

**L-METHIONINE AS IMMUNE-SUPPORTIVE SUPPLEMENT IN
HIV AND OTHER IMMUNE-DEFICIENT CONDITIONS;
A CLINICAL STUDY**

by

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DECLARATION

“I hereby declare that the thesis submitted for the degree Doctor Technologiae: Biomedical Technology, at Tshwane University of Technology, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references”.

Roy van Brummelen

Date

DEDICATION

Dedicated to my father, who is also my colleague and friend, for being a motivation and inspiration to me during this project and in life.

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The author gives all the glory to:

**THE ALMIGHTY GOD AND HIS ONLY SON, JESUS CHRIST, SAVIOUR
AND FRIEND**

ABSTRACT

The aim of this study was to investigate the essential amino acid L-methionine as a possible immune-supportive supplement, specifically by means of a clinical study.

Mechanistic studies done on a South African HIV+ subgroup confirmed the results of previous studies that glutathione levels in HIV+ patients are decreased. These results served to confirm the hypothesis that L-methionine could play a positive role in these patients' immune systems by increasing their bodies' methylation capacity and by increasing their glutathione levels. The protective and supportive role that co-factors such as vitamin B₆, vitamin B₁₂, folic acid and magnesium play in this reaction, was also further elucidated by means of the second study.

A limited study, testing the effect of this combination on healthy individuals (ultra-long distance athletes) followed. Interesting trends were observed, especially in respect to decreased cortisol levels. This could be seen to be indicative of a positive TH1 (cellular immune response). Differences were observed in incidence of illness, as well as in days of training lost owing to illness.

An initial small pilot study on HIV+ and full-blown AIDS patients presented with

all

patients showing significant increases in their CD4 count, CD4%, as well as in

their general feeling of well-being. A second pilot study was therefore initiated with 103 HIV+ patients. More than 90% of the patients again showed a definite improvement, especially in respect of an increased CD4 count, CD4% and Karnofsky score values.

Based on these positive results, a large double-blind, placebo-controlled study on HIV+ patients was initiated. Within 12 months of the study, clinically and statistically significant differences between the two groups were observed, specifically in the female group, which showed a statistically significant decreased level of decline in their CD4 counts and in Centre 1 of the four trial centres, as has been reported. No serious side effects directly associated with treatment were observed.

This study therefore confirms the positive role of L-methionine in the supportive treatment of immune compromised or deficient patients.

EKSERP

Die doel van hierdie studie was om die essensiële aminosuur L-metionien te ondersoek met die oog op moontlike immuun ondersteunende effekte, aan die hand van 'n kliniese studie.

Meganistiese studies op 'n Suid Afrikaanse HIV+ subgroep bevestig vroeëre werk dat hierdie pasiënte se glutatioonvlakke verlaag is. Hierdie resultate bevestig ook die hipotese dat L-metionien 'n positiewe rol kan speel in hierdie pasiënte se immuunsisteem deur die verhoging van hulle metilasiekapasiteit en glutatioonvlakke. Die beskermende rol wat meegaande faktore soos vitamien B₆, vitamien B₁₂, foliensuur en magnesium in hierdie reaksie speel is ook verder uitgelig aan die hand van 'n tweede studie.

Hierdie werk is gevolg deur 'n beperkte studie wat die kombinasie se effek op gesonde persone (ultralangafstand-atlete) getoets het. Interessante patrone is opgemerk, veral met betrekking tot verlaagde kortisolvlakke. Dit kan gesien word as aanduidend van 'n positiewe TH1 (sellulêre immuunreaksie). Verskille is ook aangedui ten opsigte van voorkoms van siektes, asook in dae af as gevolg van siekte.

'n Klein aanvanklike loodsstudie op HIV+ en VIGS pasiënte het betekenisvolle verhogings in hulle CD4 tellings en CD4% asook hulle algemene gevoel avn welsyn getoon. 'n Tweede loodsstudie met 103 HIV+ pasiënte is daarom begin.

Meer as 90% van die pasiënte het 'n definitiewe verbetering getoon, veral ten opsigte van hulle CD4-telling, CD4% en "Karnofski"-telling.

Gebaseer op hierdie positiewe resultate is 'n groot dubbelblinde, plasebo-gekontroleerde studie op HIV+ pasiënte geloods. Binne die eerste 12 maande van die studie kon daar reeds klinies en statisties betekenisvolle verskille tussen die twee groepe aangedui word spesifiek in die vroulike groep, wat statisties betekenisvolle vertraging in afname van hulle CD4 telling getoon het, sowel as sentrum 1 van die vier studie sentrums. Geen ernstige newe- effekte wat direk met die studie verband hou, is aangetoon nie.

Hierdie studie bevestig die positiewe rol van L-metionien in die ondersteunende behandeling van 'n ingekorte of afwesige immuunsisteem.

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ABBREVIATIONS

ACE	Angiotensin converting enzyme
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARDS	Adult respiratory distress syndrome
BP	Blood pressure
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
cm	Centimetre
cmm	Cubic centimetre
CRF	Case record form
CV	Coefficient of variation
CYS	Cysteine
dl	Decilitre
ECG	Electrocardiogram
g	Gram
G	10 ⁹
GCP	Good clinical practice
GPMP	Good Clinical Practice for Trials on Medical Products in the European Community
GSH	Glutathione
h	Hour
HARTS	Hoechst Adverse Reaction Terminology System

HCYS	Homocysteine
HDL	High density lipoprotein
ICD	International Classification of Diseases
ICD9CM	International Classification of Diseases, 9th Revision, Clinical Modification
ICH	International conference on harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
kg	Kilogram
l	Litre
LDL	Low density lipoprotein
LOCF	Last Observation Carried Forward
LPCA	Last evaluation, predefined change abnormal; a PCA occurring at the patient's final evaluation on treatment
LS Mean	Least squares mean
m	Metre
m ²	Square metre
Max	Maximum
MCC	Medicines Control Council
MD	Mean difference
mg	Milligram
Min	Minimum
min	Minute
ml	Millilitre
MOS	Medical Outcomes Study

mmHg	Millimetre mercury
mmol	Millimol
n	Number of observations
NAC	N-acetyl-cysteine
PC	Predefined change
PCA	Predefined change abnormal; an increase/decrease in a laboratory value of at least a predefined amount which is abnormally high/low
PE	Point estimate
PCYS	Plasma cysteine levels
PHOM	Plasma homocysteine
PGSH	Plasma GSH levels
SAH	S-Adenosylhomocysteine
SAM	S-Adenosyl-L-methionine
SD	Standard deviation
sec	Second
T	10^{12}
TCYS	Total cysteine levels
THOM	Total homocysteine
μmol	Micromol
UOFS	University of the Orange Free State
URTI	Upper respiratory tract infection
WCC	White cell count
WHO	World Health Organization

PAPERS PRESENTED AT INTERNATIONAL CONFERENCES

- **Tenth International Conference on AIDS
Yokohama, Japan, 7-12 August 1994**

Equimmune in Treatment of HIV-infected patients

Bissbort SH, Davis H, van Brummelen R & Miller S

- **12th World AIDS Conference
Geneva, Switzerland, 28 June – 3 July 1998**

Glutathione, cysteine and homocysteine levels in HIV+ patients,
possible immune supportive treatment

van Brummelen R, Erasmus E, Knoll DP, Mienie LJ & Miller Steven

- **7th International Congress on Amino Acids and Proteins
Vienna, Austria, 6-10 August 2001**

L-Methionine: Immune supportive supplement in HIV+ patients: A
South African study

van Brummelen R

CHAPTER 1

INTRODUCTION AND AIM OF STUDY

1.1 INTRODUCTION

Very little attention has been paid to the possible role of amino acids in medicine. Only since the effect of modern life on dietary intake has been realised, has more attention been paid to this important aspect of medicine. L-methionine is classified as one of the essential amino acids and yet most people seem to know very little about this substance and its role in health and disease.

An amino acid is an organic acid in which one of the CH hydrogen atoms has been replaced by an NH₂ group (R-CHNH₂COOH). Amino acids are generally classified into two major groups; 'essential' and 'non-essential'. This classification, however, has no reference to the importance of the amino acid, but only refers to the fact that it can or cannot be produced by the body. The so-called 'essential' amino acids, in other words, cannot be produced by the body and need to be either derived from dietary intake or obtained by means of supplementation. 'Non-essential' amino acids, however, can be produced by the body out of other 'essential' amino acids; i.e. methionine to cysteine (Table 1.1).

Table 1.1: Classification of essential and non-essential amino acids

▪ ESSENTIAL	▪ NON-ESSENTIAL
▪ Leucine	▪ Alanine
▪ Isoleucine	▪ Aspartic acid
▪ Valine	▪ Glutamic acid/Glutamine
▪ Methionine	▪ Cysteine (if methionine is sufficient)
▪ Phenylalanine	▪ Tyrosine (if phenylalanine is sufficient)
▪ Threonine	▪ Proline
▪ Lysine	▪ Serine
▪ Histidine	▪ Glycine
▪ Arginine	▪ Histidine (for adults)
▪ Tuarinine	▪ Arginine (for adults)
(For early growth and development)	

L-methionine, like most amino acids, is present in meat; very little is, however, found in soybeans. Other natural food sources of L-methionine include sunflower seeds, egg yolk, wheat germ and certain cheeses. Low levels are generally found owing to insufficient diet, inborn error, or conditions of rapid or increased metabolism (Braverman & Pfeiffer, 1987).

