuvenates, prevents emaciation.

Inochi New Medicine immune boosting products (Impilo Drugs, Isithebe in KwaZulu-Natal) are administered to HIV/AIDS patients at the hospice and care centre (Table 1.8) by the Director and co-founder of the Ikhaya Lobomi Hospice and Care Centre, Mrs Patience Mavata, a registered nurse. She works with a team of rehabilitated patients who have entered a voluntary home-based care programme and are volunteer workers for those who are still sick and bedridden at the hospice. They assist in patient management by providing nursing care to the sick and bedridden at the hospice as well as visiting them at their homes, administering medication, and providing support and hope to both in-patients and out-patients. This ensures compliance to therapy as the staff/volunteers are part of the immune booster programme themselves and this also promotes patients' commitment to the programme. This also takes away the stigma associated with the disease as everybody speaks the same language of getting better and returning back to their community to be productive.

Table 1.8 indicates physiological effects of the Inochi New Medicine. This is a herbal immune booster comprising of two herbal products, *viz*. the Clear the Heat and the Nourish the Body.

Laboratory testing was performed at Global Clinical and Viral Laboratory in Merewent, Durban, KwaZulu-Natal, South Africa (appendix 2).

The WHO HIV/AIDS antiretroviral newsletter (2000) recommended that in situations where HIV monitoring is highly costly, baseline testing followed by repeat testing after antiretroviral therapy has been started, should be performed every 3-6 months. In this study, the same frequency of testing was followed as this made easier the comparison between the efficacy of antiretroviral drugs and that of immune boosters.

Chapter 2 of this study details the aims of the study and the patient population recruited at three sites namely, Ikhaya Lobomi Care Centre and Hospice in Botha's Hill; the Tafuleni area, Inanda in Durban, KwaZulu -Natal, South Africa and at Impendle area in Pietermaritzburg, KwaZulu-Natal, South Africa. The latter two were satellite outpatients' sites of Ikhaya Lobomi Care Centre and Hospice.

CHAPTER 2

AIM OF THE STUDY AND THE PATIENT POPULATION

2.1 AIM OF THE STUDY

The aim of this study was to investigate the change in the immune status of HIV infected patients on immune boosters as measured by decrease and/or increase in the following laboratory parameters:

1. $CD3^+$ $CD4^+$ absolute count and percentage
2. $CD3^+$ $CD8^+$ absolute count and percentage
3. CD3 absolute count
4. Free HIV RNA viral load in plasma

The objective of the study was to report on the above-mentioned parameters before and after taking immune boosters for 6 months or more, so as to ascertain whether they follow the expected immune response pattern and improvement as has been documented for antiretroviral therapy. Hence, this comparison will establish the difference or similarity in the rate of change in CD4 counts, CD8 counts and viral loads in the two different treatment modalities.

After patients were recruited, baseline testing of their CD4 counts, CD8 counts, CD3 counts, and HIV RNA viral loads were performed in the laboratory before participants started the immune booster treatment. They were followed-up after 6 months of treatment. This enabled the comparing of the changes in the CD4 counts, CD8 counts,

CD3 counts and HIV RNA viral loads before, and 6 months after the herbal therapy. This comparison was to establish the difference or similarity in the rate of change in the two different treatment modalities *ie*. the Inochi New Medicine herbal therapy and antiretroviral therapy.

In addition, the quality of life of the patient was observed and the following laboratory parameters were observed:

1. Evaluating presence of anaemia (haemoglobin levels)
2. Liver enzymes assays: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).
3. Improvement in the quality of life as gathered from questionnaires (appendices 5 and 7).

2.2 PATIENT SELECTION

One hundred and thirty-one HIV infected patients with CD4 counts of less than or equal to 500 cells/mm^3, not on any anti-HIV therapy nor immune boosters, were enrolled from a total of 140 patients recruited. They were recruited from Ikhaya Lobomi Hospice and Care Centre (Botha's Hill), at the Tafuleni area (Inanda), both in Durban and at the Impendle area (Pietermaritzburg) for the study, during November 2004 to June 2006. A biostatistician from the Medical Research Council, Durban, KwaZulu-Natal, South Africa, was consulted to determine a statistically significant sample size.

The sample size calculated to be 90 had the ability to detect a 10% change in CD4 counts between baseline and follow-up with a probability of 0.05 and power of 80% (Connolly, personal communication).

Participants were selected according to the following criteria:

2.2.1 Inclusion criteria

Participants were to:

- be willing to sign a consent form (appendix 4)
- be 18 years or older
- be HIV infected (with copies of HIV laboratory reports)
- never have been on any anti-HIV treatment and immune boosters
- have a CD4 count $\leq$ 500 cells/mm^3
- be of any sex
- be of any race
- be willing to answer questions on the questionnaire (appendix 5)
- be able and commit to sustaining access to immune booster regimens
- commit to coming to the follow-up visit after 6 months on treatment

2.2.2 Exclusion criteria

- Pregnancy
- CD4 count > 500 cells/mm^3
- Not willing to comply with protocol

2.3 PATIENT RECRUITMENT

Patient recruitment took place at the Ikhaya Lobomi Hospice and Care Centre, Botha's Hill, Durban, as well as at their two satellite sites: at the Tafuleni area, Inanda and at the Impendle area (Figure 2.1). Very ill and bedridden patients are admitted at the Ikhaya Lobomi Hospice and Care Centre where they are provided clinical care by clinicians and a professional nurse (Mavata, personal communication). They are also taken care of by rehabilitated patients who either become volunteers at the centre or enter a home-based care programme as house visitors under the supervision of Mrs Mavata, the Director and co-founder of the Ikhaya Lobomi Hospice and Care Centre, to help those who are still sick and bedridden (appendix 9).

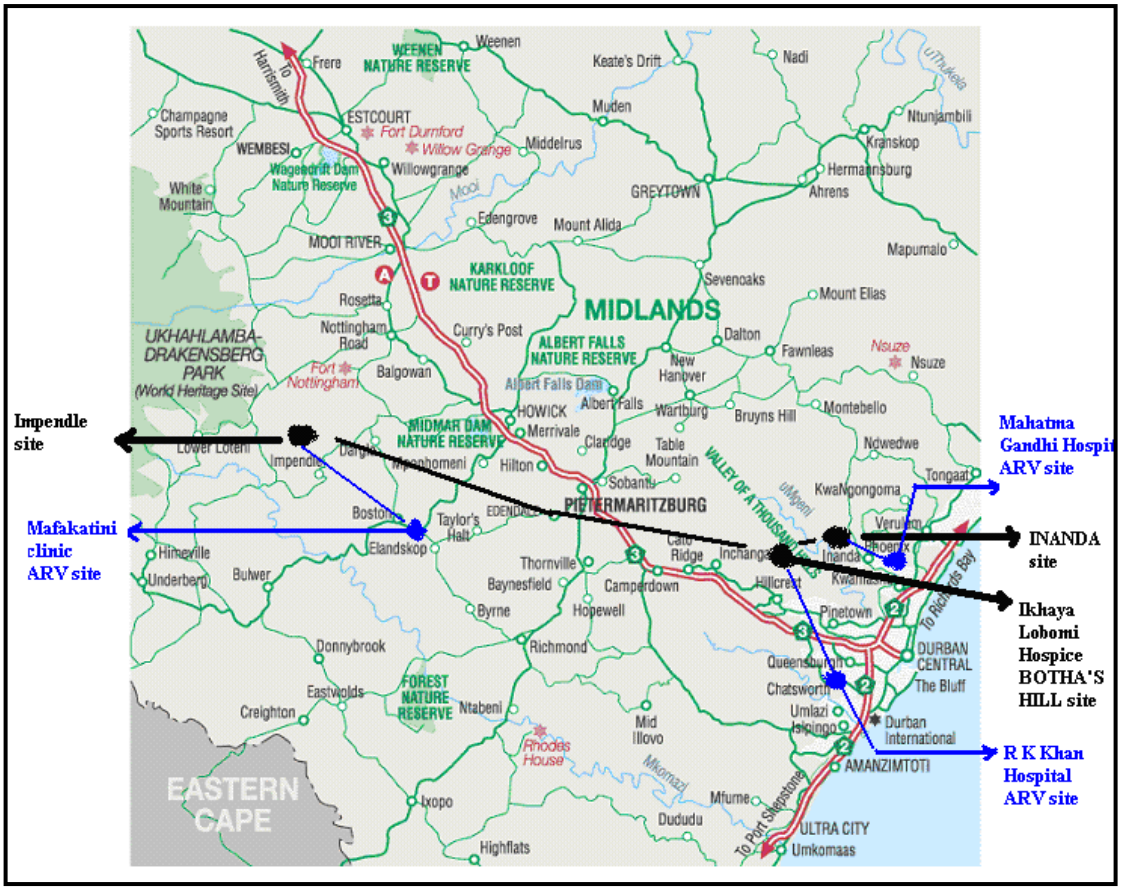

Figure 2.1: Map of KwaZulu-Natal's Durban and Midlands areas showing the locations where the study participants were recruited from, also showing the distances (in black) between Ikhaya Lobomi Hospice and Care Centre and Inanda which is approximately 80 km and between the Ikhaya Lobomi Hospice and Care Centre and Impendle which is approximately 130 km. Also shown are the antiretroviral therapy (ARV) sites and the distances between the Ikhaya Lobomi and its satellite sites and their nearest ARV sites (in blue) (Adapted from www.kzn.org.za/travelguidemaps/Midlands.gif)

The patients, both admitted and out-patient, were monitored constantly while on the Inochi New Medicine herbal medication. This is an immune boosting medication that is registered with the Medical Control Council of South Africa (appendices 10, 11 and 12) (Mavata, personal communication). The volunteers themselves were on this medication and this ensured compliance to this therapy and also promoted commitment to the study project in some patients.

The first 131 consecutive patients that met the inclusion criteria were selected into the study. There was no coercion of patients to participate in this study. If CD4 counts of selected patients fell to below 200 cells/mm^3, they were not prevented from taking antiretroviral therapy, if they so wished. Since antiretroviral therapy is the current proven therapy that is used in South Africa for HIV/AIDS treatment, patients were encouraged to use it, particularly when their CD4 counts were below 200 cells/mm^3. Such patients were thereafter excluded from the study. Some patients did not want to take antiretroviral therapy because of the following reasons:

- Some were not sure whether they would cope with taking antiretroviral drugs, and therefore they insisted on taking the Inochi New Medicine immune booster
- Some indicated that they were living with young children; therefore, they would not be able to take antiretroviral drugs
- Some had heard of side effects associated with antiretroviral drugs; therefore, preferred not to take them.

Details of the study were discussed with the willing participants, verbally, as well as by the subject information leaflet using isiZulu (appendix 3). Those who did not even know what a CD4 count was, benefited in gaining knowledge of the significance of this parameter and the importance of making sure that it is maintained in high levels. Thereafter, they signed an informed consent form (appendix 4) to indicate that they were willing to participate in the study and that they understood the benefits brought about by the study in their lives as well as understanding what is expected from them in return.

They were counselled to ensure adherence to the treatment regimen in terms of dosages, changing their eating patterns to eating healthily and changing behaviour, such as using condoms, and to stop drinking or smoking for health reasons. Appointments were made with them for follow-up medical assessments as well as for picking up their treatment. The pills given to them were counted to last them for a month, and thereafter, they had to replenish. The benefits they enjoyed from participating in the study were:

1. not having to go to a hospital far away to have their CD4, CD8 counts and viral loads tested.
2. having CD4, CD8, CD3 and viral loads performed; whereas in hospitals, only CD4 counts were done.
3. being able to have a 24 hour consultation on anything that was worrying them, clinically or emotionally, by telephoning Mrs Mavata.
4. receiving explanations on the disease, or on anything they wanted to know; such as myths and stigma issues.
5. being offered remedies for their ailments, *eg*. sores.
6. receiving advice on how to eat healthy; including reasons why they had to change their eating habits to only possibly eating what is nutritious.

They were also told of possible risks such as developing diarrhoea during the first 3 or more days of treatment. They always brought feedback on how they managed the treatment, *ie*. whether there was any discomfort or side effects. If necessary, Mrs Mavata would adjust the dosage with explanations in everything she did, ensuring that the patient fully understood all aspects of the immune booster.

AST and ALT levels were used to monitor any liver damage; haemoglobin (Hb) levels were used to monitor any anaemia developed during pre- and post-treatment.

2.4 ECONOMIC STATUS OF THE PATIENT POPULATION

In Africa, the disease is spreading very rapidly in impoverished communities. The levels of poverty in South Africa are already so great that resources for dealing with the care of the sick, the dying and the orphans are extremely scarce. Countries lose labour, and this impacts on the society and economy as subsistence farming communities, commercial plantations, mines, factories and the administration sector, lose people. This disease results in a full range of developmental issues. The little money the poor people might have is spent on burials, on treatment, on transport to health care facilities to collect treatment for opportunistic infections, and very little is left for food.

The three areas (Figure 2.1) in KwaZulu-Natal namely, Botha's Hill, Inanda and Impendle are rural, populated mainly by poor and unemployed people (Mavata, personal communication). Most of the households in these areas are headed by females and few are headed by children (Mavata, personal communication). Swartz (2001) pointed out that most of South African households, chiefly in rural areas, experience outright poverty with no access to clean water, energy, health care and education.

The population in Durban in 2005 was about 3 million people, 68% being Black (Ndimande, 2001). Black residents in rural areas of Durban are living at a poverty rate of 57% and an unemployment rate of about 43% (Casher, 2003; Ndimande, 2001; Swartz,

2001). These statistics are not far off when compared to those of Swartz (2001) who reported that South Africa's poverty rate in 2001 was 50%, affecting Blacks who constituted about 82% of the population of about 40 million people. He reported that the poverty rate in KwaZulu-Natal in 2001 was 51.9%. The population in Pietermaritzburg and surrounding areas was approximately 988 000, 87% being Black. By the end of 2004 the unemployment rate was found to be 52% (Coetzee, 2005).

Poverty goes hand in hand with malnutrition (Swartz, 2001), as is the case in these rural areas. Irwin (2001) pointed out that malnutrition results in immunosuppression with lowered CD4 counts, which translates to lowered cell mediated immune responses.

Some of the households barely survive through casual work, subsistence gardening and selling second-hand clothing, as well as on old age pensions, disability grants and assistance from non-governmental organizations with food parcels and clothes (AFSA, 2005; HIV infected patients, personal communication). The majority of people who are able to obtain an income get less than R1 500 per month (eThekwini Municipality, 2005). Some patients that qualified for disability grants because of their low CD4 counts were not receiving the grants since they did not have identity documents; whereas for others, the reasons were unknown (HIV infected patients, personal communication). This was a major drawback to them since they could not afford to buy nutritious food that would boost their immune system and thus help in slowing down the HIV disease progression to AIDS.

Mrs Mavata was invited by the political structures of the Inanda and Impendle areas to bring her herbal medication to these areas. She, therefore, established satellite sites to extend her counselling, diet management and medicating services, similar to what is available at Ikhaya Lobomi Hospice and Care Centre, except for the admitting facility which is only at the Botha's Hill site. The patients came to consult with her with ease at these satellite sites (Mavata, personal communication; personal observation). Most of these patients were reluctant to visit their local clinics because of stigma issues. This helped in providing a local accessible service to these patients, which did not require them to travel for long distances to visit hospitals or clinics.

As stated, the Ikhaya Lobomi Hospice and Care Centre is situated in Botha's Hill near Pinetown. It is utilized by people from the nearby rural areas and from other rural and urban areas as far as Pietermaritzburg. The Inanda site is utilized by people from the nearby rural and informal settlement areas. The Impendle site is utilized by people from the nearby rural area (Figure 2.1).

Botha's Hill is about 40km away from the nearest hospital, the R K Khan Hospital (Durban, KwaZulu-Natal), an antiretroviral therapy site. Some of the patients were reluctant to go to the nearest Botha's Hill clinic, citing poor service delivery and ill-treatment by the nurses (Mavata, personal communication). Patients cited that the hospital was quite far and some did not have money for transport. Inanda is approximately 20 km away from the Mahatma Gandhi Memorial Hospital (Durban, KwaZulu-Natal), an antiretroviral therapy site.

However, HIV infected patients did not have money for transport to go to this hospital (Figure 2.1). Some of the patients from the Inanda area pointed out that they were reluctant to go to the nearest clinic because of the fear of being recognized, and therefore disclosure of their status to the local community.

A similar situation existed in the Impendle area, where the nearest hospitals are Edendale, Greys and Northdale Hospitals (Pietermaritzburg, KwaZulu-Natal), which are antiretroviral therapy sites but they are about 100 km away (Figure 2.1). In the Impendle area also, some of the patients were reluctant to go to the nearest clinic because of the fear of being recognized, and therefore, their status being known by the local community: (Impendle patients, personal communication). The hospitals are usually quite far and inaccessible to people from rural areas. Some find it difficult to access the hospitals for purposes of monitoring the progress of their disease, or to obtain antibiotics in the event of opportunistic infections.

As already indicated, these patients were desperate for health care services but since they were poor they could not afford accessing private health care facilities that were situated far away, where possibly their identities were not going to be known. The intervention provided by Mrs Mavata was greatly welcomed by many patients, particularly those that were reluctant to start antiretroviral therapy and those that did not use the local health care facilities because of stigma issues.

Chapter 3 of this study details the methodology used in this study. The background of the participants enrolled in the study, which was collected by the use of a questionnaire, will be further detailed in chapter 4.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This study was undertaken at the Ikhaya Lobomi Hospice and Care Centre in Botha's Hill, Durban and its satellite sites; at Inanda (Durban) and Impendle (Pietermaritzburg), between November 2004 and June 2006. This study obtained ethical approval from the Durban Uniersity of Technology, Durban, KwaZulu-Natal (appendix 17). Laboratory testing was performed at Global Clinical and Viral Laboratory in Merewent, Durban, KwaZulu-Natal, South Africa.

3.2 PATIENT POPULATION

All individuals included in the study were out-patients or in-patients at the Ikhaya Lobomi Hospice and Care Centre and its satellite sites. Information on these patients such as their HIV status, as indicated in their laboratory or clinic reports, was collected from them as they attended the sites for care and treatment. All patients included in the study were not on any anti-HIV treatment; neither antiretroviral drugs nor immune boosters at the time of enrolment to the study. Those that had taken traditional medicine had taken it at least more than three months prior to their enrolment to the study. One hundred and thirty-one HIV infected patients with CD4 counts of less than, or equal to, 500 cells/mm^3 were enrolled into the study.

Appointments for subsequent visits for the participants were at intervals of one week after taking the Inochi New Medicine (to safeguard against toxicities), one month, three months and then after six months. The visits were for replenishing the medication as well as for follow-up assessments. They brought their pill bottles, clinic cards (appendix 13) and appointment cards (appendix 14).

3.3 PATIENT RECRUITMENT AND ENROLMENT PROCEDURE

The recruitment procedure was carried out for each patient in privacy. Strict confidentiality was upheld. Information and details of the study was given to the patient by the principal investigator (Mrs B.T. Mkhize) or by Mrs Mavata in the form of a subject information leaflet (appendix 3) as well as by verbal explanation in isiZulu. After accepting to participate in the study, an informed consent form (appendix 4) was given by the principal investigator to the patient to sign. Pre-test counselling was undertaken by Mrs Mavata, a registered nurse and counsellor. This was offered to all patients. The participant's name, date of birth, sex and date of Inochi New Medicine dispensing was logged onto a log book. All patients were given a bar code for identity. This ensured confidentiality. Assessments were then done by Mrs Mavata. A Doctor, when available, recorded the medical history of the patient. Clinic cards brought by the patient were looked at by Mrs Mavata to ascertain if there was any recent opportunistic infection; and if there was any, for how long the patient was infected with that opportunistic infection before they presented themselves at the clinic.

Many patients did not realise the importance of screening for TB, and therefore, Mrs Mavata advised them to visit clinics to screen for TB as well as to enquire about the PCP prophylactic antibiotic, if they did qualify according to their clinical presentation. Weight was measured using the weighing scale, when available.

Prior to treatment a questionnaire (appendix 5) establishing the demographic details as well as socioeconomic and clinical status of participants was issued by the principal investigator to each participant to complete. Those patients that could not read were assisted by the principal investigator in completing the form. This constituted part of the baseline data. The informed consent form (appendix 4) and the completed questionnaire (appendix 5) were filed in the data file, using the code number assigned.

Three initial coded 5ml blood samples were collected from the patients by Mrs Mavata for laboratory testing. Two blood samples were collected into ethylene-diamine tetra-acetic acid (EDTA) anticoagulated blood tubes and one into a plain tube (with no anticoagulant). Health and safety precautions were always observed. Sample tubes and a request form (appendix 8) were packaged in plastic packets. The form was separated from sample tubes in the plastic packet as a safety precaution in the event of a tube leak. The packet was packed in a cool container and transported to Global Clinical and Viral Laboratory in Merewent, within 6 hours of blood collection. If the samples were going to reach the laboratory after six hours of sample collection, the full blood count EDTA sample tube was refrigerated at 4^0C before being transported to the laboratory, the rest of the tubes were stored at room temperature. All blood samples reached the laboratory within 12 hours of collection. The patient was then given the Inochi New Medicine

immune booster treatment by Mrs Mavata. The dosages were prescribed according to the clinical presentation and the clinical history of the patient. The treatment was to be taken as prescribed uninterruptedly. All patients were given an appointment card (appendix 6) by the principal investigator. The next visit was scheduled for a week from the initial visit. Mrs Mavata advised them on the benefits of healthy eating and to give up bad habits such as smoking and drinking *etc*. She also provided them with her personal cell-phone number so that they could contact her at anytime when they needed advice and support. For instance when they experienced side effects or they found it difficult to change their bad behaviour.

3.4 LABORATORY SAMPLE PREPARATION AND TESTING

Blood samples on receipt at the laboratory were checked for patient's details against details on the request form (appendix 8). The form was logged into the laboratory database. The date and time of sample receipt was documented. The samples were checked for suitability, clotted EDTA tubes were rejected. The samples were then bar-coded and forwarded to relevant departments for sample analyses. CD4, CD8 and CD3 counts were performed on a blood sample from the one EDTA blood tube, the rest of the sample was centrifuged at 5300rpm for 15 minutes at room temperature (RT) using the Labofuge 200 (Heraeus Sepatech, Laboratory and Scientific Equipment) to separate plasma from cells. The plasma was frozen at -70^{0}C for subsequent viral load testing.

Full blood count was performed on the second EDTA blood sample (to screen for anaemia), and liver function tests (AST and ALT) were performed on the plain tube (to screen for liver toxicity). Viral load testing was done only on the plasma samples of participants enrolled in the study.

Results of CD4, CD8 counts and percentages, CD3 counts, full blood counts (FBC), AST and ALT were delivered to the Ikhaya Lobomi administration offices in sealed envelopes, for the attention of the principal investigator. They were captured in the data file using the code numbers.

3.5 FOLLOW-UP VISITS

The laboratory results of patients, *ie*. CD4, CD8 counts and their percentages, CD3 counts, FBC, AST and ALT and viral load were communicated to them on their one week's follow-up. The clinical significance of the results was explained to the patient by the principal investigator. The patient was again advised of the antiretroviral treatment. Patients with CD4 counts of less or equal to 500 cells/mm^3 were enrolled on the study. The clinical significance of the full blood count results, AST and ALT results were explained to the patients. Assessments were done again to ascertain whether medication was tolerated or not by the patients. Weight was measured, when the scale was available and recorded on the medical history form and also on the questionnaire (appendix 5). If not, weight was recorded either by observation or by asking the participants. The dose of the Inochi New Medicine medication was adjusted if necessary, according to the CD4 results and the clinical assessment. The next appointment was then scheduled to be in a

month's time. The patients were advised to bring the pill bottles and clinic cards at the next visit. After a month of medication, clinical assessments were done again and the weight was assessed. The clinic card was examined for any apparent opportunistic infections. The patient was then given a two months' supply of the Inochi New Medicine immune boosters.

The next visit was scheduled to be on the third month of treatment where clinical assessments were done and weights were assessed. The last appointment for the study was scheduled for after 6 months treatment on Inochi New Medicine immune boosters. At the 6 month follow-up session, clinical and laboratory assessments were done on the patients. They were also required to complete a follow-up questionnaire (appendix 7). Those that could not read were assisted by the principal investigator in completing the form.

Blood was also collected from each patient for a 6 month follow-up blood analysis, *ie*. CD4, CD8, CD3 counts; viral load testing; full blood count; and liver function tests (AST and ALT).

3.6 METHODS USED IN LABORATORY TESTING

3.6.1 CD4, CD8 and CD3 counts

3.6.1.1 Principle

Becton Dickinson (BD) MultiTEST™ CD3 fluorescin isothiocyanate (FITC) /CD8 phycoerythrin (PE)/ CD45 peridinin chrorophyll protein (PerCP) /CD4 allophycocyanin (APC) is a four-colour direct immunofluorescence reagent for use with the BD FACS Calibur Flow Cytometer (BD Biosciences; Becton, Dickinson and Company, USA). The reagent was used to identify and determine the percentages and absolute counts of mature human T lymphocytes ($CD3^+$), suppressor/cytotoxic T lymphocyte subsets ($CD3^+$ $CD8^+$), helper T lymphocyte subsets ($CD3^+CD4^+$) in erythrocyte lysed whole blood collected into EDTA tubes (Becton Dickinson, USA).

When whole blood was added to the reagent, the flourochrome-labelled antibodies in the reagent bound specifically to leucocyte surface antigens. During acquisition, cells travelled past the laser beam and scattered the laser light according to their size, internal complexity and relative fluorescence intensity. The stained cells fluoresced, allowing direct fluorescence gating of the lymphocyte population and unlysed or nucleated red blood cells were regarded as contaminants and were not counted. The scatter and fluorescence signals were detected by the BD FACS Calibur Flow Cytometer machine. A known volume of sample was stained directly in a TruCOUNT™ Tube which contained a lyophilized pellet. The pellet dissolved in the tube releasing a known number

of fluorescent beads. During analysis, the absolute number of positive cells in the sample was determined by comparing cellular events to the known bead events. This acted as a quality control measure since the bead count in each tube was known, so the machine reported the number of beads counted. When the bead count was low due to settling of cells, the machine picked it up, mixed the tube and then re-counted.

