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# Quantitative assessment of the prevalence of mitochondrial toxicity in HIV/AIDS patients initiated on a stavudine containing regimen in Mount Ayliff Hospital ARV clinic

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**2013-05-28**
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# QUANTITATIVE ASSESSMENT OF THE PREVALENCE OF MITOCHONDRIAL TOXICITY IN HIV/AIDS PATIENTS INITIATED ON A STAVUDINE CONTAINING REGIMEN IN MOUNT AYLIFF HOSPITAL ARV CLINIC

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A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in the partial fulfilment of the requirements for the Degree of Master of Science in Medicine (Pharmacotherapy)

Johannesburg, 2012

**Declaration**

I, Amanambu Ndudi Azubuike, declare that this research report is my own work. It is being submitted as partial fulfilment for the degree of MSc (Med) Pharmacotherapy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Signed on this day of 30th October 2012.

## ABSTRACT

Since the introduction of Anti-Retroviral Therapy (ART) worldwide, Human Immunodeficiency Virus (HIV)-related morbidity and mortality has reportedly improved for many People Living With HIV/AIDS (PLWHA). The treatment of HIV/AIDS (Acquired Immune Deficiency Syndrome) consists of taking a combination of three or more antiretroviral drugs (ARVs) known as Highly Active ART (HAART). Chronic administration of HAART can lead to various adverse events that include mitochondrial toxicity which not only impacts adherence to therapy, but also patient safety. Mitochondrial toxicity has been most commonly associated with stavudine (d4T). Mitochondrial toxicity may manifest as peripheral neuropathy, lactic acidosis/hyperlactatemia and lipoatrophy.

The South African ART guidelines launched in 2004 uses d4T as part of the first line HAART regimen in the management of HIV/AIDS [National Department of Health (NDoH), 2004].
This is a retrospective study of 803 enrolled HAART-naïve adult patients initiated on a d4T-containing regimen between January 2006 and June 2009. The aim of the research was to quantitatively assess for the prevalence of mitochondrial toxicity as a result of d4T use in their individual regimens.

A total of 120 (14.9%) of the study population (803) experienced peripheral neuropathy, with the majority reporting peripheral neuropathy within 6 months (median 5.9 months) after d4T initiation. Twelve patients on concurrent anti-TB drug treatment complained of increased severity of peripheral neuropathy with d4T use. The blood lactate level of 70 patients were reviewed, 18 (8.7%) of the study population had lactate levels > 2.2mmol/l. Fourteen patients (1.7%) of the study population experienced lipoatrophy. The mean duration before developing hyperlactatemia was 21.8 months (SD± 7.6) and 24.5 months (SD± 7.3) for lipoatrophy.

An awareness of the early detection of symptoms associated with mitochondrial toxicity is useful to enable optimal clinical outcomes, and support adherence and compliance to therapy. In-service training of clinicians in pharmacovigilance protocols may also be improved.
Since 2010, Tenofovir has been added to the first line regimen, and although d4t is no longer the first line ARV of choice, a large number of patients are still being maintained on a d4t-containing regimen. Finally, this research may be used in assessing the risk profile for patients being initiated on NRTI based regimens.

## ACKNOWLEDGEMENTS

I would like to acknowledge the assistance I received from my supervisors, Ms Neha Singh and Professor Michael Danckwerts, Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa throughout the period of research in always providing guidance and support.

I sincerely thank Mount Ayliff Hospital and ARV Clinic for the opportunity to conduct research and share the findings of this research to better hospital practice and quality of care to all patients accessing care at the institution.

Finally, a special thank you to my family and lovely wife for their unconditional support and encouragement.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| 3TC | Lamivudine |
| 5`NDPK | 5`nucleoside diphosphate kinase |
| ABC/ABV | Abacavir |
| ACTG | AIDS Clinical Trial Group |
| ADR | Adverse Drug Reaction |
| AIDS | Acquired Immune Deficiency Syndrome |
| AMPD | Adenosine monophosphate deaminase |
| AMPK | Adenosine monophosphate kinase (adenylate kinase) |
| APT | Adenosine phosphotransferase |
| APV | Amprenavir |
| ART | Anti-Retroviral Therapy |
| ARV | Anti-Retroviral |
| ATV | Atazanavir |
| AZT/ZDV | Zidovudine |
| bid | *bis in die* (twice daily) |
| BMI | Body Mass Index |
| CCMT | HIV/AIDS Comprehensive Care Management and Treatment Programme |
| $CO_2$ | Carbon dioxide |
| d4T | Stavudine |
| dCK | deoxycytidine kinase |
| dCMPK | deoxycytidinemonophosphate kinase |
| ddA, | 2`,3`-dideoxyadenosine |
| ddC | Zalcitabine |
| ddI | Didanosine |
| DEXA | Energy X-Ray Absorptiometry |
| DLV | Delavirdine |
| DNA | Deoxyribonucleic acid |
| DP | diphosphate |
| DRV | Darunavir |
| DSPN | Distal Sensory Polyneuropathy |
| EFV | Efavirenz |
| ENF | Enfuvirtide |
| FDA | Food and Drug Administration |
| FDC | Fixed-Dose Combination |
| FPV | Foramprenavir |
| FTC | Emtricitabine |
| gK | guanylate kinase |
| $H_2O$ | Water (aq) |
| HAART | Highly Active Anti-Retroviral Therapy |
| HIV | Human Immunodeficiency Virus |
| IAS | International AIDS Society |
| IDV | Indinavir |
| LA | Lactic Acidosis |
| LDH | Lactate dehydrogenase |
| LPV/r | Lopinavir/ritonavir |
| MCC | Medicines Control Council |
| mtDNA | Mitochondrial DNA |

| | |
|---|---|
| $NAD^+$ | oxidized counterpart of the dinucleotide NADH |
| NADH | Nicotinamide adenine dinucleotide |
| NDoH | South Africa National Department of Health |
| NFV | Nelfinavir |
| NNRTIs | Non-Nucleoside Reverse Transcriptase Inhibitors |
| NRTIs | Nucleoside Reverse Transcriptase Inhibitors |
| NtRTIs | Nucleotide Reverse Transcriptase Inhibitors |
| NVP | Nevirapine |
| PI | Protease Inhibitor |
| PLWHA | People Living With HIV/ AIDS |
| RTV | Ritonavir |
| SA | South Africa |
| SQV | Saquinavir |
| TB | Tuberculosis |
| TDF | Tenofovir |
| TDM | Therapeutic Drug Monitoring |
| TPV | Tripanavir |
| UNAIDS | Joint United Nations Programme on HIV and AIDS |
| UNICEF | United Nations Children's Fund |
| WHO | World Health Organisation |

# Chapter 1

## Mitochondrial Toxicities in HIV/AIDS Patients

HIV remains the leading cause of mortality in Sub-Saharan Africa. Until recently, Antiretroviral Therapy (ART) for the treatment of HIV/AIDS was largely inaccessible because of the high cost, inadequate infrastructure, and concerns about suboptimal adherence (Desclaux et al., 2003; Laurent et al., 2005).

UNAIDS (2010) estimates that there were 33.3 million (31.4 million–35.3 million) people living with HIV at the end of 2009 compared with 26.2 million (24.6 million– 27.8 million) in 1999- a 27% increase. With an estimated 5.6 million (5.4 million–5.8 million) people living with HIV in 2009, South Africa's epidemic remains the largest in the world [World Health Organisation (WHO), United Nations Children's Fund (UNICEF) & Joint United Nations Programme on HIV and AIDS (UNAIDS), 2010]. It is one of the few countries in the world where child and maternal mortality has risen since the 1990s (Every Death Counts Writing Group, 2008). HIV/AIDS is the largest cause of maternal mortality in South Africa and also accounts for 35% of deaths in children younger than five years (WHO, UNICEF & UNAIDS, 2010). AIDS-related deaths decreased by 18% in Southern Africa- an estimated 610 000 (530 000–700 000) people died from AIDS-related illnesses in Southern Africa in 2009, compared with 740 000 (670 000–820 000) five years earlier. This is due to the significant scale up of ART over the past few years (UNAIDS, 2010).

In 2009 alone, 1.2 million people received HIV antiretroviral therapy for the first time - an increase in the number of people receiving treatment of 30% in a single year. Overall, the number of people receiving therapy has grown 13-fold, more than five million people in low- and middle-income countries, since 2004. Expanding access to treatment has contributed to a 19% decline in deaths among people living with HIV between 2004 and 2009 (UNAIDS, 2010).

Treatment with a combination of two NRTIs and Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) or a Protease Inhibitor (PI) constitutes the HAART regime which is presently being adopted as the gold standard in the clinical management of HIV infections [WHO/UNAIDS/IAS (International AIDS Society), 2000]. The National ART programme in South Africa, based on the HAART regime as suggested by WHO/UNAIDS/IAS, was launched

in April 2004 (NDoH, 2004).

However, whilst drugs clearly provide many proven and potential benefits, any drug or medicine that is capable of producing a therapeutic or pharmacological effect can also produce unwanted adverse effects, either in isolation or in combination with other drugs or factors (Teck and Donald, 2002). A 'safe' drug is not risk-free, but "has reasonable risk given the magnitude of the benefit expected and the alternatives available" (FDA, 1999).

Due to the great morbidity associated with HIV-1, the degree of toxicity considered reasonable for HAART is higher than that for other drugs (Carr, 2002). The WHO defines an Adverse Drug Reaction (ADR) as "a response to a drug that is noxious and unintended and occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease or for modification of physiological function" (WHO, 1972).

ART toxicity is an increasingly important issue in the management of HIV-infected patients. The principal toxicities of the licensed ARV drugs (Table 1) include mitochondrial toxicity, hypersensitivity, and lipodystrophy. Mitochondrial toxicity includes peripheral neuropathy, lipoatrophy and lactic acidosis.

**Table 1:** Antiretroviral agents approved by the FDA and Medicines Control Council (MCC) for treatment of HIV infection

| **Drug Class** | **Generic Name** | **FDA Approval date** | **MCC Registration date** |
|---|---|---|---|
| NRTIs | Abacavir (ABC) | February 1999 | June 2001 |
| | Abacavir/zidovudine/lamivudine | November 2000 | October 2003 |
| | Didanosine (ddI) | October 1991 | July 1992 |
| | Emtricitabine (FTC) | July 2003 | |
| | Emtricitrabine/Tenofovir | August 2004 | May 2007 |
| | Lamivudine (3TC) | November 1995 | June 1996 |
| | Lamivudine/zidovudine | November 2000 | November 2000 |
| | Stavudine (d4T) | June 1994 | November 1998 |
| | Zalcitabine (ddC) | June 1992 | Not registered |
| | Zidovudine (AZT, ZDV) | March 198 | May 1989 |
| NtRTIs | Tenofovir (TDF) | October 2001 | May 2007 |
| NNRTIs | Delavirdine (DLV) | April 1997 | Not registered |
| | Efavirenz (EFV) | September 1998 | September 1999 |
| | Nevirapine (NVP) | June 1996 | February 1998 |
| Fusion Inhibitors | Enfuvirtide (ENF) | March 2003 | Not registered |
| | Maraviroc | August 2007 | Not registered |

| | | | |
|---|---|---|---|
| PIs | Amprenavir (APV) | April 1999 | September 2001 |
| | Atazanavir (ATV) | June 2003 | |
| | Foramprenavir (FPV) | November 2003 | Not registered |
| | Indinavir (IDV) | March 1996 | October 1996 |
| | Lopinavir/ritonavir (Lop/r) | September 2000 | August 2002 |
| | Nelfinavir (NFV) | March 1997 | October 1999 |
| | Ritonavir (RTV) | March 1996 | July 1997 |
| | Saquinavir (SQV) hard gel capsules | December 1995 | January 1997 |
| | Tripanavir (TPV) | June 2005 | Not registered |
| | Darunavir (DRV) | June 2006 | Not registered |

Source: Adapted from Bartlett et al., 2008

Two ART regimens are recommended for use in the South African public sector (NDoH, 2004). The first-line regimen is a d4T-containing regimen. Factors in considering d4T for first-line therapy include lower cost; availability in dual and triple ARV combinations; has high initial adherence rates due to good gastrointestinal tolerance; does not require administration with food or large amounts of fluid (WHO: 2009).