L-methionine is the initial metabolite in several fundamental, biological processes including protein synthesis, transmethylation and transsulphuration. Together with its derivatives, it plays a primary role in the normal growth and development of mammals (Finkelstein, 1990). S-Adenosyl-L-methionine (SAM) is the biproduct of methionine adenosylation. This reaction is catalysed by the enzyme methionine-adenosyltransferase (Figure 1.1). SAM can provide a methyl group to a variety of substances and S-adenosylhomocysteine (SAH) is formed as a biproduct (Mudd *et al.*, 1995). SAM is thus the methyl donor to molecules such as hormones, neurotransmitters, nucleic acids, proteins, phospholipids (at cellular level) and even certain drugs (Friedel *et al.*, 1989).

1.2 METHIONINE AND METHYLATION

This methylation plays an important role in several reactions in the body. It is involved in the metabolism of substances such as adrenaline and histamine and could thus play a role in reactions such as stress and allergies (Scott, 1995).

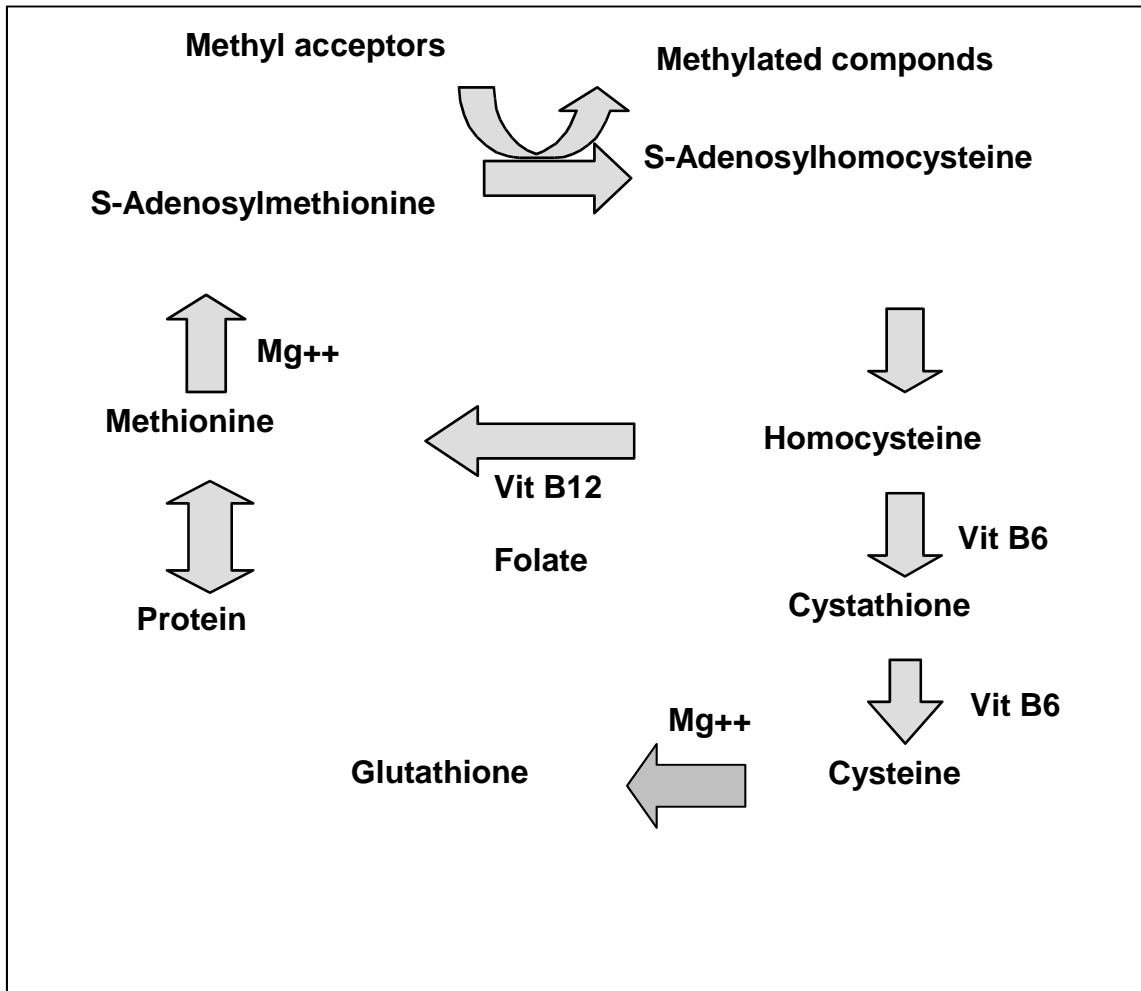


Figure 1.1: Methionine to glutathione pathway

It also plays an important role in the immune system. In genetic cases of severe combined immunodeficiencies, especially in the adenosine-deaminase deficient form, it could be shown that the mechanism of impaired immune function is mainly due to a lack of methylation capacity (Hershfield & Mitchell, 1995). In a deficiency of SAM or a low ratio of SAM in respect to SAH, a T-cell deficiency develops and in more severe cases a combined (T- and B-cells) immunodeficiency can develop (Hershfield & Mitchell, 1995; Surtees *et al.*, 1990). A large percentage of the methylation

capacity is also used for the formation of creatine, which plays an important role in the supply of energy to muscles. Thus, SAM is an indispensable compound for human survival.

The liver is the most important organ for methionine metabolism, as the transmethylation reactions occur mainly there. Methionine metabolism might be affected in the presence of liver diseases. This assumption led to investigations of the use of SAM as a therapeutic agent in various liver diseases, such as bile secretion impairment and intrahepatic cholestasis (Di Padova *et al.*, 1982; Di Padova *et al.*, 1984; Frezza *et al.*, 1988; Frezza *et al.*, 1990) lead poisoning (Paredes *et al.*, 1986; Paredes *et al.*, 1985) and porphyria cutanea tarda (Cantoni *et al.*, 1990; Del *et al.*, 1987).

The protective effect of SAM was also investigated in various extrahepatic diseases, where altered methylation may be a possible contributing factor (Glorioso *et al.*, 1985; Montrone *et al.*, 1985; Schumacher, 1987; Oriente *et al.*, 1985; Cohen *et al.*, 1988; Gatto *et al.*, 1986). SAM therapy was attempted in diseases such as osteoarthritis (Glorioso *et al.*, 1985; Montrone *et al.*, 1985; Schumacher, 1987), progressive systemic sclerosis (Oriente *et al.*, 1985), Alzheimer's disease (Cohen *et al.*, 1988) and migraine (Gatto *et al.*, 1986). Some form of association between demyelination and methylation could be shown (Hyland *et al.*, 1998). The use of SAM in the treatment of depression has

become especially popular (Lipinski *et al.*, 1984; Bell *et al.*, 1988; Potkin *et al.*, 1988; Janicak *et al.*, 1989; Kagan *et al.*, 1990; Bottiglieri *et al.*, 1990). It has been implied that both L-methionine and SAM can cross the blood-brain barrier (Baldessarini & Kopin, 1966; Rubin *et al.*, 1974; Tudball & Griffiths, 1976), and more importantly, SAM crosses the blood-brain barrier much more poorly than methionine (Baldessarini & Kopin, 1966). Despite claims of success when SAM was used as a clinical drug in various diseases, its use, instead of the more stable, cheaper L-methionine (precursor to SAM) therefore appears to be illogical. Results indicate that L-methionine should be re-evaluated as a therapeutic agent, especially in view of the successful trials reported on SAM.

Supplementation of L-methionine significantly increases circulating SAM levels in humans (Lagendijk, 1992) and subsequently the overall methylation capacity.

1.3 GLUTATHIONE SYNTHESIS

SAM, as has been pointed out, is enzymatically formed from the

amino acid L-methionine and Mg^{++} -ATP forming SAH as product. SAH is further enzymatically cleaved into L-homocysteine and adenosine. L-homocysteine can either be re-methylated to L-methionine or by two enzymatic steps be converted to L-cysteine and subsequently to glutathione (GSH) (Mudd *et al.*, 1995) (Figure 1.1).

This tripeptide protects against two kinds of metabolic stress; 1) it can non-enzymatically reduce substances such as free radicals and 2) through the enzyme glutathione S-transferase participates in detoxification of many substances (Mathews & Van Holde, 1993). GSH itself has been found to be important as a mediator of normal immune responsiveness and to have antiviral activity (Ho & Douglas, 1992; Kalebic *et al.*, 1991; Palamara *et al.*, 1996). Absorption of oral GSH, however, may be relatively poor owing to gastrointestinal enzymatic degradation. In a study by Witchi *et al.*, 1992, it was shown that oral supplementation of GSH as high as 3g had no influence on circulating GSH levels, probably as a result of hydrolysis of GSH by intestinal and hepatic glutamyltransferase. Supplementation of L-methionine (and cysteine) has on the other hand been shown to increase intracellular GSH by as much as twofold (Shih-Tsung Wang *et al.*, 1997). Supplementation of the precursor N-acetyl-cysteine (NAC) has been described with limited success to increase GSH levels in HIV-infected people (Roederer *et al.*, 1991; Roederer *et al.*, 1990;

Dröge *et al.*, 1992). It is well documented that intracellular GSH levels in patients infected with HIV are decreased (Buhl *et al.*, 1989; Buhl, 1994).

In patients suffering from lead poisoning, the blood GSH levels were found to be diminished, but increased to normal values after SAM therapy (Paredes *et al.*, 1986; Paredes *et al.*, 1985). In these cases the most probable mechanism for SAM action seems to be the elevation of GSH availability for the rapid removal of lead from different compartments, thereby facilitating the detoxification process. It is suggested that the lead is taken up and transported from the liver to the bile as a GSH conjugate, resulting in increased biliary metal excretion (Paredes *et al.*, 1986).