3.6.1.2 Method

1. EDTA whole blood was used in the analysis.
2. For each patient sample, a TruCOUNT Tube was labelled with the sample laboratory number.
3. 20 µl of MultiTEST CD3/CD8/CD45/CD4 monoclonal antibody reagent was pipetted into the bottom of the TruCOUNT tube without touching the pellet.
4. 50 µl of well mixed EDTA anticoagulated whole blood was added into the bottom of the patient's test tube.
5. 50 µl of well mixed Immuno-Trol ™ control sample was added into the bottom of the control tube.
6. The tubes were then capped and vortexed gently to mix.
7. The tubes in a rack were then incubated in the dark at RT for 15 minutes.
8. After incubation, 450 µl of FACS lysing solution was added into each tube. This ensured that red blood cells were lysed.
9. The tubes were capped and then vortexed gently to mix.
10. The tubes were incubated in the dark at RT for 15 minutes.

11. The samples loaded on a carousel were then ready to be analysed on the BD FACS Calibur flow cytometer machine. A tube of FACS Clean was placed in position 39 of the carousel and a tube of FACS Rinse placed in position 40. The FACS Clean contained diluted bleach to clean the flow cytometer machine and FACS Rinse contained distilled water to rinse the machine at the end of each run.
12. After 2 minutes, results were ready and reports were printed (appendix 15: copy of a result).

3.6.1.3 Quality control

For reliability and validity of measurement, the following quality control measures were employed:

1. A commercial Immuno-Trol quality control sample was included in each run.
2. A precision control was run at random in the morning, at midday and in the afternoon to assure reproducibility. A sample with normal values was chosen to be the precision control for the day.
3. Results of both the quality control and precision control were plotted on a Levy-Jennings chart to track random and systematic errors.
4. Flagged results were re-run for verification, unless it was a known case.
5. The flow cytometer machine was calibrated each morning.

3.6.2 Viral load testing

3.6.2.1 Principle

The viral load testing was performed by using the AMPLICOR HIV-1 MONITOR®, version 1, 5 method based on five major processes: specimen preparation; reverse transcription of target RNA to generate complementary DNA; polymerase chain reaction (PCR) amplification of target cDNA using HIV-1 specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target; and detection of the probe-bound amplified products by colorimetric determination. The AMPLICOR HIV-1 MONITOR Test, version 1.5 permitted simultaneous reverse transcription and PCR amplification of HIV-1 and HIV-1 Quantitation Standard RNA. The Master Mix reagent contained a primer pair specific for both HIV-1 and HIV-1 Quantitation Standard RNA and yielded equivalent quantitation of group M subtypes of HIV-1. The quantitation of HIV-1 viral RNA was performed using the HIV-1 Quantitation Standard. The HIV-1 Quantitation Standard was a non-infectious RNA transcript that contained the identical primer binding sites as the HIV RNA target and a unique probe binding region that allowed quantitation standard amplicon to be distinguished from HIV-1 amplicon. The quantitation standard was incorporated into each individual specimen at a known copy number and was carried through the specimen preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target and was amplified together with the HIV-1 target. HIV-1 RNA levels in the test specimens were determined by comparing the HIV-1 signal to the quantitation standard signal for each specimen.

The quantitation standard compensated for effects of inhibition and controlled the amplification process to allow the accurate quantitation of HIV-1 RNA in each specimen.

3.6.2.2 Method

1. Lysis buffer was removed from the fridge and placed at 37^0 C for 2 hours for crystals to dissolve.
2. A stock solution of 70% ethanol was prepared by mixing 11ml of 95% ethanol with 4ml of distilled water.
3. A standard working lysis reagent was prepared by adding 100µl of HIV-1 QS (Quantitation Standard), version 1.5 to one vial of HIV-1 LYS (lysis reagent) and was well mixed.
4. 600µl of the standard working lysis reagent was added into each tube.
5. 200µl of each patient specimen was then added to the same tubes. The tubes were capped and then mixed by vortexing.
6. The tubes were then incubated at RT for 10 minutes.
7. 800µl of isopropanol was added to the tubes and separation of the solution into two layers was observed. The tubes were mixed by vortexing.
8. The tubes were then centrifuged using the Spectrofuge microcentrifuge (Labnet) for 15 minutes at 14000rpm at RT.
9. The supernatant was carefully removed and discarded and the pellet, which was not so visible, was not disturbed. The tubes were dabbed to remove all the remaining liquid, leaving the pellet untouched.

10. 1ml of 70% ethanol was added into each tube at RT and the tubes were then vortexed.
11. The tubes were then centrifuged using the Spectrofuge microcentrifuge for 10 minutes at 14000 rpm at RT.
12. The supernatant was removed and discarded without disturbing the visible pellet. All ethanol was removed by dabbing the tube since it interferes with amplification.
13. The tubes were pulse centrifuged for 2 minutes at 14000rpm to remove any remaining ethanol. The pellet was dried by opening the tube and letting ethanol evaporate.
14. 400µl of diluent was added into each tube and to break up the insoluble precipitate containing RNA. The tubes were mixed by vortexing.
15. The tubes were centrifuged at 10 000rpm for 5 minutes at RT. RNA was in the supernatant.
16. While the tubes were centrifuging, the working master mix was prepared as follows:
    - 16.1 100µl HIV-1 Manganese solution, v1.5, was added to one vial of HIV-1 MMX (master mix), v1.5. The solution was well mixed by inversion.
    - 16.2 50µl working master mix was added into each polymerase chain reaction (PCR) tube.
17. 50µl of sample containing viral RNA was aspirated from the supernatant of the centrifuged sample, in the vicinity of the pellet, but the pellet was not touched and added into the PCR tube containing the working master mix.

18. The tubes were then taken to the 2720 thermal cycler block (Applied Biosystems) for the polymerase chain reaction which ran for 1 hour 40 minutes and allowed to run as follows:

    2 minutes at 50^0C

    30 minutes at 60^0C

    10 seconds at 95^0C, 10 seconds at 52^0C, 10 seconds at 72^0C (this cycle was repeated 8 times)

    10 seconds at 90^0C, 10 seconds at 55^0C, 10 seconds at 72^0C (this cycle was repeated 23 times)

    15 minutes at 72^0C

19. The tubes were then removed from the thermal cycler and 100µl of Monitor DN (denaturation solution), was added into each reaction tube to stop the amplification process.

20. A working wash solution was prepared by adding 100µl 1 volume of 10X WB (ten times wash concentrate) to 9 volumes of distilled water (1 in 10 dilution).

21. After HIV-1 Monitor Microwell plates were allowed to warm to RT, 100µl of Monitor HYB (hybridization buffer) was added to each well to be tested. Rows A to F were coated with the HIV-1-specific oligonucleotide probe, rows G and H coated with the HIV-1 quantitation standard-specific oligonucleotide probe.

22. 25µl of denatured amplicon was added to the HIV-1 wells in row A of the microwell plate and mixed. 5-fold serial dilutions were then made in the wells in rows B through to F by transferring 25µl from row A to row B, mix

and then continue through until row F and the last 25µl from row F was discarded (Table 3.1).

23. 25µl of denatured amplicon was added into the quantitation standard wells in row G of the microwell plate and mixed and 25µl from row G was added into row H, mixed and the last 25µl from row H discarded.

Table 3.1: Microplate wells showing dilutions in each well of sample or control

Rows	Sample 1 dilution factor	Sample 2 dilution factor
A	1:1	1:1
B	1:5	1:5
C	1:25	1:25
D	1:125	1:125
E	1:625	1:625
F	1:3125	1:3125
G	1:1 control	1:1 control
H	1:5: control	1:5: control

24. The microwell plate was covered with a lid and then incubated for 1 hour at 37^0C.

25. After incubation, the wells were washed with the working wash solution five times, tapping the plate dry each time using paper towel. Air bubbles were avoided since they give a false reading.

26. 100µl of Solution 3, AV-HRP (avidin- horseradish peroxidase conjugate) was added to each well, starting with the quantitation standard wells first and the plate was incubated for 15 minutes at 37^0C.

27. While the plate was incubating, the working substrate solution was prepared by mixing 12ml 4A SUB A (substrate A) and 3ml 4B SUB B (substrate B) and stored at room temperature away from light.

28. After the plate was incubated, it was washed four times using the working wash buffer, patting the plate dry each time using a paper towel.

29. 100µl of working substrate solution was added into each well in minimized light since the solution is light sensitive.

30. The microwell plate was incubated in the dark for 15 minutes at RT to allow for colour development.

31. 100µl of solution 5, the Stop solution was added to each well.

32. Optical density (OD) was then measured within 10 minutes of adding the stop solution, using the BP 800 spectrophotometer (Biohit) at wavelength 450 nm, and the absorbance values of each well recorded.

33. Results were calculated and reported as follows using a spreadsheet:

    33.1 All HIV-1 OD values < 0.20 were reported as <50 copies/ ml HIV RNA.

    33.2 All HIV-1 OD values > 2.0 were reported as invalid and re-tested using the original plasma.

    33.3 All quantitation standard OD values < 0.35 were regarded as invalid and therefore the result of that specimen not reported and the sample re-tested.

33.4 All quantitation standard OD values > 2.0 were regarded as invalid and therefore the result of that specimen not reported and the sample re-tested.

33.5 When the OD value of the quantitation standard was acceptable and the HIV-1 OD value was within the cut-off values, then the result was calculated by multiplying the OD value of the selected quantitaion standard well by the dilution factor associated with that well.

3.6.2.3 Quality control

1. The Amplicor HIV-1 Monitor negative control was included in each test run to pick up contamination in the run. The OD value was expected to be <0.20.
2. Patient specimens and controls were expected to yield quantitation standard OD values that meet the cut-off criteria, therefore demonstrating that the specimen processing, reverse transcription, amplification, amplification and detection steps were performed correctly.
3. When any quantitation standard OD value did not meet the cut-off criteria, the result for that specimen was invalid.

3.6.3 Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) assays

3.6.3.1 Principle

Spectrophotometric methods used in the CX5 Beckman SYNCHRON (Beckman Coulter Inc, USA) are based on the principle that when a patient's serum sample or control is mixed with one or more appropriate chemical reagents, a substance which is a chromophore, is produced that has the ability to absorb light at a specific wavelength. The assay method for AST, the Karmen method, incorporates a coupled enzymatic reaction using malate dehydrogenase as the indicator reaction and monitors the change in absorbance at 340 nm continuously as NADH is oxidized to NAD^+.

The assay method for ALT incorporates a coupled enzymatic reaction using lactate dehydrogenase as the indicator enzyme, which catalyzes the reduction of pyruvate to lactate with the simultaneous oxidation of NADH. The change in absorbance at 340 nm measured continuously is directly proportional to ALT activity. According to Beer's Law, the amount of light absorbed at the completion of the reaction (endpoint) is proportional to the concentration of the AST or ALT being measured.

3.6.3.2 Method

1. The sample identification on the tube and request form was verified.
2. Machine was programmed for each sample.

3. Sample sectors were then placed onto the autoloader assembly.
4. The start button was pressed to initiate the analysis process.
5. Results were printed after sample analysis was completed.

3.6.3.3 Quality control

1. Three Synchron® commercial quality control samples (level 1, level 2 and level 3 controls) were included in each run.
2. A precision control was run at random in the morning, at midday and in the afternoon to assure reproducibility. A sample with normal values was chosen to be the precision control for the day.
3. Results of both the quality control and precision control were plotted on a Levy-Jennings chart to track random and systematic errors.
4. Flagged results were re-run for verification, unless it was known cases.

3.6.4 Full blood count

3.6.4.1 Principle

The Coulter method accurately counts and sizes cells (red cells, white cells and platelets) by detecting and measuring changes in electrical resistance when a cell in a conductive liquid passes through a small aperture. As each cell goes through the aperture, it impedes the current and causes a measurable pulse. The number of pulses signals the number of cells. The height of each pulse is proportional to the volume of that cell. The amplitude

of the electrical pulse produced depends on the cell's volume. During red cell counting, pulses that represent cells of 36 fl or greater are classified as red cells, and pulses from 2 fl to 20 fl are classified as platelets. During white cell counting, pulses that represent cells of 35 fl or greater are classified as white cells. Haemoglobin is determined from the lysed white cell dilution. The absorbance of light from a lamp is measured at 525 nm through the optical path length of the white cell bath. A beam of light from the lamp passes through the sample, through a 525 nm filter, and is measured by a photodiode. The signal is amplified and the voltage is measured and compared to the blank reference reading.

3.6.4.2 Method

1. The sample identification on the tube and request form was verified.
2. The EDTA whole blood sample was mixed.
3. The machine probe was placed into the tube and the aspirate switch was pressed to aspirate the sample into the machine.
4. 12 µl of whole blood was drawn by the aspiration syringe into the white cell bath and a dilution made.
5. 100 µl of the dilution was dispensed into the red cell bath for the red cell and platelet enumeration by the machine.
6. A lytic agent was added into the white cell bath to lyse red cells for white cell enumeration by the machine.

7. The machine used the lysed white cell dilution for Hb measurement. It measured the transmitted beam of light from a lamp through the sample by means of a photodiode.
8. Results were printed after sample analysis was completed.

3.6.4.3 Quality control

1. Coulter 4C® Plus commercial quality control samples (low, normal and high controls) were included in each run.
2. A precision control was run at random in the morning, at midday and in the afternoon to assure reproducibility. A sample with normal values was chosen to be the precision control for the day.
3. Results of both the quality control and precision control were plotted on a Levy-Jennings chart to track random and systematic errors.
4. Flagged results were re-run for verification, unless it was known cases.
5. The Coulter machine counted a sample three times and compared the counts. If there was no agreement among all three counts then a 'vote out' flag was displayed. The sample was then re-run.

3.7 ASSESSMENT OF THE QUALITY OF LIFE

This was done in an interview conducted by the principal investigator in the presence of Mrs Mavata, a registered nurse, while filling a baseline data collection questionnaire (appendix 5). The interview was held in privacy and strict confidentiality was upheld.

Participants were not coerced or badgered. They offered information freely. The following was gathered:

1. the baseline clinical status of the participants before taking the medication,
2. the general well-being of the participants since the diagnosis of HIV, whether there had been opportunistic infections presenting. The clinic cards they brought with them also assisted in indicating any opportunistic infections recently presented.
3. where the participant lived, *ie*. in an urban or rural area, for the purpose of indicating if this had an influence on the response to the medication
4. the number of children the participant had, if female, for the purpose of identifying if this had an influence on the response to the medication
5. whether the participant was working and if not working, whether anyone in the household was, for the purpose of finding out whether they could afford healthy (nutritious) food to help boost the immune system.

On subsequent follow-up visits, improvements presented were recorded on the follow-up data collection questionnaire (appendix 7). This questionnaire gathered information such as weight gain, appetite, strength, subsiding pain in the lower limbs *etc*. since taking the medication.

Chapter 4 details the results that were generated by this study.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

The aim of this study was to investigate the change in the immune status of HIV infected patients that were on the Inochi New Medicine immune boosters, as well as, to assess the safety and efficacy of these immune boosters in improving the patients' quality of life. The rationale of the study was based on a typical/conventional antiretroviral therapy monitoring programme where laboratory safety and efficacy parameters were determined *viz*. aspartate aminotransferase (AST), alanine aminotransferase (ALT), haemoglobin (Hb); CD4 and CD8 parameters as well as viral load, respectively. These laboratory parameters are used for monitoring safety and efficacy of commonly used antiretroviral drugs as described in the Comprehensive HIV and AIDS Care, Management and Treatment Plan (2003).

In a total number of 131 patients enrolled, a CD4/ CD4%; CD8/ CD8%; CD3 count; viral load; Hb; AST and ALT were performed on the first visit sample, called baseline testing (pre-immune booster administration). The laboratory parameters were re-assayed at follow-up visit 6 months later.

Biostatistical evaluations (Cathy Connolly, Medical Research Council) revealed that 90 participants recruited would be sufficient to produce significant findings (probability of 0.05 assuming baseline of CD4 counts to be 500 cells/mm^3).

One hundred and forty participants were recruited for screening; consideration was given to loss to follow-up as well as the possibility of patients entering the antiretroviral rollout programme.

4.2.1 Participants recruited and enrolled to the study

Table 4.1 List of participants recruited to the study (n = 140); showing their age and gender profile

Code	Age (yrs)	M/F	Code	Age (yrs)	M/F	Code	Age (yrs)	M/F
dit001	33	M	dit095	61	F	dit052	28	M
dit002	29	M	dit096	41	F	dit053	28	F
dit003	41	F	dit097	31	F	dit054	29	F
dit004	36	F	dit100	45	M	dit058	35	F
dit005	37	F	dit101	34	F	dit059	36	F
dit006	42	M	dit104	46	M	dit060	34	F
dit007	39	F	dit105	27	M	dit061	26	F
dit008	30	F	dit107	unknown	F	dit062	41	F
dit009	37	F	dit120	28	F	dit064	29	M
dit010	34	F	dit125	37	F	dit065	35	M
dit012	45	F	dit130	46	F	dit066	27	F
dit013	43	F	dit145	30	F	dit067	18	M
dit016	34	F	dit205	26	F	dit070	27	F
dit017	18	M	dit01I	34	F	dit071	27	F
dit018	33	M	dit02I	44	F	dit072	31	F
dit019	37	M	dit04I	28	F	dit074	36	F
dit020	43	F	dit05I	42	M	dit075	36	F
dit021	46	F	dit06I	23	F	dit076	34	F
dit023	42	F	dit08I	39	F	dit077	45	F
dit024	27	F	dit09I	26	M	dit078	29	F
dit025	44	F	dit11I	unknown	M	dit080	42	F
dit027	42	M	dit16I	34	M	dit083	34	F
dit029	59	M	dit19I	34	F	dit085	28	M
dit030	unknown	F	dit20I	39	F	dit088	21	F
dit033	37	F	dit21I	37	F	dit090	31	F
dit034	unknown	M	dit22I	33	M	dit091	42	F
dit035	35	F	dit23I	36	M	dit092	42	F
dit037	30	F	dit26I	43	F	dit093	42	F
dit039	28	F	dit27I	48	M	dit094	32	F
dit040	47	M	dit29I	28	F	dit12M	64	M

dit041	32	F	dit1M	40	M	dit13M	47	F
dit042	47	F	dit02M	48	F	dit14M	50	F
dit043	32	F	dit03M	40	F	dit15M	24	F
dit044	32	F	dit4M	40	F	dit16M	29	F
dit045	42	F	dit08M	36	F	dit17M	32	M
dit050	27	F	dit09M	30	F	dit18M	29	F
dit051	38	F	dit10M	35	F	dit19M	19	F
dit20M	47	M	Hp4	30	F	Hosp3	36	F
dit22M	46	F	Hp9	37	M	Hosp4	33	F
dit23M	unknown	M	Hp10	35	F	Hosp5	33	F
dit01TR	37	F	Hp11	32	M	Hosp6	40	F
dit01PM	28	F	Hp12	19	F	Hosp7	38	M
Hp1	29	F	Hosp1	38	F	dit069	24	F
Hp7	37	M	Hosp2	36	F	dit082	34	F
dit098	37	M	dit13I	32	M	dit10I	34	F
dit099	33	F	dit14I	33	M	dit18I	34	F
Hp5	42	F	dit11M	18	F			

Table 4.1 illustrates the list of 140 participants that were recruited into the study. This population comprised of 73.6% females (n = 103) and 26.4% males (n = 37). However, only 93.6% (n = 131) of the recruited population met the inclusion criteria and were therefore enrolled into the study.

Table 4.2: List of participants enrolled into the study (n = 131), showing their age and gender profile as well as the CD4 counts used as inclusion criteria into the study

Code	Age yrs)	M/F	CD4 count	Code	Age (yrs)	M/F	CD4 count
dit001	33	M	21	dit059	36	F	477
dit002	29	M	311	dit060	34	F	381
dit003	41	F	199	dit061	26	F	20
dit004	36	F	408	dit062	41	F	314
dit005	37	F	211	dit064	29	M	162
dit006	42	M	467	dit065	35	M	140
dit007	39	F	97	dit066	27	F	474
dit008	30	F	211	dit067	18	M	486
dit009	37	F	238	dit070	27	F	13
dit010	34	F	455	dit071	27	F	379
dit012	45	F	133	dit072	31	F	397
dit013	43	F	168	dit074	36	F	118
dit016	34	F	144	dit075	36	F	184
dit017	18	M	4	dit076	34	F	222
dit018	33	M	3	dit077	45	F	345
dit019	37	M	98	dit078	29	F	22
dit020	43	F	111	dit080	42	F	63
dit021	46	F	119	dit083	34	F	352
dit023	42	F	229	dit085	28	M	261
dit024	27	F	3	dit088	21	F	325
dit025	44	F	23	dit090	31	F	446
dit027	42	M	28	dit091	42	F	110
dit029	59	M	370	dit092	42	F	71
dit030	unknown	F	448	dit093	42	F	403
dit033	37	F	112	dit094	32	F	127
dit034	unknown	M	5	dit095	61	F	74
dit035	35	F	106	dit096	41	F	257
dit037	30	F	215	dit097	31	F	73
dit039	28	F	126	dit100	45	M	47
dit040	47	M	148	dit101	34	F	176
dit041	32	F	55	dit104	46	M	427
dit042	47	F	17	dit105	27	M	2
dit043	32	F	61	dit107	unknown	F	360
dit044	32	F	442	dit120	28	F	32
dit045	42	F	16	dit125	37	F	399
dit050	27	F	2	dit130	46	F	462
dit051	38	F	303	dit145	30	F	6
dit052	28	M	71	dit205	26	F	493
dit053	28	F	453	dit01I	34	F	79
dit054	29	F	139	dit02I	44	F	173
dit058	35	F	256	dit04I	28	F	370

dit05I	42	M	371	dit15M	24	F	178
dit06I	23	F	315	dit16M	29	F	178
dit08I	39	F	247	dit17M	32	M	7
dit09I	26	M	377	dit18M	29	F	432
dit11I	unknown	M	67	dit19M	19	F	322
dit16I	34	M	308	dit20M	47	M	188
dit19I	34	F	381	dit22M	46	F	104
dit20I	39	F	243	dit23M	unknown	M	5
dit21I	37	F	50	dit01TR	37	F	219
dit22I	33	M	265	dit01PM	28	F	496
dit23I	36	M	289	Hp1	29	F	221
dit26I	43	F	464	Hp4	30	F	237
dit27I	48	M	114	Hp7	37	M	216
dit29I	28	F	58	Hp9	37	M	237
dit01M	40	M	316	Hp10	35	F	105
dit02M	48	F	104	Hp11	32	M	43
dit03M	40	F	152	Hp12	19	F	13
dit04M	40	F	210	Hosp1	38	F	162
dit08M	36	F	97	Hosp2	36	F	292
dit09M	30	F	151	Hosp3	36	F	480
dit10M	35	F	139	Hosp4	33	F	211
dit11M	18	F	27	Hosp5	33	F	315
dit12M	64	M	61	Hosp6	40	F	144
dit13M	47	F	224	Hosp7	38	M	207
dit14M	50	F	234				

The enrolled participants (n = 131) consisted of 74% females (n = 97) and 26% males (n = 34). The mean CD4 count for this population was 206 cells/mm^3.

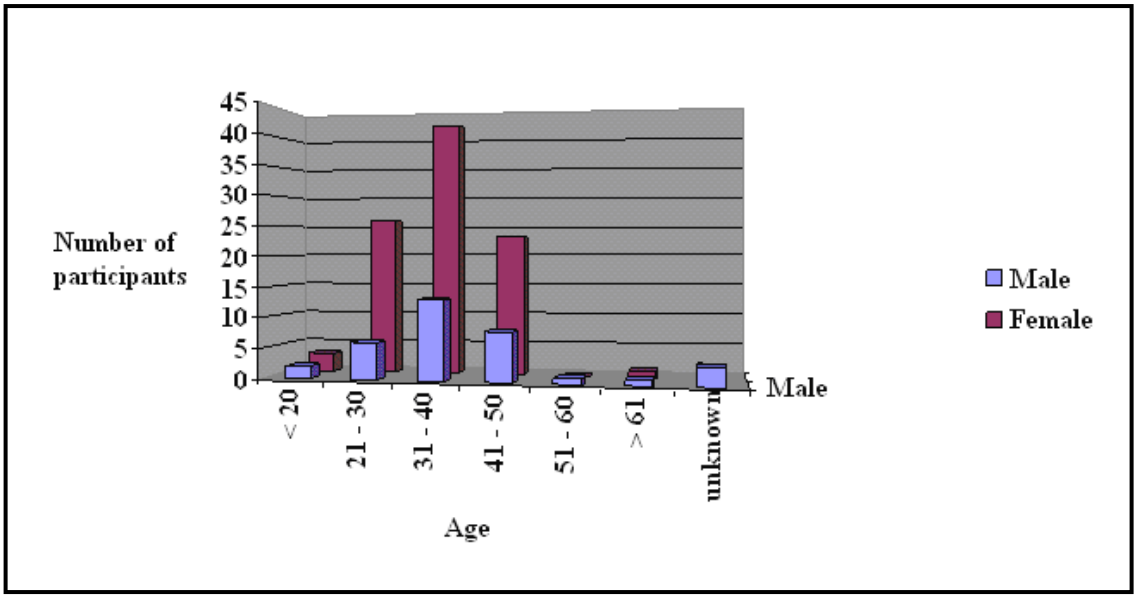

Figure 4.1: Age and gender profile of the participants that were enrolled into the study

Figure 4.1 illustrates that the majority of the participants enrolled were females, in the 21 to 40 years age group. It also shows that the majority of males were in the 31 to 40 years age group. However, 2 females and 3 males did not know their age.

Of the 131 participants enrolled, only 47 participants (36%) were successfully followed-up after 6 months on Inochi New Medicine immune boosters (Table 4.3).

Table 4.3: Comparison of the number of participants successfully followed-up against the number of those that were lost to follow-up; indicating reasons for those that were lost to the study

Number of participants successfully followed-up, n = 47 (36 %)	**Number of participants lost to follow-up, n = 84 (64 %)**	
n = 47	n = 56 (43 %)	reasons not known
	n = 24 (18 %)	participants died
	n = 4 (3 %)	participants changed to antiretroviral drugs
Total number of participants (n) = 131		

Table 4.3 illustrates that 47 participants (36%) were successfully followed-up. These participants returned for the after 6 months' visit. Table 4.3 also illustrates that 64 % (n = 84) of the participants were lost to follow-up. Fifty six participants (43%) were lost to follow-up. However, the reasons were not known, as they did not state why they pulled out of the study. Twenty four participants (18%) died, whereas 4 participants (3%) reported opting for antiretroviral drugs, and therefore, they were excluded from the study.