The recommended ART regimens are:

**Table 2:** The South African National Department of Health 2004 recommended ART regimens

| **Regimen** | **ART Regimen** |
|---|---|
| **1a** | Stavudine (d4T) 40mg (or 30mg if < 60kg) 12 hourly<br>Lamivudine (3TC) 150mg 12 hourly<br>Efavirenz (EFV) 600mg nocte |
| **1b** | Stavudine (d4T) 40mg (or 30mg if < 60kg) 12 hourly<br>Lamivudine (3TC) 150mg 12 hourly<br>Nevirapine (NVP) 200mg 12 hourly |
| **2** | Zidovudine (AZT) 300mg 12 hourly<br>Didanosine (ddI) 400mg daily (250mg daily if <60kg)<br>Lopinavir/ritonavir (LPV/r) 400/100mg 12 hourly |

D4T is used as the first-line therapy in the HAART-naïve adult patient

Mount Ayliff Anti-Retroviral Clinic, located in Mount Ayliff town, Eastern Cape Province, started in January 2006. It receives patients from surrounding public health facilities and a hospital viz, Tabankulu Health Centre, Mhlotsheni Clinic, Dundee Clinic, Lubaleko Clinic,

Mount Ayliff Gateway clinic, Ntsizwa clinic, Tela clinic and Sipetu Hospital.

A descriptive study assessing the prevalence of peripheral neuropathy, lipoatrophy and hyperlactatemia/lactic acidosis in adult HIV/AIDS patients initiated on a d4T-containing regimen was conducted for all patients enrolled in the HIV/AIDS Comprehensive Care, Management and Treatment (CCMT) Programme.

### 1.1 Aim and Objectives

The aim of this study was to determine the prevalence of mitochondrial toxicity in adult HIV/AIDS patients initiated on a d4T-containing regimens.

Due to the limited research available on the rural population of South Africa who access the public healthcare system, the impact of such toxicity on the success of the ART programme and quality of life of patients needs to be evaluated.

The objectives in this study were as follows:

1. To determine the prevalence of side-effects in patients initiated on a d4T-containing regimen and the effect of concurrent anti-TB drug use with d4T
2. To determine the severity of side-effects in patients initiated on a d4T-containing regimen
3. To determine the prevalence of the increase in severity of peripheral neuropathy with use of d4T
4. To determine the prevalence for hyperlactatemia/lactic acidosis in pregnant patients and patients who weigh > 70kg on a d4T-containing regimen
5. To determine the timeline between the initiation of d4T and the onset of clinical signs and symptoms of mitochondrial toxicity

A patient risk profile will be compiled from the data above and used to decide on what the future initiation of HAART-naïve patients should be.

### 1.2 Research Outcomes

This study hoped to create an awareness of the early detection of clinical signs and symptoms associated with mitochondrial toxicity. Early recognition and management will optimize the clinical outcomes of these patients whilst maintaining adherence and compliance to therapy and

reducing the significance of cosmetic changes if any. In-service training of clinicians in routine monitoring of patients on ART will promote safety awareness and adverse drug reporting as well as identify preventable risk factors associated with mitochondrial toxicity (Pharmacovigilance).

**Conclusion**

The high HIV-associated morbidity and mortality reported in Sub-Saharan Africa affects therapeutic and clinical outcomes. South Africa is reported to have the largest number of HIV infections worldwide and the SA government has made a concerted effort since 2004 to increase access and availability to treatment. Global access to treatment has contributed to a 19% decline in deaths among people living with HIV between 2004 and 2009. ART toxicity is an increasingly important issue in the management of HIV-infected patients and includes mitochondrial toxicity, hypersensitivity, and lipodystrophy. Research indicates that the prevalence of mitochondrial toxicity manifests as peripheral neuropathy, lipoatrophy and lactic acidosis. The prevalence of mitochondrial toxicity in the rural population at Mount Ayliff Hospital has not been investigated since the inception of the programme and the rollout of ART. The impact of such toxicity on the success of the ART programme and quality of life of these patients would assist all health professionals in supporting and managing the clinical outcomes of the programme.

# Chapter 2

## Literature Review

Toxicities related to ART make long-term adherence to therapy difficult for patients and present challenges to providers, especially those in the resource-poor setting who work with a limited formulary. Anti-Retroviral agents commonly used in resource-poor settings, such as d4T and ddI, have been associated with adverse events that are likely related to mitochondrial toxicity, whereas newer medications with fewer metabolic effects, like Tenofovir (TDF) and Abacavir (ABC), often are not available (Richard et al., 2007). Although the prevalence and severity of treatment-associated toxicities have decreased in the developed world, the incidence remains relatively high in the developing world, primarily because of the use of older antiretroviral agents (Subbaraman et al., 2007).

### 2.1 NRTI Pharmacology

After Nucleoside Reverse Transcriptase Inhibitors (NRTIs) are tri-phosphorylated intracellularly to nucleotides, they are incorporated in the growing Deoxyribonucleic Acid (DNA) chain by the viral enzyme reverse transcriptase, which prohibits further DNA elongation (NRTIs lack the critical hydroxyl group at the 3'-position of the sugar residue of the nucleotide). Other enzymes also capable of DNA formation (DNA-polymerases) can theoretically use these nucleotides as substrate and indeed, of the five known human DNA polymerases, both DNA polymerase β and γ have a high affinity for these compounds (Brinkman et al., 1998). The only exception for the inhibition of DNA polymerase γ is possibly Lamivudine (3TC), which at this moment is the only NRTI that acts as an inhibitor of the polymerase activity and concomitantly as a substrate of the integral 3`-5` exonuclease activity of this enzyme: incorporation is immediately followed by excision, enabling continuation of the DNA chain elongation (Gray et al., 1995).

The cellular activation of NRTIs produces at least two distinct sets of pharmacokinetic dispositions, one for the biologically inactive drug in plasma and the other for the active NRTI phosphate in cells (Stretcher et al., 1994). Although underlying relationships probably exist between plasma NRTI concentrations and intracellular NRTI phosphate concentrations, these relationships are currently unpredictable in patients (Hoggard et al., 2002; Anderson et al., 2003). This is likely the result of rate-limiting or saturated-phosphorylation steps and the overall biological complexity of the system. A simplified diagram of the intracellular activation of the currently available NRTIs is shown in Table 3 (Kakuda, 2000; Van Rompay et al., 2000; Stein et

al., 2001). Most of the clinical manifestations of NRTI toxicities resemble mitochondrial diseases, and histologic evidence demonstrates abnormal mitochondria and/or mitochondrial DNA (mtDNA) depletion in affected tissues (Kakuda, 2000; Dalakas et al., 2001; Shikuma et al., 2001; Bonnet et al., 2003). Studies show that NRTI triphosphates competitively inhibit mtDNA polymerase γ in vitro (Johnson et al., 2001). This, in turn, may decrease the number of mitochondrial respiratory chain proteins, inhibit aerobic respiration, induce oxidative stress, increase mutation in mtDNA, and result in mitochondrial and/or tissue failure (Lewis, 2001)

**Table 3**: Cellular chemistry of NRTIs and NtRTIs

| | | |
|---|---|---|
| **Thymidine analogs** | ZDV → ZDV MP → ZDV DP → *(Thymide kinase 1 and 2; Thymidylate kinase; 5'NDPK)*<br>d4T → d4T MP → d4T DP → | ZDV TP<br>d4T TP |
| **Cytidine analogs** | ddC → ddC MP → ddC DP →<br>FTC *(dCK; dCMPK; 5'NDPK)*<br>3TC → 3TC MP → 3TC DP → | ddC TP<br>FTC TP<br>3TC TP |
| **Adenosine Analogs** | ddI *(5'NT)* → ddl MP → ddA MP *(AMPD)* → ddA DP →<br>TDF → PMPA → PMPA MP *(AMPK)* *(Cellular Esterase)* | ddA TP<br>*5'NDPK*<br>→ PMPA DP |
| **Guanosine analogs** | ABV → ABV MP → CBV MP → CBV DP → *(APT; AMPD; gk; 5'NDPK)* | → CBV TP |

Host-cell-mediated sequential enzymatic phosphorylation steps required for activating the nucleotide- and nucleoside-analogue reverse transcriptase inhibitors (NRTIs) to the triphosphate moiety. ABV, abacavir; AMPD, adenosine monophosphate deaminase; AMPK, adenosine monophosphate kinase (adenylate kinase); APT, adenosine phosphotransferase; CBV, carbovir; dCK, deoxycytidine kinase; dCMPK, deoxycytidinemonophosphate kinase; ddA, 2`,3`-dideoxyadenosine; ddI, didanosine; DP, diphosphate; d4T, stavudine; FTC, emtricitabine; gK, guanylate kinase; MP, monophosphate; PMPA, tenofovir (PMPA DP is a triphosphate analogue); TDF, tenofovir disoproxil fumarate; TP, triphosphate; ZDV, zidovudine; 3TC, lamivudine; 5`NDPK, 5`nucleoside diphosphate kinase; 5`NT, 5`nucleotidase (Source: Anderson et al. Clinical Infectious Disease. 2004; 38:743-53)

NRTIs are important drugs in the treatment of HIV infection. They disrupt the function of the viral reverse transcriptase enzyme, which results in the premature termination of viral DNA synthesis. NRTIs do not affect cellular DNA duplication because the enzymes implicated in this process have a proofreading mechanism. However, NRTIs can affect the mitochondrial genes (Lewis et al., 1994; Nusbaum et al., 1996).

A pathway proposed to explain a link between the nucleoside analogues and toxicities such as lactic acidosis, peripheral neuropathy and lipoatrophy is mitochondrial toxicity (Cote et al., 2002). In vitro evidence suggests that d4T, ddI, and to a lesser extent AZT are inhibitors of mitochondrial DNA polymerase, whereas ABC and TDF are less potent inhibitors (Kakuda, 2000; Reiss et al., 2004). The use of d4T and ddI in combination appears to confer an especially high risk of complications (particularly peripheral neuropathy) and should be avoided (Robbins et al., 2003).

Mitochondria replicate in cells independently of cell proliferation, according to the energy needs of the cell. The high sensitivity of mitochondrial DNA to NRTIs depends on a particular DNA polymerase (DNA polymerase γ), which directs DNA mitochondrial duplication. DNA polymerase γ has no proofreading function, so NRTIs can inhibit this enzyme in a manner similar to their effect on viral reverse transcriptase. When about 70% of mitochondrial DNA is damaged, cells begin to suffer from energy deficiencies and increase their anaerobic processes (glycolysis), with the production of lactic acid (Caffrey, 2000). Clinical expression of mitochondrial toxicity is determined by the organ system affected. For example, mitochondrial toxicity in the adipose tissue promotes lipoatrophy, whereas mitochondrial toxicity in liver promotes lactic acidosis (Kakuda, 1999).

Mitochondrial toxicity may be covert or overt, which implies a threshold level of depletion for occurrence of clinical sequelae. The NRTI effect also may interact with other factors such as co-morbid diseases. There also are genetic predispositions, as found in substudy of AIDS Clinical Trial Group (ACTG) 384, in which patients received the combinations of 3TC/AZT or d4T/ddl, each combined with Nelfinavir , Efavirenz, or both (Robbins et al., 2003). The result showed a highly significant over-representation of mitochondrial haplotype T in patients who developed peripheral neuropathy compared with control subjects (23% vs. 5%; P = 0.009; odds ratio: 5.4; 95% confidence interval: 1.4-25.0), and the haplotype T was independently associated with the development of neuropathy, regardless of treatment received (Hulgan et al., 2005).