1.4 HOMOCYSTEINE METABOLISM

The correlation between homocysteine, an intermediate in this methionine metabolism, and vascular disease has recently become a popular topic (Ueland *et al.*, 1989) and the possibility that elevated homocysteine levels might contribute to the development of coronary heart disease (CHD), has already been investigated to a large extent (Ubbink *et al.*, 1991). The biochemical mechanism explaining elevated circulating homocysteine levels in patients with CHD is, however, still uncertain. The prevalence of elevated plasma homocysteine

levels in CHD was also investigated in South Africa and the prevalence of hyperhomocysteinemia in coronary heart disease patients was 41.9% (Ubbink *et al.*, 1991). Homocysteine is either re-methylated to methionine by a vitamin B₁₂ and folate-dependent enzyme (5-methyltetrahydrofolate-homocysteine ethyltransferase), or is irreversibly catabolised by the transsulphuration pathway, which utilises vitamin B₆ (pyridoxal-5'-phosphate) in at least one enzyme-catalysed reaction (Figure 1.1). Defects in either of these pathways will result in hyperhomocysteinemia. Such a defect can either be caused by a) a deficiency of one of the essential cofactors for normal homocysteine metabolism; vitamin B₁₂, vitamin B₆ or folate, or b) certain enzyme variants, which may also cause hyperhomocysteinemia. For efficient homocysteine metabolism, an adequate supply of vitamin B₁₂, vitamin B₆, and folic acid is required. However, during food refinement and processing, losses of these nutrients may occur (Herbert, 1963; Hurdle *et al.*, 1968; Leklem, 1991).

1.5 VITAMIN AND MINERAL SUPPLEMENTATION

A daily vitamin supplement (containing vitamin B₆, folic acid and vitamin B₁₂) normalised elevated circulating homocysteine levels in patients with homocysteine urea within six weeks of treatment

(Ubbink *et al.*, 1992). This was in agreement with Brattstrom's studies (Brattstrom *et al.*, 1988), which investigated the effect of vitamin B₁₂, vitamin B₆ and folic acid on circulating homocysteine levels. Magnesium is also an essential cofactor for the enzyme methionine adenosyl transferase, which forms SAM from L-methionine. It is thus clear that the vitamin and mineral status is an important determinant of circulating homocysteine levels.

1.6 METHIONINE, D OR L?

In previous studies uncertainties with regard to dosage ranges and the form of methionine to use were obvious shortcomings. The recommended dietary allowance of L-methionine in humans is estimated at 0.92 g/day (Wallur *et al.*, 1957). Research on the effect of D-methionine, in comparison to L-methionine, after ingestion is sparse and there have been some inconsistencies regarding the body's utilisation of D-methionine (Friedman, 1991). D-methionine must first be converted to the L-form before it can be utilised. However, the extent of oxidation of the D-form by D-amino acid oxidase is not the only factor governing utilisation. Other factors that could influence utilisation include rates of transport, action of intestinal enzymes, rates of absorption, renal clearance and possible toxic effects. These factors have not yet been investigated (Friedman, 1991). It is even possible that D-methionine may act as an enzyme inhibitor and thus have an

adverse effect on biological methylation. L-methionine thus seems to be the preferred form to use.

1.7 THE HIV CONNECTION

The fact that GSH levels are decreased in HIV patients and seem to play an important role in the regulation of the immune system, was confirmed by several reports presented at the Oxidative Stress and Redox Regulation Conference 1996, Paris, with Dr Luc Montagnier in the chair, as well as by several recent publications (Skurnick *et al.*, 1996; Delmas-Beauvieux *et al.*, 1996; Barbaro *et al.*, 1996). Findings suggest that GSH redox disturbances may be of pathogenic importance (Aukrust *et al.*, 1996). Low plasma GSH levels in children with HIV were found to be associated with low CD4 cell count values, as well as an increased viral load (Rodriguez *et al.*, 1998). The most recent publication by Herzenberg *et al.* implicates GSH levels as predictive of survival in HIV patients (Herzenberg *et al.*, 1997). Results seem to suggest that GSH could inhibit the reverse transcriptase process of HIV-1 and so directly influence virus levels (Kameoka *et al.*, 1996; Sen & Packer, 1996). GSH was also shown to be effective in reducing the proviral DNA load in the first period of infection in murine AIDS (Palamara *et al.*, 1996; Rossi *et al.*, 1996). In another study it could be shown that exogenous GSH strongly suppresses the production of p24gag protein, as well as the virus infectivity

(Palamara *et al.*, 1996). All this serves to highlight the important role GSH plays in HIV patients and the possible role it could play in the treatment of this disease. Already results have indicated the importance of antioxidant treatment in HIV patients to revert the impaired proliferative activity of their CD4 cells (Cayota *et al.*, 1996).

What role does methionine play in this process? The well-known pharmacological reference book, Martindale, states the following about methionine; "Methionine enhances the synthesis of GSH" and "GSH, a peptide involved in intracellular defense mechanisms, is depleted in patients infected with HIV" (Martindale 31 edition). Results provide evidence that the depletion of GSH also leads to a methionine depletion, which injures the methylation processes (Lertratanangkoon *et al.*, 1996). This was confirmed by a study which indicated low concentrations of methionine in the plasma of HIV infected patients (Muller *et al.*, 1996).

In the treatment of neuropathy and myeloneuropathy, as induced by nitrous oxide and AIDS associated myelopathy (vascular), methionine has also been postulated as a possible first line treatment (Di Rocco *et al.*, 1998).

1.8 SUMMARY

The importance of L-methionine and its role as an essential amino

acid is clear. It is therefore imperative to ensure sufficient intake of this essential amino acid. It is also evident that, if supplemented, the nutritional status of other vitamins such as vitamin B₆, vitamin B₁₂ and folic acid, as well as minerals such as magnesium, could play a critical role. It is therefore important to confirm the role of vitamin B₆, vitamin B₁₂, folic acid and magnesium in the metabolism of methionine on a clinical level. The GSH and related metabolite levels of the HIV+ patient also needs investigating.

In this study the effect of L-methionine supplementation, specifically in combination with vitamin B₆, vitamin B₁₂, folic acid and magnesium, will be investigated. Specifically the possible beneficial effect this combination might have on the support of the immune system of immune-compromised or deficient patients, will be investigated. In order to do this, the possible mechanism of action should be further investigated, with special attention to safety.

This could have a possibly positive impact clinically on not only the HIV+ patient, but also on any person with a low or deficient immune system, whether due to stress or disease. Stress in this case could also be defined as the severe metabolic stress experienced during an ultra-long distance marathon. The long-term safety of supplementation with L-methionine and any other

possible positive or negative effects this might have, also need to be investigated.

Fully elucidating the possible positive effect L-methionine could have on the immune system, might have a dramatic impact on the way one views immune-supportive treatments. This holds true not only for South Africa, but also worldwide, for conditions from those as common as flu and colds to those as serious as AIDS.

1.9 AIM AND DESIGN OF STUDY

The aim of this study is to investigate the role of L-methionine as an immune-supportive supplement in HIV and other immune-deficient conditions by means of clinical studies.

Mechanistic studies will be done by:

- Investigating GSH and other related metabolite levels in a South African HIV+ population
- Investigating the possible protective role of co-factors, e.g. vitamin B₆, vitamin B₁₂, folic acid and magnesium, in preventing homocysteine accumulation during methionine supplementation.

Before testing the possible role of L-methionine in 'sick' patients (HIV+ patients), a study will first be done on a 'healthy' population. Ultra-long distance athletes were chosen, as their immune system

may be negatively affected by over-training and the physiological stress of such gruelling events.

Owing to the serious nature of a disease such as HIV and AIDS, a small pilot study with less than 20 patients will initially be done. Only if the results prove promising, will it be followed by a second slightly larger study with approximately 100 patients. In the case of positive results in both these studies, the main double-blind placebo-controlled clinical study will be done with more than 200 HIV+ patients, carefully monitoring them for any possible side effects and adverse events, while investigating the possible positive effects of the formulation.

CHAPTER 2

METABOLIC STUDIES AND ROLE OF L-METHIONINE IN HEALTH AND PREVENTION

2.1 GLUTATHIONE, CYSTEINE AND HOMOCYSTEINE LEVELS IN HIV+ PATIENTS

2.1.1 INTRODUCTION

GSH and subsequently its precursors cysteine (CYS) and homocysteine (HCYS), seem to play an important role in the immune system of the HIV+ patient (Rodriguez *et al.*, 1998; 41; Muller *et al.*, 1996). Determining these imbalances and their consequent treatment could play an important role in the supportive management of the patient.

The aim of this study was to do a comparative study, to establish GSH levels and their precursors, CYS and HCYS, in a subgroup of HIV+ patients enrolling for the main study, compared to an HIV-control group. This was to see whether the results of previous studies, indicating decreased GSH levels, could be confirmed in the particular sub-population. This was also done to help establish a working model for the possible mechanism of action of

the formula to be tested in the main study.

Confirming decreased GSH levels in the study population will help confirm the hypothesis that L-methionine supplementation might be of assistance as an immune-supportive supplement in these patients, as discussed in Chapter 1.3.

2.1.2 MATERIAL AND METHODS

2.1.2.1 Trial design

An open prospective pilot study was designed (n=85).

2.1.2.2 Selection of patients

Patients enrolling for the main double-blind, placebo-controlled clinical trial planned, were randomly selected (n = 41).