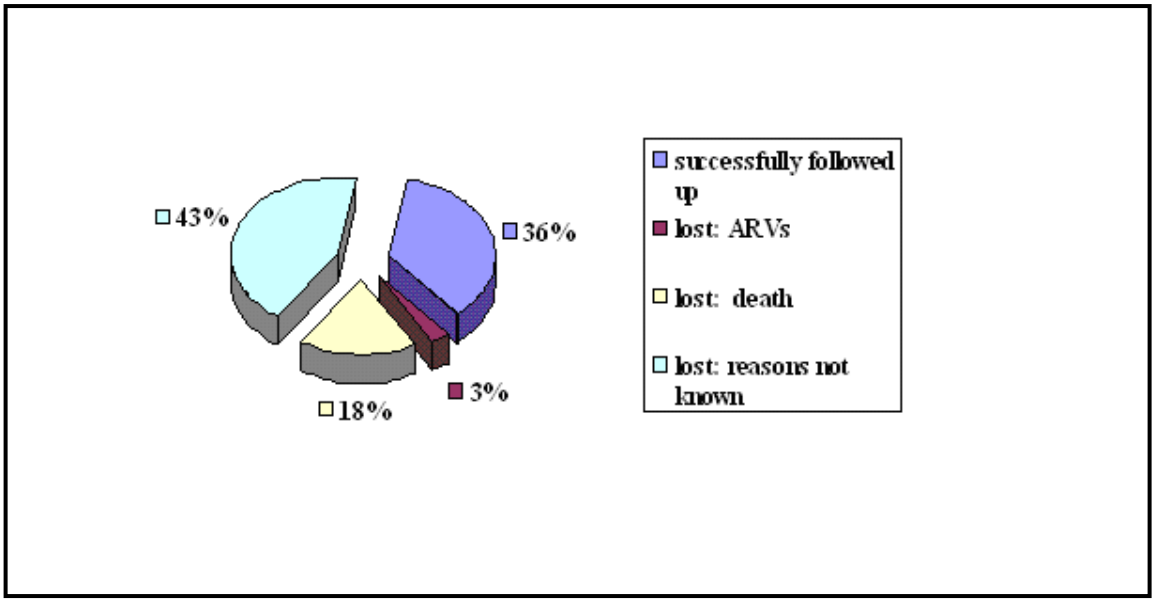

Figure 4.2: Participants successfully followed-up compared to those lost to follow-up, illustrating that the majority of participants enrolled were lost to follow-up

Figure 4.2 illustrates that only 36% of participants (n = 47) were successfully followed-up. A very high percentage, 43% (n = 56), did not return and the reasons were not known. The mortality rate was high as it was 18% (n = 24). Very few participants, 3% (n = 4), reported that they were changing to antiretroviral drugs.

4.2.2 Participants successfully followed-up

Forty-seven participants were successfully followed-up as they returned for the post-treatment 6 months follow-up visit and their blood samples were collected for laboratory analyses. Their laboratory results were used as an indicator to monitor the HIV disease whilst measuring the efficacy of the Inochi New Medicine immune boosters.

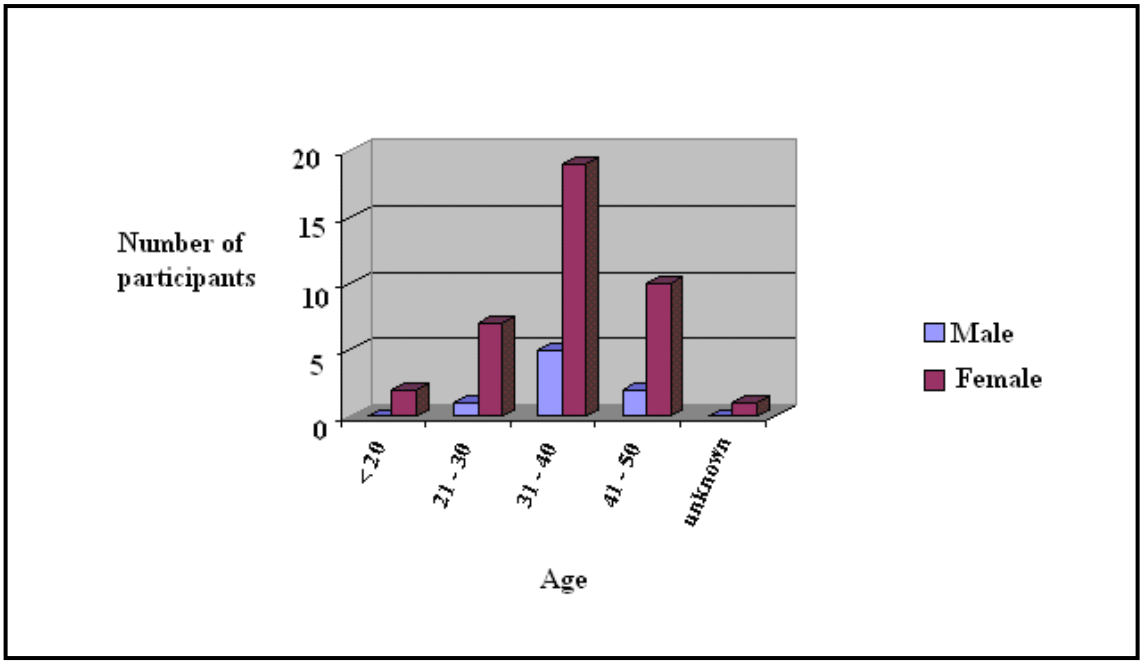

Figure 4.3: Age and gender profile of the participants that were successfully followed-up in the study; showing that the majority of these participants were female in the 31 to 40 years age group

The participants successfully followed-up (n = 47) consisted of 80.9% females (n = 38) and 19.1% males (n = 9). Figure 4.3 shows that participants were mostly females who were in the 31 to 40 years age group. This was a general finding in the population enrolled, as seen in Figure 4.1. It also illustrates that most of the males in this cohort were in the 41 to 50 years age group, whereas Figure 4.1 indicates that males that were enrolled were mostly in the 31 to 40 years age group.

4.3 QUALITATIVE RESULTS

Questionnaires (appendices 5 and 7) assessed the quality of life and the socioeconomic status of the patients before and after taking these immune boosters. The impact of the immune boosters on the general wellbeing of the patient was determined by these

questionnaires. However, limited data was obtained from these questionnaires as they were not filled by all participants.

The baseline questionnaire (appendix 5) was filled by 72 recruited participants (51.4%). It was gathered from this questionnaire that 4 out of 72 participants (5.6%) lived in an urban area whereas the majority of the participants lived in rural areas. Thirteen out of 72 participants (18.1%) indicated that they were employed and 10 out of 72 participants (13.9%) indicated that they had some financial assistance, namely from their children's grants, their own disability grants or there was a member of the family helping them financially. The rest of the participants cited difficulty in securing a stable supply of healthy food since they did not have money.

Eleven out of 72 (15.3%) participants presented with no opportunistic infections clinically, the rest reported that they were suffering from upper respiratory infections such as bronchitis, some had TB, some had shingles, some had oral and/or vaginal thrush *etc*.

Although weight was not measured all the time, it was assessed by observation and the patients were trusted when they said they had gained weight and their clothes could no longer fit. Only 62 participants (86.1%) were weighed at baseline, and had a mean weight of 61.3kg (range 36kg to 122kg).

The follow-up questionnaire (appendix 7) was filled by 11 out of the 47 participants (23.4%), the mean weight gained was 70.6kg (range 44kg to 124kg). The participants

reported feeling well, and that they were no longer tired and could do heavy work such as ploughing fields and walking for long distances without tiring. Most reported that their appetite had improved and diarrhoea had subsided. Only 1 participant dropped her weight from 54kg to 44kg, and was subsequently diagnosed with a TB infection.

The socioeconomic status of the participants was low. Even though some of them lived in urban areas, they were not advantaged in any way as they were unemployed. Their status did not improve even after 6 months, as most were still not employed. Most of those that were working were earning basic wages of less than R1500. Therefore, they were unable to cope.

4.4 LABORATORY QUANTITATIVE RESULTS

4.4.1 Results of laboratory tests used to monitor safety of the immune booster

Haemoglobin (Hb), AST and ALT assays were performed to monitor safety of the immune booster (Table 4.4). Forty-four participants (93.6%) were tested for Hb levels at baseline out of the 47 participants followed-up and 43 participants (91.5%) were tested for follow-up Hb levels. Thirty participants (63.8%) out of the 47 participants were tested for baseline AST levels whereas 37 participants (78.7%) were tested for follow-up AST levels. Thirty one participants (66.0%) out of the 47 participants were tested for baseline ALT levels whereas 37 participants (78.7%) were tested for ALT levels at follow-up.

Table 4.4: Laboratory parameters and their results that were used to monitor the safety of the Inochi New Medicine immune booster

laboratory parameter	Improved	No change	Unsafe
Hb levels (n = 43)	2 participants (4.6%) had anaemia at baseline, Hb normal at follow-up; 5 participants (11.6%) had severe anaemia at baseline, Hb improved at follow-up even though there was still anaemia	10 participants (23.3%) had normal baseline Hb, Hb normal at follow-up; 15 participants (34.9%) had anaemia at baseline, anaemia at follow-up *ie*. no change	4 participants (9.3%) had normal baseline Hb, anaemia at follow-up; 7 participants (16.3%) had mild anaemia at baseline, severe anaemia at follow up
AST levels (n = 30)	4 participants (13.3%) had increased baseline AST levels, normal AST levels at follow-up; 3 participants (10%) had markedly increased baseline AST levels, with improved AST levels at follow-up even though still high	18 participants (60%) had normal baseline AST levels, normal AST levels at follow-up; 1 participant (3.4%) had increased baseline AST levels, increased AST levels at follow up *ie*. No change	4 participants (13.3%) had normal baseline AST levels, increased AST levels at follow-up
ALT levels (n = 31)	2 participants (6.5%) had increased baseline ALT levels, normal ALT levels at follow-up; 2 participants (6.5%) had markedly increased ALT levels, with improved ALT levels at follow-up even though still high	25 participants (80.5%) had normal baseline ALT levels, normal ALT levels at follow-up	2 participants (6.5%) had normal baseline ALT levels, increased ALT levels at follow-up

Table 4.4 illustrates that the Inochi New Medicine immune booster was unsafe to only few of the participants. 25.6% of the participants developed anaemia as indicated by their follow-up Hb levels; 13.3% of the participants had developed increased AST levels at follow-up and 6.5% of the participants had developed increased ALT levels at follow-up. The majority of the participants had either improved their baseline results at follow-up or the results remained the same. Therefore, indicating that the parameters/markers were not affected by the immune booster in most of the participants.

Participant dit05I and participant Hp 7 had markedly high baseline AST and ALT results, even though they remained high at follow-up, however, they showed improvement. Participant dit05I had baseline AST level of 217 IU/L with follow-up AST level of 76 IU/L (reference range: 10 – 45 IU/L) and baseline ALT level of 275 IU/L with follow-up ALT level of 91 IU/L (reference range: 10 – 60 IU/L). Participant Hp 7 had baseline AST level of 113 IU/L with follow-up AST level of 82 IU/L and baseline ALT level of 84 IU/L with follow-up ALT level of 64 IU/L. However, both participants had normal Hb levels for both baseline and follow-up results.

Participant dit29I had increased levels for both baseline AST and ALT and normal levels for both parameters at follow-up. Participant dit11M had increased baseline AST and ALT levels and had improved AST level at follow-up, though it remained high and ALT level was normal at follow-up. Both participant dit29I and participant dit11M had anaemia at baseline that worsened at follow-up.

Participant Hosp2 had normal baseline AST level of 27 IU/L that increased to 105 IU/L at follow-up and normal baseline ALT level of 16 IU/L that increased to 111 IU/L at follow-up. Participant Hp11 had normal baseline AST level of 35 IU/L that increased to 112 IU/L at follow-up as well as a normal baseline ALT level of 14 IU/L which increased to 92 IU/L at follow-up. Both these participants had mild anaemia both at baseline and at follow-up.

4.4.2 Laboratory results used to monitor efficacy of the immune booster

Table 4.5: Laboratory results of the baseline and follow-up parameters of participants that were successfully followed-up

Code	Bslin CD4 count	f-up CD4 count	Bslin CD4%	f-up CD4%	Bslin CD8 count	f-up CD8 count	Bslin CD8%	f-up CD8%	Bslin CD3 count	f-up CD3 Count	Bslin viral load	Bslin HIV log	f-up viral load	f-up HIV log
dit005	211	157	12	10	1015	918	55	58	1232	1108	86354	4.9	45213	4.6
dit008	211	171	15	15	1000	765	69	66	1221	955	4949	3.7	3250	3.5
dit009	238	205	12	11	1202	1219	63	67	1570	1552	1611	3.2	898	3.0
dit013	168	196	8	8	1147	1724	58	67	1375	2004	106215	5	300000	5.5
dit020	111	163	15	9	592	1046	79	60	753	1295	2563	3.4	1726	3.2
dit033	112	114	12	15	523	448	58	58	680	593	12200	4.1	4210	3.6
dit035	106	118	7	8	1128	1252	77	80	1288	1434	45121	4.7	29600	4.5
dit051	303	475	22	17	869	1857	63	65	1215	2469	490000	5.7	210000	5.3
dit085	261	226	14	11	800	916	44	46	1076	1213	80000	4.9	300000	5.5
dit090	446	700	28	29	803	1235	51	51	1283	2000	88200	4.9	4900	3.7
dit092	71	117	14	18	215	181	26	29	355	335	1100000	6.0	155000	5.2
dit093	403	293	26	24	813	671	53	55	1257	1010	2010	3.3	1083	3.0
dit094	127	96	13	12	467	460	48	59	632	584	89000	4.9	n/a	n/a
dit104	427	391	9	9	2781	2482	58	59	3277	2925	31000	4.5	74343	4.9
dit107	360	223	14	16	1654	938	66	68	2065	1192	76800	4.9	950000	6.0
dit125	399	296	27	32	654	394	44	42	1089	712	31410	4.5	14000	4.2
dit145	6	7	1	2	218	118	35	37	270	147	140000	5.2	1000000	6.0
dit205	493	490	27	30	920	733	50	45	1437	1249	9600	4.0	12000	4.1
dit02I	173	191	25	19	338	605	48	59	506	776	1495000	5.2	n/a	n/a
dit05I	371	484	15	17	1279	1667	52	57	1699	2225	29000	4.5	19000	4.3
dit16I	308	233	7	8	2932	2108	67	70	3403	2445	127699	5.1	330000	5.5

dit19I	381	411	21	24	1084	971	59	57	1515	1409	15182	4.2	126343	5.1
dit21I	50	177	5	6	700	2489	72	80	772	2713	2100	3.3	210000	5.3
dit26I	464	351	21	27	1274	761	59	58	1795	1140	220000	5.3	74000	4.6
dit29I	58	112	9	15	336	373	54	49	394	533	29037	4.5	210000	5.3
dit01M	316	305	36	17	362	511	41	34	685	932	144500	5.2	13000	4.1
dit02M	104	220	5	8	1448	1954	69	67	1576	2202	n/a	n/a	n/a	n/a
dit04M	210	151	7	7	1725	1277	55	61	2014	1482	3300000	6.5	37000	4.6
dit11M	27	57	4	4	503	1192	7	77	551	1297	67000	4.8	2200000	6.3
dit13M	224	191	10	9	1445	1312	63	62	1779	1597	40393	4.6	85312	4.9
dt14M	234	137	13	8	1287	1363	69	78	1620	1597	16389	4.2	430000	5.6
dit16M	178	218	7	7	1726	2555	66	78	1985	2852	220108	5.3	3500000	6.5
dit17M	7	5	1	1	721	506	70	62	817	600	n/a	n/a	33000	4.5
dit22M	104	76	6	6	1012	704	61	57	1144	808	88000	4.9	160000	5.2
Hp1	221	370	8	6	1830	4149	64	69	2415	4675	1200000	6.1	8400000	6.9
Hp7	216	238	22	15	492	791	50	49	761	1138	36000	4.6	32000	4.5
Hp4	237	288	13	15	1189	1123	68	67	1465	1370	170000	5.2	72000	4.9
Hp9	237	210	9	11	1697	1330	68	73	1988	1589	510000	5.7	730000	5.9
Hp10	105	96	10	9	734	667	67	64	898	848	4400	3.6	15000	4.2
Hosp1	162	221	18	16	476	826	54	61	664	1076	19000	4.3	80000	4.9
Hosp2	292	468	19	26	998	857	66	47	1304	1439	15000	4.2	51000	4.7
Hosp3	480	454	19	24	1661	1208	66	64	2215	1715	15000	4.2	22000	4.3
Hosp4	211	152	16	12	839	n/a	65	n/a	1072	n/a	<50	0	3300	3.5
Hp12	13	103	1	5	628	1476	66	65	670	1639	82000	4.9	<50	0
Hosp5	315	524	24	19	575	1407	44	51	921	1987	150000	5.2	38000	4.6
Hosp6	144	292	12	13	808	1639	67	70	974	1960	210000	5.3	220000	5.3
Hosp7	**207**	**179**	**14**	**13**	**804**	**841**	**54**	**59**	**1065**	**1088**	**62000**	**4.8**	**570000**	5.8

Key: bslin: baseline; f-up: follow-up; n/a: results not available

Table 4.5 lists baseline and follow-up laboratory results of the parameters commonly used for monitoring the efficacy of antiretroviral drugs (Comprehensive HIV and AIDS Care, Management and Treatment Plan, 2003). Some results showed a significant change in certain parameters.

Bartlett (2005) suggested that an expected mean increase of CD4 counts is approximately150 cells/mm^3 in the first year on antiretroviral therapy. He indicated that treatment failure will be when there is a failure to increase the CD4 count by 25 to 50 cells during the first year of therapy. The same benchmark was used in this study, *ie*. a significant change was recorded as when the increase in CD4 count was more than 50 cells/mm^3 after 6 months on the Inochi New Medicine medication. Treatment failure was defined as when there was a drop from baseline CD4 counts by 50 cells/mm^3 as well as when there was no increase at all or when the increase was below 25 cells/mm^3 after 6 months on the Inochi New Medicine immune booster.

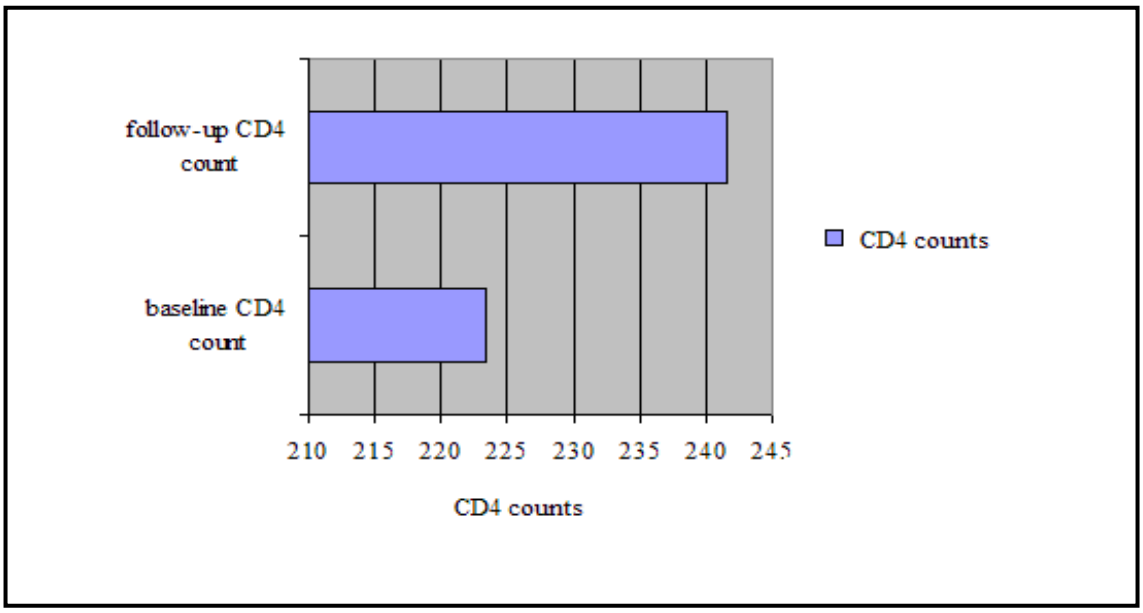

Figure 4.4: Rate of change between the mean baseline CD4 count and the mean follow-up CD4 count; showing an increase in the follow-up CD4 counts

Figure 4.4 shows an increase in the mean CD4 count at follow-up. The mean CD4 count (n = 47) at baseline for the followed-up cohort was 223.4 cells/mm^3 and the mean CD4 count (n = 47) at follow-up was 241.5 cells/ mm^3 (p = 0.166). Data was analysed using the Statistical Package for Social Sciences (SPSS) (version 14) statistical package, where the p value of <0.05 indicated statistical significance in the difference/change tested with 95% confidence interval (appendix 16).

Table 4.6: Absolute and percentage difference between baseline CD4 counts and follow-up CD4 counts that showed a significant change, *ie*. either increased or dropped by more than 50 cells/mm^3

Code	Baseline CD4 count	Follow-up CD4 count	Absolute difference	Percentage difference (%)	CD4 counts increased/ dropped
dit005	211	157	-54	-25.6	Dropped
dit020	111	163	+52	46.8	Increased
dit051	303	475	+172	56.8	Increased
dit090	446	700	+254	57.0	Increased
dit093	403	293	-110	- 27.3	Dropped
dit107	360	223	-137	-38.1	Dropped
dit125	399	296	-103	-25.8	Dropped
dit05I	371	484	+113	30.5	Increased
dit16I	308	233	-75	-24.4	Dropped
dit21I	50	177	+127	254.0	Increased
dit26I	464	351	-113	-24.4	Dropped
dit29I	58	112	+54	93.1	Increased
dit02M	104	220	+116	111.5	Increased
dit04M	210	151	-59	-28.1	Dropped
dit14M	234	137	-97	-41.5	Dropped
Hp1	221	370	+149	67.4	Increased
Hp4	237	288	+51	21.5	Increased
Hosp1	162	221	+59	36.4	Increased
Hosp2	292	468	+176	60.3	Increased
Hosp4	211	152	-59	-28.0	Dropped
Hp12	13	108	+95	730.8	Increased
Hosp5	315	524	+209	66.3	Increased
Hosp6	144	292	+148	102.8	Increased

Twenty three out of 47 participants (48.9%) showed a significant change in CD4 counts by either an increase or decrease in their follow-up results compared to the baseline CD4 counts ($p = 0.105$). Results were classified as being significant when they showed either an increase or a decrease, by more than 50 cells/mm^3.

Fourteen out of the 23 participants (60.9%) had significant increases in the CD4 counts ($p = 0.000$); whereas 9 out of 23 participants (39.1%) showed significant decreases in their CD4 counts after 6 months on the Inochi New Medicine ($p = 0.000$). The cohort that increased their CD4 counts ($n = 14$) had a mean CD4 count at baseline of 201.9 cells/mm^3 and the mean CD4 count at follow-up was 328.7 cells/mm^3, whereas the cohort that decreased their CD4 counts ($n = 9$) had a mean CD4 count of 311.1 cells/mm^3 at baseline and the mean CD4 count at follow-up was 221.4 cells/mm^3.

Twenty four out of the 47 participants (51.1%) showed no significant changes to their CD4 counts. These CD4 counts were categorized as showing immunological non response since the baseline results failed to increase by at least 50 cells/mm^3 (Lawn *et al.*, 2006). Twenty-eight out of the 47 participants (59.6%) showed treatment failure since their CD4 counts did not increase or if increased, it was by less than 25 cells/mm^3 after 6 months on treatment.

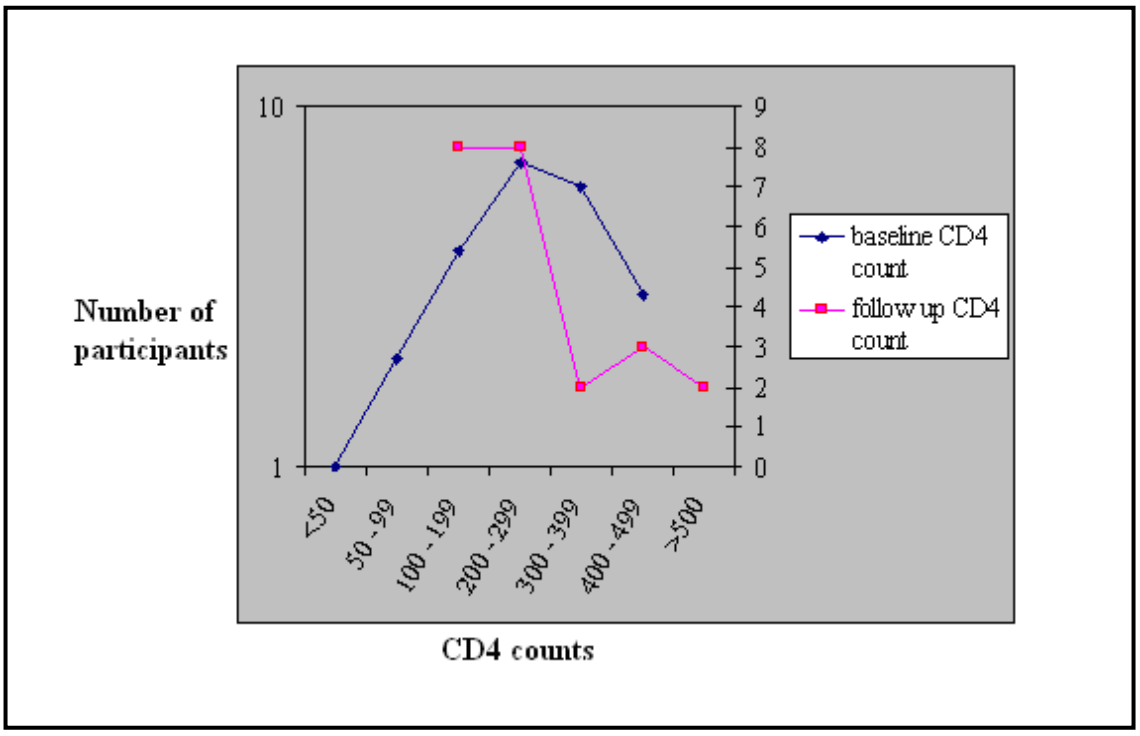

Figure 4.5: Diagram showing the rate of change of baseline CD4 counts compared to follow-up CD4 counts, indicating a shift to the right of the graph representing follow up results

Figure 4.5 illustrates a shift to the right in the follow-up graph. It was observed that in baseline CD4 counts there were no results that were greater than 500 cells/mm^3, whereas in the follow-up CD4 counts there were results greater than 500 cells/mm^3. It was also observed that in the baseline CD4 counts there were results as low as less than 50 cells/mm^3, whereas in the follow-up CD4 counts, they were greater than 100 cells/mm^3.