## 2.2 Peripheral Neuropathy

Peripheral neuropathy, or distal sensory polyneuropathy (DSPN), is the most common neurological problem in HIV disease. DSPN also represents a complex symptom that occurs because of peripheral nerve damage related to advanced HIV disease and in association with the use of antiretroviral therapy-particularly in individuals treated with dideoxynucleosides (Nicholas et al., 2007). Distal painful sensorimotor polyneuropathy is clinically apparent in 10-30% of patients with AIDS. Subclinical forms occur in many more patients who are HIV positive (Morgello et al., 2004). DSPN is now commonly seen as a complication of ART, and it is particularly associated with the use of d4T or ddI (Cherry et al., 2006).

From 10% to 21% of persons exposed to d4T developed peripheral neuropathy in developed countries (Scarselle et al, 2002). Although symptoms usually resolve after prompt discontinuation of d4T therapy, persistent symptoms in a subset of patients may be problematic in developing countries, where many persons rely on physical labour for survival and usually do not have disability insurance. Cohort studies from Cameroon, India, and Thailand found peripheral neuropathy rates that were similar to, or, surprisingly, lower than those in developed countries (Kumarasamy et al, 2004; Laurent et al, 2004; Pujari et al, 2004). It is hard to establish whether these lower rates reflect under ascertainment biases (e.g. short follow-up periods and insensitive screening tools).

In India, investigators reported that 13% of 183 patients receiving d4T, 3TC, and Nevirapine (NVP) who changed therapy, did so because of peripheral neuropathy, but the overall prevalence of DSPN was not reported (Kumarasamy et al., 2006).

Of 264 patients in Malawi receiving d4T, 3TC, and NVP for at least 6 months, symptoms of numbness or pain in the lower extremities was reported in 56% (van Oosterhout et al., 2005).
The authors noted that one-third of their patients had a body weight <60 kg, for which a 30mg dose of d4T is recommended; however, only the 40-mg dose was available in the fixed-dose combination (FDC) used in the study. This highlights the necessity of the availability of FDCs with varied doses to minimize toxicity, especially in malnourished patients.

In Uganda, Forna et al. (2007) reported that peripheral neuropathy was by far the most commonly reported toxicity among 1029 subjects receiving home-based ART in Tororo district, with 36% developing anti-retroviral toxic neuropathy, 9% severe.

In South Africa, there are surprisingly few data. One study reported that 6% of patients put onto a d4T-containing regimen switched due to peripheral neuropathy (Boulle et al., 2007).

In a retrospective analysis of an observational cohort conducted between March 2003 and December 2010 in Cambodia, out of 2581 adult patients initiating a d4T-containing regimen, d4T was replaced in 276 (10.7%) patients for suspected d4T related neuropathy. The main early side effect was peripheral neuropathy (7.0% by 1 year), with a cumulative incidence of 16.6% and 19.0% by 3 and 6 years respectively (Phan et al., 2012). From 2006 on, d4T was prescribed as 30mg bid (*bis in die:* twice daily), irrespective of body weight. Prior to that, dosing was done according to body weight with a higher dose (40 mg bid) for individuals with a body weight above 60 kg (WHO, 2006).

HIV suppression was found to be similar in adult patients treated in South Africa with either the 30mg or 40mg dose (Hoffmann et al., 2009); the incidence of peripheral neuropathy in adults treated in South Africa was significantly lower in the 30mg group than in the 40mg group, but the overall incidence was considered to be unacceptably high (Pahuja et al., 2010).

In a longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects, the proportion of patients diagnosed with peripheral neuropathy was significantly higher in the group receiving d4T-based therapy (17.1% vs. 11.2%; $p < 0.001$) compared to those on non-d4T based therapy. Peripheral neuropathy was reported equally in both genders (approximately 17%), with no difference in time to development between the drug groups (Menezes et al., 2011).

## 2.3 Lactic Acidosis

Lactic acidosis, a potentially fatal adverse effect of the nucleoside analogues, has emerged as an important toxicity, particularly in countries using d4T in the first-line therapy. The growing evidence that d4T is closely linked with this toxicity is consistent with prior evidence from the developed world implicating this drug (Cornejo-Juarez et al., 2003; Geddes et al., 2006).

Lactate is a normal metabolic end-product of glycolsis. It is formed when pyruvate reacts with reduced nicotinamide adenine dinucleotide (NADH) and is converted back to pyruvate by reactions with the oxidized counterpart of the dinucleotide ($NAD^+$). Both reactions are catalyzed by the ubiquitous enzyme lactate dehydrogenase (LDH). Under normal aerobic conditions, pyruvate is further metabolized in the mitochondrion by oxidative phosphorylation to $CO_2$, $H_2O$, and ATP (Figure 1). During resting conditions, but especially during exercise, skeletal muscle is the most important producer of lactate, but in normal situations the liver (~50%) and, to a lesser extent, the renal cortex (~20%) guarantee an efficient clearance of this lactate from the circulation, which leads to a stable lactate concentration of ~1 mmol/L. Persistent hyperlactatemia (measured under resting conditions), therefore, only develops when this clearance mechanism is hampered (Madias, 1986).

Increased lactate production can be induced either by anaerobic glycolysis or by a defect in the oxidative phosphorylation of peripheral tissue (e.g., muscle), but the finding of a persistently elevated lactate concentration in patients who are receiving NRTI therapy is more a reflection of an impaired hepatic lactate clearance than of an increased production. The sole pathway for lactate utilization is conversion back to pyruvate, which depends on efficient oxidative phosphorylation in the liver. An impaired lactate-clearance, therefore, can only be the result of a mitochondrial dysfunction in the hepatocytes (Fromenty et al., 1997).

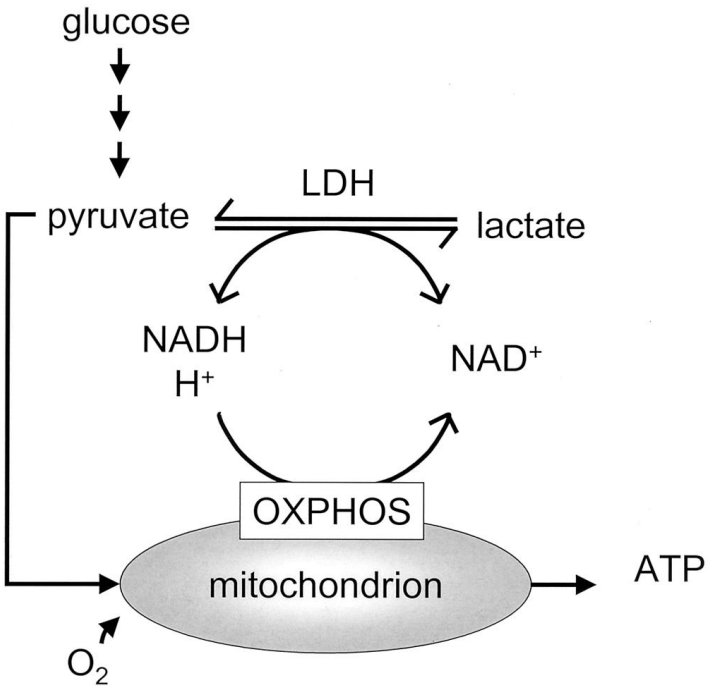



**Figure 1:** Schematic representation of the cytosolic equilibrium between the conversion of pyruvate to lactate and the reconversion of lactate to pyruvate, mediated by the enzyme lactate dehydrogenase (LDH). (Source: Brinkman K. 2000. Clinical Infectious Diseases. 31:167-9)

In South Africa, a report described the incidence of lactic acidosis in a cohort of 891 patients treated with d4T, 3TC, and EFV as being 19 cases/1000 person-years of treatment after an average of 7.5 months of receiving d4T. All cases occurred in female patients (median body weight, 81kg), and the case mortality rate was 29% (Geddes et al., 2006). Pregnancy, renal and liver dysfunction may also place patients at elevated risk of lactic acidosis (Ivers et al., 2006).
More recently, asymptomatic or mildly symptomatic hyperlactatemia without acidosis has been observed in 8–21% of persons receiving treatment with a NRTI and, less commonly, in HIV-infected persons who have never received Combined ART (John et al., 2001).

In another South African study, a significantly higher proportion of patients on stavudine-based therapy presented with symptomatic hyperlactatemia when compared to those on non-stavudine based therapy (5.75 vs. 2.2%, $p < 0.0005$), with females more frequently affected than males (7.1% vs. 2.5%; $p < 0.0001$). Although the rate of development of lactic acidosis was the same for both drug groups, it was experienced more frequently in females than males (3.3% vs. 0.8%; $p < 0.0001$), (Menezes et al., 2011).

In a cross-sectional analysis of 880 patients in a Swiss cohort, again, Stavudine treatment, especially in combination with Didanosine, was found to give an increased risk for elevated lactate compared with Zidovudine (Boubaker et al., 2001). However, when Stavudine was studied without Didanosine, the statistical significance of this finding got lost. In their cohort of 2069 patients, Moyle et al., (2001) also found the highest prevalence of hyperlactatemia in Stavudine/Didanosine-treated patients (17.1%), but in their uni- and multivariate analysis, they conclude that especially current Didanosine use is the most significant risk factor, whereas no difference in risk factors was found for current Zidovudine or Stavudine use (Moyle et al., 2001).

Phan et al., (2012), reported that in Cambodia, out of 2581 adult patients initiated on a d4T-containing regimen, d4T was replaced in 14 (0.5%) patients due to symptomatic hyperlactatemia/lactic acidosis. Symptomatic hyperlactatemia/lactic acidosis was mainly seen after the first six months but remained rare overall, with a cumulative incidence of 1% by 6 years.

### 2.4 Lipoatrophy

Lipoatrophy is characterized by a loss of subcutaneous fat in the extremities, buttocks, and face and is closely associated with the use of nucleoside analogues that cause mitochondrial toxicity, such as d4T and ddI. There is lower risk associated with AZT (Cornejo-Juarez et al., 2003).
It occurs most often in patients treated with NRTIs with the greatest propensity to inhibit mDNA polymerase γ (Mallal et al., 2000). This study provided convincing substantiation of a quantitative link between mtDNA depletion and lipoatrophy and a further link to thymidine analogue therapy. Several clinical trials in antiretroviral-naive subjects have shown that treatment with thymidine analogue NRTIs leads to more lipoatrophy compared with treatment with other NRTIs (Gallant et al., 2004; Pozniak et al., 2006) as shown in Table 4.

**Table 4:** GS 903 (Gilead 903 Study) and GS 934 (Gilead 934 Study):
Differential effect of NRTIs on total limb fat

| | **Mean Total Limb Fat, Kg (n)** | | |
|---|---|---|---|
| | **Week 48** | **Week 96** | **Week 144** |
| **Study 903** (Gallant, 2004) | | | |
| TDF + 3TC + EFV | - | 7.9* (128) | 8.6* (115) |
| d4T + 3TC + EFV | - | 5.0 (134) | 4.5 (117) |
| **Study 934** (Pozniak, 2006) | | | |
| TDF + FTC + EFV | 7.4† (51) | 8.1*‡ (49) | - |
| ZDV/3TC + EFV | 6.0† (49) | 5.5*§ | - |

*P< .001, †P= .034, ‡P= .01, §P= .001

Studies of NRTIs involving enzyme assays and cell cultures have demonstrated that the hierarchy of mDNA polymerase γ inhibition is Zalcitabine ≥ Didanosine ≥ Stavudine > Lamivudine > Zidovudine > Abacavir (Kakuda. 2000).

Consistent with this hierarchy, the NRTI, d4T has been shown clinically to place patients at a 2.5-fold higher risk of developing lipoatrophy than does AZT (Mallal et al., 2000), and, that risk increases with duration of d4T treatment, concurrent elevation of serum lactate levels, and concurrent administration of Protease Inhibitors (PIs), (John, 2000). Stavudine is also believed to cause lipoatrophy by inducing adipocyte apoptosis (Caron et al., 2003).