The inclusion criteria were:

- Age > 18 years
- Laboratory confirmation of HIV positivity
- CD4 count at entry < 500 cmm and > 200 cmm
- Prophylactic treatment of any possible secondary problem
- Patients giving their written consent and agreeing to the described protocol.

The exclusion criteria were:

- Pregnancy

- Women who refuse to use contraception
- Underlying/concomitant renal disease
 - Concomitant significant hepatic disease (liver enzymes > 3 times upper limit of normal)
 - Life expectancy on clinical grounds < 6 months
 - Known inborn error(s) of metabolism
 - Concomitant use of other NAC or GSH products
 - Haemoglobin < 8 g/dl, white cell count < 750/ml at entry
 - Concomitant use of corticosteroids or other potentially immunosuppressive agents, e.g. adriamycin, atoposide, doxorubicin, cyclophosphamide
 - Concomitant use of interferon, or any other immunological agent
 - Any patient who, on clinical grounds, needed or would need other antiviral treatment within the next six months.

A HIV- control group, consisting of men and women of different ages and races testing negative for HIV, was used (n = 44).

The GSH, CYS and HCYS levels of all patients were measured in the plasma and whole blood and compared to the control group. The samples were all collected in sample tubes that contained N-ethylmaleimide to stabilise the relevant metabolites. Electrospray ionisation mass spectrometry was carried out using a VG Quattro II quadrupole (Micromass, UK). Data were acquired in the neutral

loss mode of operation, scanning over the relevant mass range in the first mass spectrometer and keeping the second mass spectrometer static, monitoring the collisional-induced dissociation neutral fragments at 102, 131, 177 and 191 m/z (mass-to-charge ratio). These neutral fragments allowed for the selective detection of butylated amino acids, GSH, CYS and HCYS respectively. Quantification was carried out using the corresponding deuterated isotopes of the metabolites and calibration curves were obtained using fixed concentrations of the isotopes and various concentrations of GSH, CYS and HCYS.

2.1.3 RESULTS

The plasma GSH levels were found to be decreased in the HIV+ group; 3.5 $\mu\text{mol/l}$, compared to that of the control group; 16.6 $\mu\text{mol/l}$. The mean difference (MD) in baseline values (HIV group - control group) was -13.1 $\mu\text{mol/l}$, with the 95% confidence interval (CI) for difference; -19.1 to -7.18, $p < 0.05$.

Differences were also found in the levels of GSH precursors levels between the two groups. The plasma CYS (MD; -205 $\mu\text{mol/l}$, CI; -333 to -76.2) and HCYS (MD; -42.5 $\mu\text{mol/l}$, CI; -75 to -9.93) levels were found to be significantly lower in the HIV+ group ($p < 0.05$). These differences were also reflected in the total blood values of CYS (MD; -81 $\mu\text{mol/l}$, CI; -152 to -10.6, $p < 0.05$) and HCYS (MD; - 8.83 $\mu\text{mol/l}$, CI; -16.1 to -1.57, $p < 0.05$) (Table 2.1).

Table 2.1: Blood levels of GSH and its precursors

		PGSH	PCYS	PHOM	TCYS	THOM
n	- Control	44	44	44	44	44
	- HIV patients	41	41	41	41	41
Mean	- Control	16.6	359.1	58.2	198.0	26.6
	- HIV patients	3.5	154.3	15.7	116.7	17.7
Median	-Control	8.8	287.3	35.1	158.6	25.1
	- HIV patients	2.6	122.2	9.0	63.6	8.5
SD	- Control	18.8	357.9	102.6	134.7	19.8
	- HIV patients	3.6	215.5	21.7	237.5	23.1
Mean difference		-13.1	-205	-42.5	-81.4	-8.83
95% CI of difference		-19.1 to	-333	-75.0	-152	-16.1
		-7.18	to	to	to	to
p-value		<0.05	<0.05	<0.05	<0.05	<0.05

Plasma GSH levels	-	PGSH
Plasma cysteine levels	-	PCYS
Total cysteine levels	-	TCYS
Plasma homocysteine	-	PHOM
Total homocysteine	-	THOM

2.1.4 DISCUSSION

These results confirm previous studies showing decreased GSH levels in HIV+ patients and present additional evidence implicating GSH as an important role player in HIV infection and disease progression. These results are of importance, not only in

evaluating the South African HIV+ patients, but also in understanding the mechanism of the disease better on a biochemical level, so as to better equip researchers in dealing with the disease.

The decreased levels of GSH in the HIV+ group by implication, point towards an increase in demand or use of this tri-peptide in the body. Therefore supplementation with L-methionine is justified as a possible method of replenishing GSH levels, thus supplying the body with what it needs. Based on these results, further research is necessary to investigate the possible immune-supportive role L-methionine might play in such immune-compromised patients.

The role of other co-factors, which might also be involved in the process, also needs further investigation, so as to find the optimum and safest formula possible.

2.2 THE USE OF L-METHIONINE ALONE AS COMPARED TO L-METHIONINE COMBINATION

2.2.1 INTRODUCTION

As L-methionine supplementation could lead to HCYS accumulation in certain people, with the ensuing health risk coupled to this as described in section 1.4 and 1.5, it was decided that L-methionine should be given in combination with vitamin B₆,

vitamin B₁₂, folic acid and magnesium. This would ensure the best and safest formula or combination possible.

To test this hypothesis, the aim of this study was to test the difference in bio-availability of L-methionine if given alone, compared to when it is given in combination, so as to investigate the possible difference this might have on the L-methionine bioavailability and HCYS levels.

It was postulated that, if given in combination with vitamin B₁₂ and folic acid, not only will HCYS not accumulate, but will be re-methylated back to L-methionine. This could therefore result in more optimum use of L-methionine by the body (Figure 1.1), resulting in lower dosages required for the same effect.

2.2.2 MATERIALS AND METHODS

2.2.2.1 Description of investigational products

Product A: L-methionine combination (BioBoost)

Active ingredients per capsule:	L-methionine	- 405 mg
	Folic acid	- 0.36 mg
	Vitamin B ₆	- 1.575 mg
	Vitamin B ₁₂	- 11.25 µg

Magnesium lactate - 135 mg

Dosage: Six capsules as bolus dosage at time 0

Description: Pale white powder in
a clear-clear, size
0, hard gelatin capsule

Product B: L-methionine alone

Active ingredients per capsule: L-methionine - 405 mg

Dosage: Six capsules as bolus dosage at time 0

Description: Pale white powder in a clear-clear, size
0, hard gelatin capsule

2.2.2.2 Trial design

An open prospective pilot study was designed (n=7).

2.2.2.3 Procedures and measurements

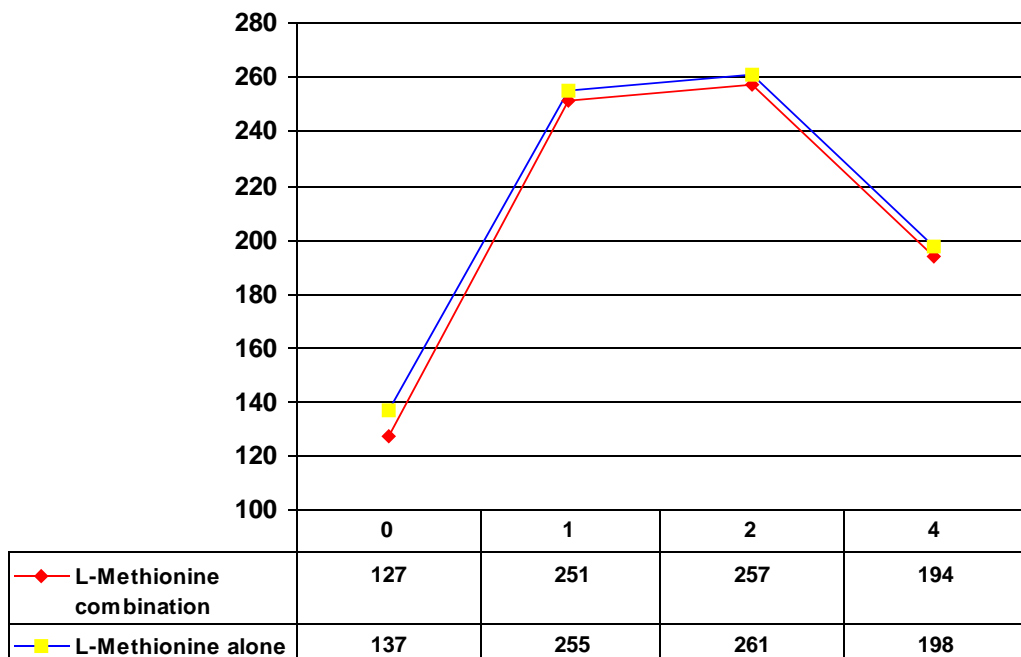
Seven normal healthy individuals, five male and two female, were randomly divided into two groups. Four were placed on Product A (L-methionine combination) and three on Product B (L-methionine alone). For 12 hours prior to the treatment, the seven individuals were only allowed water intake and no food or dietary intake. This was also applicable for the four-hour period of the study itself. One person from group A was subsequently excluded because of disease, leaving two equal groups.