Table 4.7: Absolute and percentage difference between baseline CD4% results and follow-up CD4% results; showing whether they were increased or decreased

Code	Baseline CD4%	Follow-up CD4%	Absolute Difference	Percentage difference (%)	CD4% increased/ dropped
dit005	12	10	-2	16.7	Dropped
dit008	15	15	0	0	Not changed
dit009	12	11	-1	8.3	Dropped
dit013	8	8	0	0	Not changed
dit020	15	9	-6	40	Dropped
dit033	12	15	+3	25	Increased
dit035	7	8	+1	14.3	Increased
dit051	22	17	-5	22.7	Dropped
dit085	14	11	-3	21.4	Dropped
dit090	28	29	+1	3.6	Increased
dit092	14	18	+4	28.6	Increased
dit093	26	24	-2	7.7	Dropped
dit094	13	12	-1	7.7	Dropped
dit104	9	9	0	0	Not changed
dit107	14	16	+2	14.3	Increased
dit125	27	32	+5	18.5	Increased
dit145	1	2	+1	50	Increased
dit205	27	30	+3	11.1	Increased
dit02I	25	19	-6	24	Dropped
dit05I	15	17	+2	13.3	Increased
dit16I	7	8	+1	14.3	Increased
dit19I	21	24	+3	14.3	Increased
dit21I	5	6	+1	20	Increased
dit26I	21	27	+6	28.6	Increased
dit29I	9	15	+6	66.7	Increased
dit01M	36	17	-19	52.8	Dropped
dit02M	5	8	+3	60	Increased
dit04M	7	7	0	0	Not changed
dit11M	4	4	0	0	Not changed
dit13M	10	9	-1	10	Dropped
dt14M	13	8	-5	38.5	Dropped
dit16M	7	7	0	0	Not changed
dit17M	1	1	0	0	Not changed
dit22M	6	6	0	0	Not changed
Hp1	8	6	-2	25	Dropped
Hp7	22	15	-7	31.8	Dropped

Hp4	13	15	+2	15.4	Increased
Hp9	9	11	+2	22.2	Increased
Hp10	10	9	-1	10	Dropped
Hosp1	18	16	-2	11.1	Dropped
Hosp2	19	26	+7	36.8	Increased
Hosp3	19	24	+5	26.3	Increased
Hosp4	16	12	-4	25	Dropped
Hp12	1	5	+4	400	Increased
Hosp5	24	19	-5	20.8	Dropped
Hosp6	12	13	+1	8.3	Increased
Hosp7	14	13	-1	7.1	Dropped

Table 4.7 illustrates that 21 out of the 47 (44.7%) participants had significantly increased their follow-up CD4% results compared to baseline CD4% results. The cohort that increased their CD4% results (n = 21) had a mean CD4% of 13.6% at baseline and a mean CD4% of 16.6% at follow-up (p = 0.000). Eighteen out of 47 (38.3%) participants had significantly decreased their baseline CD4% results. The cohort that decreased their CD4% had a mean CD4% of 17.2% at baseline and a mean CD4% of 13.1% at follow-up (p = 0.001). However, 8 out of 47 (17.0%) participants had their baseline CD4% results not changed.

The CD4% is the percentage of CD4 lymphocytes in the total number of lymphocytes. Guarner *et al*. (1997) and Hare (2004) suggested that a CD4 percentage must be used to avoid suspecting treatment failure when there is a drop in the absolute number of CD4 T lymphocytes during therapy as a result of normal variations and not due to the progression of HIV disease. Absolute CD4 counts vary on repeated measurements.

Therefore, a CD4% is suggested as there are smaller variations on repeated measurements compared to absolute CD4 counts (Dayama *et al*., 2003; Guarner *et al*., 1997; Kunkl 1997). This suggests that CD4 percentages are more stable than absolute CD4 counts since they are not affected by diurnal variations.

It was observed that 14 out of 47 (29.8%) participants showed increased follow-up CD4 counts compared to 21 out of 47 (44.7%) participants that showed increased follow-up CD4% results. Therefore, CD4% results showed more participants had increased their follow-up results than those that showed increased absolute CD4 counts at follow-up. It must be noted that treatment decisions in South Africa are based on CD4 counts.

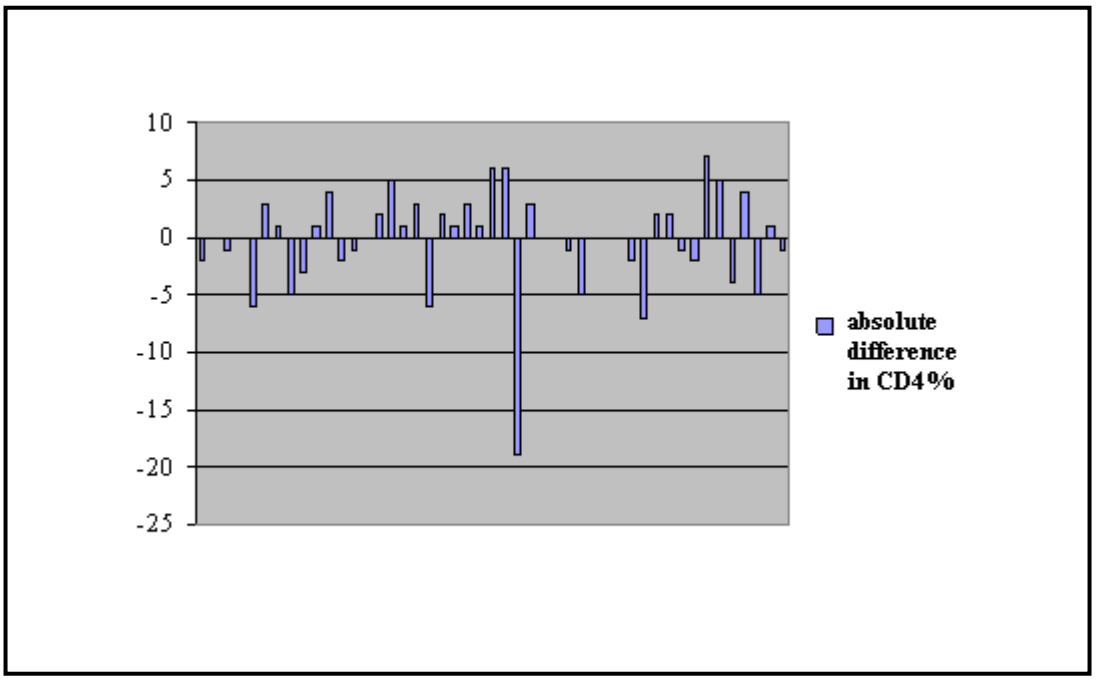

Figure 4.6: Diagram showing absolute differences between baseline CD4% results and follow-up CD4% results, indicating that most were increased

It is evident from Figure 4.6 and Table 4.7 that the majority of participants increased their follow-up CD4% results. The average rate of increase is noted to be 3%. The USA Department of Health and Human Services (2005) cited that a significant change in CD4% is a 3 percentage point change. Most of those that decreased their baseline CD4% results, did by an absolute difference of not more than -6, except participant dit01M who dropped his results from baseline CD4% of 36% to follow-up CD4% of 17% (absolute difference of -19). It is noted that his baseline absolute CD4 count had dropped from 316 cells/mm^3 to follow-up absolute CD4 count of 305 cells/mm^3.

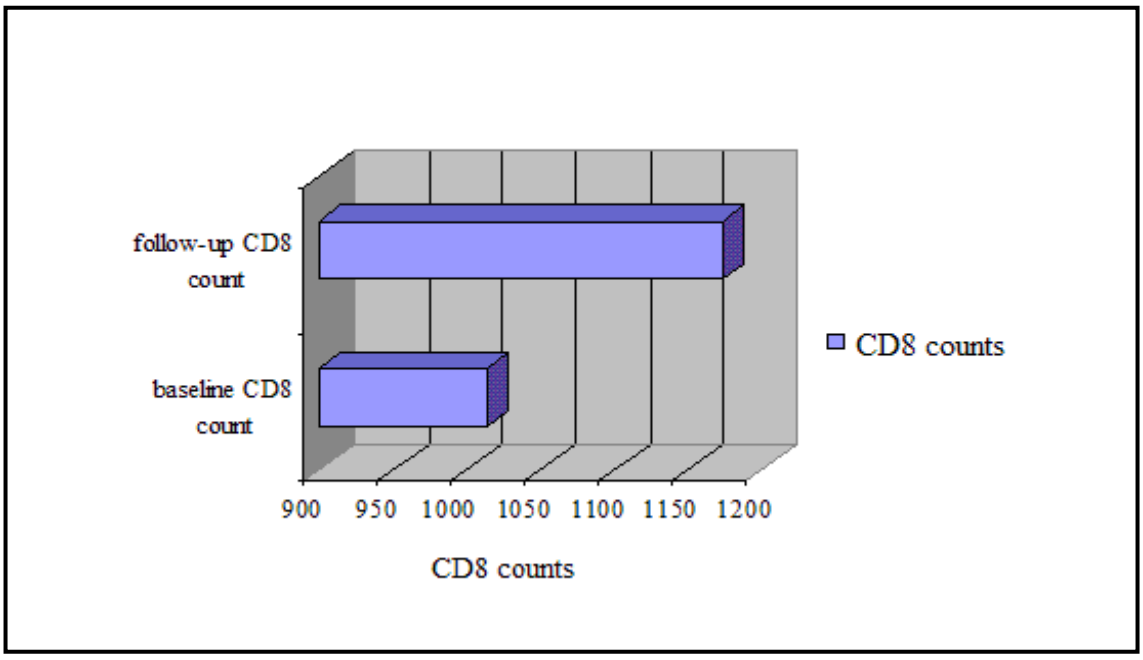

Figure 4.7: Rate of change of the mean baseline CD8 count and the mean follow-up CD8 count, showing an increase in the follow-up CD8 counts

Figure 4.7 illustrates an increase in the mean follow-up CD8 count compared to the baseline CD8 counts. The mean baseline CD8 count was 1015 cells/mm^3 and the mean follow-up CD8 count was 1174 cells/mm^3.

Table 4.8: Absolute and percentage difference between baseline CD8 counts and follow-up CD8 counts; showing whether they were increased or decreased

Code	Baseline CD8 count	Follow-up CD8 count	Absolute difference	Percentage difference (%)	CD8 count increased/ dropped
dit005	1015	918	-97	9.6	Dropped
dit008	1000	765	-235	23.5	Dropped
dit009	1202	1219	+17	1.4	Increased
dit013	1147	1724	+577	50.3	Increased
dit020	592	1046	+454	76.7	Increased
dit033	523	448	-75	14.3	Dropped
dit035	1128	1252	+124	11.0	Increased
dit051	869	1857	+988	113.7	Increased
dit085	800	916	+116	14.5	Increased
dit090	803	1235	+432	53.8	Increased
dit092	215	181	-34	15.8	Dropped
dit093	813	671	-142	17.5	Dropped
dit094	467	460	-7	1.5	Dropped
dit104	2781	2482	-299	10.8	Dropped
dit107	1654	938	-716	43.3	Dropped
dit125	654	394	-260	39.8	Dropped
dit145	218	118	-100	45.9	Dropped
dit205	920	733	-187	20.3	Dropped
dit02I	338	605	+267	80	Increased
dit05I	1279	1667	+388	30.3	Increased
dit16I	2932	2108	-824	28.1	Dropped
dit19I	1084	971	-113	10.4	Dropped
dit21I	700	2489	+1789	255.6	Increased
dit26I	1274	761	-513	40.3	Dropped
dit29I	336	373	+37	11.0	Increased
dit01M	362	511	+149	41.2	Increased
dit02M	1448	1954	+506	34.9	Increased
dit04M	1725	1277	-448	26.0	Dropped
dit11M	503	1192	+689	137	Increased
dit13M	1445	1312	-133	9.2	Dropped
dt14M	1287	1363	+76	5.9	Increased
dit16M	1726	2555	+829	48.0	Increased
dit17M	721	506	-215	29.8	Dropped
dit22M	1012	704	-308	30.4	Dropped
Hp1	1830	4149	+2319	126.7	Increased
Hp7	492	791	+1219	60.8	Increased

Hp4	1189	1123	-66	5.6	Dropped
Hp9	1697	1330	-367	21.6	Dropped
Hp10	734	667	-67	9.1	Dropped
Hosp1	476	826	+350	73.5	Increased
Hosp2	998	857	-141	14.1	Dropped
Hosp3	1661	1208	-453	27.3	Dropped
Hosp4	839	n/a	n/a	n/a	n/a
Hp12	628	1476	+848	135.0	Increased
Hosp5	575	1407	+832	144.7	Increased
Hosp6	808	1639	+831	102.8	Increased
Hosp7	804	841	+37	4.6	Increased

Table 4.8 illustrated that 23 out of the 46 (50%) participants had significantly increased their follow-up CD8 counts compared to baseline CD8 counts. The cohort that increased their CD8 counts had a mean CD8 count of 875.3 cells/mm^3 at baseline and a mean CD8 count of 1438.5 cells/mm^3 at follow-up ($p = 0.000$). Twenty three out of 46 (50%) participants had significantly decreased their baseline CD8 count results. The cohort that decreased their CD8 counts had a mean CD8 count of 1162.2 cells/mm^3 at baseline and a mean CD8 count of 910.0 cells/mm^3 at follow-up ($p = 0.000$).

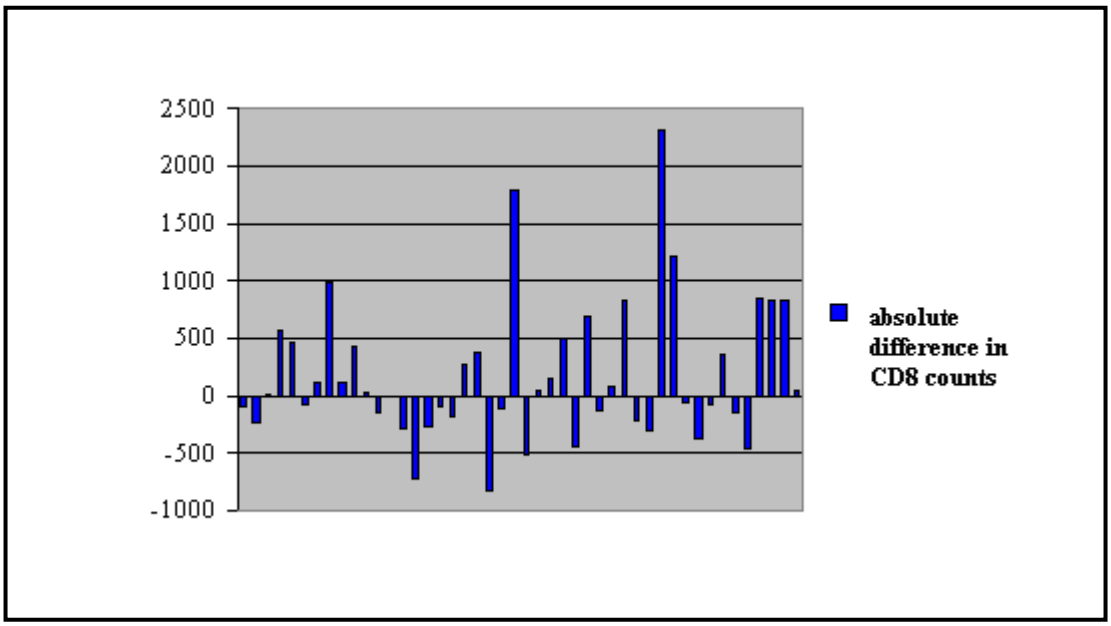

Figure 4.8: Absolute differences between baseline CD8 count results and follow-up CD8 count results; indicating that most were increased

Figure 4.8 and Table 4.8 illustrate that the majority of participants increased their follow-up CD8 counts compared to baseline CD8 counts. The average rate of increase was noted to be 67.9%. Those that decreased their baseline CD8 counts, did so by an absolute difference of not more than -513, except for participants dit107 and dit16I who dropped their results from baseline CD8 counts of 1654 and 2932 to follow-up CD8 counts of 938 and 2108 respectively (absolute difference of -716 and -824 respectively).

Table 4.9: Absolute and percentage difference between baseline CD8% results and follow-up CD8% results; showing whether they were increased or decreased

Code	Baseline CD8%	Follow-up CD8%	Absolute difference	Percentage difference (%)	CD8% increased/ dropped
dit005	55	58	+3	5.5	Increased
dit008	69	66	-3	4.3	Dropped
dit009	63	67	+4	6.3	Increased
dit013	58	67	+9	15.5	Increased
dit020	79	60	-19	24.1	Dropped
dit033	58	58	0	0	not changed
dit035	77	80	+3	3.9	Increased
dit051	63	65	+2	3.2	Increased
dit085	44	46	+2	4.5	Increased
dit090	51	51	0	0	not changed
dit092	26	29	+3	11.5	Increased
dit093	53	55	+2	3.8	Increased
dit094	48	59	+11	22.9	Increased
dit104	58	59	+1	1.7	Increased
dit107	66	68	+2	3.0	Increased
dit125	44	42	-2	4.5	Dropped
dit145	35	37	+2	5.7	Increased
dit205	50	45	-5	10	Dropped
dit02I	48	59	+11	22.9	Increased
dit05I	52	57	+5	9.6	Increased
dit16I	67	70	+3	4.5	Increased
dit19I	59	57	-2	3.4	Dropped
dit21I	72	80	+8	11.1	Increased
dit26I	59	58	-1	16.9	Dropped
dit29I	54	49	-5	9.3	Dropped
dit01M	41	34	-7	17.1	Dropped
dit02M	69	67	-2	2.9	Dropped
dit04M	55	61	+6	10.9	Increased
dit11M	7	77	+70	1000	Increased
dit13M	63	62	-1	1.6	Dropped
dt14M	69	78	+9	13.0	Increased
dit16M	66	78	+12	18.2	Increased
dit17M	70	62	-8	11.4	Dropped
dit22M	61	57	-4	6.6	Dropped
Hp1	64	69	+5	7.8	Increased
Hp7	50	49	-1	2	Dropped

Hp4	68	67	-1	1.5	Dropped
Hp9	68	73	+5	7.4	Increased
Hp10	67	64	-3	4.5	Dropped
Hosp1	54	61	+7	13.0	Increased
Hosp2	66	47	-19	28.8	Dropped
Hosp3	66	64	-2	3.0	Dropped
Hosp4	65	n/a	n/a	n/a	n/a
Hp12	66	65	-1	1.5	Dropped
Hosp5	44	51	+7	15.9	Increased
Hosp6	67	70	+3	4.5	Increased
Hosp7	54	59	+5	9.3	Increased

Table 4.9 illustrates that 26 out of 46 (55.3%) participants had significantly increased their baseline CD8% results ($p = 0.006$). Their mean CD8% was 55.1% at baseline and the mean CD8% was 62.8% at follow-up. Eighteen out of 46 (39.1%) participants significantly decreased their CD8% ($p = 0.002$). Their mean CD8% was 61.1% at baseline and the mean CD8% was 56.3% at follow-up. Two out of 46 (4.3%) participants did not change their baseline CD8% results.

It was observed that 55.3% participants increased their CD8% results compared to just 52.2% participants who increased their CD8 counts. Therefore, there were more participants that increased their CD8% results at follow-up than those that showed increased absolute CD8 counts. It was noted that this observation was consistent with that made for CD4 counts and CD4% results.

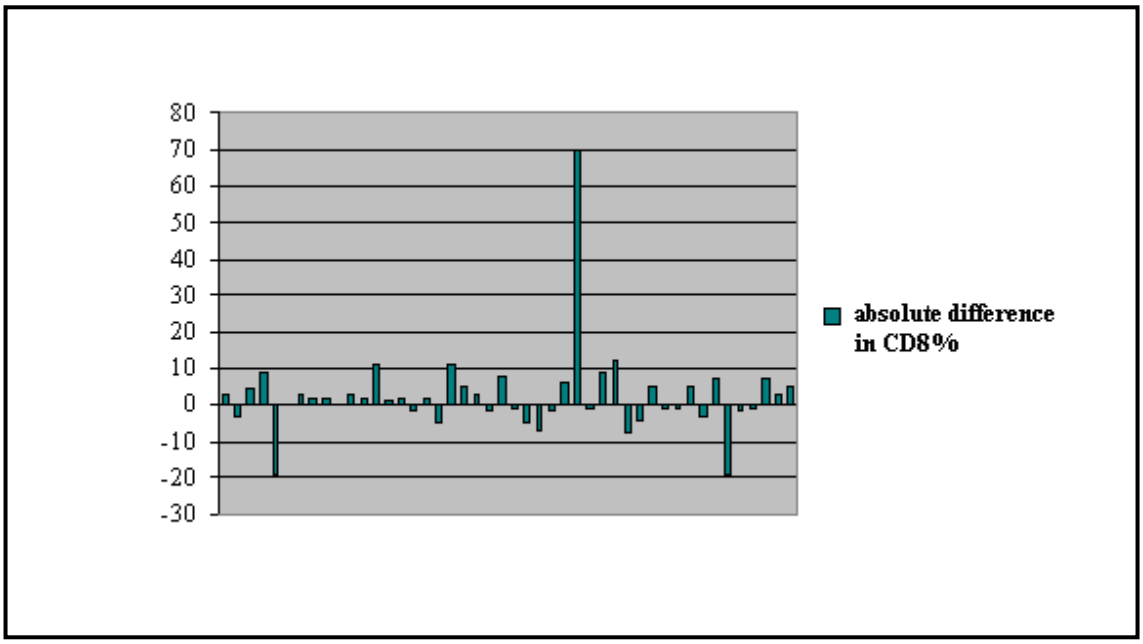

Figure 4.9: Absolute differences between baseline CD8% results and follow-up CD8% results; indicating that most were increased

Figure 4.9 and Table 4.9 shows that the majority of the participants increased their follow-up CD8% results compared to their baseline CD8% results. The average rate of increase of CD8% results was noted to be 5.2%. Most of those that decreased their baseline CD8% results, did so by an absolute difference by not more than -8, except participant dit020 and

participant Hosp2 who dropped their results from baseline CD8% of 79% to follow-up CD8% of 60% and from baseline CD8% of 66% to follow-up CD8% of 47%, respectively (absolute difference of -19). It is, however, noted that the baseline absolute CD8 counts for participant dit020 had increased from 592 cells/mm^3 to 1046 cells/mm^3 at follow-up, whereas, for participant Hosp2 it had dropped from baseline CD8 count of 998 cells/mm^3 to follow-up absolute CD8 count of 857 cells/mm^3. However, participant dit11M had increased her CD8% results by 1000%, from baseline CD8% of 7% to follow-up CD8% of 77% (an absolute increase of 70%).

Table 4.10: Absolute and percentage difference between baseline CD3 counts and follow-up CD3 counts; showing whether they were increased or decreased

Code	Baseline CD3 count	Follow-up CD3 count	Absolute Difference	Percentage difference (%)	CD3 count increased/ dropped
dit005	1232	1108	-124	10.1	Dropped
dit008	1221	955	-266	21.8	Dropped
dit009	1570	1552	-18	1.2	Dropped
dit013	1375	2004	+629	45.7	Increased
dit020	753	1295	+542	71.8	Increased
dit033	680	593	-87	12.8	Dropped
dit035	1288	1434	+146	11.3	Increased
dit051	1215	2469	+1254	103.2	Increased
dit085	1076	1213	+137	12.7	Increased
dit090	1283	2000	+717	55.9	Increased
dit092	355	335	-20	5.6	Dropped
dit093	1257	1010	-247	19.6	Dropped
dit094	632	584	-48	7.6	Dropped
dit104	3277	2925	-352	10.7	Dropped
dit107	2065	1192	-873	42.3	Dropped
dit125	1089	712	-377	34.6	Dropped
dit145	270	147	-123	45.6	Dropped
dit205	1437	1249	-188	13.1	Dropped
dit02I	506	776	+270	53.4	Increased
dit05I	1699	2225	+526	31.0	Increased
dit16I	3403	2445	-958	28.2	Dropped
dit19I	1515	1409	-106	7.0	Dropped
dit21I	772	2713	+1941	251.4	Increased
dit26I	1795	1140	-655	36.5	Dropped
dit29I	394	533	+139	35.3	Increased
dit01M	685	932	+247	36.1	Increased
dit02M	1576	2202	+626	39.7	Increased
dit04M	2014	1482	-532	26.4	Dropped
dit11M	551	1297	+746	135.4	Increased
dit13M	1779	1597	-182	10.2	Dropped
dt14M	1620	1597	-23	1.4	Dropped
dit16M	1985	2852	+867	43.7	Increased
dit17M	817	600	-217	26.6	Dropped
dit22M	1144	808	-336	29.4	Dropped
Hp1	2145	4675	+2530	117.9	Increased
Hp7	761	1138	+377	49.5	Increased
Hp4	1465	1370	-95	6.5	Dropped

Hp9	1988	1589	-399	20.1	Dropped
Hp10	898	848	-50	5.6	Dropped
Hosp1	664	1076	+412	62.0	Increased
Hosp2	1304	1439	+135	10.4	Increased
Hosp3	2215	1715	-500	22.6	Dropped
Hosp4	1072	n/a	n/a	n/a	n/a
Hp12	670	1639	+969	144.6	Increased
Hosp5	921	1987	+1066	115.7	Increased
Hosp6	974	1960	+986	101.2	Increased
Hosp7	1065	1088	+23	2.2	Increased

Table 4.10 illustrates that 22 out of 46 (47.8%) participants significantly increased their baseline CD3 counts ($p = 0.000$). Their mean CD3 count was 1075.5 cells/mm^3 and the mean CD3 count was 1770.3 cells/mm^3 at follow-up. 24 out of 46 (52.2%) participants significantly decreased their baseline CD3 counts ($p = 0.000$). Their mean CD3 count was 1489.0 cells/mm^3 at baseline, and the mean CD3 count was 1206 cells/mm^3 at follow-up.