In the Mitochondrial Toxicity (MITOX) study, 111 patients receiving AZT or d4T- containing regimen were randomised to remain on the thymidine analogue or switch to ABC. A modest increase in the limb fat was observed in the ABC arm after 24 weeks. Limb fat continued to recover during extended follow-up, and 2 years after switching, approximately one third of limb fat loss had been recovered (Martin et al., 2004). In the Renal Atherososclerotic reVascularization Evaluation (RAVE study), 105 virologically suppressed patients receiving a d4T or AZT- containing regimen and who had clinical evidence of lipoatrophy were randomized to replace the thymidine analogue with either TDF or ABC. After 48 weeks of follow-up, limb fat measured by dual x-ray absorptiometry had increased significantly from baseline in both treatment arms and to a similar extent. Lipid changes were significantly more favorable in the TDF arm compared with the ABC arm (Moyle et al., 2006).

Several clinical trials in antiretroviral-naive subjects have shown that treatment with thymidine analogue NRTIs leads to more lipoatrophy compared with treatment with other NRTIs (Gallant et al., 2004.

In India, among 150 patients who initiated therapy with a regimen that included d4T and were followed for a mean of 19 months, investigators found lipoatrophy in 27%, compared with 10% of those initiating therapy with a regimen that included AZT (Joly et al., 2002).

A retrospective analysis of an observational cohort in Cambodia, out of 2581 adult patients initiating a d4T-containing regimen, d4T was replaced in 957 (37.1%) for lipoatrophy. After the first year, lipoatrophy became the predominant side effect, with a cumulative incidence of 56.1% and 72.4% by 3 and 6 years respectively (Phan et al., 2012).

In Rwanda, among 141 patients' (112 of whom were receiving d4T, 3TC, and NVP) body fat changes were found on physical examination in 24% (lipoatrophy 12%, mixed pattern 8%, lipohypertrophy 4%), (van Griensven et al., 2006).

In a South African cohort of 8,497 initiated on a d4T-based therapy, 7.3% presented with lipoatrophy, compared to 4.6% ($p < 0.05$) patients on non-stavudine based therapy. The median time to development of lipoatrophy was similar across the two treatment groups. Lipoatrophy was more predominantly seen in female than male patients (10.0% vs. 1.6%; $p < 0.0001$) (Menezes et al., 2011).

**Conclusion**

Stavudine has been associated with adverse events of mitochondrial toxicity and evidence suggests that it acts as an inhibitor of mitochondrial DNA polymerase, thereby increasing the susceptibility to these adverse effects. Peripheral neuropathy is reported to be the most common neurological problem in HIV disease, and is associated with d4T use. Severity of neuropathy ranges across patients with some reporting resolution after discontinuation of d4T therapy. Lactic acidosis has been most often reported in female patients and is a potential fatal adverse effect. Asymptomatic or mildly symptomatic hyperlactatemia without acidosis has been observed in patients receiving treatment with d4T. Another adverse effect with risks reported to increase with d4T use is lipoatrophy. This has shown to be related to HIV infection and use of thymidine analogues.

# Chapter 3

## Methodology

Since the inception of the Mount Ayliff Hospital ARV Clinic in January 2006 until June 2009, there were 1300 patients initiated on ART with 822 adult patients on a d4T-containing regimen. The patient population was distributed as follows:

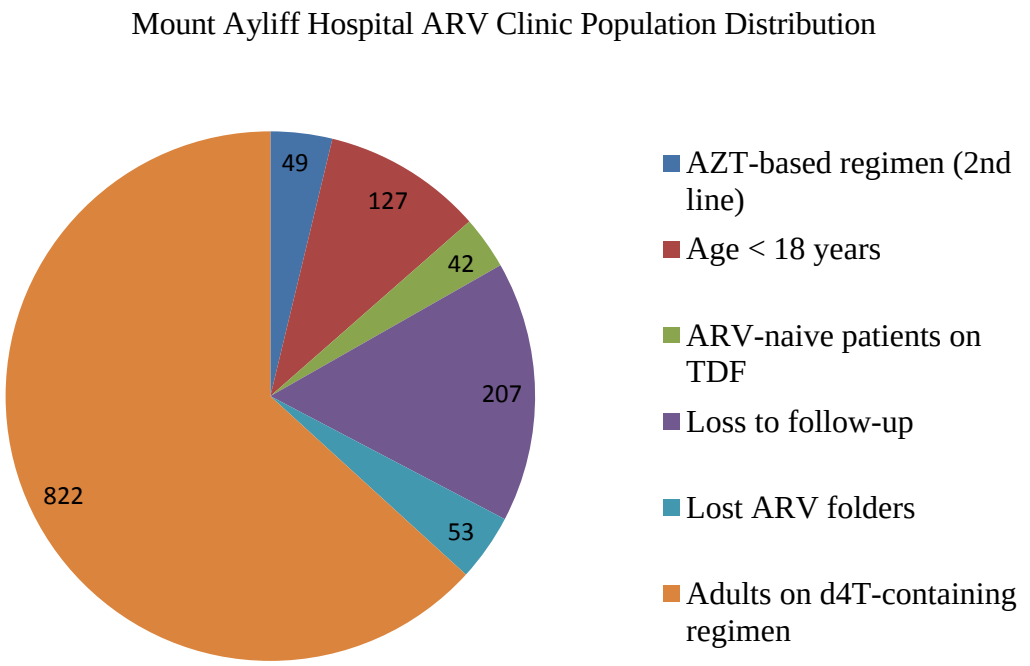



**Figure 2**: Pie chart of Mount Ayliff Hospital ARV Clinic Population Distribution

From January 2006 d4T was used as the first line therapy in the HAART-naïve adult patient (d4T 40mg or 30mg if < 60kg), (Table 1). During 2008, the use of a reduced dose of d4T (30mg), as recommended by WHO, was phased in for all patients on d4T 40mg. Also in 2008, TDF was available strictly on a named patient basis for patients with a positive Hepatitis B surface antigen result.

### 3.1 Study Population

A retrospective study of all adult patients who were initiated from January 2006 to June 2009 on a d4T-containing regimen was done. Only patients who attended the Mount Ayliff Hospital ARV Clinic and who were treatment naïve to ART before initiation of triple ART (HAART) were reviewed.

Patient screening included, patients on d4T, the concurrent administration of anti-TB drugs, pregnancy and weight >70kg. Lactic acid tests were performed for pregnant females, females with weight >70kg and patients presenting with signs and symptoms of hyperlactatemia/lactic acidosis. Lactic acid tests were not routine. In addition, the patients had to have had at least two follow-up visits after initiating HAART. The patients' history taken at each follow-up visit was assessed for the presence of:

1. Peripheral neuropathy – The study investigated whether there was presence of symptoms before initiation of ART, presence with initiation of TB treatment, after initiation of ART, increased severity with concurrent use of ART and TB treatment. The diagnosis of peripheral neuropathy was made by a clinician who determined the characteristics of the pain (burning, tingling sensation) as experienced by the patients. As specified by the South African National Antiretroviral Treatment guidelines (2004), the severity of peripheral neuropathy is classified as follows:
   a). Mild - if the discomfort is mild and no treatment is required
   b). Moderate - if the discomfort is constant, but is relieved by narcotic analgesia
   c). Severe - if the discomfort is severe and is not relieved by narcotic analgesia
2. Hyperlactatemia/Lactic acidosis – presence of symptoms (weakness, nausea, vomiting, abdominal pain etc). In asymptomatic patients, the lactic acid levels were recorded if pregnant and/or weight >70kg
3. Lipoatrophy – presence of loss of subcutaneous fat in the face and extremities. This is diagnosed by symptom and signs of loss of subcutaneous fat e.g. prominent zygomatic arch on the face etc.

Patients excluded from the study included patients on AZT and age < 18 years. Other risk factor for hyperlactatemia like- AZT, hepatitis C/ hepatitis B co-infection, liver disease, was not considered.

### 3.2 Data Collection and Analysis

Data analyzed was collected through the routine hospital monitoring system and the Pharmacovigilance form (Eastern Cape Regional Training Centre) (refer to Appendix C).

The data was analyzed for variables using a checklist (refer to Appendix A). This included age; sex; weight; concurrent use of TB treatment, pre- existing peripheral neuropathy, pregnancy and d4T-containing regimen.

Descriptive statistics was used to calculate the frequency, mean, median and standard deviation (SD) of the presence of peripheral neuropathy, lactic acidosis, lipoatrophy in patients on a d4T-containing regimen including those who are pregnant and/or weight >70kg.

The mean ± SD was used for variables that are normally distributed; and the median, interquartile range (IQR), and range, if required, was calculated for variables that were influenced by extreme values like death and hospitalization.

The Adult Clinical Record and Pharmacy Order Form (refer to Appendix B) was used to monitor the patients' progress, adherence and side-effect profiles after initiation of ART.

### 3.3 Ethical Considerations

To pursue this study, permission was sought from the management and quality assurance committee of the hospital through the medical manager after approval by the Departmental Research Committee and the University Ethics Committee (refer to Appendix F).

Patient Informed Consent form and Consent for the Use of Pharmacy Folders for Perusal are attached in the Appendices D and E respectively as approved by the managing Responsible Pharmacist at Mount Ayliff Hospital ARV Clinic.

# Chapter 4

## Results

This chapter describes how data has been analyzed and categorizes the prevalence of mitochondrial toxicity within the study population. It consists of 6 tables and figures with descriptions.

### 4.1 Demographic and weight data of patients

The folders of 822 patients on a d4T-containing regimen were screened for inclusion into the study, 803 folders were selected and reviewed. Nineteen did not return for follow- up and were excluded from the study.

Two hundred and eighteen (27.1%) of the patients were males while 585 (72.9%) were females yielding a male-female ration of 1 to 2.7.

The mean age of the patients in the sample was 38.4years, mode of 34.5years and a median age of 37.3years (interquartile range [IQR], 42.7- 57.1 years). Standard deviation (SD) ± 10years.

The mean weight of the patients was 53.6kg± 11kg. The modal weight was found to be 54.5kg and the median weight 52.8kg (IQR, 45.5- 59.3). The majority of the patients (68.8%) were in the 40-59kg weight range. Table 5 demonstrates the distribution of weight in the study population at initiation.

**Table 5:** Distribution of patients by weight

| **Weight (kg)** | **Number of patients** | **Percentage** |
|---|---|---|
| 30-39 | 57 | 7.1 |
| 40-49 | 240 | 29.9 |
| 50-59 | 312 | 38.9 |
| 60-69 | 137 | 17.0 |
| 70-79 | 34 | 4.2 |
| 80-89 | 16 | 2.0 |
| 90-99 | 7 | 0.9 |
| **TOTAL** | 803 | 100 |

### 4.2 Lipoatrophy

Lipoatrophy is the presence of loss of subcutaneous fat in the face and extremities. This is diagnosed by symptom and signs of loss of subcutaneous fat e.g. prominent zygomatic arch on the face etc. From the study population (803), the number of patients that complained of lipoatrophy and the diagnosis was made by a clinician after examination, was 14 (1.7%) with a 95% confidence interval of 0.8 -2.6%. Six of these were males (42.9%), while 8 were female (57.1%). Table 6 and Figure 3 list the number of patients complaining of symptoms of lipoatrophy after 36 months on treatment.

**Table 6:** Duration on d4T-containing regimen before complaining of Lipoatrophy

| Duration on treatment | Number of patients | Percentage |
|---|---|---|
| 1 – 6 months | 0 | 0 |
| 7 – 12 months | 1 | 0.1 |
| 13 – 18 months | 1 | 0.1 |
| 19 – 24 months | 6 | 0.8 |
| 25 – 30 months | 2 | 0.2 |
| 31 – 36 months | 4 | 0.5 |
| **TOTAL** | 14 | 1.7 |

The mean duration on treatment before diagnosis of lipoatrophy was 24.5 months 95% CI (19.3-28.9), median of 23.5 months, a mode of 21.5 months (IQR, 21- 43.25), SD± 7.3.