Table 2.2: Sampling schedule

Time	Blood samples	Urine samples
▪ Time 0	1 x EDTA tube	Sample
▪ 1 hour	1 x EDTA tube	None
▪ 2 hours	1 x EDTA tube	None
▪ 4 hours	1 x EDTA tube	Sample

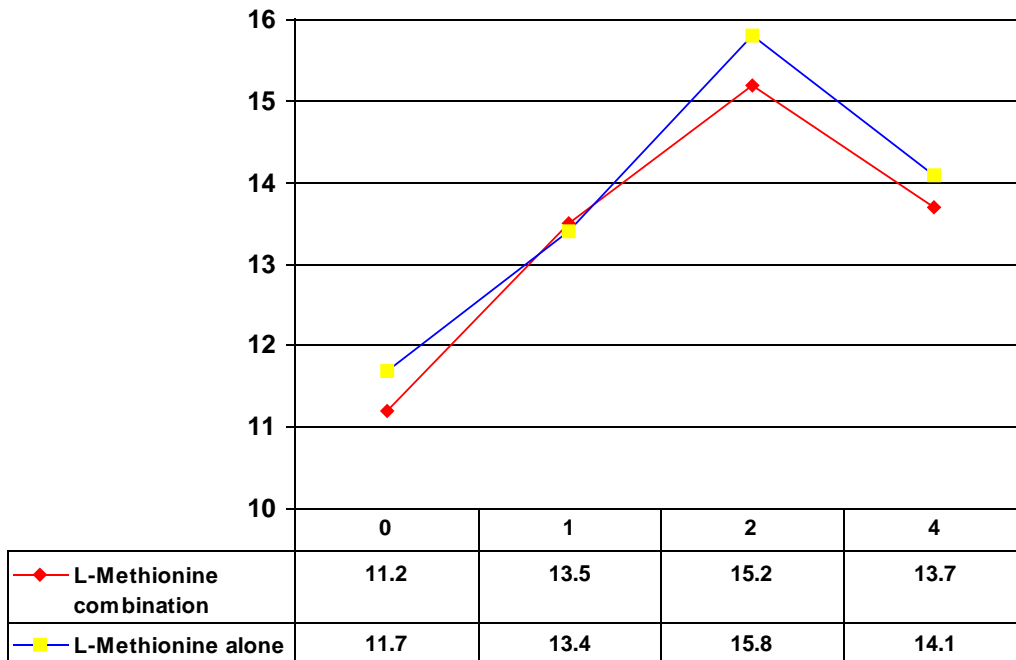
2.2.2.4 Results

L-methionine plasma levels increased in the first hour. From one



hour to two hours only a small further increase could be shown.

From two hours to four hours the L-methionine plasma levels started decreasing. No obvious differences in the plasma L-methionine levels between the two groups could be shown (Figure 2.1).



The plasma homocysteine levels (creatinine corrected) also showed an increase in up to two hours and then decreased to four hours. The increase in group B seems to be slightly higher than that in group A (Figure 2.2).

μm
o/l

Figure 2.2: Homocysteine plasma levels after treatment

Figure 2.1: L-methionine plasma levels after treatment

2.2.2.5 Discussion

The L-methionine levels in the plasma of the 'L-methionine combination' (Product A) group and the 'L-methionine alone' (Product B) group were almost exactly the same over the treatment period. The peak plasma levels differed by as little as 1,5%; (L-methionine combination 257 $\mu\text{mol/l}$, L-methionine alone 261 $\mu\text{mol/l}$). It therefore does not seem as if the combination therapy dramatically influenced the absorption or bioavailability of L-methionine in this study.

The HCYS levels between the two groups presented with a slightly larger difference, namely 3,8% (L-methionine combination 15,2 $\mu\text{g/mg creatinine}$, L-methionine alone 15,8 $\mu\text{g/mg creatinine}$). Although this difference was not significant, it is still worth taking note of, taking into consideration the similarity seen in L-methionine levels and the possible risk HCYS accumulation might have in patients.

Even though this only represented a limited pilot study, the trend of the results seemed to confirm the hypothesis that L-methionine, in combination with vitamin B₆, B₁₂, folic acid and magnesium, might be safer than L-methionine alone. L-methionine plasma levels could be increased to the same extent with the

L-methionine combination, compared to L-methionine alone, with less of an increase in HCYS levels. These results are in accordance with other studies, proving the importance of supplementing with especially vitamin B₁₂ and folic acid in high HCYS levels.

It would be of importance to confirm these results in a larger study, as the inter-patient plasma variations of these substances are quite large. The plasma levels should also be controlled over a longer period to get a more accurate picture of the absorption rate of L-methionine, as well as considering the intracellular levels of this amino acid and its metabolites.

Owing to the negative role HCYS plays as a possible risk factor in heart disease, it is thus preferable to combine L-methionine with vitamin B₆, vitamin B₁₂, folic acid and magnesium in future studies.

2.3 L-METHIONINE SUPPLEMENTATION IN HEALTHY LONG-DISTANCE ATHLETES

2.3.1 INTRODUCTION

It is a well-known fact that ultra-distance athletes that run very long distances in preparation for their event are often prone to infections owing to a compromised immune system. It is also

thought that an ultra-marathon such as the Comrades (± 90 km) causes considerable damage to the athlete's physiological system – the extent of this damage has not yet been determined. Lastly, anecdotal reports from runners indicate that it takes up to three months to recover fully from such an event, and it is believed that the runners are at increased risk of developing upper respiratory tract (URTI) infections during this 'open window' period following the race. This theory is, however, not fully accepted yet (Nieman & Pedersen, 1999).

The purpose of the trial was to determine the value of L-methionine as supplement and more specifically immune-supportive supplement for ultra-distance runners.

2.3.1.1 Major objectives

- To determine the effect of L-methionine supplementation on the immune systems and exercise performance of well-trained ultra-distance runners preparing for the Comrades marathon.
- To determine whether or not supplementation with L-methionine will protect the immune systems of well-trained ultra-distance runners while they are competing in the Comrades marathon.
- To determine whether or not supplementation with L-methionine will enhance the recovery rate of well-trained ultra-distance runners after they have completed the Comrades

marathon.

This double-blind, placebo-controlled study investigated the effect of L-methionine in combination with vitamin B₆, vitamin B₁₂, folic acid and magnesium (BioBoost) supplementation on various immune markers, including URTI (e.g. common cold, sore throat) and performance of runners in response to endurance exercise.

2.3.2 MATERIALS AND METHODS

2.3.2.1 Description of investigational products

Product A: L-methionine combination (BioBoost)

Active ingredients per capsule:	L-methionine	- 405 mg
	Folic acid	- 0.36 mg
	Vitamin B ₆	- 1.575 mg
	Vitamin B ₁₂	- 11.25 µg
	Magnesium lactate	- 135 mg

Dosage: Six capsules per day taken in a divided dosage

Description: Pale white powder in a clear-clear, size 0, hard gelatin capsule

Product B: Placebo

Active ingredient per capsule: No active ingredient. (Only potato starch and magnesium carbonate)

Dosage: Six capsules per day taken as a divided dosage

Description: Pale white powder in a clear-clear, size 0, hard gelatin capsule

2.3.2.2 Trial design

A paired, double-blind, placebo-controlled study was designed.

2.3.2.3 Procedures and measurements

Twenty-one (males n=17 and females n=4) healthy, well-trained, ultra-marathon runners aged 25 to 51 years were monitored during preparation for 76 days and recovery from the 2000 Comrades marathon (84 days). Subjects randomly received either the active therapy or placebo supplementation. Measures for immune status (full blood count, cortisol, CD4, CD8 and CD4/CD8 ratio) and performance (VO_2max % VO_2max at 4 mmol/l⁻¹, body composition and running economy) were taken at various intervals during the study.

Subjects recorded their training and the incidence of URTI in a logbook for the duration of the study.

2.3.2.4 Results

(1) Pre-Comrades

Considering changes seen in the 76 days during intensive training before Comrades, the following differences were observed:

Both groups showed an increase in haemoglobin and red blood cell levels of between 3 and 5% and slight decreases in the mean corpuscular volume (red blood cell size). There was, however, a significant difference ($p < 0,01$) in basophil percentage between the two groups, the active group decreasing from 0,64 (SD 0,29) to 0,41 (SD 0,25) and the placebo group increasing from 0,55 (SD 0,25) to 0,9 (SD 0,42) during preparation.

The endurance fitness, measured as the percentage VO_2 max at 4 mmol/l⁻¹ lactate concentration, was higher in the supplement group (87, SD4) compared to the placebo group (83, SD5), $p < 0,05$, prior to the start of the race.

(2) Comrades

Comparing levels from before to directly after Comrades, the following was seen:

A general increase in white blood cell counts (about 180 to 190%), neutrophil counts (about 330 to 350%) and monocyte counts (about 140 to 150%) was observed in both groups. A decrease in lymphocyte counts was observed in both groups, but more so in the placebo group (31%) is compared to the active group (17.4%). An increase in cortisol levels was found in both groups; active group 262% and placebo group 301%. The active (L-methionine) group showed significantly ($p < 0.05$) less increase in the mean percentage difference in creatine kinase values (1 005 units), compared to 1 698 units in the placebo group.

(3) Post-Comrades

Comparing levels in the recovery period after Comrades with pre-Comrades levels, the following differences were seen:

A significant decrease in mean cortisol values (24%) and increase in lymphocyte count values (12.6%) were found in the active group.

(4) Incidence of Illness

The active group reported having lost 19 days total of training as a results of illness, while the placebo group lost 42 days. The incidence of illness in the active group was 36% (four of the 11 subjects) and 80% (eight of the ten subjects) in the placebo group. The mean duration of the illness was five days (SD three days) in

the active group and three days (SD one day) in the placebo group. However, this did not appear to affect the performance parameters that were measured significantly during preparation for Comrades nor the performance during Comrades (race time). An appropriate CD4+/CD8+ cell ratio is important to immune defenses. Prior to Comrades the active group had a higher CD4+/CD8+ ratio (1.8 ± 1.4) than the placebo group (1.5 ± 0.7), but not significantly so. After the recovery period, compared to directly after Comrades, both groups showed improvement in the CD4 and CD8 levels, but more so in the active group (active group: CD4 increase of 84% and CD8 increase of 74%; placebo group: CD4 increase of 60% and CD8 increase of 52%).