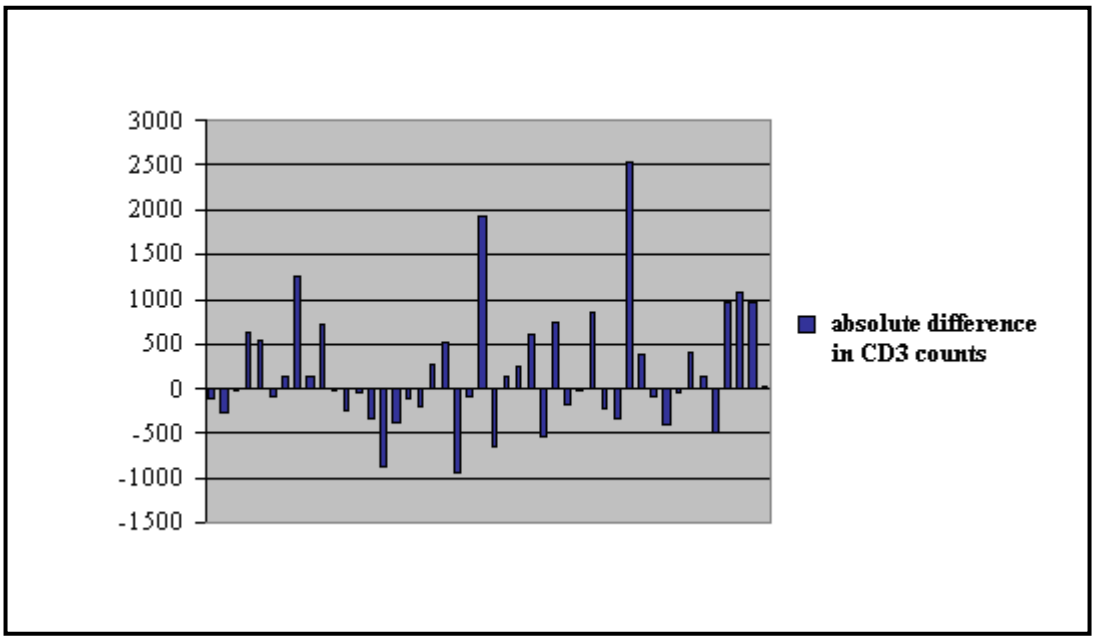

Figure 4.10: Absolute differences between baseline CD3 count results and follow-up CD3 count results; indicating that most were decreased

Figure 4.10 and Table 4.10 illustrate that most of the participants had their follow-up absolute CD3 counts decreased when compared to baseline absolute CD3 counts. However, those that were increased were by an average of 69.6% from baseline CD3 counts. Those that were decreased were by an average of 18.6% from baseline CD3 counts.

Table 4.11: Significant changes in viral load results from baseline to after 6 months on Inochi New Medicine immune boosters; significant changes were defined as an increase or drop in viral load by greater than 0.5 log copies/ml

Code	Baseline viral load in log	Follow-up viral load in log	Absolute Difference	viral load (log) increased/dropped
dit085	4.9	5.5	0.6	Increased
dit090	4.9	3.7	1.2	Dropped
dit092	6.0	5.2	0.8	Dropped
dit107	4.9	6.0	1.1	Increased
dit145	5.2	6.0	0.8	Increased
dit19I	4.2	5.1	0.9	Increased
dit21I	3.3	5.3	2.0	Increased
dit26I	5.3	4.6	0.7	Dropped
dit29I	4.5	5.3	0.8	Increased
dit01M	5.2	4.1	1.1	Dropped
dit04M	6.5	4.6	1.9	Dropped
dit11M	4.8	6.3	1.5	Increased
dit14M	4.2	5.6	1.4	Increased
dit16M	5.3	6.5	1.2	Increased
Hp1	6.1	6.9	0.8	Increased
Hp10	3.6	4.2	0.6	Increased
Hp12	4.9	0	4.9	Dropped
Hosp1	4.3	4.9	0.6	Increased
Hosp 4	0	3.5	3.5	Increased
Hosp5	5.2	4.6	0.6	Dropped
Hosp7	4.8	5.8	1.0	Increased

A change in viral load of greater than 0.5 log copies/ml, which is approximately a 3-fold change, may be considered to represent a true biological event, and therefore, a significant change (Hare, 2004). A change of less than 0.5 log copies/ml may be due to random variability and diurnal variation in stable HIV viral loads and is said to be approximately 0.4 log copies/ml (Hare, 2004). Significant change was observed in 21 out of 43 (48.8%) participants. Seven out of the 21 (33.3%) participants had their viral loads dropped

significantly *ie*. by > 0.5 log copies/ml. Their mean viral load was 5.4 copies/ml at baseline and their mean viral load was 3.8 copies/ml at follow-up (p = 0.032). Fourteen out of the 21 (66.7%) participants had their viral loads increased significantly *ie*. by > 0.5 log copies/ ml. Their mean viral load was 4.3 at baseline and their mean viral load was 5.4 at follow-up (p = 0.000). Participant Hosp 4 had a baseline viral load result of <50 copies/ml and the follow-up result increased to 3300 copies/ml (3.5 log copies/ml). This is known as a 'blip' (Huff, 2004) or transient viraemia (Mocroft *et al*., 2006). The number 0 (zero) was used instead of the result < 50 viral copies/ml for calculation purposes rather than reporting the result as a 'not applicable' log copies/ml. Participant Hp12 had a baseline viral load result of 82000 (4.9 log copies/ml) and a follow-up result decreased to < 50 copies/ml (0 log copies/ml). Twenty two out of 43 (51.2%) participants did not significantly change their viral loads. They showed a decrease or an increase of less than 0.5 log copies/ml and participant Hosp6 had both baseline and follow-up viral load results not changed.

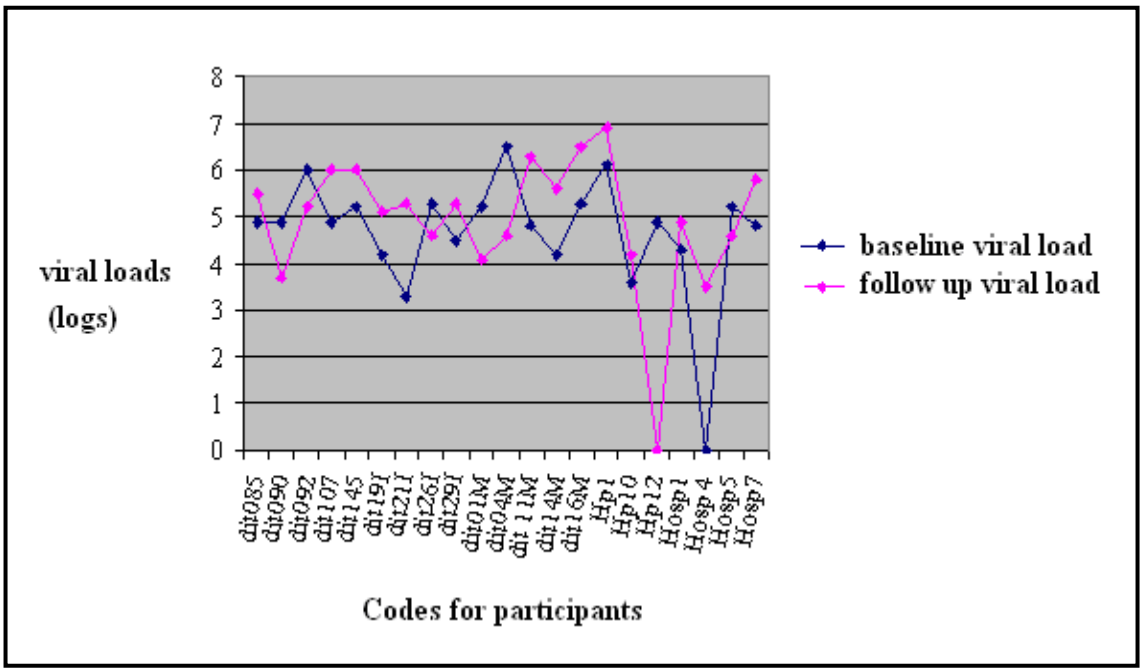

Figure 4.11: Rate of change of baseline viral loads compared to follow up viral loads (in logs) for the viral load results that a showed significant change, *ie*. an increase by more than 0.5 log or a decrease by more than 0.5 log

It is evident that most of the viral loads increased from baseline to follow-up (Figure 4.11 and Table 4.11). The mean significant drop was by 1.6 log copies/ ml (n = 7) and the mean significant increase was by 1.2 log copies/ml (n = 14). The remaining 22 (51.2%) participants had a mean baseline viral load of 4.5 copies/ml and a mean follow-up viral load of 4.5 copies/ml *ie*. unchanged.

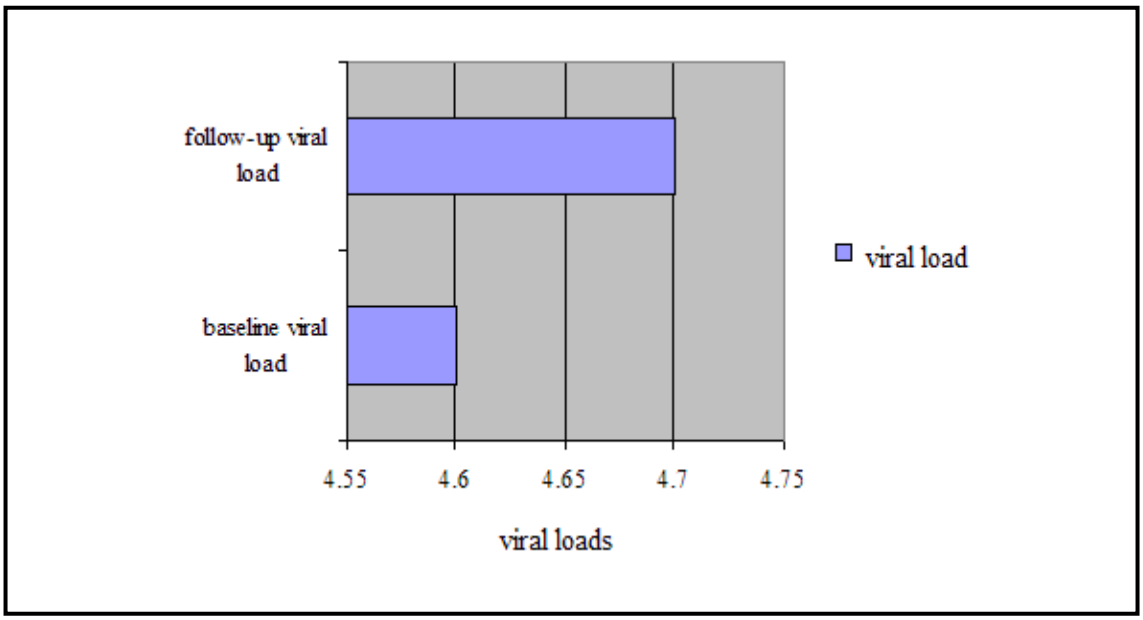

Figure 4.12: Rate of change between mean baseline viral loads and mean follow-up viral loads

Figure 4.12 shows that viral loads were increased on average. The mean baseline viral load was 4.6 copies/ml and the mean follow-up viral load was 4.7 copies/ml. However, this is not classified as a significant increase as it is below 0.5 copies/ml, since the increase was by 0.1.

4.5 PARTICIPANTS LOST TO THE STUDY

4.5.1 Participants lost to the study due to unknown reasons

Of the 131 participants enrolled, a total of 87 participants (66.4%) were lost to follow-up. Fifty nine participants were lost for unknown reasons, and they comprised 67.8% of the population (n = 87) that were lost to follow-up (Figure 4.2, Table 4.3, Table 4.12 and Figure 4.13).

Table 4.12: List of participants (n = 59) lost to follow-up due to unknown reasons; showing their CD4 counts and CD4 percentages

Code	Age (years)	M/F	CD4 counts	CD4%	Code	Age (years)	M/F	CD4 counts	CD4%
dit003	41	F	199	13	dit072	31	F	397	22
dit004	36	F	408	30	dit074	36	F	118	8
dit006	42	M	467	23	dit075	36	F	184	11
dit007	39	F	97	4	dit076	34	F	222	13
dit010	34	F	455	19	dit077	45	F	345	18
dit012	45	F	133	15	dit080	42	F	63	4
dit016	34	F	144	22	dit083	34	F	352	11
dit023	42	F	229	15	dit088	21	F	325	14
dit025	44	F	23	2	dit091	42	F	110	8
dit029	59	M	370	13	dit096	41	F	257	10
dit030	unknown	F	448	17	dit097	31	F	73	13
dit034	unknown	M	5	1	dit100	45	M	47	3
dit037	30	F	215	12	dit101	34	F	176	10
dit039	28	F	126	8	dit130	46	F	462	19
dit043	32	F	61	11	dit01I	34	F	79	7
dit044	32	F	442	15	dit04I	28	F	370	14
dit054	29	F	139	13	dit06I	23	F	315	28
dit058	35	F	256	14	dit08I	39	F	247	29
dit059	36	F	477	24	dit09I	26	M	377	20
dit060	34	F	381	23	dit11I	unknown	M	67	11
dit061	26	F	20	5	dit20I	39	F	243	13
dit062	41	F	314	24	dit22I	33	M	265	13

dit064	29	M	162	8	dit23I	36	M	289	16
dit065	35	M	140	10	dit09M	30	F	151	7
dit066	27	F	474	26	dit12M	64	M	61	8
dit067	18	M	486	19	dit15M	24	F	178	4
dit070	27	F	13	3	dit17M	32	M	7	1
dit071	27	F	379	21	dit18M	29	F	432	13
dit19M	19	F	322	14	dit20M	47	M	188	42
dit01PM	28	F	496	23					

Table 4.12 lists the 59 participants that were lost to follow-up for unknown reasons, including their CD4 counts and CD4% results. Their mean CD4 count was 242 cells/mm^3 and mean CD4% was 14.2%.

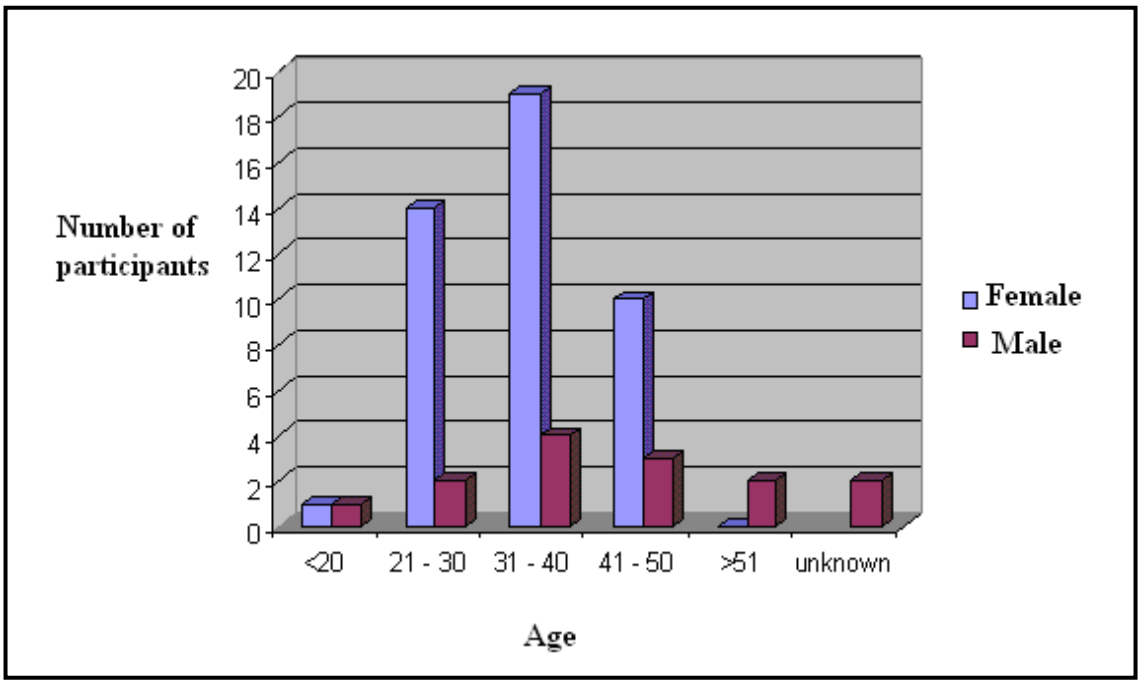

Figure 4.13: Age and gender profile of the participants that were lost to follow-up for unknown reasons; indicating that it was significantly more females in the 21 to 40 age group that made this cohort

Participants that did not come back comprised of 76.3% females and 23.7% males. Generally, in most of the age groups the majority were females rather than males, the highest number in the 31 to 40 years age group. The exception was in the greater than 51 years' age group where males were the majority, and in the group of those that did not know their age comprised of only males (Figure 4.13).

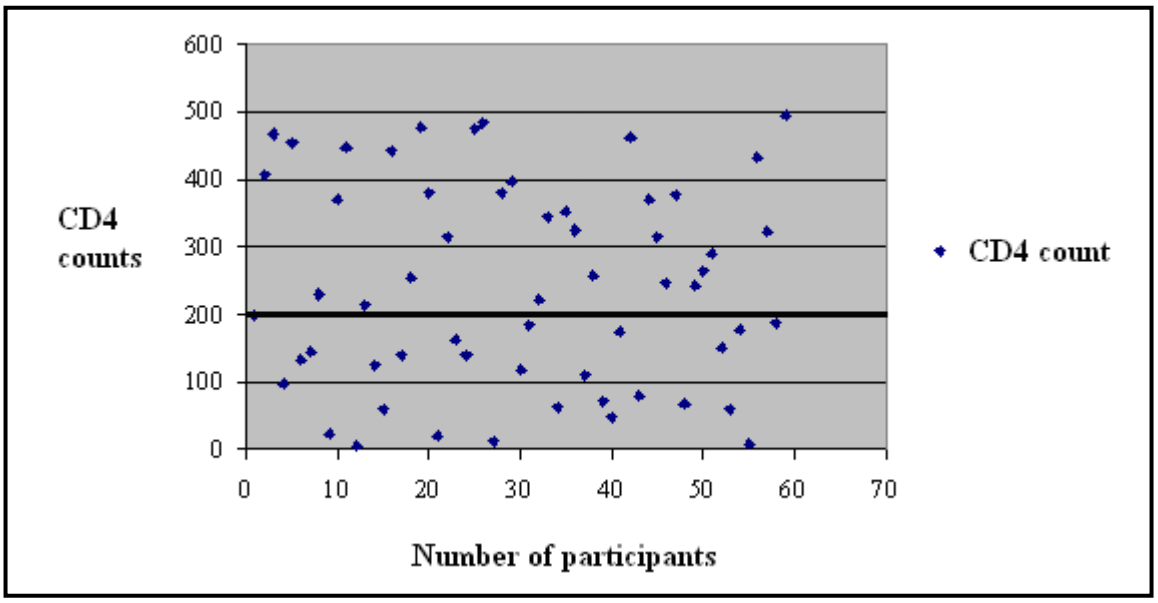

Figure 4.14: Scatter plot of CD4 counts for the participants lost to follow-up for unknown reasons. The bold line in the 200 cells/mm^3 CD4 count region is separating those participants with CD4 count < 200 cells/mm^3 from those with CD4 count > 200 cells/mm^3

Figure 4.14 illustrates that most of the CD4 counts were more than 200 cells/mm^3. 55.9% (n = 33) of this population lost to follow-up for unknown reasons had CD4 counts > 200 cells/ mm^3 and 44.1% (n = 26) of them had CD4 counts < 200 cells/mm^3 (Figure 4.14). Figure 4.14 displays a bold line separating the group with CD4 counts < 200 cells/mm^3 with those with CD4 counts > 200 cells/mm^3. The mean CD4 count was 242 cells/mm^3. AIDS is defined as when the CD4 count is less or equal to 200 cells/ mm^3 (WHO, 2005). A confluent scatter is noted in Figure 4.14, indicating that the CD4 counts had little or no influence to loss to follow-up. 61% (n = 36) participants had CD4% of $\leq$ 14% and 39% (n = 23) participants had CD4% of $\geq$ 15%, with a mean CD4% of 14.2%. AIDS is defined as when the CD4% is less or equal to 14% (Hare, 2004).

4.5.2 Participants lost to follow-up due to antiretroviral drug programme enrolment

Table 4.13: Age and gender profile as well as CD4 counts of participants that changed to antiretroviral drugs

Code	Age	Gender	CD4 count baseline	CD4 count follow-up	Time at which follow-up done
dit03M	40	F	152	208	1 month
dit08M	36	F	97	104	2 weeks
dit09M	30	F	151	n/a	
dit01TR	37	F	219	n/a	

Key: n/a - not available

Four out of the 87 (4.6%) participants, all female, were lost to follow-up due to the ARV rollout. Their mean CD4 count was 154.8 cells/mm^3. Table 4.13 shows that most of the participants had baseline CD4 counts less than 200 cells/mm^3 and that follow-up CD4 counts (those that were done) had increased. Participant dit03M had significantly increased her CD4 count by 56 cells/mm^3 within a month of taking the Inochi New Medicine immune boosters.

4.5.3 Participants lost to follow-up due to death

Table 4.14: List of participants lost to follow-up due to death; showing their age and gender profile, their CD4 counts and CD4% results

Code	Age	M/F	CD4 count	CD4 %
dit 001	33	M	21	3
dit 002	29	M	311	16
dit 017	18	M	4	1
dit 018	33	M	3	1
dit 019	37	M	98	12
dit 021	46	F	119	15
dit 024	27	F	3	5
dit 027	42	M	28	5
dit 040	47	M	148	10
dit 041	32	F	55	3
dit 042	47	F	17	9
dit 045	42	F	16	3
dit 050	27	F	2	1
dit 052	28	M	71	11
dit 053	28	F	453	18
dit 078	29	F	22	2
dit 095	61	F	74	9
dit 105	27	M	2	0
dit 120	28	F	32	10
dit 27 I	48	M	114	13
dit 10 M	35	F	139	12
dit 15 M	23	F	178	4
dit 23 M	unknown	M	5	3
Hp 11	32	M	43	3

Twenty four out of 87 (27.6%) participants who were lost to follow-up, were lost due to death.

This cohort comprised of 50% (n = 12) females and 50% males (n = 12) (Table 4.14).

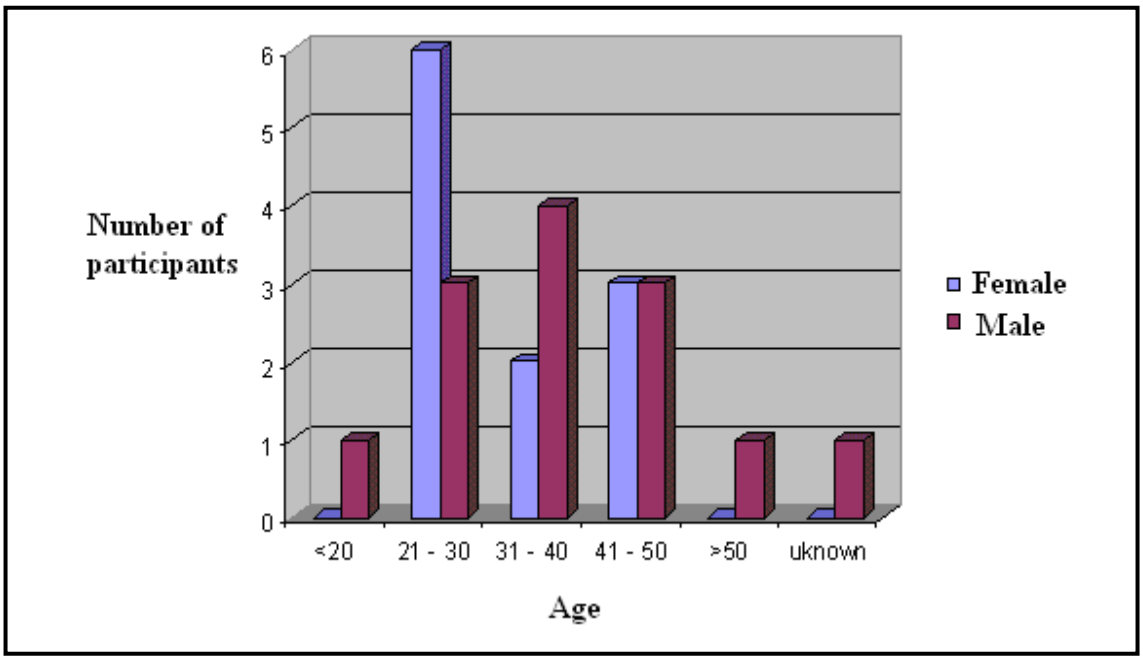

Figure 4.15: Diagram showing the age and gender profile of the participants that were lost to follow-up due to death, indicating that females in the 21 to 30 age group were in the majority

Most of the participants that died were females in the 21 to 30 years age group (Figure 4.15).

The majority of males that died were in the 31 to 40 years age group.

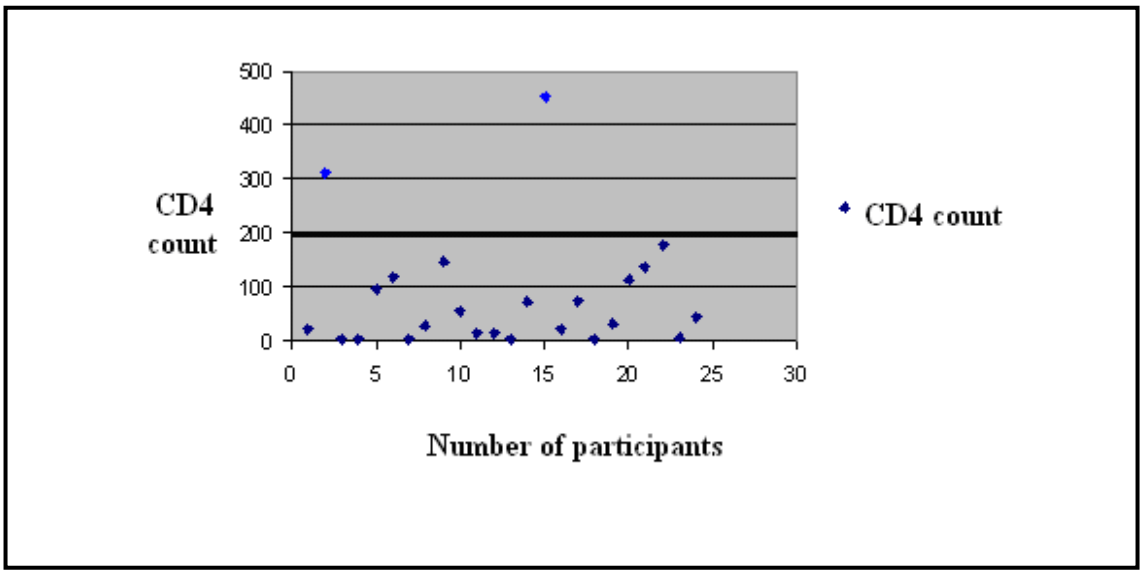

Figure 4.16: Scatter plot of CD4 counts for the participants lost to follow-up due to death. The bold line in the 200 CD4 count region is separating those participants with CD4 count <200 cells/mm^3 from those with CD4 count > 200 cells/mm^3

It is evident that 91.7% of the participants that died (n = 22) had baseline CD4 counts <200 cells/mm^3 (Figure 4.16) and the mean CD4% of 7.0%. This confirms that they were in the AIDS stage. Two out of the 24 (8.3%) participants had baseline CD4 counts > 200 cells/mm^3 (Figure 4.16). Participant dit002 had a baseline CD4 count of 311 cells/mm^3 and participant dit053 had a baseline CD4 count of 453 cells/mm^3. Participant dit002 died of pneumonia and participant dit053 died of poisoning (Mavata, personal communication).