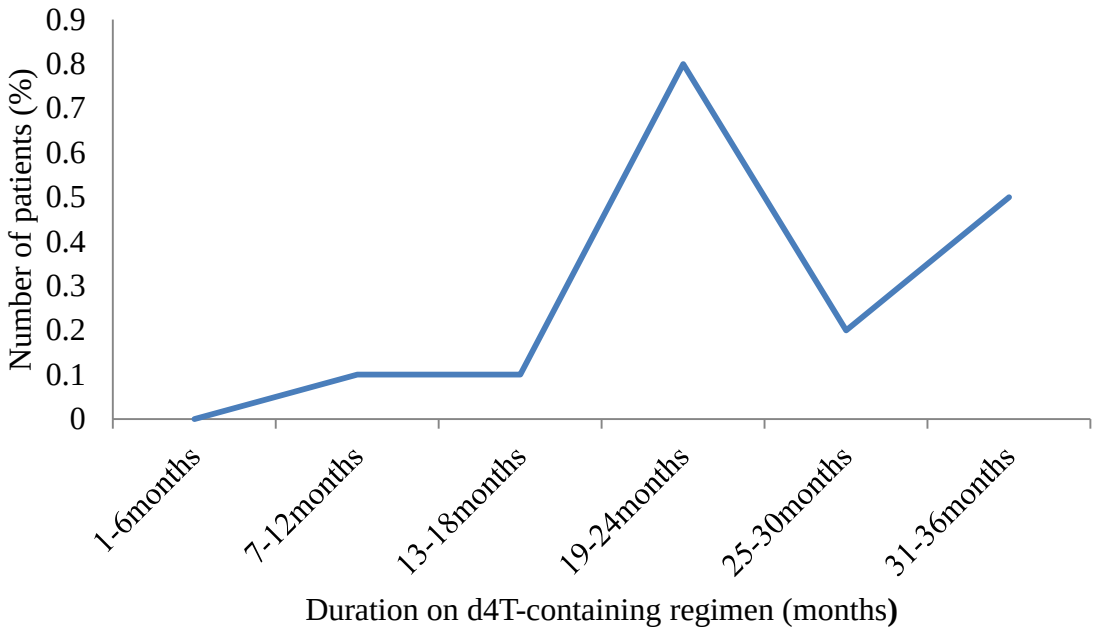



**Figure 3:** Graph of duration on d4T-containing regimen before developing lipoatrophy

As can be seen in Table 6 and Figure 3, most patients tended to develop lipoatrophy from 19-24 months. Lipoatrophy was found to be more prevalent in the female population, and to be more likely to manifest two years after being on a d4T-containing regimen.

### 4.3 Lactic Acid/Hyperlactatemia

Of the 803 patients on d4T-containing regimen, 70 were tested for hyperlactatemia/lactic acidosis; of which 8 (11.4%) was males and 62 (88.6%) was females. The normal range for venous blood lactate level is 0.5 – 2.2mmol/l. These 70 patients had symptoms of hyperlactatemia, pregnant, or weight > 70kg. 18 patients out of the 70 tested for hyperlactatemia/lactic acidosis had lactate level >2.2mmol/l. This represents 2.2% [95% CI (1.2-3.2)] of the study population (803). This is shown in Table 7.

**Table 7:** Distribution of lactate levels within study population

| | **Lactate level** | |
|---|---|---|
| | <2.2mmol/l | >2.2mmol/l |
| Male | 4 (5.7%) | 4 (5.7%) |
| Female | 48 (68.6%) | 14 (20.0%) |
| **Total** | 52 (74.3%) | 18 (27.7%) |

68.6% (48) of the women (n = 62), had lactic level <2.2mmol/l and 20% (14) had lactic level >2.2mmol/l. There were 16 pregnant women screened and all had lactic acid <2.2mmol/l. Fifty-seven patients weighed >70kg which 81.4% of those tested for hyperlactatemia/lactic acidosis. Forty four (77.2%) out of these 57 patients had lactic acid levels <2.2mmol/l while 13 (22.8%) had lactic acid level >2.2mmol/l. This showed that majority of patients who weighed more than > 70kg had lactate levels within the normal range.

The number of patients presenting with symptoms of nausea, vomiting, abdominal pain, etc was 10 (7 females and 3 males), out of which 2 (20%) had lactic acid level <2.2mmol/l and 8 (80%) had lactic acid >2.2mmol/l.

The mean duration of months before developing lactic acidosis/hyperlactatemia is 21.8 months 95% CI (18- 25.6), a median of 21.5 months and a mode of 21.5 months (IQR, 16.25- 27.5), SD±7.6. None of the patients was diagnosed with lactic acidosis.

Table 8 and Figure 4 list the number of patients who had elevated lactate level after 36 months on treatment.

**Table 8:** The duration on d4T-containing regimen before developing Lactic Acidosis /Hyperlactatemia (lactate level > 2.2mmol/l).

| Duration on Treatment | Number of patients | Percentage |
|---|---|---|
| 1 -6 months | 0 | 0 |
| 7 – 12 months | 2 | 0.2 |
| 13 – 18 months | 4 | 0.5 |
| 19 – 24 months | 6 | 0.7 |
| 25 – 30 months | 3 | 0.4 |
| 31 – 36 months | 3 | 0.4 |
| **Total** | 18 | 2.2 |

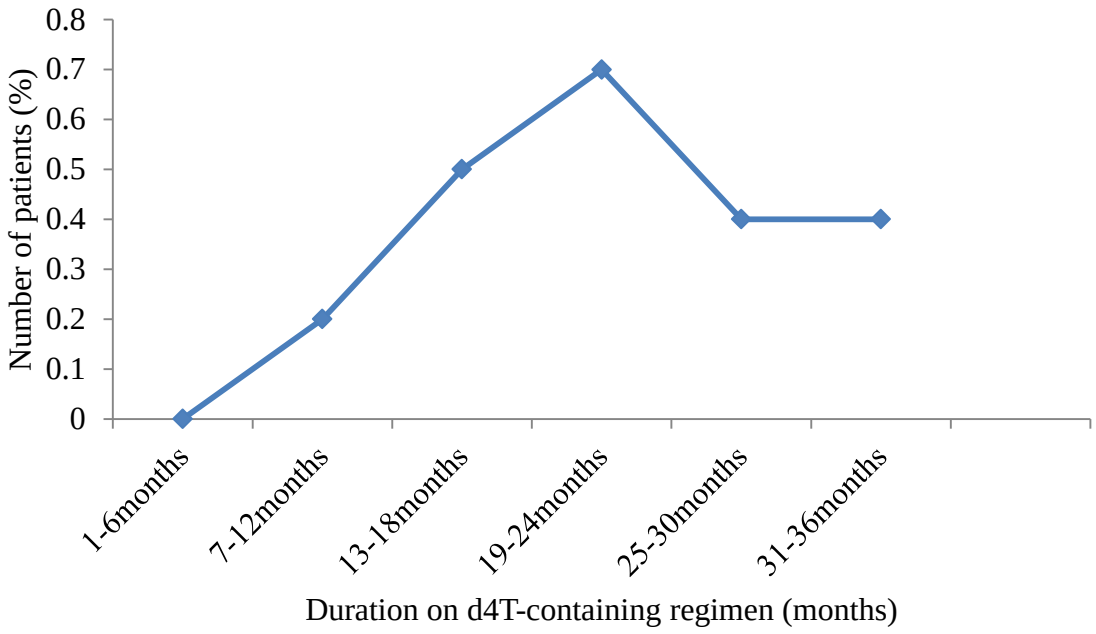



**Figure 4:** The duration on d4T-containing regimen before developing Lactic Acidosis /Hyperlactatemia (lactate level >2.2mmol/l

The risk of increased lactic acid levels appeared to be more prevalent 18-24 months after being initiated on a d4T-containing regimen.

### 4.4 Peripheral Neuropathy

Out of the 803 patients initiated on a d4T-containing regimen, 303 (37.7%) complained of peripheral neuropathy before initiation of ARVs, 120 (14.9%) 95% CI (12.4- 17.4) complained of peripheral neuropathy after initiation of ARVs and 16 (2.0%) with no prior peripheral neuropathy, complained of peripheral neuropathy after initiation of TB treatment. Table 9 and Figure 5 show the prevalence of peripheral neuropathy.

**Table 9:** Distribution of the presence of Peripheral Neuropathy

| Complaints | Number of patients | Percentage (%) |
|---|---|---|
| No peripheral neuropathy reported | 364 | 45.4 |
| Peripheral neuropathy before initiating ARV | 303 | 37.7 |
| Peripheral neuropathy after initiating ARV | 120 | 14.9 |
| Peripheral neuropathy after starting TB treatment | 16 | 2.0 |

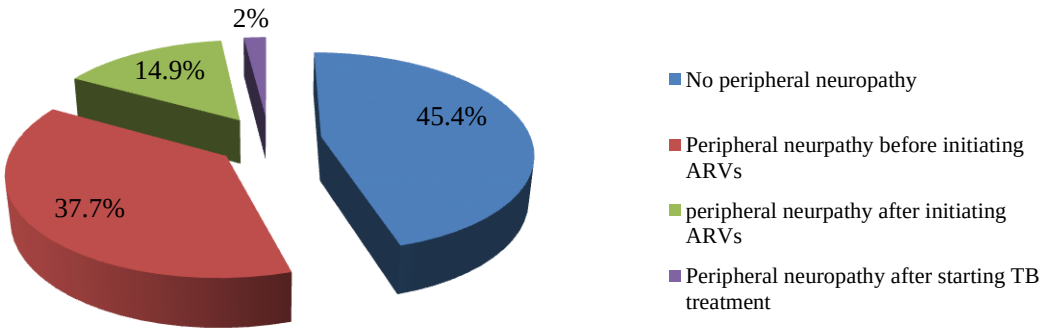



**Figure 5:** Pie chart of patients presenting with Peripheral Neuropathy

Of the 303 patients who complained for peripheral neuropathy before initiation of ARVs, 47 (15.5%) of them complained of increase in severity in peripheral neuropathy with use of ARVs and 256 (84.5%) had no increase in severity with use of ARVs. The effect of concurrent use of anti- TB drugs on peripheral neuropathy was evaluated. The number of patients who were on ARVs and anti- TB drugs was one hundred and fifty-nine. Twelve (7.5%) had an increase in the severity of peripheral neuropathy while 147 (92.5%) had no increased effect.

Table 10 shows the effects of ART on existing peripheral neuropathy prior to ART initiation.

**Table 10:** Effects of d4T on existing Peripheral Neuropathy

| Severity of Peripheral neuropathy | Number of patients |
|---|---|
| Increase in severity | 47 |
| No increase in severity | 256 |
| **TOTAL** | 303 |

The presence of peripheral neuropathy after the initiation of ARVs was seen in 120 patients. The severity was classified into mild (if the discomfort is on/off and no treatment is required), moderate (if the discomfort is constant, but is relieved by narcotic analgesia), and severe (if the discomfort is severe and is not relieved by narcotic analgesia). This distribution is shown in Figure 6. The majority of these patients 73 (60.8%) complained of mild peripheral neuropathy, 37 (30.8%) complained of moderate symptoms and 10 (8.4%) with severe peripheral neuropathy.

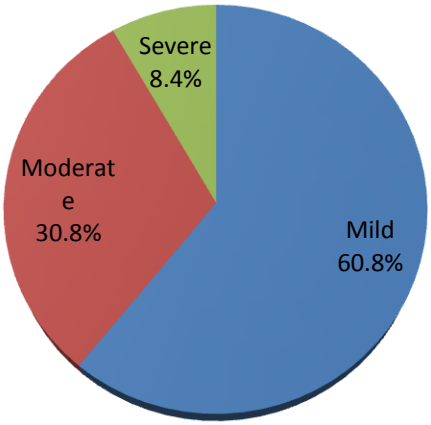



**Figure 6:** Pie chart showing the severity of Peripheral Neuropathy after starting d4T

Of the 120 patients who presented with peripheral neuropathy after initiation of d4T, 67 presented with peripheral neuropathy within the first six months on therapy while 25 presented with peripheral neuropathy within seven to twelve months on treatment. The mean of the duration on ARVs before the complaint of peripheral neuropathy is 9 months 95% CI (8.4- 10.4), the median of 5.9 months and mode of 3.5 months, (IQR, 3.2- 12), SD±8.

The duration on a d4T-containing regimen before presenting with Peripheral Neuropathy is shown below in Figure 7. Peripheral Neuropathy presented within the first six months on treatment for patients initiated on a d4t-containing regimen, and diminished with time.