Although supplementation did not reflect any significant differences in race times between the two groups, a significant improvement ($p=0,05$) in race times when compared with previous race times was observed in the active group (42 min, SD 60 min) but not in the placebo group (21 min, SD 54 min.).

2.3.2.5 Discussion

The first impression remains the dramatic impact an event of this nature has on the physiological system of athletes and specifically their immune systems. It should, however, be noted that immunological parameters, i.e. CD4 values, even though changing, still remained within normal ranges.

The cortisol levels presented lower in the active (L-methionine treatment) group. Increased cortisol levels cause TH1 levels (cellular immune response) to decrease. This in turn causes an increase in the TH2 (humoral immune response). The TH1 response is what is generally required in viral infections etc. The active group also had a more limited decrease in lymphocyte count and made a faster recovery compared to the placebo group. A further important difference found, was the significantly lower increase in serum creatine kinase levels in the active (L-methionine treatment) group. This is indicative of possible protection by the supplementation against muscle damage during this gruelling event. This result is of obvious importance for any athlete.

The results also indicated that L-methionine supplementation reduced the number of training days lost owing to illness and the incidence of illness. This was reflected in improved performance (42min improvement, $p = 0.05$) in the active group compared to their previous Comrades times.

The results of this study are of dramatic importance for any long- and ultra-distance athlete. Not only do these results indicate L-methionine supplementation as playing a positive role in the immune system of these athletes, but also as having a protective

effect against muscle damage. This amino acid thus seems to be of importance not only in disease, but also in health and prevention of illness.

In conclusion, this study is of importance not only due to the positive effects that could be shown in long distance athletes, but also as it is essential to first test the effect of a treatment in healthy individuals, before testing it in a serious condition such as HIV/AIDS. This study will be followed by a pilot study in HIV+ patients before following it up with a larger clinical study.

CHAPTER 3

THE ROLE OF L-METHIONINE IN DISEASE – PILOT STUDIES

3.1 PILOT STUDY NO. 1

3.1.1 INTRODUCTION

An initial pilot study was set up to test the effectiveness of the combination treatment in a small group of HIV patients. The aim of this open prospective evaluation of the combination was to test whether the dosage and treatment regime would be effective and whether an impact could be shown on the immune system of a small group of patients, before continuing with a possible larger study.

3.1.2 METHODS

3.1.2.1 Description of investigational products

Product A:

Active ingredients per capsule:	L-methionine	- 467.5
	mg	
	Folic acid	- 1.44 mg
	Vitamin B ₆	- 1.8 mg

	Vitamin B12	- 0.036 mg
Description:	Fine cream or pale yellow powder in a clear-clear, size 0, hard gelatin capsule.	
Dosage:	Take in combination, three capsules (A) and one tablet (B) on an empty stomach (approximately 30 minutes before meals).	

Product B:

Active ingredient per tablet:	Magnesium chloride	- 535 mg
Description:	Cherry-red, biconvex, round, enteric coated tablet, no markings.	
Dosage:	Taken in combination, three capsules (A) and one tablet (B) on an empty stomach (approximately 30 minutes before meals).	

3.1.2.2 Trial design

Seventeen adult patients with HIV infection consented to enroll in an open prospective pilot study for evaluation of the L-methionine combination.

3.1.2.3 Inclusion and exclusion criteria

None of the patients received anti-retroviral therapy, nor were they taking other forms of NAC or GSH supplementation.

Individuals with CD4 cell counts < 200/ml all received primary

pneumocystis prophylaxis in the form of cotrimoxazole or dapson; antifungal agents (topical miconazole, oral ketoconazole, fluconazole) were prescribed as needed. No other vitamin or mineral supplements were allowed during the period of the pilot study.

Two groups were defined according to entry CD4 counts and followed up for 52 weeks:

- Group 1 - CD4: < 200/ml (n=6)
- Group 2 - CD4: 250-500/ml (n=11)

3.1.2.4 Procedure and measurements

All patients were followed up every six weeks for the first 24 weeks and thereafter approximately every eight weeks. Blood was drawn at each visit and a CD4 count, as well as a CD4% done on every patient. At the start, as well as at 24 weeks, β -2 Microglobulins levels were also determined and based on the clinical condition of the patient and his/her general feeling of wellbeing, the Karnofsky score of every patient was determined. This is done by the physician based on standard questions relating to energy levels and possible activities.

All determinations were done by a private pathology laboratory. CD4 counts were done by means of a Flow cytometer. The β -2 Microglobulin determinations were done by means of commercial

kit microparticle enzyme immunoassays.

3.1.3 RESULTS

All the patients showed an improvement in the CD4 count. This seemed to be sustainable for at least 52 weeks (Figure 3.1 and 3.2). The CD4%, as well as the Karnofsky score values of these pilot study patients also improved (Table 3.1), confirming an improvement not only in the immune system, but also in the patients' feeling of wellbeing.

No adverse effects, severe enough to warrant discontinuation, were reported by any of the patients, with almost all reporting diminution in fatigue and improvement in energy levels. This was confirmed by the increases seen in their Karnofsky scores, used as an indicator of their productivity and feeling of wellbeing. The β -2 Microglobulins levels seemed to stay relatively stable with a possible small decrease. The patient numbers were too small for statistical evaluation.

Table 3.1: Change in immunological & productivity parameters (Pilot study No.1)

Weeks	Mean Median	CD4	CD4%	Karnofsky score	β-2 micro globulins
0	N	17	17	17	17
	Mean	256.6	19.9	84.1	2.7
	Median	290.0	24.0	85.1	2.6
6	N	17	17		
	Mean	281.9	20.9		
	Median	313.0	25.0		
12	N	17	17		
	Mean	312.8	22.4		
	Median	367.0	27.0		
18	N	17	17		
	Mean	322.4	23.6		
	Median	368.0	28.0		
24	N	17	17	17	17
	Mean	322.3	23.9	93.2	2.6
	Median	370.0	28.0	95.0	2.6
32-36	N	17	17		
	Mean	321.8	23.6		
	Median	363.0	27.0		
40-45	N	17	17		
	Mean	324.7	24.1		
	Median	368.0	27.0		
48-52	N	17	17		
	Mean	325.4	24.2		
	Median	360.0	27.0		