4.6 FOLLOW-UP RESULTS OF PARTICIPANTS NOT ENROLLED INTO THE STUDY

Nine out of the 140 (6.4%) participants recruited were not enrolled into the study as their CD4 counts were greater than 500 cells/mm^3, and therefore, they did not meet the inclusion criteria. Therefore, they were excluded from the study. 66.7% (n = 6) were females and 33.3% (n = 3) were males. The reason of including this set of data was to ascertain whether the patients that had >500 cells/mm^3 CD4 counts benefited from the Inochi New Medicine immune booster better than the <500 cells/mm^3 CD4 count cohort.

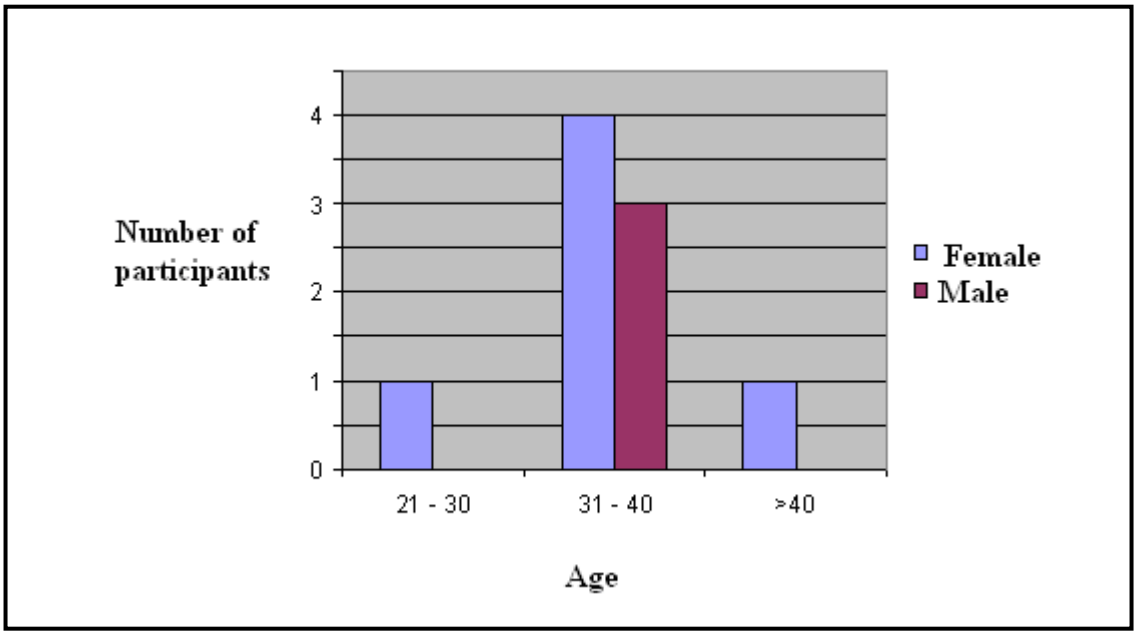

Figure 4.17: Age and gender profile of the participants that were excluded from the study as their CD4 counts were > 500 cells/mm^3

Figure 4.17 shows that this cohort predominantly comprised of females, most of them in the 31 to 40 age group. This was a general observation in the < 500 cells/mm^3 CD4 count cohort as well. Males were only in the 31 to 40 age group.

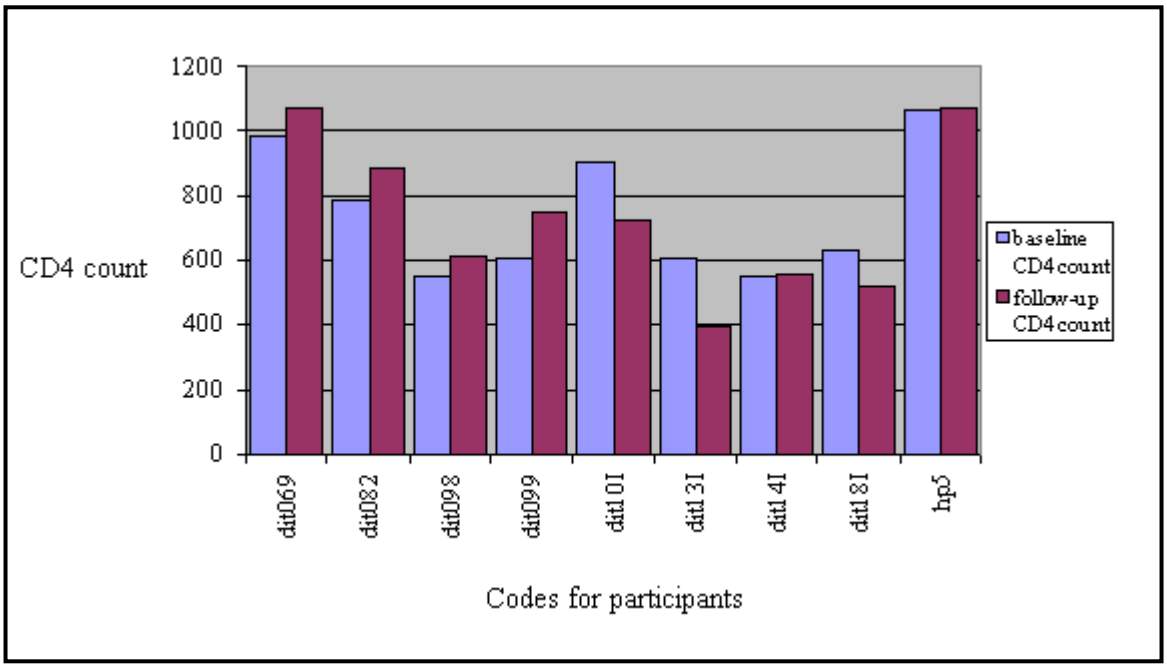

Figure 4.18: Difference between baseline CD4 counts and follow-up CD4 counts of participants excluded from the study

The mean baseline CD4 count for this cohort was 742.7 cells/mm^3 and the mean CD4 count at follow-up was 730.2 cells/mm^3. Four patients (44.4%), as indicated in Figure 4.18, significantly increased their CD4 counts at follow-up compared to the baseline CD4 counts *ie*. by more than 50 cells/mm^3. The average increase of their CD4 counts was 92.8 cells/mm^3.

The 3 patients (33.3%) that dropped their CD4 counts, decreased them by more than 100 cells/mm^3. The average decrease of their CD4 counts was 164.7 cells/mm^3.

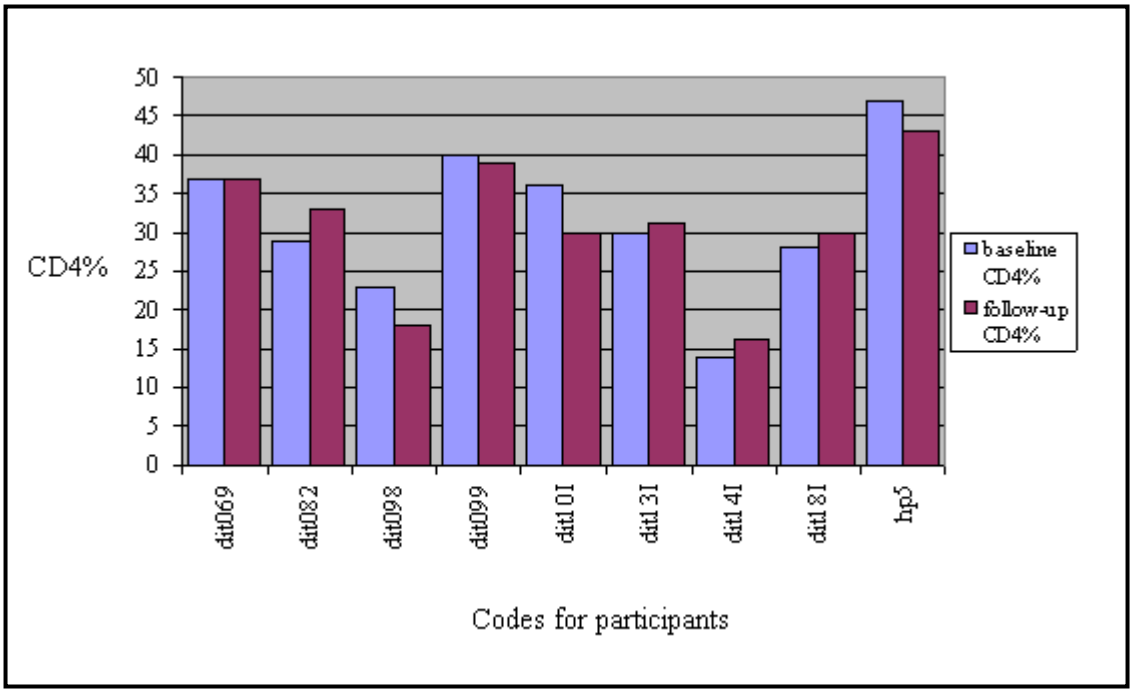

Fig 4.19: Difference between baseline CD4% and follow-up CD4% of participants excluded from the study

The mean baseline CD4% for this cohort was 31.6% and the mean CD4% at follow-up was 30.8%. Figure 4.19 indicates that 4 patients (44.4%) increased their CD4% results at follow-up, by an average of 2.3%. The 4 patients (44.4%) that dropped their CD4% decreased them by an average of 4%.

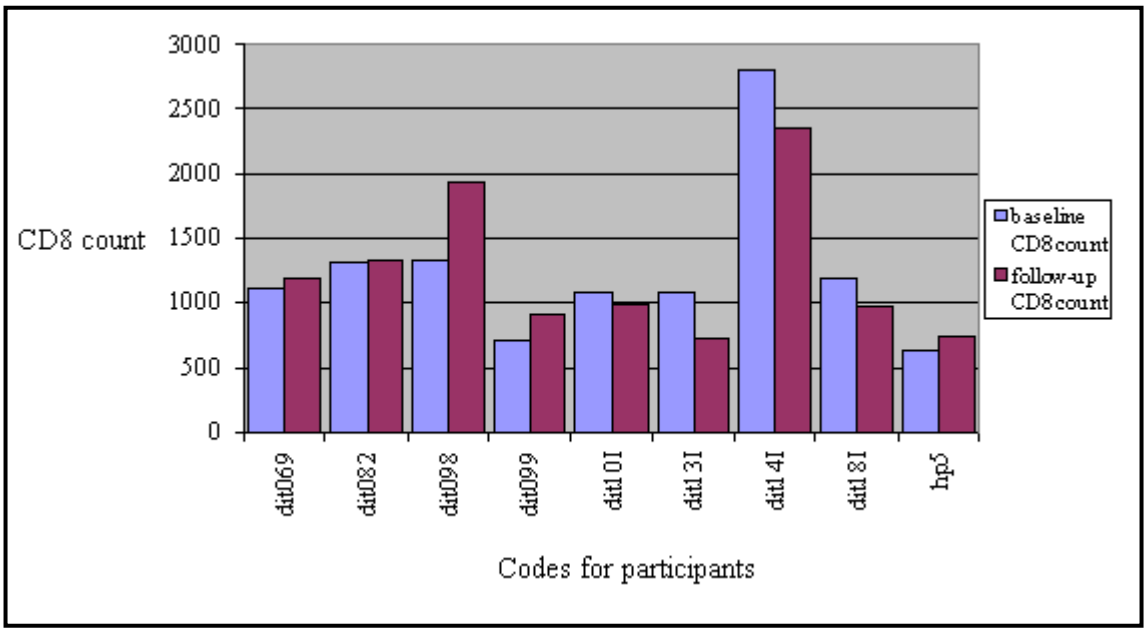

Figure 4.20: Difference between baseline CD8 counts and follow-up CD8 counts of participants excluded from the study

The mean baseline CD8 count for this cohort was 1248.4 cells/mm^3 and the mean CD8 count at follow-up was 1237.4 cells/mm^3. Figure 4.20 indicates that 5 patients (55.6%) increased their CD8 counts at follow-up, by an average of 204.4 cells/mm^3. The 4 patients (44.4%) that dropped their CD8 counts decreased them by an average of 280.3 cells/mm^3.

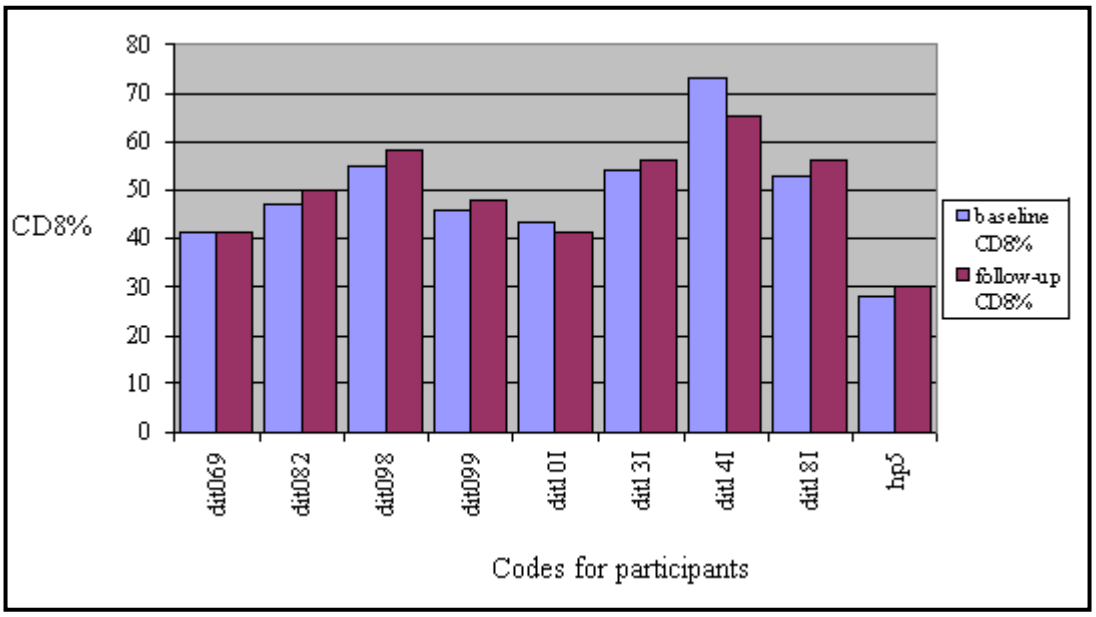

Figure 4.21: Difference between baseline CD8% results and follow-up CD8% results of participants excluded from the study

The mean baseline CD8% for this cohort was 48.9% and the mean CD8% at follow-up was 49.4%. Six patients (66.7%), as indicated in Figure 4.21, increased their CD8% results at follow-up compared to the baseline CD8% results. The average increase of their CD8% was 2.5%. The 2 patients (22.2%) that dropped their CD8% decreased them by 5%. One patient's CD8% results did not change at follow-up.

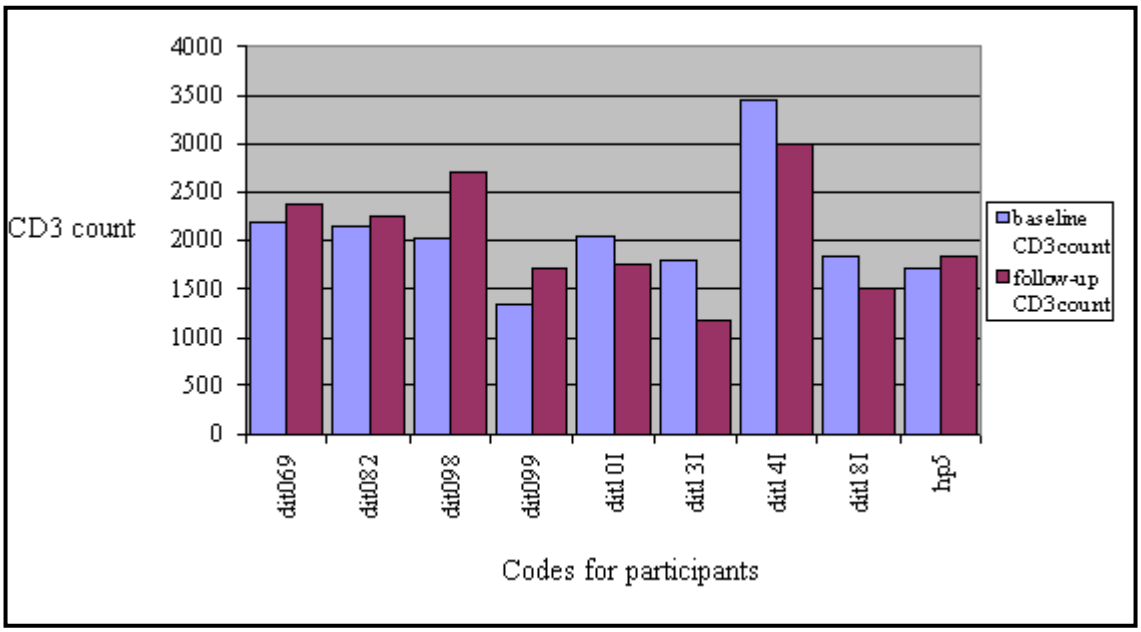

Figure 4.22: Difference between baseline CD3 counts and follow-up CD3 counts of participants excluded from the study

The mean baseline CD3 count for this cohort was 2056.8 cells/mm^3 and the mean CD3 count at follow-up was 2033.4 cells/mm^3. Figure 4.22 indicates that 5 patients (55.6%) increased their CD3 counts at follow-up, by an average of 293.6 cells/mm^3. The 4 patients (44.4%) that dropped their CD3 counts decreased them by an average of 419.5 cells/mm^3.

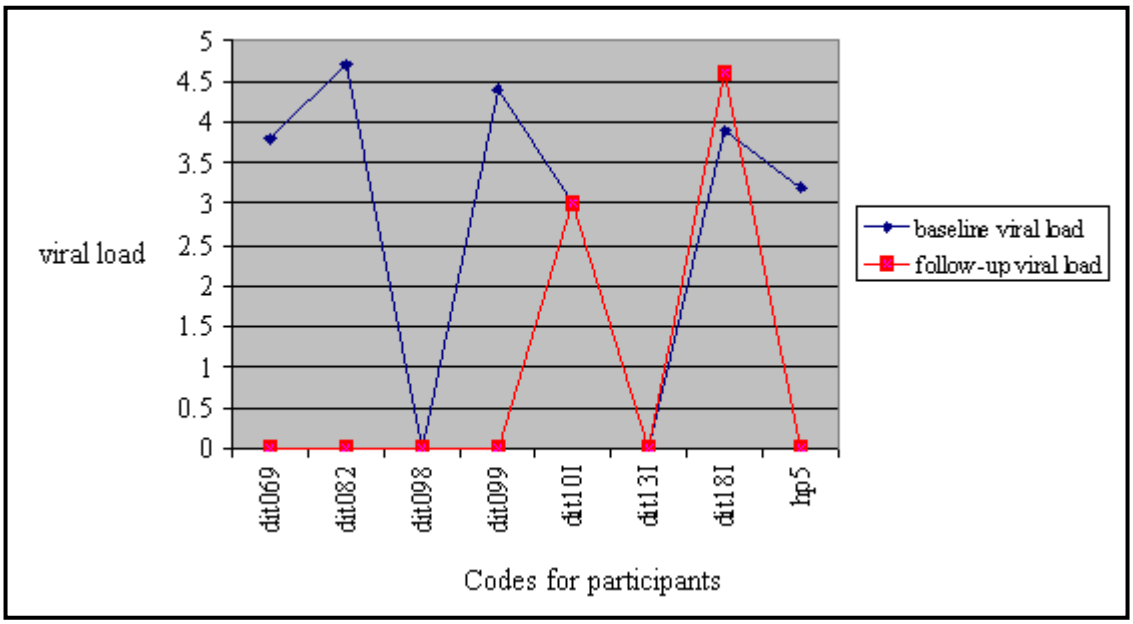

Figure 4.23: Rate of change of baseline viral loads compared to follow-up viral loads (in logs) for the participants that were excluded from the study

Figure 4.23 shows that the mean baseline viral load was 2.9 log copies/ml and the mean viral load at follow-up was 1 log copies/ml. Four patients (44.4%) decreased their follow-up viral loads by 4 log copies/ml, 1 patient increased her follow-up viral load by 0.7 log copies/ml (a significant increase). Two patients did not change their viral loads: *ie*. both baseline and follow-up viral loads were < 50 copies/ml and 1 patient did not change her baseline viral load of 3 log copies/ml.

Table 4.15: Rate of change in the laboratory parameters used to measure the efficacy of the Inochi New Medicine immune booster for the followed-up participants (< 500 cells/mm^3 cohort)

Parameter	Average rate of increase	Average rate of decrease	Significant change
CD4 count	126.8 cells/mm^3 (60.9% participants) p = 0.000	89.7 cells/mm^3 (39.1% participants) p = 0.000	more than 50 cells/mm^3, showed by 23 out of 47 participants (48.9%)
CD4%	3% (44.7% participants) p = 0.000	4.1% (38.3% participants) p = 0.001	3%, showed by 39 out of 47 participants (82.9%)
CD8 count	563.2 cells/mm^3 (50% participants) p = 0.000	252.2 cells/ mm^3 (50% participants) p = 0.000	67.9%, showed by 23 out of 46 participants (50%)
CD8%	7.7% (55.3% participants) p = 0.006	4.8% (39.1% participants) p = 0.002	7.7%, showed by 26 out of 46 participants (55.3%)
CD3 count	694.8 cells/mm^3 (47.8% participants) p = 0.000	283 cells/mm^3 (52.2% participants) p = 0.000	64.6% showed by 22 out of 46 participants (47.8%)
Viral load	1.2 copies/ml (66.7% participants) p = 0.000	1.6 copies/ml (33.3% participants) p = 0.032	A decrease of > 0.5 copies/ml, showed by 7 out of 21 participants (33.3%)

It is evident that CD4 count, CD4% and CD8% were significantly increased by the immune booster, as observed in Table 4.15, in the majority of participants that were followed-up. CD8 counts were increased for half the number of participants, whereas, CD3 counts were decreased for the majority of the participants.

The 9 patients with CD4 counts > 500 cells/mm^3 were followed-up but were not included in the study since their baseline CD4 count was more than that forming part of the inclusion

criteria. They were mostly females, comprising 66.7% of this cohort. Two of these patients maintained their baseline viral load of < 50 copies/ml at follow-up. However, it was observed that the immune booster did not increase or decrease their cells as observed in the < 500 cells/mm^3 cohort. 44.4% of the patients that increased their CD4 counts did so by an average of 92.8 cells/mm^3, as opposed to the 126 cells/mm^3 increase observed in the < 500 cells/mm^3 cohort. Those that decreased (33.3%) did so by 164.7 cells/mm^3 as opposed to the 89.7 cells/mm^3 decrease, observed in the < 500 cells/mm^3 cohort. Similar results were observed with CD4%, in that the CD4% rate of increase was less (2.3%) compared to that of the < 500 cells/mm^3 cohort (3%) and the rate of decrease (4%) was less than that observed in the < 500 cells/mm^3 cohort (4.1%). The rate of increase of the CD8 count (204.4 cells/mm^3) was less than in the < 500 cells/mm^3 cohort (563.2 cells/mm^3), and the rate of decrease (280.3 cells/mm^3) was more than in the < 500 cells/mm^3 cohort (252.2 cells/mm^3). The rate of increase of the CD8% (2.5%) was less than in the < 500 cells/mm^3 cohort (7.7%), and the rate of decrease (5%) was more than in the < 500 cells/mm^3 cohort (4.8%). The rate of increase of the CD3 count (293.6 cells/mm^3) was less than in the < 500 cells/mm^3 cohort (694.8 cells/mm^3), and the rate of decrease (419.5 cells/mm^3) was increased more than in the < 500 cells/mm^3 cohort (283 cells/mm^3). The rate of decrease in the viral load (4 log copies/ml) was more than that observed in the < 500 cells/mm^3 cohort (1.6 log copies/ml), and the rate of increase (0.7 log copies/ml) was less than that observed in the < 500 cells/mm^3 cohort (1.2 log copies/ml).

CHAPTER 5

DISCUSSION AND OBSERVATIONS

5.1 DISCUSSION

5.1.1 Quality of life

It was observed that most of the recruited and the subsequently enrolled participants were females, in the range of 73.6% and 74% respectively (Table 4.1 and Table 4.2). This confirms the findings that it is mostly females that are infected and it is mostly females that seek help when there are opportunities for assistance (AFSA, 2005). Ndimande (2001) suggested that it is women that carry a heavier burden of poverty since they are responsible for housekeeping services for their families. Most of the females enrolled were in the 31 to 40 years age group, the so-called socially and economically active group. Cohen (not dated) reported that HIV infection is concentrated in the socially and economically productive groups aged 15 to 45, with slightly more women infected than men and the infection for girls occurring at younger ages. Ndimande (2001) stated that KwaZulu-Natal is among the provinces that have a larger proportion of females than males, many of them living in rural areas and are African.

Ndimande (2001) further stated that 60% of these women are unemployed. Cohen (not dated) indicated that the poorest households are often female-headed and that poverty is associated with low levels of education. He also indicated that for the poor it is the "here and now" that prevails, which often leads to risky sexual behaviour.

The literacy and numeracy levels were observed to be low as some could not read or write. Some did not know their age and yet they were in their youth. It was evident from their responses to the questionnaires that their socioeconomic status was low as most were unemployed and had no financial support whatsoever. Most of them indicated not being able to secure for themselves a healthy diet, a probable reason that made them reluctant to enrol into the ARV programme. Swartz (2001) stated that most South African households experience outright poverty or vulnerability to being poor. Giarelli & Jacobs (2003) indicated that KwaZulu-Natal is home to 8.4 million South Africans and poverty is common. Ndimande (2001) indicated that most KwaZulu-Natal households have no access to a telephone service and have unsatisfactory access to clean water, energy, health care and education. Ndimande (2001) further indicated that levels of education are low, stating that most women from KwaZulu-Natal are less educated than men. South Africa is among the countries with the highest levels of income inequality in the world, and compares poorly, in most social indicators, to countries with similar income levels. In South Africa, 24% of the population lives below poverty lines (Ndimande, 2001). Poverty increases the risk of HIV infection and other poverty related diseases such as TB. Unemployment is twice as high in rural areas than

in metropolitan areas (Ndimande, 2001). In this study, most participants were from rural areas, few from urban areas. Most of those that were from urban areas were, however, not employed. They, therefore, were not advantaged in any way in terms of accessing healthy diets nor being more educated or informed. It was also deduced that most of the people from the communities around the sites were living in extreme poverty and in low socioeconomic conditions.