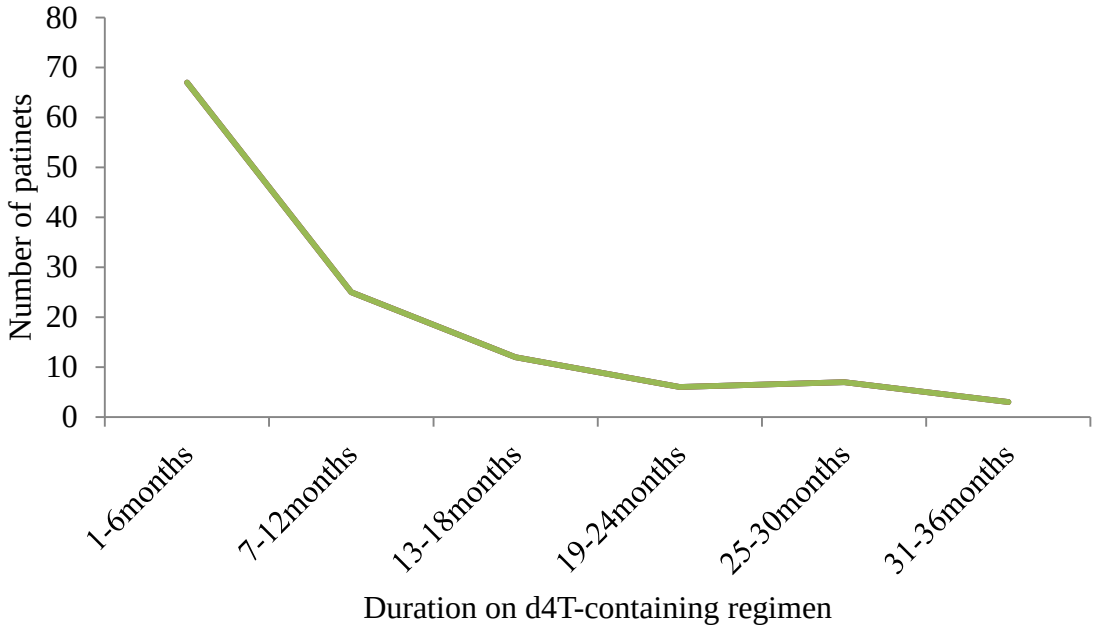



**Figure 7:** Duration on a d4T-containing regimen before presenting with Peripheral Neuropathy

Figure 8 represents a combination of the signs and symptoms of mitochondrial toxicity as experienced by the patients. The trend for lactic acidosis /hyperlactatemia and lipoatrophy manifestations appeared to increase with time.

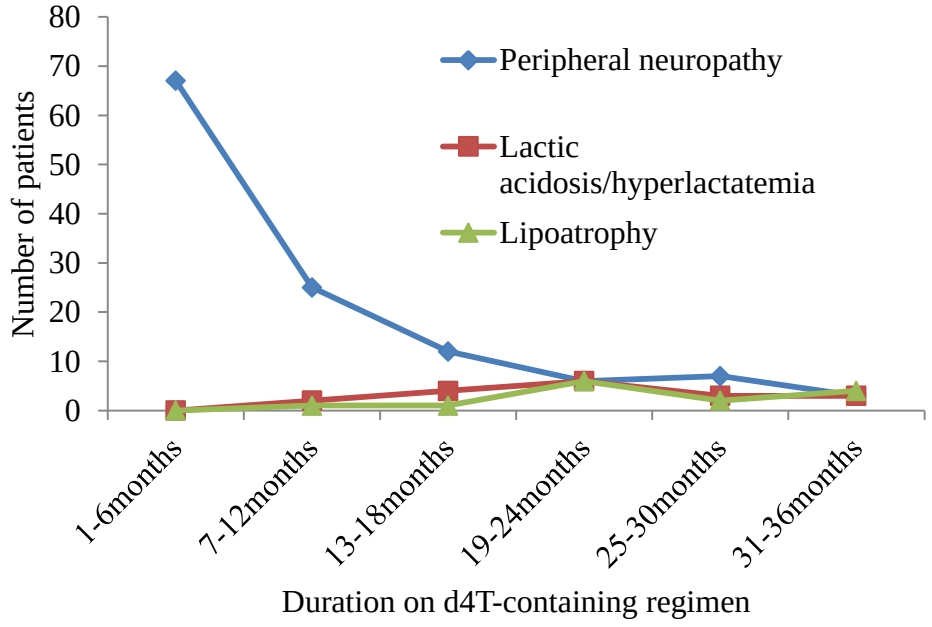



**Figure 8:** Duration on d4T-containing regimen before presenting with Peripheral Neuropathy, Lipoatrophy and Lactic Acidosis/Hyperlactatemia

**Conclusion**

The results of the 803 patients reviewed show the majority of the patients are female with a mean weight of 53.6kg. This means that a majority of the patients would have being taking 30mg of d4T. 1.7% of these patients experienced lipoatrophy 19-24 months after starting a d4T-containing regimen. Hyperlactatemia was seen in 18 (2.2%) of the study population. The pregnant female patients had a normal lactate level (0.5 – 2.2mmol/l) which differed from the literature which demonstrated pregnancy as a risk factor for hyperlactatemia. This may be due to the ddI/d4T combination regimens used in pregnant patients as reported in the literature. 14.9% of the patients presented with peripheral neuropathy after d4T initiation, of which 8.4% was severe in nature. Collectively the data showed that peripheral neuropathy occurred mostly in the first six months of treatment and the incidence declined as treatment progressed. Hyperlactatemia and lipoatrophy tend to occur after about 12 months after initiation as shown in Figure 8.

# Chapter 5
## Discussion

In this chapter, a summary of the study is provided. The patient profile and prevalence of mitochondrial toxicity in the study population is discussed and compared with the literature.

### 5.1 Population Profile

Mount Ayliff Antiretroviral clinic started initiating patients on ART in January 2006. In line with the WHO recommendations, the South African National Antiretroviral treatment guidelines, first edition 2004, indicated the use d4T 40mg every 12 hours (30mg every 12 hours if body weight<60 kg) in adults and adolescents. A total of 822 adult patients on a d4T-containing regimen, 803 were selected and reviewed for this study.

The mean weight of this population was 53kg. 609 (75.8%) of the study population had weight between 30- 59 kg. These patients were started on a d4T dose of 30mg. A meta-analysis from randomised control trials and cohort studies showed that switching from higher to lower doses of d4T, or starting at lower doses, is associated with improvement in d4T toxicity without loss of efficacy (Hill et al., 2007).

Clinical experience with d4T has been extensive. It has a favourable resistance profile, good short-term tolerability, does not require dietary restrictions and, due to its relatively low cost, is still widely used. However, d4T has been associated with a higher risk of mitochondrial DNA depletion and adipocyte metabolic dysfunction compared with other nucleoside with other NRTIs (Nolan et al., 2003) which may contribute to the pathogenesis of subcutaneous fat wasting, and the development of lipoatrophy and metabolic complication (Dube et al., 2005).

The following two main approaches have been used to reduce the toxic effects of d4T: replacement of d4T by potentially less toxic agents, usually Tenofovir (Domingo et al., 2004; Moyle et al., 2006) or Abacavir (Carr et al., 2002; Martin et al., 2004); or more recently a d4T dose reduction (Martin et al., 2004; Moyle et al., 2006). Results obtained mainly from cohort studies, show that a d4T dose reduction strategy may lead to a modest reversal of side-effects (Sanchez-Conde et al., 2005).

Switching to lower doses of d4T was associated with decreased drug exposure, mitochondrial DNA repletion, partial reversal of lipoatrophy, improvement in lactate and lipids (Hill et al., 2007; McComsey et al., 2008; Ait-Mohand et al., 2008).

Out of 2190 adults (median follow-up: 1.5 years) in Rwanda, d4T was replaced in 175 patients (8.0%) for neuropathy, 69 (3.1%) for lactic acidosis and 157 (7.2%) for lipoatrophy, which was the most frequent toxicity by 3 years of antiretroviral treatment (ART). Use of d4T 40 mg was associated with increased risk of lipoatrophy and early (<6 months) neuropathy (van Griensven et al., 2009).

It seems that d4T toxicity is cumulative, as it is shown to be dose related and is associated with prolonged duration on treatment (Hill et al., 2007). Following a meta-analysis showing lower doses were safer and as effective, WHO issued a statement that only low dose d4T (30 mg) should be used (Hill et al, 2007). In compliance with this finding, the Eastern Cape province recommended that all patients on d4T 40mg be changed to d4T 30mg and all new patients be initiated on d4T 30mg irrespective of weight in 2008. The 40mg d4T was gradually phased out.

**5.2 Lipoatrophy**

The result of this study showed that d4T- associated lipoatrophy was seen in 1.7% of patients. This was less than observed by Joly et al, 2002; Pujari et al, 2005. In a South African cohort of forty seven participants, 34% had lipoatrophy (Sinxadi et al., 2010). A meta-analysis from randomised control trials and cohort studies showed that switching from higher to lower doses of d4T, or starting at lower doses, is associated with improvement in d4T toxicity without loss of efficacy (Hill et al.,2007). Switching to lower doses of d4T was associated with decreased drug exposure, mitochondrial DNA repletion, partial reversal of lipoatrophy, improvement in lactate and lipids (Hill et al, 2007; McComsey et al, 2008; Ait-Mohand et al, 2008).

The patients at Mount Ayliff Antiretroviral clinic were on 30mg d4T irrespective of the weight. This might explain the lower number of lipoatrophy that was reported in this study when compared to other studies.

Benefits of a d4T dose reduction and /or switching to TDF were also observed by total body Dual- Energy X-Ray Absorptiometry (DEXA) scan, showing a median increase in peripheral and total fat compared with baseline values. Although the median increase was higher in the d4T

(30mg) arm compared with the TDF arm, only those changes in the TDF arm reached statistical significance when compared with the d4T (40mg) arm. Values in subcutaneous fat continued to decrease in the d4T (40mg) arm, compared with baseline, but did not reach statistical significance (Milinkovic et al., 2007).

The effect of reduced d4T dosage on lipoatrophy and viral failure was evaluated in an observational cohort of 80 patients in Thailand (Hanvanich et al., 2003). Before reducing the dose of d4T all patients developed lipoatrophy. In patients weighing ≥ 60 kg the d4T dose was reduced to 30 mg 12 hourly and in patients weighing < 60 kg the d4T dose was reduced to 20 mg 12 hourly. After 93 weeks, all patients maintained a plasma viral load of < 50 copies/mL. All patients had an improvement in lipoatrophy with the d4T dose reduction. The improvement was much faster for mild lipoatrophy cases than for moderate and severe lipoatrophy cases.

The data observed at Mount Ayliff demonstrates that lipoatrophy did not often present in the first six months after d4T initiation. The median duration of d4T exposure is 23.5 months. This is higher than reported by Sinxadi et al (2010) which reported a median of 14.5 months. According to Urbina et al. (2005), 71% of patients taking low dose d4T were free of lipodystrophy after 5 years on treatment. This showed that long term use of low dose d4T is well tolerated.

In our study at Mount Ayliff Antiretroviral Clinic more women were diagnosed with lipoatrophy than men, further studies can be conducted to assess the trend of lipoatrophy amongst men and women. The reason for variation in lipoatrophy rates found in this study compared to others could be due to varying diagnostic criteria.

**5.3 Lactic acid/Hyperlactatemia**

8.7% (70) of the study population met the criteria for lactate evaluation and were tested for hyperlactatemia/lactic acidosis. Eighteen of these patients had a lactic acid level > 2.2 mmol/l. This is less than reported by Bolhear et al, 2007. The high incidence of lactate in their study may be due to the use 40mg d4T and the concomitant use of d4T and ddI. This possibility is supported by observations that patients on higher dose stavudine (40 mg twice daily) have a higher incidence of elevated lactate than those who receive lower doses (20 or 30 mg twice daily) (van Griensven et al., 2009).