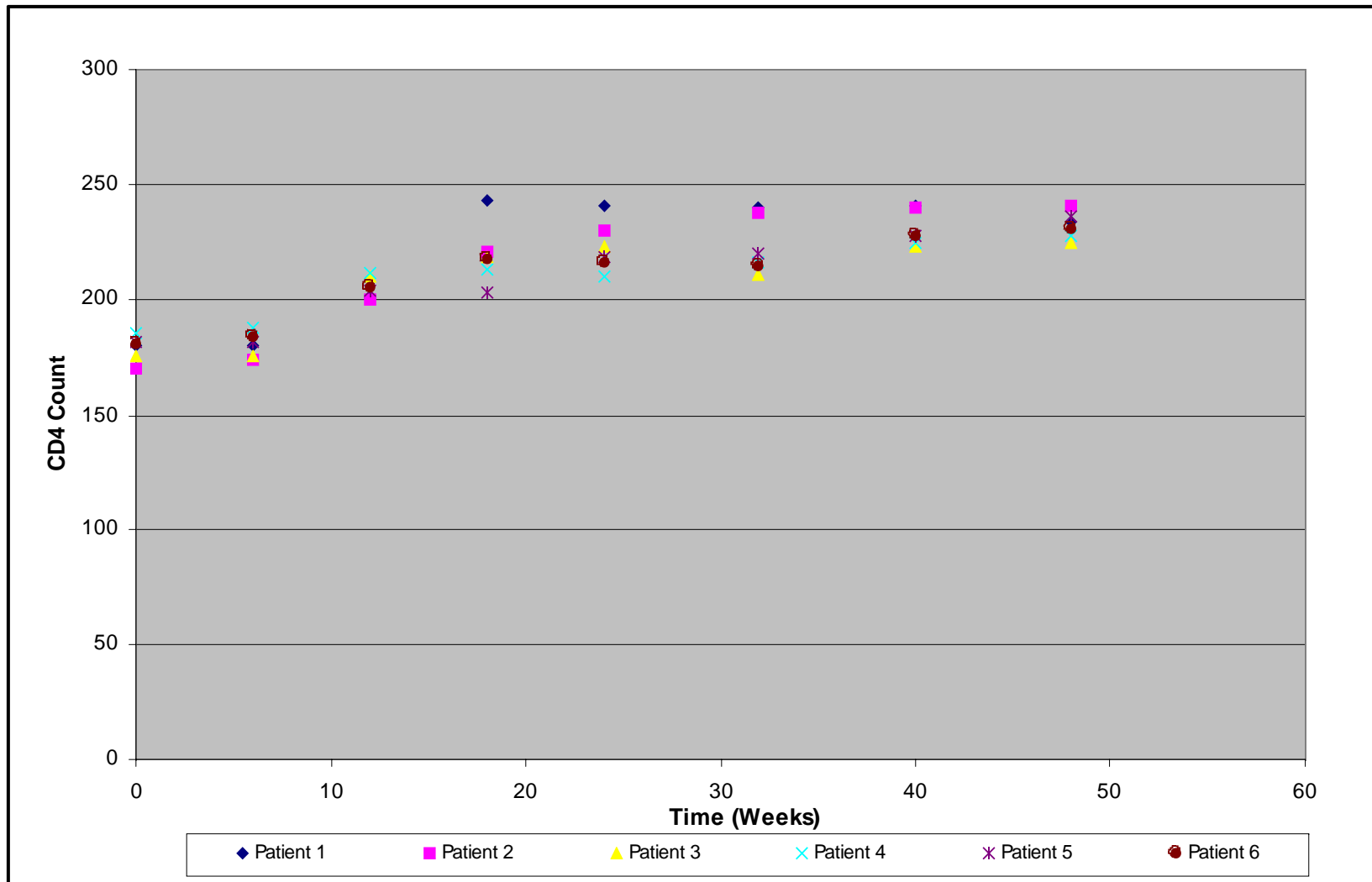
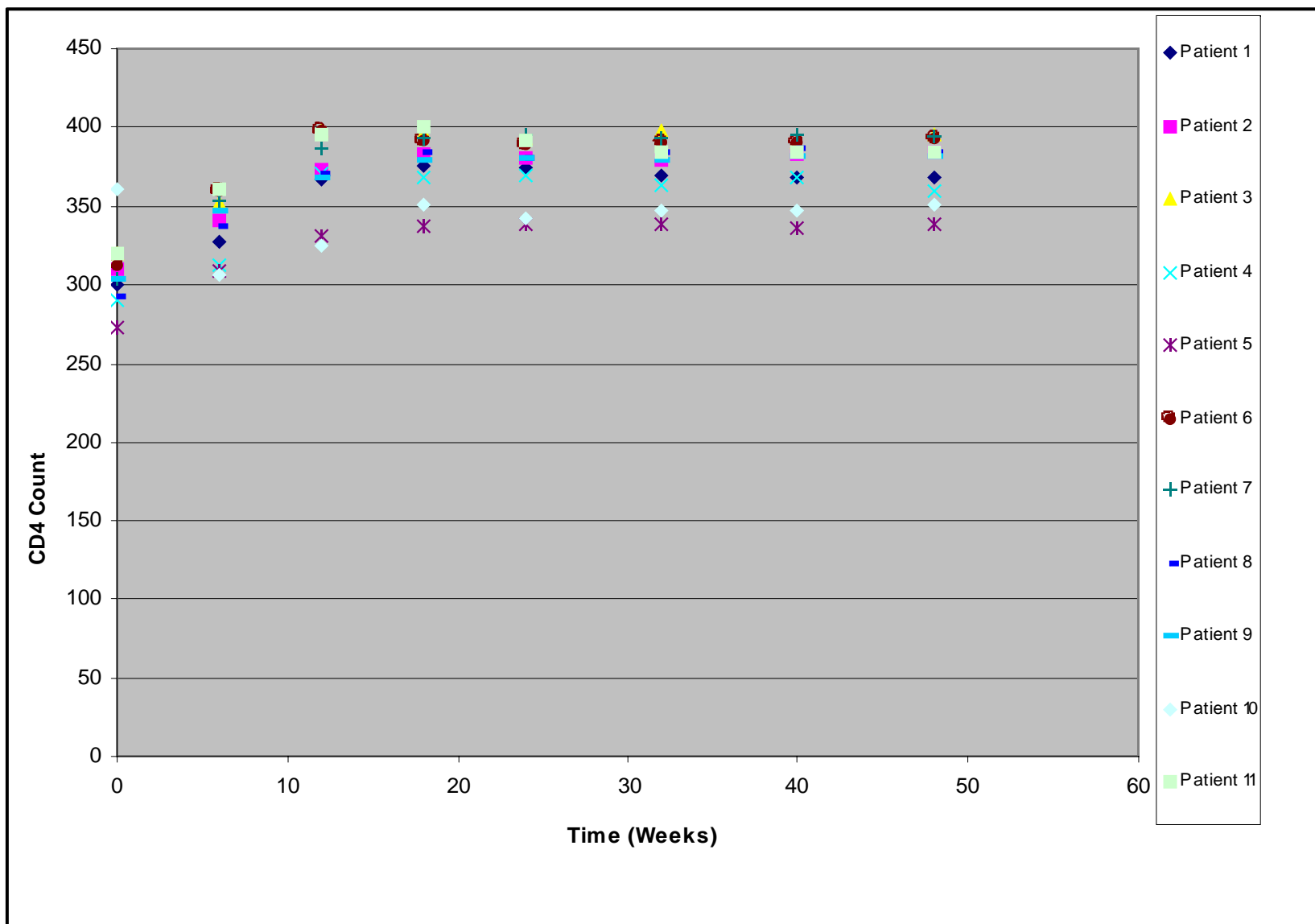


Figure 3.1: Change in CD4 count (Group A)



3.1.4 **DISCUSSION** **Figure 3.2: Change in CD4 count (Group B)**

A rise in CD4 count, as well as in the CD4%, indicating an improvement in their immune system, was observed in all patients. There was an associated improvement in the Karnofsky score and thus subjective sense of wellbeing.

The study concentrated on patients with a CD4 count at the onset of between 200 and 500. Results presented in this study show that the immune-supportive effect could be sustained for at least 12 months, without any major side effects. All results should be considered against the declining background that is usually found in HIV patients.

Even though this is an open prospective study, concentrating on a relatively small group of patients, the positive results found are still indicative of the effectiveness of the combination. This warrants further investigation by means of a larger study.

3.2 PILOT STUDY NO. 2

3.2.1 INTRODUCTION

In reaction to the first pilot study, a larger study was devised to test the effectiveness of the L-methionine combination in HIV+ patients.

3.2.2 METHODS

3.2.2.1 Description of investigational products

Product A:

Active ingredients per capsule: L-methionine -
467.5 mg

Folic acid - 1.44 mg

Vitamin B₆ - 1.8 mg

Vitamin B₁₂ 0.036 mg

Description: Fine cream or pale yellow powder in a clear-clear, size 0 hard gelatine capsule.

Dosage: Take in combination, three capsules (A) and one tablet (B) on an empty stomach (approximately 30 minutes before meals).

Product B:

Active ingredient per tablet: Magnesium chloride - 535mg

Description: Cherry-red, biconvex, round, enteric coated tablet, no markings.

Dosage: Take in combination, three capsules (A) and one tablet (B) on an empty stomach (approximately 30 minutes before meals).

In this study L-methionine was administered daily over a 24-month period to HIV+ and AIDS patients in a divided dosage, twice a day on an empty

stomach. Blood tests were performed on a monthly basis to evaluate the potential effect of L-methionine combination during the first six months.

3.2.2.2 Trial design

An open, multi-centre, prospective, clinical trial. A hundred and three adult patients with HIV infection consented to enroll in a prospective evaluation of the L-methionine combination. Of the 103 patients on the trial, 63 were from Dr S Miller (Johannesburg), and 38 from Dr M Tyrer (Johannesburg). The patients were evaluated at four-weekly intervals over a six-month period. The planned ten patients from Cape Town had to be omitted because of practical problems with transporting the blood samples, causing aging of the samples.

3.2.2.3 Procedure and measurements

The inclusion criteria were:

- Age > 18 years
- Laboratory confirmation of HIV positivity
- CD4 count at entry < 650/ml

The exclusion criteria were:

- Pregnancy
- Women who refuse to use contraception
- Underlying/concomitant renal disease

- Concomitant significant hepatic disease (liver enzymes > 3 times upper limit of normal)
- Life expectancy on clinical grounds < 6 months
- Known inborn error/s of metabolism
- Haemoglobin < 8g/dl, WCC < 750/ml at entry
- Concomitant use of corticosteroids or other potentially immunosuppressive agents, e.g. adriamycin, atropine, doxorubicin, cyclophosphamide
- Concomitant use of interferon, G-CSF, GM-CSF
- Concomitant use of other NAC or GSH products or similar supplements.

Use of antiretroviral therapies was not an exclusion criterion. No changes in existing antiretroviral therapy were, however, made during the trial. The results of ten patients had to be omitted because of transport problems that lead to aging of the blood samples. All the patients were, however, included for the evaluation of side effects (n=103). Patients were not selected on grounds of race or sex, but randomly on availability for the trial.

3.2.2.4 Parameters tested

Literature seems to indicate CD4 as the most relevant surrogate marker in trials of this nature. Other surrogate markers

suggested by literature, namely CD4%, serum β -2 Microglobulin levels and p24 antigen levels, were also investigated, and it is believed that the improvements seen in these markers should be seen as a whole.

All determinations were done by a private pathology laboratory. CD4 counts were done by means of a Flow cytometer. The β -2 Microglobulin determinations were done by means of microparticle enzyme immunoassays and the HIV p24 antigen determinations by enzyme immunoassays. This was only available as a qualitative test and not a quantitative one.

Just as important as the magnitude of the change in CD4 count and other parameters, is the direction of change, as it is generally accepted that the median values of these parameters show a constant decline over time, as is pointed out in these and other publications. The Karnofsky score, one of the most commonly used parameters of productivity and feeling of wellbeing in patients of this nature, should ideally correlate with the observed changes in the immunological parameters. These parameters, each time evaluated by the same trial doctor for each patient, give an indication of the clinical improvement of the patient.

3.2.3 RESULTS

The CD4 status of more than 96/103 (93.2%) of the patients of the trial showed a definite improvement. The results are summarised in table 3.2. The CD4 count increased by a median of 51 cells ($p < 0.0001$) and seemed to stabilise at this level (Fig. 3.3). The CD4% also showed an increase ($p < 0.0001$), with a decrease in the β -2 microglobulin values ($p < 0.0001$).