This study offered opportunities for participants to be educated about the pathogenesis of HIV; about laboratory monitoring of the disease; the clinical significance of laboratory results as well as changes in lifestyle that are necessary to improve their quality of life. Participants were expected, as part of showing compliance, to change their diet to healthy foods. Those that smoked and drank alcohol had to abandon these bad habits. The following dietary requirements were recommended to all participants:

a) well balanced nutritious diet *eg*. vegetables, chicken, fish

b) lots of water; mineral drinks to be avoided

c) 100% fruit juice

d) addition of turmeric powder in stews as it is has antiseptic/antibiotic properties (Canadian AIDS Treatment Information Exchange 2005; Pothitirat & Gritsanapan, 2006)

e) the usual household food. However, the following to be avoided:

    i) cooking oil

    ii) sugar

    iii) yoghurt, maas and cheese; buttermilk instead

iv) processed or raw foods

v) red meat, as it is not easily digested.

Generally, the majority of patients were unemployed. There was also no other income generating family member. Some, however, were able to access food from neighbours, and sometimes from the hospice in the form of food parcels. Some either reported going hungry for days or opted for eating unhealthy foods instead. Some ate food that was not nourishing as they were unable to afford healthy foods. Some feared that, if they were to tell their families that they needed to change their diet, they would have to reveal their HIV status. They were not willing to do so as they would risk being ostracized.

It was evident that education on the details of HIV and the associated disease is important as this can influence change of behaviour and encourage adherence to the treatment programme. Condom usage was encouraged to prevent re-infections. Majority of patients indicated that they were compliant. The protocol specified that patients should take the Inochi New Medicine only, by their own choice. It was evident that patients tended to use other alternative treatment (OAT) modalities *eg*. those advertised on radio. This was disclosed when adverse effects were seen, when using these OATs. These patients could not be included in the study.

Successful follow-up was low. It was at 36%. It is believed that if there was a monetary incentive, loss to follow-up could have been lessened. Thornton (not dated) reported from his

study that even when testing is available, most people do not take advantage. However, when monetary incentives are afforded, follow-up numbers increase. She further indicated that even the smallest amount of money was able to increase attendance at follow-up visits by 50%. Laboratory testing and the immune boosters were offered free of charge. This was probably not sufficient as it did not put food on the table for the participants, and there was no transport fee incentive. This was probably the reason for the poor follow-up. Forty three percent of the participants were lost to follow-up and the reasons were not known. It was mostly females (80.9%) that returned for follow-up visits. One of the possible reasons for the high loss to follow-up could be the inability of the study team to contact participants that did not honour scheduled visits, only trusting that they would return. Most patients did have access to telecommunication services. The recorded loss to follow-up was 21%, *ie*. those participants that died (18%) and those that changed to antiretroviral drugs (ARVs) (3%).

In this study, the mortality rate was high, at 18% and the loss to follow-up for non-recorded reasons (unknown) was 43%. Lawn *et al*. (2006) reported that among ARV programmes in sub-Saharan Africa, rates of non-death programme losses range from less than 5 to greater than 50%. Lawn *et al*. (2006) suggested that patients termed as lost to follow-up because of not attending clinic appointments, patient death may not be ruled out. Kumarasamy *et al*. (2005) indicated that "patients with CD4 counts less than 200 cells/mm^3 are 19 times more likely to die than those with CD4 counts greater than 350 cells/mm^3". Hogg *et al*. (2001) concurred with Kumarasamy *et al*. (2005) in stating that those with CD4 counts less than 200

cells/mm^3 were 3.85 times more likely to die than those with CD4 counts of 200 cells/mm^3 or more. In this study, 91.7% of participants that died had baseline CD4 counts of less than 200 cells/mm^3, which correlated with published data (Hogg *et al*., 2001; Kumarasamy *et al*., 2005).

5.1.2 Safety of the immune booster

Haemoglobin levels, AST and ALT levels were assayed to ascertain and monitor the safety of the immune booster (Table 4.4). Bishop, Fody & Schoeff (2005) indicated that increased AST and ALT levels are used to assess hepatocellular damage. These parameters were, therefore, used to indicate whether the immune booster caused any liver damage. It was evident from the results that the Inochi New Medicine immune booster did not cause any major adverse effects. 9.3% of the participants developed anaemia at follow-up, and 16.3% who were mildly anaemic at baseline, were moderately anaemic at follow-up. 13.3% and 6.5% participants increased their AST and ALT levels by more than 3 times, respectively, at follow-up.

Two participants *ie*. Hosp2 and Hp11 developed increased AST and ALT levels at follow-up, whilst their mild anaemia was neither improved nor worsened at follow-up. However, 58.2% participants did not change their Hb levels from baseline to follow-up values and 16.2% improved their Hb levels at follow-up. The immune booster was, therefore, observed to be safe to a majority of participants.

5.1.3 Efficacy of the immune booster

There were no interruptions in the immune booster supply. Most participants that were successfully followed-up reported that they felt better after starting the medication, even though it was not always evidenced by an increased CD4 count or reduced viral load, as expected. This is in keeping with literature which indicates that CD4 counts do not always represent the clinical wellness of patients (Havens, 2003). Most participants presented with opportunistic infections at baseline which were reported to have disappeared after taking the Inochi New Medicine immune boosters. Suspected TB cases were referred to the public treatment facilities. Participants reported feeling well and were no longer feeling tired and sick. Most reported that their appetite had improved, diarrhoea had subsided and aching feet felt better. They reported that they had gained weight as their clothes were no longer fitting. Patients were trusted when they said they had gained weight and their clothes were no longer fitting. Physical observation also confirmed this.

5.1.3.1 Laboratory results evaluating efficacy

Fourteen out of 23 participants (60.9%) showed significant increases in their CD4 counts and 24 out of the 47 participants showed no significant changes. In summary, only 9 (19.1%) out of the 47 participants showed a negative hypothesis. However, at this stage, the cause for the negative hypothesis cannot be fully defined.

An extensive follow-up study will follow in order to rule out potential sources such as compliance, other infections, diet, taking other herbal remedies, *etc*.

Fourteen out of the 47 (29.8%) followed-up participants had increased their baseline CD4 counts at follow-up, by a significant number (by more than 50 cells/mm^3). The mean increase in CD4 count was by 126 cells/ mm^3. Erb *et al*. (2000) defined antiretroviral treatment efficacy as a decrease of viral load of at least 1 log copy/ml and an increase of the CD4 count by at least 50% of the baseline value. Ten of the 14 (71.4%) showed a > 50% increase in CD4 counts from baseline. A study performed by Erb *et al*. (2000) reported that almost 60% of participants on HAART showed a CD4 count increase of at least 50% of the baseline value. Five participants (35.7%) significantly increased their viral loads at follow-up despite the increased CD4 counts. This was not in keeping with the typical HIV disease progression pattern. It was noted that only 1 participant significantly decreased viral load from 4.9 log copies/ml to undetectable levels at follow-up. Demeter *et al*. (2001) suggested that indicators of a successful therapy are a decrease in the RNA HIV viral load to undetectable levels in plasma, an increase in CD4 counts, a decline in opportunistic infections as well as progressive weight gain. However, it has been observed that antiretroviral therapy may produce discordant results (Jamil *et al*., 2002; Spritzler *et al*., 2003). Seven of the participants (16.3%) successfully followed-up showed a significant decline in their viral loads, *ie*. by > 0.5 log copies/ml.

Twelve participants had increased their CD8 counts at follow-up. The mean increase in CD8 count was by 563.2 cells/mm^3. Lee *et al*. (2002) indicated that high levels of HIV specific CD8 T cells are demonstrable throughout HIV disease and elimination of these cells results in higher viral burden and rapid disease progression. It was observed that 50% of the participants that were followed-up had increased their CD8 counts by an average of 67.9% and those that dropped their CD8 counts did so by 21.6%. It was also observed that out of the 14 participants that had increased CD4 counts at follow-up, 12 participants (85.7%) had increased CD8 counts as well. Similar results were also demonstrated by Gray *et al*. (1998). Their study reported an increase in both CD4 and CD8 cells in patients on HAART for 6 months. Gray *et al*. (1998) also reported a strong correlation between the number of activated CD8 cells and viral load. This was, however, not the case in this study, as it was observed that only 4 participants out of the 12 (33.3%) that had increased their CD8 counts had significantly increased viral loads as well.

According to Chinen *et al*., 2001 and Kunkl, 1997, when AIDS develops, CD3 counts decrease in numbers. Twenty four out of 46 participants (52.2%) decreased their CD3 counts at follow-up. However, the decrease was by an average of 18.6% of the baseline CD3 counts, whereas the increase was by an average of 69.6% of the baseline CD3 counts.

According to King (1998), it is expected that when a person is on antiretroviral therapy, their CD4 counts increase dramatically in the first few months**.** The increase then becomes gradual in the following months. This was observed in some participants, where their CD4 counts and CD4 percentages; CD8 counts and CD8 percentages and CD3 counts were increased.

According to Schlossberg (2001), it is expected that viral loads will decrease in antiretroviral therapy. This was also observed in 33.3% of the participants, where their viral loads were decreased. Demeter *et al*. (2001) observed a viral load suppression rate of only 40 to 60% in 60 to 90% of patients on antiretroviral therapy. It can thus be concluded that not all patients taking ARVs have a decline in viral load.

Mocroft *et al*. (2006) reported that patients with CD4 counts of more than 650 cells/mm^3 experienced significantly lower changes in CD4 counts compared to patients with lower CD4 counts. This was also observed in this study as the cohort with > 500 cells/mm^3 had lower rates of increase for CD4, CD8 and CD3 counts compared to the < 500 cells/mm^3 cohort. However, the rate of decrease of viral loads was more in the > 500 cells/mm^3 compared to the < 500 cells/mm^3 cohort. Mocroft *et al*. (2006) suggested that there is a possibility of CD4 counts approaching normal levels to display limited capacity for increasing further.

5.2 POSSIBLE STRENGTHS OF THE STUDY

I, (Mrs B T Mkhize, study investigator) was in attendance at the clinics when patients visited the Ikhaya Lobomi sites. This offered first hand experience on the devastation, desperation and frustration created by the HIV epidemic. A 26 year old participant reported that when he showed his mother the laboratory results stating his HIV status diagnosis, she burnt the results sheet as she could not deal with the news, as it meant her son was going to die. Another participant reported having to engage in unprotected sex with her partner because she could not tell the partner of her HIV status. She had been informed of the benefits of using a condom to prevent cross infections. Another participant reported having to breastfeed her child because she could not justify to her family the need to abstain from breastfeeding without disclosing her status.

The study group encouraged community members to attend voluntary testing centres. Many took the advice and got tested. People expressed reservations of attending local clinics where they would be identified by family and neighbours. They felt comfortable to come to Ikhaya Lobomi, where people did not know them.

Participants were able to openly discuss their frustrations and concerns with the study team. The following issues were identified:

1. Some suggested that it would benefit them if the counsellors at public clinics and public hospitals were HIV infected themselves. They felt that since some were not HIV positive, they do not treat them with dignity. They reported that some nurses did not want to touch them as they feared contracting the disease.
2. Some reported that they were unable to access jobs in the careers they were trained for, as the corporate world sometimes shuns away from employing HIV positive individuals.
3. One participant reported that she had visited a public hospital where her CD4 count was found to be 186 cells/mm^3. She was asked to return the following year to be enrolled into the antiretroviral programme. She reported that she was ridiculed by the nurse who attended to her. Furthermore, her mother, when drunk, would swear and shout at her about her HIV status.
4. Another participant reported of being told by a nurse in a public hospital that her CD4 count was too high and she should return the following year when it had dropped. She was not told of what the actual count was. She was also not counselled on the importance of maintaining a high CD4 count.
5. One participant reported that she could not use antiretroviral drugs as she was living an unstable life, a life of prostitution and drugs. She felt that she would not manage antiretroviral drugs and therefore, she opted for the immune booster.

6. Most participants were not aware of the public health system services available to them regarding ARV treatment options and the facilities that were available to treat opportunistic infections.
7. A married couple was counselled to use condoms when they reported that they were unable to be intimate with each other as they hated the sexual intercourse. They blamed their infection to the act.
8. Some were discouraged by their religious denominations in using condoms. They were told that they were evil and had worms. They were being counselled on re-infections and multiple strains of HIV and were encouraged to use condoms.
9. Some reported that they were being abused by community workers and community organisations taking their pictures for soliciting donations and food for them. They reported that they were either offered spoilt and stale foodstuffs or little food parcels that were not enough for their families.

5.3. LIMITATIONS OF THE STUDY

The patient follow-up was undertaken over a 6 months' period. The 6 months testing interval appeared to be inadequate in providing sufficient data to provide conclusive evidence of the use of the product. Twenty four out of 47 participants showed little or no difference. This could be due to the 6 months' data analysis timeline. Successful follow-up of participants was a major challenge. Patients were counselled and encouraged to attend all study visits.

However, the loss to follow-up rate was high (refer Figure 4.2). As previously discussed, lack of resources to provide financial support for travel could be a major contributor.

The questionnaires (appendices 5 and 7) as quality analysis instruments were not effective, as not all patients adequately filled in the questionnaires. Most of the participants' literacy levels were low: they did not know their dates of birth nor when they were accurately diagnosed. They could not fill in the questionnaires themselves, so the principal investigator needed to assist them. It was not possible for the principal investigator to be there at all times as counselling sessions were also held simultaneously. There was no funding for support structures.

The intention of the questionnaires was to gather data on the general background of the participants; for instance, whether they could afford and access a healthy diet, whether they were presented with opportunistic infections, whether they lived in a rural or urban setting, as well as the impact of the area where they lived, on their general well-being. This data would have been a very useful instrument to assess the effect of poor diet and poverty as a contributing factor to the HIV epidemic.

It was difficult to measure compliance, hence the participants' assurance of compliance had to be trusted. This is usually a consideration for all clinical trials. It was also difficult to gather whether the increases in parameters were due to the Inochi New medicine only or as a result of

other interventions not declared. A proper placebo group was not used in this study, and data was compared to already established norms seen in ARV treatment.

A major challenge was when patients did not want to improve their CD4 counts as they would not qualify for disability grants: when CD4 counts increased to more than 200 cells/mm^3, some opted for antiretroviral therapy as they would automatically qualify for disability grants. However, when they experienced side effects, they returned wanting to resume the Inochi New Medicine medication. They were excluded from this study. They were advised that they could not mix medications as this would be detrimental to their health and this would have an impact on both ARV treatment and the Inochi New Medicine.

5.4 OBSERVATIONS

CD4 counts, CD4 percentages and viral loads are useful markers in managing the HIV disease. However, HIV infected individuals should be educated on the significance of these results and the importance of maintaining high CD4 counts. Patients should be intensely and adequately counselled on behaviour and/or lifestyle change, as an influential factor in maintaining higher CD4 counts. This definitely is an essential component of any HIV treatment programme as it has been proven that quality of life improves when ther is psychosocial support (Au *et al*., 2003).

From personal observation, it was evident that HIV infected individuals require continuous social support. Mrs P Mavata at Ikhaya Lobomi Hospice and Care Centre has an excellent psychosocial support programme where patients call or consult regularly, discussing their progress. The immune booster dosages were changed as per the patients' progress or lack of, as determined by their laboratory results. The significance of every laboratory result was explained and patients developed faith in the treatment regimen when they observed improvements. Patients were advised on how to utilize healthily foodstuffs which were available to them if they were not able to afford a healthy diet. Cooking lessons were held where patients were shown how to cook foods that were nutritious instead of being cooked in cooking oil.

5.5 CONCLUSION AND FUTURE RESEARCH

ARV treatment options are not readily available to the public sector as a whole. Proper controlled clinical trials on immune boosters undertaken on a larger scale, to establish efficacy and toxicities, will avoid people being misled. There is evidence that some HIV infected individuals prefer other alternate therapies. The Inochi New Medicine immune booster indicated qualitative and quantitative improvements in most participants.

An extensive follow-up study is necessary to investigate other influencing factors in this improvement/ lack of improvement; such as diet, other herbal remedies, compliance, other infections, psychosocial support *etc*. A follow-up study will be undertaken on the participants of this study at 9 months and at 12 months, to ascertain progress for publication purposes.

CHAPTER 6

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Personal communication:

Mavata P.N. Ikhaya Lobomi Hospice and Care Centre

Connolly C. Medical Research Council

HIV positive patients

APPENDICES

Appendix 1

Ikhaya Lobomi Ministry
"Home of Life"

Date: 30/09/04

To: Mrs. Brenda Mkhize

We hereby grant permission to Mrs. Brenda Mkhize for use of Ikhaya Lobomi Hospice and Care Centre for purposes of the Masters Degree.

Names of the patients will not be disclosed to you, to maintain patient confidentiality.

Your Sincerely

Mrs P.N Mavata
Manager

Zimele & Patience Mavata
P.O. Box 583, Bothas Hill, 3660, South Africa
Tel: (27) (31) 7022979 (h) / 082 8345956 (cell) / (27) (31) 0724612 (fax)
Email: mavata@mweb.co.za

Appendix 2

GLOBAL CLINICAL & VIRAL LABORATORY

Jacobs Health & Industrial Clinic
217 Quality Street
P.O. Box 13026, Jacobs
Jacobs 4026

Tel: (031) 461 5509
Fax: (031) 461 4583
Emergency Telephone
073 237 9275
082 333 8230

Date: 30/09/04

To: Mrs. Brenda Mkhize

We hereby grant permission to Mrs. Brenda Mkhize for use of Laboratory data for purposes of the Masters Degree.

Names of patients will not be disclosed to you, to maintain patient confidentiality.

Yours Sincerely

Dr.L.Madurai

DURBAN INSTITUTE of TECHNOLOGY

Appendix 3a
(English version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

SUBJECT INFORMATION

Dear Patient

I would like to inform you of the study performed by a student from Durban Institute of Technology (DIT) for purposes of obtaining a Master's degree in Technology.

The title of the study is an investigation of the rate of change of CD4 and CD8 T lymphocyte counts and viral loads in HIV infected patients on immune boosters.

The study is going to assess how immune boosters affect the HIV status as determined by the CD4, CD8 tests and viral loads in HIV infected people. No additional blood samples will be taken from you other than those taken by the Doctor. The cost of the CD4 and CD8 counts will be paid for by the principal investigator (student).

The questionnaire you will be given is confidential and your details will not be disclosed to any other person other than the principal investigator. You will be given a confidential reference number so that you cannot be identified by your name.

The study will also entail a review of your medical records.

You can withdraw from this study at anytime. You can contact me (Principal Investigator) at anytime to discuss any issues that may be bothering you.

If you require further information or details about this study you can contact the following person:

Mrs Brenda Mkhize	Dr Lorna Madurai	Dr J K Adam
Principal Investigator	Supervisor	Supervisor
Tel: 031 308 5297	031 462 6691	031 308 5291

Thank you for your participation

Appendix 3b
(Zulu version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

ULWAZI NGOCWANINGO

Siguli esithandekayo

Ngicela ukukwazisa ngalolucwaningo oluzokwenziwa umfundi waseDurban Institute of Technology (DIT) ukuze azuze iziqu eziphakeme zemfundo.

Isihloko socwaningo siwukuhlolwa kwezinga lokushintsha kwenani leCD4 ne CD8 nenani legciwane kubantu abaphethwe igciwane lengculazi abadla imithi yokulekelela amasosha omzimba.

Lolucwaningo luzohlola ukuthi imithi yokulekelela amasosha omzimba iyishintsha kanjani impilo yomuntu ophethwe igciwane lengculazi, imiphumela yalokho ezovezwa yiCD4, CD8 nenani legciwane kubantu abaphethwe igciwane lengculazi. Alizukuthathwa igazi elingaphezu kwalelo elizothathelwa ukuhlola kukaDokotela. Ukuhlolwa kwamanani eCD4 ne CD8 kuzokhokhelwa uMcwaningi omkhulu (umfundi).

Iphepha elinemibuzo ozolinikwa liyimfihlo, imininingwane yakho ngeke idalulwe kumuntu ngaphandle komcwaningi omkhulu. Uzonikwa inombolo eyimfihlo ozokwaziwa ngayo, hhayi ngegama lakho.

Lolucwaningo luzohlola imininingwane yeziguli yakho.

Ungakhetha ukuyeka ukuzimbandakanya nalolucwaningo noma yinini uma ufuna. Ungangithinta (mina uMcwaningi oMkhulu) noma yinini uma ufuna ukucasiselwa nganoma yiluphi udaba olukuhluphayo.

Uma udinga olunye ulwazi noma incazelo ngalolucwaningo, ungathintana nalona olandelayo:

Mrs Brenda Mkhize	Dr Lorna Madurai	Dr J K Adam
Umcwaningi omkhulu	Umphathi	Umphathi
Tel: 031 308 5297	031 462 6691	031 308 5291

Siyabonga ngokuzimbandakanya kwakho

DURBAN INSTITUTE of TECHNOLOGY

Appendix 4a
(English version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

INFORMED CONSENT

STUDY TITLE: An investigation of the rate of change of CD4 and CD8 T lymphocyte counts and viral loads in HIV infected patients on immune boosters.

I ---------- (full name) have understood the details provided by the Doctor/ Nurse/Investigator about my participation in this study. I agree that the blood drawn for my routine laboratory testing be used for research purposes. I am aware that my details will be kept STRICTLY CONFIDENTIAL.

I agree that my previous results be used. Yes ☐ No ☐

I am willing to allow access to

all my medical records Yes ☐ No ☐

Under these conditions, I am willing to participate in this study and I am aware that I can withdraw at anytime.

---------- ----------
Signature of Patient Witness
Date: ---------- Date: ----------

Signature of Clinician/Nurse Date: ----------

If you require further information or details about this study, you can contact the following person:

Mrs Brenda Mkhize	Dr Lorna Madurai	Dr J K Adam
Principal Investigator	Supervisor	Supervisor
Tel: 031 308 5297	031 462 6691	031 308 5291

Thank you for your participation

DURBAN INSTITUTE of TECHNOLOGY

Appendix 4b
(Zulu version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

IMVUME ENOKUQONDA

ISIHLOKO SOCWANINGO: Ukuhlolwa kwezinga lokushintsha kwenani leCD4 ne CD8 nenani legciwane kubantu abaphethwe igciwane lengculazi abadla imithi yokulekelela amasosha omzimba.

Mina ---------- (igama eliphelele) ngiyayiqonda imininingwane engiyinikwe uDokotela/ uNesi/uMcwaningi ngokuzimbandakanya nalolucwaningo. Ngiyavuma ukuthi igazi elithathelwe ukuhlolwa okujwayelekile lisetshenziswe kulolucwaningo. Ngiyazi ukuthi imininingwane yami izogcinwa IYIMFIHLO. Ngiyavuma ukuthi imiphumela yami emidala isetshenziswe.

Yebo ☐ Cha ☐

Ngizimisele ukuvumela ukusetshenziswa

kwemininingwane yeziguli yakho Yebo ☐ Cha ☐

Kulesisimo, ngizimisele ukumbandakanywa kulolucwaningo, futhi ngiyazi ukuthi ngingakhetha ukuyeka noma yinini uma ngifuna.

---------- ----------
Ukusayina kwesiguli Ufakazi
Usuku: ---------- Usuku: ----------

Ukusayina kukaDokotela/ uNesi Usuku: ----------

Uma udinga olunye ulwazi noma incazelo ngalolucwaningo, ungathintana nalona olandelayo:

Mrs Brenda Mkhize	Dr Lorna Madurai	Dr J K Adam
Umcwaningi omkhulu	Umphathi	Umphathi
Tel: 031 308 5297	031 462 6691	031 308 5291

Siyabonga ngokuzimbandakanya kwakho

DURBAN INSTITUTE of TECHNOLOGY

Appendix 5a
(English version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

DATA COLLECTION QUESTIONNAIRE (baseline)

To be filled by the Investigator

Patient Identification number ----------
Date ----------

For statistical purposes only
To be filled by the patient

1. Are you HIV infected? Yes ☐ No ☐
2. Which year were you diagnosed with HIV? ----------
3. Are you taking any antiretroviral treatment? Yes ☐ No ☐
4. Are you taking immune boosters? Yes ☐ No ☐
5. Where do you live (area) ----------
6. What would you classify the area as? Rural ☐ Urban ☐
7. Date of birth (day/month/year) ☐ ☐ ☐
8. Gender Female ☐ Male ☐
9. Weight ----------
10. If female, how many children do you have? ----------

11. How old are the children? ----------

12. Race Black ☐ White ☐ Indian ☐ Coloured ☐

13. Are you employed? Yes ☐ No ☐

14. If yes, what is your income? ----------

15. How many members are employed in your family? ----------

16. What is your total household income? ----------

17. How has your general health been since you were diagnosed? ----------
(prompt for STIs, TB and any other indications)

18. Will you give me permission to collect your laboratory results and to evaluate these results? Yes ☐ No ☐

19. Do you want to ask any questions? Yes ☐ No ☐

20. Are you willing to allow the study team to use your blood for research purposes? Yes ☐ No ☐

21. Have you had a CD4 test done previously? Yes ☐ No ☐
If yes, what was the value? ----------

22. Have you had a viral load test done previously? Yes ☐ No ☐
If yes, what was the value? ----------

Questionnaire completed by:

Full name ----------

Designation ----------

Signature ----------

Your details will be kept strictly confidential. Your name does not appear on this form.