In Botswana ARV related toxicities were assessed in 650 patients, who were required to be on ARVs for ≥ 2 years at the start of the study. There were 19 events in which patients had a serum lactate > 4.4 mmol/L, 15 of which were moderate or worse in severity. These episodes occurred in females who were on combined Antiretroviral Therapy for a mean of 10.2 months. Twelve of these 15 patients were on d4T. There were seven episodes of lactic acidosis. All seven patients were receiving d4T and/or ddI containing therapy (Wester et al. 2007).

This was different from our clinic where all patients received 30mg d4T irrespective of the weight, and concomitant use of d4T and ddI were not used as part of the recommended treatment guidelines. This was consistent with findings of another study conducted in Spain which reported that d4T- low doses resulted in a significantly better outcome in terms of efficacy and toxicity profile than d4T-standard doses (Ribera et al., 2005).

Gerard and colleagues described 14 cases of hyperlactatemia in a cohort of 871 patients; the incidence was 0.8% per year which rose to 1.2% per year if only patients on d4T-containing regimens were included (Gerard et al., 2000).

None of the pregnant women (n=16) in our clinic study had elevated lactate levels. There was no confirmed case of lactic acidosis, most likely due to the absence of laboratory results for serum bicarbonate level and arterial pH. These two results are important to diagnose lactic acidosis. 8 (1%) of the 70 patients had symptomatic hyperlactatemia.

A Spanish study concluded that symptomatic hyperlactatemia was reported in 0.2 to 2.5% of infected adults and the syndrome of lactic acidosis /hepatic steatosis was rare (Falco et al 2002).
Another study conducted in Botswana reported that approximately 1% of individuals starting antiretroviral therapy with a d4T-containing regimen developed lactic acidosis (Wester et al, 2007). Carter (2007) commented that the incidence of lactic acidosis found in Botswana was significantly higher than that seen in industrialized countries. The findings in this study are contradictory with the following findings in studies conducted in Europe (Gerard, 2000; Vrouenraets, 2002; Falco, 2002).
These complaints were more likely to be reported by females, and it appears that females are more likely to be pre-disposed to lactic acid level > 2.2 mmol/l than males.

**5.4 Peripheral Neuropathy**

Peripheral neuropathy, or DSPN is the most common neurological problem in HIV disease (Nicholas et al., 2007) and is a well described side-effect of treatment with d4T (Browne et al., 1993; Skowron et al., 1995) and other antiretroviral drugs (Carr et al., 2000).

It is also the most frequent side-effect reported in our clinic. 14.9% of patients complained of peripheral neuropathy after initiation with d4T which is less than reported by Forna et al. (2007); Cherry et al. (2009); but in line with Kumarasamy et al. (2006); and Scarselle et al. (2002). According to Markison et al (2008) in resource limited settings, results shows that first line ARV containing d4T was used for clinically eligible patients. 13% of patients were diagnosed with peripheral neuropathy and 6% switched to AZT as it is used as a substitute for d4T.

The lower prevalence of peripheral neuropathy in our patients compared to those reported by studies in Malawi and Uganda may be due to the use of lower dose d4T (30mg). Peripheral neuropathy occurred after a median of 5.9 months, which is longer than previously described by Simpson and Tagliati (1995). 8.4% of the peripheral neuropathy were severe (if the discomfort is severe and is not relieved by narcotic analgesia) which is similar to that reported by Forna et al. (2007); van Griensven et al. (2009).

In our cohort, HIV-associated peripheral neuropathy was reported by 37.7% (303) of patients. This was similar to that observed by Morgello et al. (2004). Symptoms of peripheral neuropathy reported by patients, included numbness/tingling of extremities, varied levels of discomfort and some pain.

It is important to communicate to patients that they may also experience drug-induced peripheral neuropathy which may exacerbate the condition. In those patients with pre- existing peripheral neuropathy, 15.5% reported worsening of peripheral neuropathy with initiation of d4T. The high number of patients who report peripheral neuropathy prior to ART initiation also infers that the prevalence of peripheral neuropathy in patients may be much higher and may have an impact on patient clinical outcomes and adherence to HAART.

Concomitant administration of TB treatment and HAART is common in sub-Saharan Africa, especially at HAART initiation. Peripheral neuropathy is caused by both Isoniazid (Subbaraman et al., 2007) and d4T (Breen et al., 2000; Dean et al., 2002; Amoroso et al., 2007; Boulle et at.,

2007; Hawkins et al., 2007), through different mechanisms; use of both drugs may lead to an additive or cumulative effect and increase the severity of symptoms.

7.5% (12) of patients on concomitant administration Isoniazid and d4T complained of increase in severity of peripheral neuropathy. These results may understate the true impact of concomitant d4T and TB treatment because the great majority of patients with TB in South Africa are prescribed vitamin B6 (pyridoxine) at the time of initiation of TB treatment for the prevention of peripheral neuropathy, and in addition, Amitriptyline is frequently prescribed to manage incident peripheral neuropathy. It is possible that the effect of TB treatment on the risk of d4T substitution would be even higher in settings where these drugs were not routinely used; conversely, rates of peripheral neuropathy may be further reduced with additional micronutrient supplementation (Villamor et al., 2008).

Close to 2% reported peripheral neuropathy after starting TB treatment; however the study did not ascertain in total how many patients were tested and treated for TB, but merely the number who reported. This might be explained by the fact that the National TB Programme was not integrated into the ART programme. Peripheral neuropathy has no permanent cure, and is symptomatically managed using pyridoxine, tri-cyclic antidepressants and Non-Steroidal Anti-Inflammatory Drugs (NSAIDS). Reporting of peripheral neuropathy is erratic and as patients adapt to it, it is not commonly reported. Co-infected HIV and TB patients must be routinely screened and managed for peripheral neuropathy. The subjective measurement of peripheral neuropathy severity is not reliable as tolerance and pain levels are different for any individual. The majority of patients reported no increase in severity of pre- existing peripheral neuropathy after initiation.

# Chapter 6

## Conclusion and Recommendations

The study has shown that mitochondrial toxicity is prevalent in adult HIV/AIDS patients initiated on a d4T-containing regimen at Mount Ayliff Antiretroviral Clinic. A retrospective study conducted using 803 patients' records were reviewed, of which 1.7% had lipoatrophy, 2.2% hyperlactatemia and 14.9% peripheral neuropathy.

Peripheral neuropathy was the side-effect most prevalent amongst the study population across both genders. Hyperlactatemia, though not actively observed, must be screened for regularly especially in pregnancy and weight >70kg which are high risk factors for lactic acidosis. Lipoatrophy was found to be most likely prevalent amongst females initiated on d4T.

The prevalence rates of mitochondrial toxicity appeared to be much lower than other studies, and this lower prevalence may be due to the use of d4T 30mg dose in all patients initiated on HAART and the non-concomitant use of d4T and ddI in therapy. WHO recommended the use of lower dose d4T because of the evidence from several studies that showed that lower dose d4T had a better side-effect profile.

The study showed that peripheral neuropathy had an early onset, within the first 6 months on therapy, which could be exacerbated by concurrent administration of anti-TB drugs. Hyperlactatemia and lipoatrophy manifested with long-term d4T use.

The importance of screening for mitochondrial toxicity in patients on d4T-containing regimens, and the early detection of possible risk factors for mitochondrial toxicity as a routine clinical assessment, may work towards improving clinical outcomes, adherence to therapy and quality of life for patients on therapy.

A patient risk profile compiled from ART preparation to initiation and continued throughout therapy would be useful in creating individual patient care plans and side-effect profiles and as tools for managed care, pharmacovigilance and in-service mentoring. This would also be useful as an indicator in the HIV/AIDS CCMT programme outcomes for many patients initiated who transfer, default or are a loss to follow-up due to the debilitating effects of mitochondrial toxicity.

Newer drugs with safer side-effect profiles, like Tenofovir, means that the use of d4T will decrease, however stable patients on d4T will need to be monitored regularly. Since 2010, Tenofovir has been added to the first line regimen, and although d4t is no longer the first line ARV of choice, a large number of patients are still being maintained on it. Continuous follow- up of these patients is important for detection of symptoms and management of mitochondrial toxicity.

Following this study, the proposed recommendations for best practice are:

1. Individual side-effect profiles for all patients should be routinely updated and monitored for changes in severity of side-effects experienced, frequency and treatment management
2. Establishment of a functional Pharmacovigilance committee within the ARV Clinic to promote safety awareness and side-effect and ADR reporting
3. Implementation of screening protocols to assist health care workers in health promotion activities and patient education of HAART
4. Implementation of revised standard treatment guidelines of newer drugs with safer side-effect profiles
5. On-going in-service education and clinical mentoring in HIV management

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**Appendices**

A. Patient Checklist for inclusion into study

B. Adult Clinical Record

C. Adverse drug reaction and product quality problem report form: HIV/AIDS Treatment Programme

D. Patient Informed Consent Form

E. Consent for the Use of Pharmacy Folders for Perusal

F. Ethical Clearance form

Appendix A. Patient Checklist for inclusion into study

# MOUNT AYLIFF HOSPITAL

Batho Pele /Abantu Kuqala/People First

ISEBE LEZEMPILO DEPARTMENT OF HEALTH

*Ingxowa Eyodwa/Private Bag X 504, MOUNT AYLIFF 4735*

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ARV CLINIC

CHECKLIST FOR INCLUSION INTO STUDY

ARV Clinic number:

Date of ART initiation:

CD4 at treatment initiation:

Regimen:

Age:

Sex:

Weight at initiation:

BMI at initiation:

Pregnancy:

Concurrent use of TB treatment:

Pre-existing peripheral neuropathy or DSN:

Lactate level >2mmol/l:

Loss of subcutaneous fat:

Appendix B. Adult Clinical Record

**MOUNT AYLIFF HOSPITAL ARV CLINIC**
**ADULT CLINICAL RECORD**

**HOSPITAL/CLINIC:**
**PATIENT NO. :**

| | |
|---|---|
| **NAME** | |
| **DATE OF BIRTH** | |
| **ID NUMBER** | |
| **ADDRESS**<br><br>**CONTACT NUMBER (INCLUDE SUPPORTER)** | |

| | |
|---|---|
| **GENDER ☐ M ☐ F** | **HEIGHT (in cm)** |
| **ALLERGY (ies)** | |
| **DATE OF FIRST HIV TEST** | |
| **WHO stage** (at enrolment) | |

| |
|---|
| **Past Medical and Drug History –** concurrent illness and medications |

| ***REASON FOR CHANGING MEDICATION*** | | | |
|---|---|---|---|
| 1. TOXICITY / SIDE-EFFECT 2. TREATMENT FAILURE 3. POOR ADHERENCE<br>4. DRUG INTERACTION 5. PREGNANCY 6. PATIENT PREFERENCE<br>7. DRUG O/S 8. TRANSFER TO ANOTHER PROGRAM<br>9. IRIS 10. OTHER (specify) | | | |
| ** ARV REGIMEN | START DATE | STOP DATE | REASON* |
| | | | |
| | | | |

| | | | |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| ** ANTI-RETROVIRAL ABBREVIATIONS | | | |
| NRTI | d4T = Stavudine | 3TC = Lamivudine | AZT = Zidovudine ddI = Didanosine |
| NRTI | EFV = Efavirenz | | NVP = Nevirapine |
| PI | LPV/r = Lopinavir/ritonavir | | RTV = Ritonavir |

| **ARV REGIMEN** | |
|---|---|
| Regimen 1a | d4T / 3TC / EFV |
| Regimen 1b | d4T / 3TC / NVP |
| Regimen 2 | AZT / ddI / LPV/r |

| | |
|---|---|
| **ARV** TREATMENT START DATE | |
| **CD4** AT START OF TREATMENT | |
| **VIRAL LOAD** AT START OF TREATMENT | |
| **WHO** STAGE AT START OF TREAMENT | |