Thank you for your participation

DURBAN INSTITUTE of TECHNOLOGY

Appendix 5b
(Zulu version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

IMIBUZO YOKUQOQA IMININGWANE (eyokuqala)

Igcwaliswa uMcwaningi
IPatient Identification number ----------
Date ----------

For statistical purposes only
Igcwaliswa isiguli

1. Unalo igciwane lengculazi? Yebo ☐ Cha ☐
2. Waze ngamuphi unyaka ukuthi unegciwane? ----------
3. Uyayisebenzisa imishanguzo yegciwane lengculazi? Yebo ☐ Cha ☐
4. Uyayisebenzisa imithi yokulekelela amasosha omzimba? Yebo ☐ Cha ☐
5. Uhlalaphi? ----------
6. Indawo ohlala kuyo injani? Isemakhaya ☐ Isedolobheni ☐
7. Uzalwe nini? (usuku/inyanga/unyaka ☐ ☐ ☐
8. Ubulili Owesifazane ☐ Owesilisa ☐
9. Isisindo ----------

10. Uma ungowesifazane, zingaki izingane? ☐

11. Mingakanani iminyaka yezingane? ----------

12. Uhlanga Umnyama ☐ Umhlophe ☐ Uyindiya ☐ Uyikhaladi ☐

13. Uyasebenza? Yebo ☐ Cha ☐

14. Uma usebenza, uhola malini? ----------

15. Bangaki abasebenzayo ekhaya lakho? ----------

16. Ingakanani imali seyihlangene eniyithola ekhaya lakho? ----------

17. Injani impilo yakho selokhu latholakala igciwane lengculazi kuwena? ----------
(prompt for STIs, TB and any other indications)

18. Uzovuma ukuthi imiphumela yakho yegazi iqoqwe bese icutshungulwa? Yebo ☐ Cha ☐

19. Uyafuna ukubuza noma yimiphi imibuzo? Yebo ☐ Cha ☐

20. Uyavuma ukuthi ithimba labacwaningi lisebenzise igazi lakho kucwaningo labo? Yebo ☐ Cha ☐

21. Ukewahlolelwa inani le CD4 ngaphambili? Yebo ☐ Cha ☐
Uma sewake, lithini inani? ----------

22. Ukewahlolelwa inani legciwane ngaphambili? Yebo ☐ Cha ☐
Uma sewake, lithini inani ----------

Iphepha lemibuzo ligcwaliswe ngu:

Igama eliphelele ----------

Isikhundla ----------

Ukusayina ----------

Imininingwane yakho izogcinwa iyimfihlo. Igama lakho aliveli kuleliphepha.

Siyabonga ngokuzimbandakanya kwakho

Appendix 6

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

Patient ID No_____

Date _____

Schedule of Events

	Day 0	2 weeks	1month	3 month	6 month
1. Informed about Study					
2. Signing of Informed Consent Form					
3. Bloods Taken Full Blood Count CD4/CD8/CD3 count Liver Function Test Lipase Amylase Urea & Electrolytes Viral Load					
4. Clinical Assessment					
5. Issuing of Antibiotics					
6. Issuing of Referral Letters					
7. Issuing of Immune Boosters					
8. Issuing of Appointment Card					

General Comment _____
_____

DURBAN INSTITUTE of TECHNOLOGY

Appendix 7a
(English version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)
DATA COLLECTION QUESTIONNAIRE (follow-up)

To be filled by the Investigator

Patient Identification number ----------
Date ----------

For statistical purposes only
To be filled by the patient

1. Weight ----------

2. Are you taking any antiretroviral treatment? Yes ☐ No ☐

3. How has your general health been since taking immune boosters? ----------
(prompt for any indications)

4. Has your income changed since you started taking immune boosters?

Yes ☐ No ☐

If yes, indicate how ----------

5. Do you want to ask any questions? Yes ☐ No ☐

Questionnaire completed by:

Full name ----------

Designation ----------

Signature ----------

Your details will be kept strictly confidential. Your name does not appear on this form.

Thank you for your participation

DURBAN INSTITUTE of TECHNOLOGY

Appendix 7b
(Zulu version)

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)
DATA COLLECTION QUESTIONNAIRE (follow-up)

To be filled by the Investigator
Patient Identification number ----------
Date ----------

For statistical purposes only
To be filled by the patient

1. Isisindo ----------

2. Uyayithatha imishanguzo yegciwane lengculazi? Yebo ☐ Cha ☐

3. Injani impilo yakho selokhu uqale ukuthatha imithi yokulekelela amasosha omzimba? ----------
(prompt for any indications)

4. Seyishintshile yini imali oyiholayo seloku uqale ukuthatha imithi elekelela amasosha omzimba? Yebo ☐ Cha ☐
Uma ishintshile, yisho ukuthi kanjani ----------

5. Uyafuna ukubuza umbuzo? Yebo ☐ Cha ☐

Iphepha lemibuzo ligcwaliswe ngu:
Igama eliphelele ----------

Isikhundla ----------

Ukusayina ----------

Imininingwane yakho izogcinwa iyimfihlo. Igama lakho aliveli kuleliphepha
Siyabonga ngokuzimbandakanya kwakho

Appendix 8

GLOBAL CLINICAL & VIRAL LABORATORY

CLINICAL PATHOLOGY, H.I.V. TESTING, MOLECULAR TESTING

1 Jullander Place
Phunjab Circle
Merewent 4052
P.O. Box 13026, Jacobs
Jacobs 4026

Tel: (031) 462 6693
(031) 462 6691
Fax: (031) 462 6692
Emergency Tel.
082 333 8230
083 412 0932

DURBAN INSTITUTE OF TECHNOLOGY

DOCTORS DETAILS: DATE: ----------

DOCTOR: ----------

PATIENT DETAILS: LAB NUMBER:

SURNAME: ----------

NAME: ----------

REF NUMBER:

ID NO: (DOB) ----------

SEX: ----------

MARK WITH X FOR APPROPRIATE TESTING

FBC	
CD4/CD8/CD3 COUNT	
HIV VIRAL LOAD	
AST (SGOT)	
ALT (SGPT)	
OTHER	

I give consent for the above tests to be performed on myself.

DIRECTOR: W. GOPAUL

DURBAN INSTITUTE of TECHNOLOGY

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

Appendix 9

IKHAYA LOBOMI HOSPICE and CARE CENTRE: The Background

The Ikhayalobomi is the site that was used in this study for patient recruitment and data collection as indicated in the study design.

The Ikhayalobomi Hospice and Care Centre is situated in the Kwa-Nyuswa area, in the Valley of Thousand Hills, Botha's Hill, Kwa-Zulu Natal. It was founded in 2000 by Mr and Mrs Mavata. Mr Zimele Mavata is a minister in a local Christian church. Mrs Patience Mavata is a registered nurse.

The Hospice operates entirely on donations. There is no permanent funding. It offers free accommodation and immune boosting medication to HIV infected patients. It also offers free home-based care to patients and their families in the area, which covers 300 square metres, providing them with food parcels and paying school fees for children of deceased patients.

The Hospice is managed by Mrs Mavata with the assistance of volunteer home-based carers and medical professionals.

The Hospice provides a 21 bed facility as well as an outpatient facility. Medical assessment is done by a doctor, if available or by a health worker on admission of in- patients. Outpatients come to the Hospice as per their follow-up appointments or as they require to be seen by the

Doctor. If patients cannot come to the Hospice on their own, arrangements are made by the Hospice for them to be picked up.

CD4 counts, viral loads and patients' clinical presentations determine the dosages of immune boosters that will be prescribed by Mrs Mavata and antibiotics prescribed by the doctor. They are advised to take the immune boosters continuously.

Most of the patients comply with the medication. Some patients belong to support groups, others join the volunteers' team. Their families are also made to participate. This helps in improving patients' lives and also motivates the patients themselves to be positive about the programme provided by the Hospice. The families are advised on suitable diet for the patients, eg low sugar to prevent the development of oral thrush etc.

Appendix 10

To whom it may concern

We are a Pharmacuetical Manufacturing Company in South Africa
We are currently the manufacturers of:
Inochi new medicine – Nourish the Body
Inochi new medicine – Clear the Heat
For soundings

As Pharmaceutical Manufacturers we have to abide by the standards and regulations set out by the Medicines Control Council in South Africa

We are audited regularly by the MCC to ensure that we manufacture products which are of a high quality, safe and effective.

Attached is a copy of our MCC license and our GMP certificate

Kind regards

Tracy Ingle
Q..A. Manager

Impilo Drugs (1966)(Pty) Ltd • Reg No: 1966/000027/07
Directors: A.M. Tully B.Soc. Sci. - C.A. Tully B.Pharm. M.P.S. (Managing)
5 Green St, Isithebe, KwaZulu Natal • PO Box 3322, SUNDUMBILI 4491
Tel: (032) 459 1529 • Fax: (032) 459 1423

Appendix11

MEDICINES CONTROL COUNCIL

Licence number: 0000000170

LICENCE TO MANUFACTURE MEDICINES

In terms of section 22C (1) (b) of the Medicines and Related Substances Act, 1965

This licence is granted to:

License Holder
Impilo Drugs (1966) (Pty) Ltd
Site 218 & 219 – 7 & 5 Green Street Isithebe 4490

On the following terms and conditions:

The licence holder and the persons described and named in Annexure 1 shall at all times ensure that all medicines manufactured in this facility, irrespective of its registration status, comply with all the provisions of the Medicines and Related Substances Act, 1965, as amended and in particular with sections 14, 18, 18A, 18B, 18C, 19, 20, 22A, 22C, 22G, 33, Regulations 8, 9, 10, 12, 13, 37, 40, 43, 44, 45, 48 and all relevant Medicines Control Council Guidelines.

This facility is authorised to perform the manufacturing activities depicted in Annexure 1 to this licence.

REGISTRAR OF MEDICINES

ISSUE DATE: 06 July 2004

EXPIRY DATE: 06 July 2009

Reference number: B7.3 C4. 28 May 2004

I HEREBY CERTIFY THAT THIS IS A TRUE COPY OF THE ORIGINAL DOCUMENT AND THAT THERE ARE NO INDICATIONS THAT ALTERATIONS HAVE BEEN MADE THEREIN.

Post Office
POSTMASTER
2004 07 22
S.A. POST OFFICE LTD
ZIMBALI

Page 1 of 1

Appendix 12

Licence number: 0000000170

ANNEXURE 1

AUTHORISED MANUFACTURING AND MATERIAL HANDLING ACTIVITIES

	YES	NO
1. MANUFACTURING ACTIVITIES		
Sterile, Non-Biological Manufacture *(includes filling, but not cartoning* or labelling)		
Large volume parenteral products		NO
Small volume parenteral products		NO
Other sterile dosage forms		NO
Non-Sterile Manufacture		NO
Tablets	YES	
Capsules	YES	
Liquids	YES	
Semi-solids	YES	
Suppositories		NO
Other non-sterile dosage forms		NO
Biological Manufacture		
Vaccines		NO
Sera and other immunologicals		NO
Blood and other blood products		NO
Other biological products		NO
Medical Gas Manufacture		NO
Radioactive Medicines Manufacture		NO
Complementary Medicines Manufacture	YES	
2. PACKAGING ACTIVITIES		
Packaging of bulk product and labelling	YES	
Re-labelling or redressing	YES	
Cartoning or secondary packaging	YES	
3. TESTING ACTIVITIES		
Analytical	YES	
Microbiological		NO
Sterility		NO
Stability	YES	
Animal		NO
Other		NO
4. DISTRIBUTION ACTIVITIES		
Bulk distribution to wholesale pharmacies	YES	
Fine distribution to retail pharmacies and other clients	YES	
5. MATERIALS HANDLED OR STORED AT THIS SITE		
Penicillins		NO
Cephalosporins		NO
Hormones		NO
Cytostatics/Cytotoxics		NO
Bulk Pesticides, Herbicides or Rodenticides		NO
Potent Steroids		NO
Other potent, toxic, sensitising or hazardous materials		NO
6. IMPORT		NO
7. EXPORT		NO

Licence number: 0000000170

8. PARTICULARS OF THE PERSONNEL RESPONSIBLE FOR OPERATIONS ON THE PREMISES ON BEHALF OF THE LICENCE HOLDER.

Responsible Pharmacist	Production	Quality Control
Colleen Amy Tully	Rashid Joosub	Tracy Verna Ingle
B. Pharm	Dip. Pharm	B.Sc

9. PARTICULARS OF THE NATURAL PERSON RESPONSIBLE TO THE MEDICINES CONTROL COUNCIL TO ENSURE COMPLIANCE WITH THE MEDICINES AND RELATED SUBSTANCES ACT, 1965.

Responsible Person	DESIGNATION	POSTAL/ RESIDENTIAL ADDRESS
Colleen Amy Tully	Site Contact	P.O.Box 3322 Sundumbili 4491
B. Pharm		

10. LICENSE SPECIFIC CONDITIONS

1. *The holder of the licence shall conduct all manufacturing, wholesaling or distribution operations in respect of those medicines for which a registration certificate has not been obtained, so as to ensure that the medicine shall conform to the standards of quality, strength and purity applicable to them in accordance with the specification made by the person to whose order they are manufactured, wholesale or distributed or the specifications under which the medicine are sold or supplied.*

2. *Medicine for export for which a registration certificate has not been obtained from the Medicines Control Council may not be exported without the relevant "Certificate of a Pharmaceutical Product" or alternatively a "Licensing Status of a Pharmaceutical Product" issued by the Council in terms of the WHO Certification Scheme on the Quality of Pharmaceutical Products moving in International Commerce.*

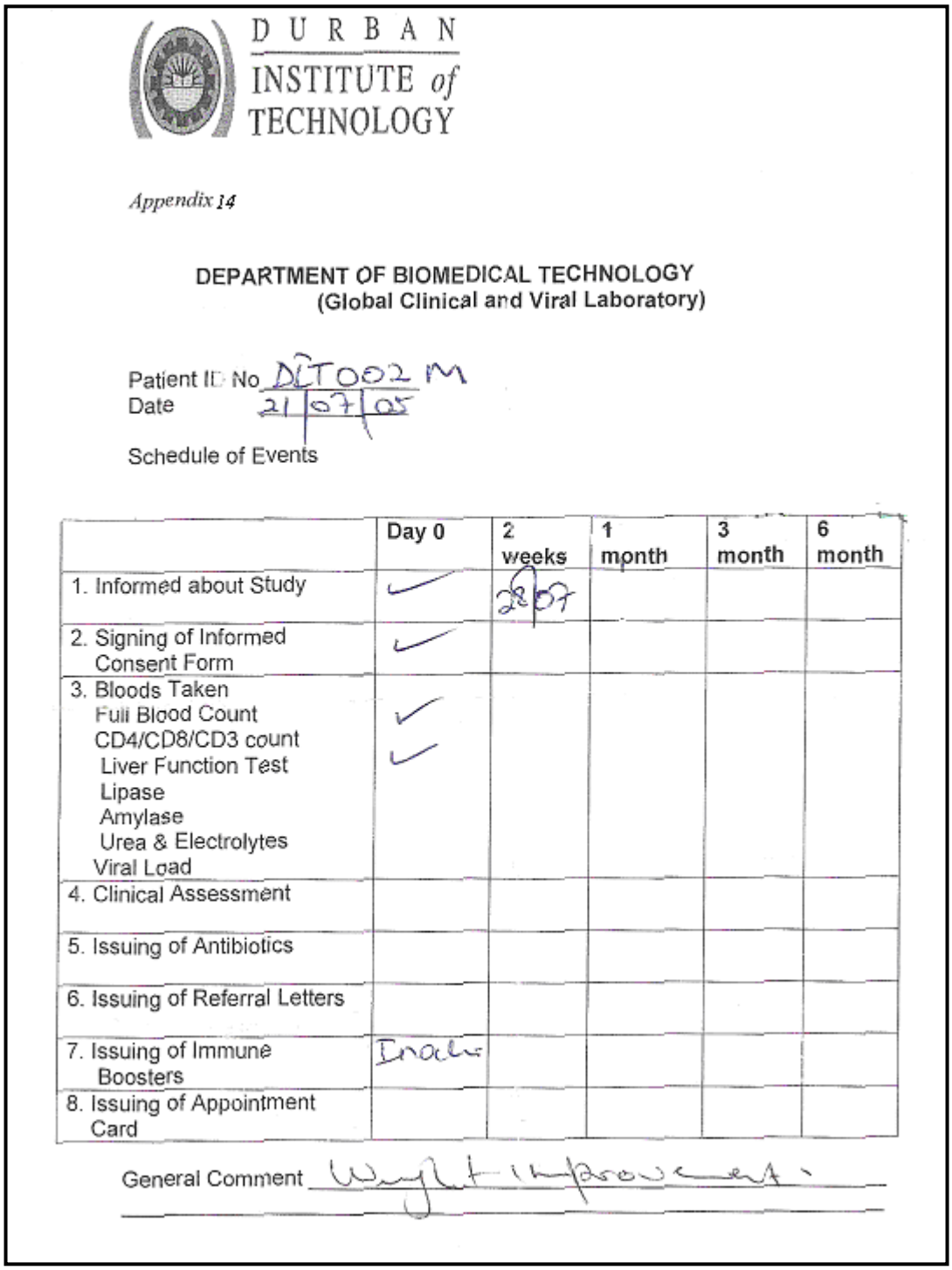
Appendix 13

SURNAME	X	FIRST NAME	X	CLINIC RES	
ADDRESS	X			TEL	
EMPLOYER					Age: 25 yrs

DATE & TIME	FINDINGS, DIAGNOSIS & TREATMENT	NAME & SIGNATURE
2. 9.	Severe diarrhea also cough OE Very much waisted young man. - Vit B co. + Doxy - Flagyl 400mg Stat 0 TDS. - Bactrim ½ POW. 800 x 2 pks.	
5/09/	Malaise: wt 45kg mouth sores Chest has slightly wheezy sounds on the Rt lung Mouth washed with Glycothymol. Daktarin oral gel.	

I.W.11

Appendix 14



DURBAN INSTITUTE of TECHNOLOGY

Appendix 14

DEPARTMENT OF BIOMEDICAL TECHNOLOGY
(Global Clinical and Viral Laboratory)

Patient ID No DLT002 M
Date 21/07/05

Schedule of Events

	Day 0	2 weeks	1 month	3 month	6 month
1. Informed about Study	✓	28/07			
2. Signing of Informed Consent Form	✓				
3. Bloods Taken Full Blood Count CD4/CD8/CD3 count Liver Function Test Lipase Amylase Urea & Electrolytes Viral Load	✓ ✓				
4. Clinical Assessment					
5. Issuing of Antibiotics					
6. Issuing of Referral Letters					
7. Issuing of Immune Boosters	Enada				
8. Issuing of Appointment Card					

General Comment Weight improvement.

Appendix 15

Global Viral & Clinical Laboratories

MultiSET™ Lab Report

Director:
Operator:

Software: MultiSET V1.1.2
Cytometer: FACSCalibur (#E14600010)

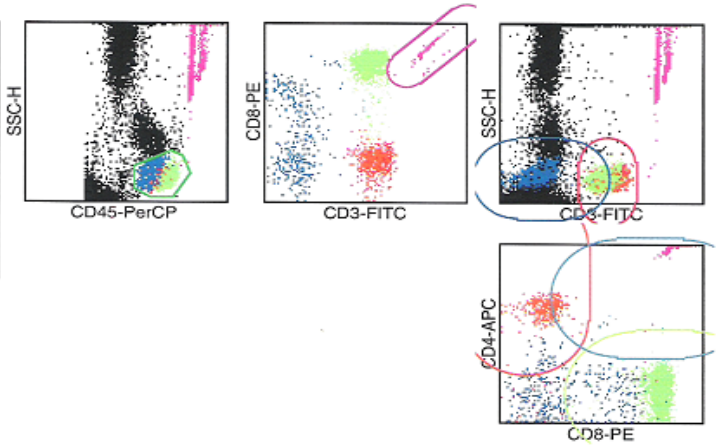
Sample Name
Sample ID
Case Number
Panel Name: Multitest CD4/CD8/CD3/CD45

Date Acquired:
Date Analyzed:
Ref. Range Type: BD

CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: C3435915.01
Reagent Lot ID: 00000 Events Acquired: 15000 Abs Cnt Bd Lot ID: 01622 Attr Def File: 3/8/45/4 MLT/TruC v2.0
Beads Per Pellet: 49889

Lymph Events	5156	
Bead Events	2591	
CD3+ %Lymph	69	
CD3+ Abs Cnt	1375	
CD3+CD8+ %Lymph	58	Hi
CD3+CD8+ Abs Cnt	1147	Hi
CD3+CD4+ %Lymph	8	Lo
CD3+CD4+ Abs Cnt	168	Lo
CD3+CD4+CD8+ %Lymph	0	
CD3+CD4+CD8+ Abs Cnt	2	
CD45+ Abs Cnt	1984	
T H/S Ratio	0.15	Lo



QC Messages:
Code 4: The CD3+CD8+ %Lymph value lies outside the normal reference range.
Code 4: The CD3+CD8+ Abs Cnt value lies outside the normal reference range.
Code 4: The CD3+CD4+ %Lymph value lies outside the normal reference range.
Code 4: The CD3+CD4+ Abs Cnt value lies outside the normal reference range.
Code 4: The T H/S Ratio value lies outside the normal reference range.

Multi-tube QC

T Helper/Suppressor Ratio: 0.15

Page 1 of 1

Appendix 16

Paired Samples Test

		Paired Differences					t
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	
Pair 1	bslincd4 - fupcd4	-18.08511	88.03528	12.84126	-43.93323	7.76302	-1.408

Paired Samples Test

		df	Sig. (2-tailed)
Pair 1	bslincd4 - fupcd4	46	.166

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	incdc4bsl	201.9286	14	131.02053	35.01671
	inccd4fup	328.7143	14	178.71181	47.76274

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	incdc4bsl & inccd4fup	14	.965	.000

Paired Samples Test

		Paired Differences					t
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	
Pair 1	incdc4bsl - inccd4fup	-126.78571	62.57202	16.72308	-162.91373	-90.65770	-7.58

Paired Samples Test

		df	Sig. (2-tailed)
Pair 1	incdc4bsl - inccd4fup	13	.000

Paired Samples Test

		Paired Differences				
					95% Confidence Interval of the Difference	
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper
Pair 1	inccd4pbsl - inccd4pfup	-3.00000	1.89737	.41404	-3.86367	-2.13633
Pair 2	deccd4pbsl - deccd4pfup	4.05556	4.24918	1.00154	1.94249	6.16862

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	inccd4pbsl - inccd4pfup	-7.246	20	.000
Pair 2	deccd4pbsl - deccd4pfup	4.049	17	.001

T-Test

[DataSet0]

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	inccd8bsl	875.3478	23	432.27018	90.13456
	inccd8fup	1438.5652	23	819.47111	170.87154
Pair 2	deccd8bsl	1162.2609	23	692.17701	144.32888
	deccd8fup	910.0870	23	554.23632	115.56626

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	inccd8bsl & inccd8fup	23	.763	.000
Pair 2	deccd8bsl & deccd8fup	23	.964	.000

Paired Samples Test

		Paired Differences					
					95% Confidence Interval of the Difference		
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t
Pair 1	inccd8bsl - inccd8fup	-563.21739	563.98073	117.59811	-807.10095	-319.33383	-4.789
Pair 2	deccd8bsl - deccd8fup	252.17391	216.02455	45.04423	158.75789	345.58993	5.598

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	inccd8pbsl	55.1154	26	15.22452	2.98577
	inccd8pfup	62.8077	26	12.61751	2.47450
Pair 2	deccd8pbsl	61.1667	18	9.95431	2.34625
	deccd8pfup	56.3889	18	9.76873	2.30251
Pair 3	inccd3bsl	1075.5455	22	475.05867	101.28285
	incrcd3fup	1770.3182	22	903.37844	192.60093
Pair 4	incvldbsl	4.2929	14	1.42260	.38021
	incvldfup	5.4929	14	.89826	.24007
Pair 5	decvldbsl	5.4286	7	.59921	.22648
	decvldfup	3.8286	7	1.75187	.66214

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	inccd8pbsl & inccd8pfup	26	.571	.002
Pair 2	deccd8pbsl & deccd8pfup	18	.840	.000
Pair 3	inccd3bsl & incrcd3fup	22	.777	.000
Pair 4	incvldbsl & incvldfup	14	.874	.000
Pair 5	decvldbsl & decvldfup	7	.534	.217

Paired Samples Test

		Paired Differences				
					95% Confidence Interval of the Difference	
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper
Pair 1	inccd8pbsl - inccd8pfup	-7.69231	13.09891	2.56891	-12.98307	-2.40154
Pair 2	deccd8pbsl - deccd8pfup	4.77778	5.57891	1.31496	2.00345	7.55210
Pair 3	inccd3bsl - incrcd3fup	-694.77273	612.29474	130.54168	-966.24901	-423.29645
Pair 4	incvldbsl - incvldfup	-1.20000	.77261	.20649	-1.64609	-.75391
Pair 5	decvldbsl - decvldfup	1.60000	1.51877	.57404	.19537	3.00463

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	inccd8pbsl - inccd8pfup	-2.994	25	.006
Pair 2	deccd8pbsl - deccd8pfup	3.633	17	.002
Pair 3	inccd3bsl - incrcd3fup	-5.322	21	.000
Pair 4	incvldbsl - incvldfup	-5.811	13	.000
Pair 5	decvldbsl - decvldfup	2.787	6	.032

T-Test

[DataSet0]

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	deccd3bsl	1489.0833	24	776.88525	158.58104
	deccd3fup	1206.7500	24	629.82518	128.56253

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	deccd3bsl & deccd3fup	24	.952	.000

Paired Samples Test

		Paired Differences					t
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	
Pair 1	deccd3bsl - deccd3fup	282.33333	261.99032	53.47855	171.70452	392.96214	5.279

Paired Samples Test

		df	Sig. (2-tailed)
Pair 1	deccd3bsl - deccd3fup	23	.000

Paired Samples Test

		df	Sig. (2-tailed)
Pair 1	inccd8bsl - inccd8fup	22	.000
Pair 2	deccd8bsl - deccd8fup	22	.000

Appendix 17

Faculty of Health Sciences
Tel: 204 2701
Fax: 204 2407

DURBAN UNIVERSITY of TECHNOLOGY

TO WHOM IT MAY CONCERN

Student Name : MRS BRENDA THABISILE MKHIZE

Student No. : 18551046

Qualification : M-TECH: BIOMEDICAL TECHNOLOGY

Date Research Proposal Approved : 07 FEBRUARY 2005

Research Title:

AN INVESTIGATION OF THE RATE OF CD4 AND CD8 T LYMPHOCYTE COUNTS AND VIRAL LOADS IN HIV INFECTED PATIENTS ON IMMUNE BOOSTERS.

The proposal meets the professional code of ethics of the Researcher

Yes ☑ No ☐

A. The proposal also meets the following ethical requirements

	YES	NO
❖ Provision has been made to obtain informed consent of the participants	✓	
❖ Potential psychological and physical risks have been considered and minimised	✓	
❖ Provision has been made to avoid undue intrusion with regard to participants and community	✓	
❖ Rights of participants will be safe-guarded in relation to:	✓	
- Measures for the protection of anonymity and the maintenance of Confidentiality.	✓	
- Access to research information and findings.	✓	
- Termination of involvement without compromise	✓	
- Misleading promises regarding benefits of the research	✓	

Signature of Student ---------- Date 07/02/05

Signature of Supervisor ---------- Date 07/02/05

Signature of Head of Department ---------- Date 7/02/05

Signature of Chairperson of the Faculty Of Health Sciences Research Committee ---------- Date 07/02/2005

Signature of Executive Dean of The Faculty Of Health Sciences ---------- Date ----------