| | |
|---|---|
| Date of visit | |
| Month / year on Rx | |
| Social Grant Y/N | |
| Nutrition support Y/N | |
| Pregnancy Y/N | |
| LMP | |
| Weight | |
| **VITAL SIGNS** | |
| Temperature | |
| BP | |
| Pulse | |
| Respiration Rate | |
| **ADHERENCE** | |
| # of missed doses last week | |
| # of missed doses last month | |
| Rx interruption (unintentional) # of days | |
| **LAB RESULTS** | |
| CD4 Count / CD4 % | |
| Viral Load | |
| ALT | |
| AST | |
| Creatinine | |
| Haemoglobin | |
| AFB | |
| Cholesterol | |
| Blood Sugar | |
| RPR | |
| **PRESENTING COMPLAINT** | |
| None | |
| Recent weight loss | |

| | |
|---|---|
| Fever | |
| Night sweats | |
| Headache | |
| Cough > 2 weeks | |

| | |
|---|---|
| Shortness of breath | |
| Nausea / vomiting | |
| Chronic diarrhoea | |
| Pain when swallowing | |
| Numbness / tingling | |
| New visual problems | |
| New swelling | |
| **OPPORTUNISTIC INFECTIONS or AIDS DEFINING ILLNESS** | |
| None | |
| Pulmonary TB | |
| Extrapulmonary TB | |
| PCP | |
| Pneumonia | |
| Oral candidiasis | |
| Oesophageal candidiasis | |
| Diarrhoea / wasting | |
| Herpes zoster | |
| Herpes Simplex | |
| Cryptococcal meningitis | |
| Encephalopathy / dementia | |
| Urethritis / cervicitis | |
| Genital ulcerative disease | |
| Steven-Johnsons Syndrome | |
| PID | |
| Cutaneous Karposi's Sarcoma | |

| **ARV SIDE-EFFECT yes/no** | |
|---|---|
| None | |
| Headache | |
| Anaemia | |
| Severe nausea and vomiting | |
| Diarrhoea | |
| Hyperlipidaemia | |
| Hyperglycaemia | |
| Rash | |
| Steven- Johnsons Syndrome | |
| Hepatic toxicity | |
| Liver failure | |
| Peripheral neuropathy | |
| Lipoatrophy | |
| Pancreatitis | |
| Lactic acidosis | |

| | |
|---|---|
| Insomnia | |
| Psychosis or hallucinations | |
| Suicide attempt | |
| Renal insufficiency | |
| Renal failure | |
| **ASSESSMENT on day of visit** | |
| Improving / stable | |
| Active OI | |
| Drug toxicity | |
| Non-adherence | |
| WHO stage | |
| WAB | |
| **PLAN** | |
| Continue current Rx | |
| Restart Rx | |
| Start new Rx ( naïve patient) | |
| Change regimen* (indicate code) | |
| Stop Rx* (indicate code) | |
| Regimen | |
| **MEDICATIONS** | |
| TB Medication | |
| Vitamins | |
| Inj contraceptives | |
| Insomnia | |
| Psychosis or hallucinations | |
| Condoms | |
| Other | |

| | |
|---|---|
| Appointment date | |
| Attending clinician / nurse | |
| Signature | |

Appendix C. Adverse drug reaction and product quality problem report form: HIV/AIDS Treatment Programme

ADVERSE DRUG REACTION AND PRODUCT
QUALITY PROBLEM REPORT FORM

**HIV/AIDS Treatment Programme**

***Eastern Cape Regional Training Centre (ECRTC)***

Main number Tel (047) Main fax # (047)

In collaboration with the Provincial HIV/AIDS Programme and MCC

***(Please Place Original Form in the patient records and Send duplicate to the RTC)***

PATIENT DETAILS

Name (or initials)…… Age…….

Weight (kg) ……

Gender M/F

Allergy ……

Height (cm) ……...

ADVERSE DRUG REACTION/PRODUCT QUALITY PROBLEM

Date of onset of reaction..…../…./…….

Time of onset of reaction ……..h……min

☐ Adverse reaction and/or

☐ Product quality problem

Description of reaction or problem (circle all relevant reactions) – Attach additional information as required

- Pain/tingling/numbness in extremities
- Abdominal or back pain
- Nausea/vomiting/diarrhoea
- Persistent muscle pain
- Anxiety/depression
- Unusual fatigue
- Chills/fever
- Unusual bruising/bleeding
- Rash
- Dizziness
- Insomnia
- Head ache

- Other_____
- Other_____

**1.MEDICINES (CONCOMITANT MEDICINES INCLUDING HERBAL PRODUCTS IF KNOWN)**

| Trade name and batch no. * Suspected drug | Daily dosage | Route | Date Started | Date Stopped | Reasons of Use | Prescriber Doctor, Nurse or Other |
|---|---|---|---|---|---|---|
| **1a, 1b, 2** | **NA, NP, AD** | PO | | | HIV Tx, | |
| | | | | | | |
| | | | | | | |

Keys: **1a** = d4T/3TC/EFZ, **1b** = d4T/3TC/NVP, **2** = AZT/ddI/lop-Rit (Circle one of the ARV Tx regimen). **NA** = Normal adult dose, **NP** = Normal paediatric dose, **AD** = adjusted dose (please specify on the back page)

**ADVERSE REACTION OUTCOME (MAKE A MARK ON THE CHECK-BOX OF ALL THAT APPLY)**

| **Action Taken** | **Patient Outcome** | **Intervention Required** |
|---|---|---|
| ❑ Discontinued Suspected drug<br>❑ Decreased dose<br>❑ Other drug used to treat ADR (Name and dose)<br>❑ Other (specify) …… …….. | ❑ Symptoms--- Improved----resolved<br>❑ Permanent significant disability<br>❑ Congenital abnormality<br>❑ Life threatening<br>❑ Death<br>❑ Other | ❑ Patient counselled<br>❑ Referred to expert for input<br>❑ Additional Clinic Visit<br>❑ Additional labs requested<br>❑ Hospitalisation<br>❑ Other |

**Important Laboratory Values: Circle abnormal one and write the values: BL=baseline Cur=current**

| ***Hgb*** | ***ALT: Neutro*** | ***Cholesterol*** | ***LACT:*** | ***$K^+$*** | ***Serum Cr.*** | ***Other_____*** | ***Other_____*** |
|---|---|---|---|---|---|---|---|
| **BL:** | | | | | | | |
| **Cur:** | | | | | | | |

## Relevant clinical history (Attach additional information)

- How long has patient been diagnosed of HIV: _____ years
- How long has patient been on ARV treatment: _____ months
- Other concomitant conditions (Circle one): HTN, Diabetes, KS, others _____

Additional Information_____

**2.PRODUCT QUALITY PROBLEM (PRODUCT AVAILABLE FOR EVALUATION Y/N)**

| Trade name | Batch no. | Registration no. | Dosage form and strength | Expiry date | Size/Type of container |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

Reporting Doctor/Pharmacist/Nurse etc:

Name _____ Qualifications _____

Facility _____

Tel (____)_____

Signature _____ Date _____

*This report does not constitute an admission that the medical personnel or product caused the event*

Appendix D. Patient Informed Consent Form

MOUNT AYLIFF HOSPITAL
Batho Pele /Abantu Kuqala/People First



ISEBE LEZEMPILO DEPARTMENT OF HEALTH
*Ingxowa Eyodwa/Private Bag X 504, MOUNT AYLIFF 4735*

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**PHARMACY DEPARTMENT**

Informed consent form for a study on the Quantitative assessment of the prevalence of mitochondrial toxicity in HIV/AIDS patients initiated on a Stavudine containing regimen in Mount Ayliff Hospital ARV clinic

**INFORMATION SHEET**:

INTRODUCTION: The South African ART guideline employs d4T as the first line treatment in the management of HIV/AIDS. The prevalence of the toxicities on this patient population impact on the success of the ART programme, and affect the quality of life of the patients. The study aims to establish a quantitative assessment for the prevalence of mitochondrial toxicity as a result of d4T use in ARV regimes.

PURPOSE: The study hopes to create an awareness of the early detection of symptoms associated with mitochondrial toxicity, thus enable optimization of clinical outcomes, whilst maintaining adherence and compliance to therapy. Furthermore, in-service training of clinicians for Pharmacovigilance protocols can be improved. Finally, this research may be used in assessing the risk profile before initiating patients on a d4T-containing regimen.

PARTICIPANT SELECTION: Participants on d4T-containng regimens have been identified for selection at the Mount Ayliff Hospital ARV clinic.

VOLUNTARY PARTICIPATION: Participation is voluntary and it is the participant's choice to be involved in the study. Standard treatment will continue, regardless of participation.

PROCEDURES AND PROTOCOLS: A descriptive study will be conducted using data collected from all participants experiencing peripheral neuropathy, lipoatrophy and lactic acidosis in Mount Ayliff Hospital ARV clinic. The data will be obtained from participant patient files, based on subjective assessment of participants by the supervising health care professional.

CONFIDENTIALITY: Confidentiality is ensured for all data employed. Information regarding the participants' status, ARV therapy, etc will be employed for statistical purposes only.

RIGHT TO REFUSE OR WITHDRAW: Participation is entirely voluntary and participants have the right to withdraw or refuse involvement in the study at any time.
This proposal has been reviewed and approved by management and quality assurance committee of the hospital through the medical manager after approval by the Departmental Research Committee and the University Ethics Committee.

1. I agree to take part in the above research. I have read the Participant Information Sheet which is attached to this form. I understand what my role will be in this research, and all my questions have been answered to my satisfaction.

2. I understand that I am free to withdraw from the research at any time, for any reason and without prejudice.

3. I have been informed that the confidentiality of the information I provide will be safeguarded.

4. I am free to ask any questions at any time before and during the study.

5. I have been provided with a copy of this form and the Participant Information Sheet.

Name of participant (print)…….Signed……..….Date……

Name of witness (print)……..Signed……..….Date……

YOU WILL BE GIVEN A COPY OF THIS FORM TO KEEP

*I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research and understand that I have the right to withdraw from the research at any time without in any way affecting my medical care.*

*If illiterate*

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team).

*I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.*

Print name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

*I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.*

Print Name of Researcher_____
Signature of Researcher _____

Date _____
A copy of this Informed Consent Form has been provided to participant _____ (initialled by the researcher/assistant).

Appendix E. Consent for the Use of Pharmacy Folders for Perusal

MOUNT AYLIFF HOSPITAL
Batho Pele /Abantu Kuqala/People First



ISEBE LEZEMPILO DEPARTMENT OF HEALTH
*Ingxowa Eyodwa/Private Bag X 504, MOUNT AYLIFF 4735*

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**PHARMACY DEPARTMENT**

**Consent for the use of pharmacy folders for perusal**

Research topic:

1. I agree to assist in the above research by providing appropriate and relevant patient information. I understand my role as a pharmacist in this research.

2. I understand that I am free to withdraw a patient folder and information, for any reason and without prejudice.

3. I am bound by the pharmaceutical legislation of my profession to maintain patient confidentiality and dignity at all times.

4. I have been provided with a copy of this form and the Participant Information Sheet.

Print Name of Pharmacist_____
Signature of Pharmacist _____
Date _____
A signed copy of this Informed Consent Form will be added to the patient folder for future reference.

F. Ethical Clearance form

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

Division of the Deputy Registrar (Research)

+ minute participant amendments 26/5/09 attached Consent is not OK - needs to be more personal

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**
R14/49 Dr Ndudu A Amanambu

| | |
|---|---|
| **CLEARANCE CERTIFICATE** | **M090716** |
| **PROJECT** | Quantitative Assessment of the Prevalence of Mitochondrial Toxicity in HIV/AIDS Patients Initiated on a Stavudine Containing Regimen in Mount Ayliff Hospital Anti-Retroviral Clinic |
| **INVESTIGATORS** | Dr Ndudu A Amanambu. |
| **DEPARTMENT** | Pharmacy & Pharmacology |
| **DATE CONSIDERED** | 09.07.31 |
| **DECISION OF THE COMMITTEE*** | Approved unconditionally |

**Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.**

**DATE** 09.08.25 **CHAIRPERSON**.....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Ms N Singh

---

**DECLARATION OF INVESTIGATOR(S)**

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...
.....