These improvements were confirmed by the increases seen in their Karnofsky scores ($p < 0.0001$), used as an indicator of their productivity. These increases seem particularly promising, considering the decreases usually seen in untreated patients. The changes in CD4 count, CD4%, β -2 microglobulin values and the Karnofsky score values over the six months for all the individual patients are shown in figures 3.4, 3.5, 3.6 and 3.7. Approximately 70% of the patients who tested positive for HIV p24 antigen at the onset tested negative after 12 weeks. This was maintained up to week 24.

No adverse effects, severe enough to warrant discontinuation, were reported by any of the patients, with almost all reporting diminution in fatigue and improvement in energy level. The only side effects recorded were persistent dyspepsia, (2.65%), insomnia (1.77%) and a pruritic skin rash in one patient. Only two patients on the trial died. The first patient (CD4 count at

onset of only 10 cmm) died of complications of Kaposi's sarcoma, which was diagnosed before the onset of the trial. The second patient (CD4 count at onset of 125 cmm) died after developing a chest infection.

Table 3.2: Change in immunological & productivity parameters

Weeks	Mean	CD4	CD4%	Karnofsky	β-2 micro
	Median				globulins

0	N	103	103	113	101
	Mean	327.4	20.9	91.9	2.8
	Median	367.0	23.0	95.0	2.9
4	N	103	103		
	Mean	367.8	23.4		
	Median	397.0	26.0		
8	N	99	99		
	Mean	375.0	24.3		
	Median	401.0	27.0		
12	N	98	98	105	95
	Mean	369.1	24.2	95.8	2.8
	Median	400.5	27.0	100	2.8
16	N	94	94		
	Mean	394.1	25.2		
	Median	411.5	28.0		
20	N	85	85		
	Mean	396.2	25.8		
	Median	418.0	28.0		
24	N	78	76	85	78
	Mean	398.1	25.2	96.4	2.7
	Median	417.0	26.0	100	2.7

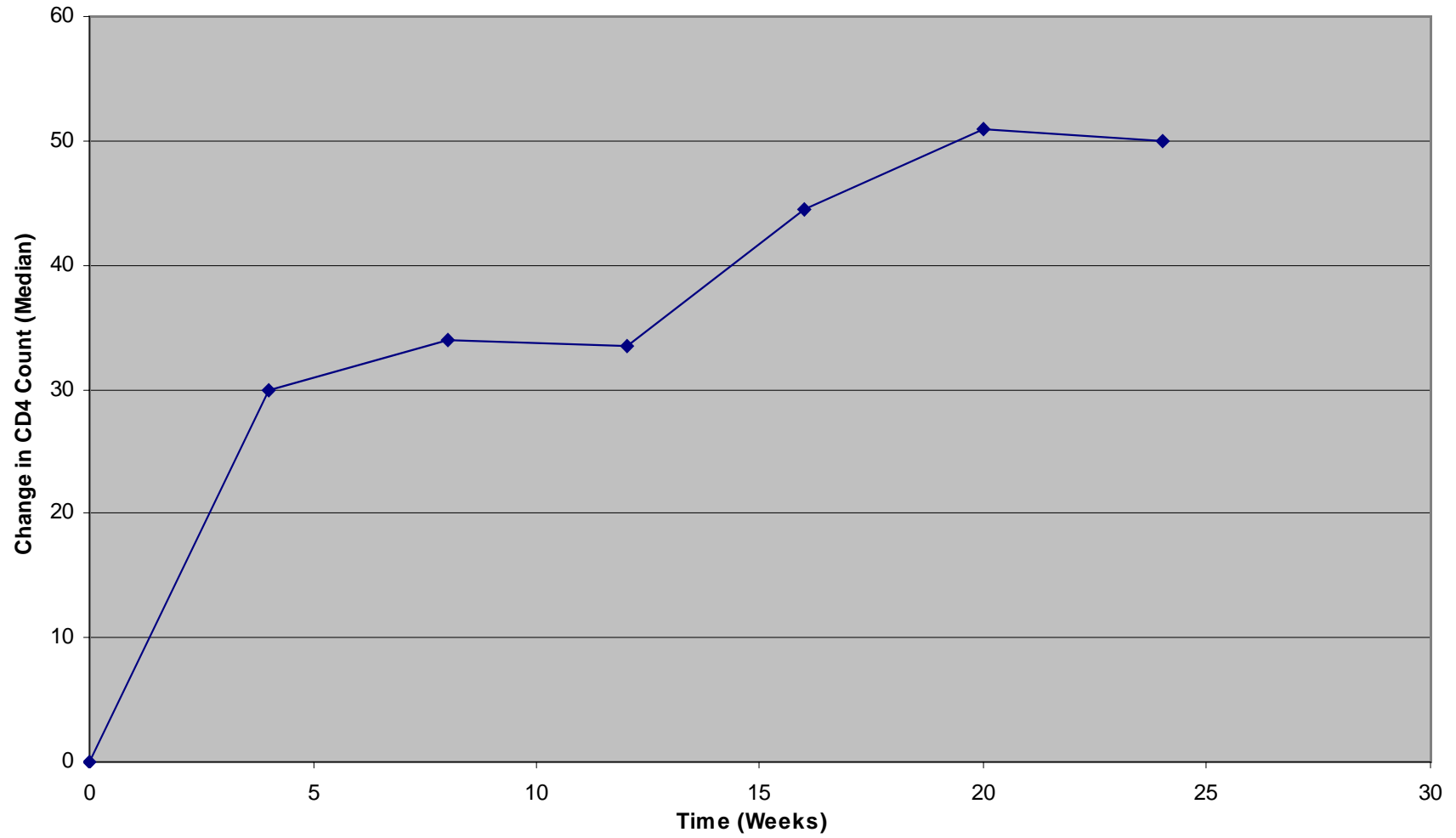


Figure 3.3: Change in CD4 count (Median) over time

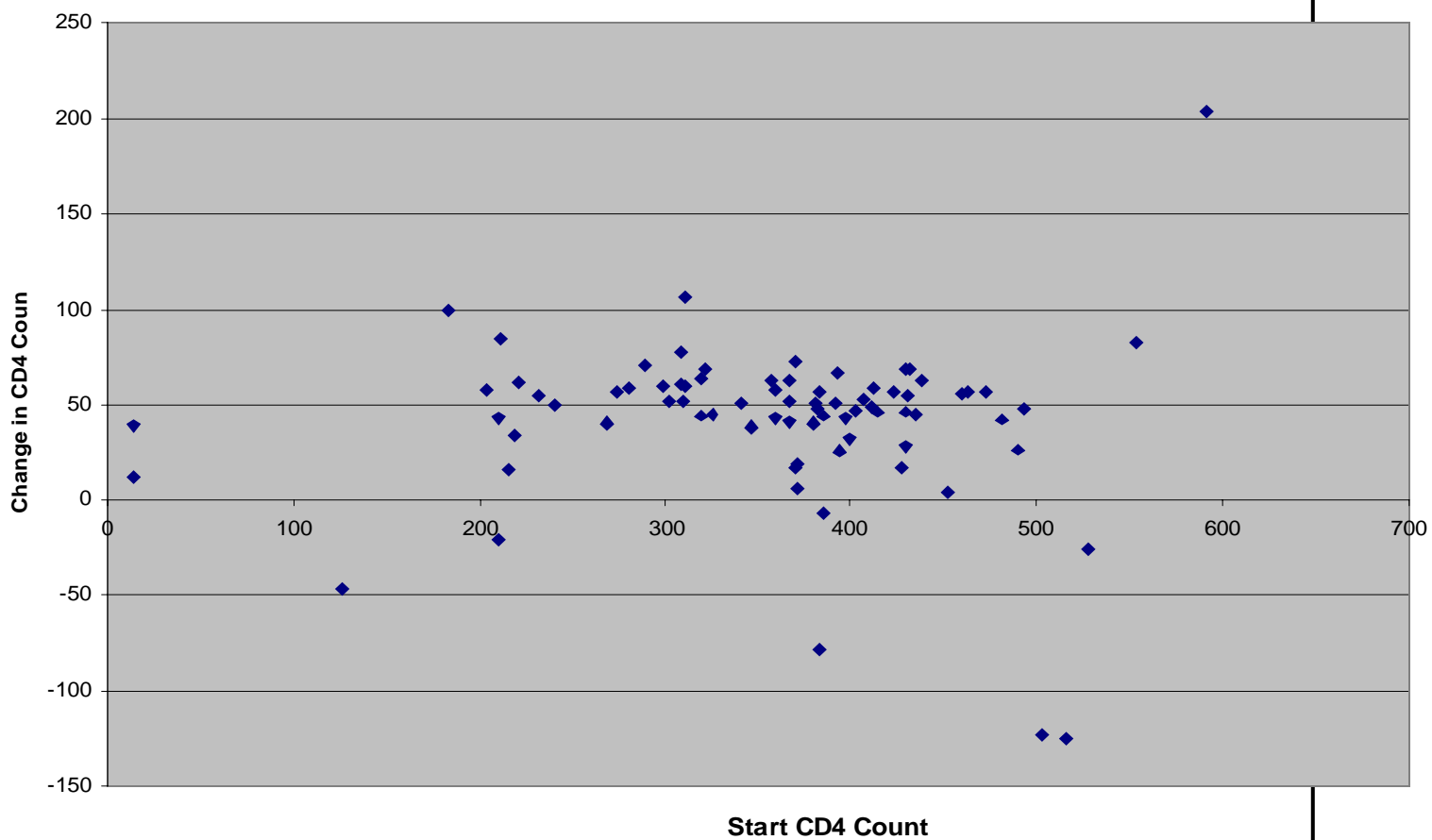


Figure 3.4: Change in CD4 count in 24 weeks

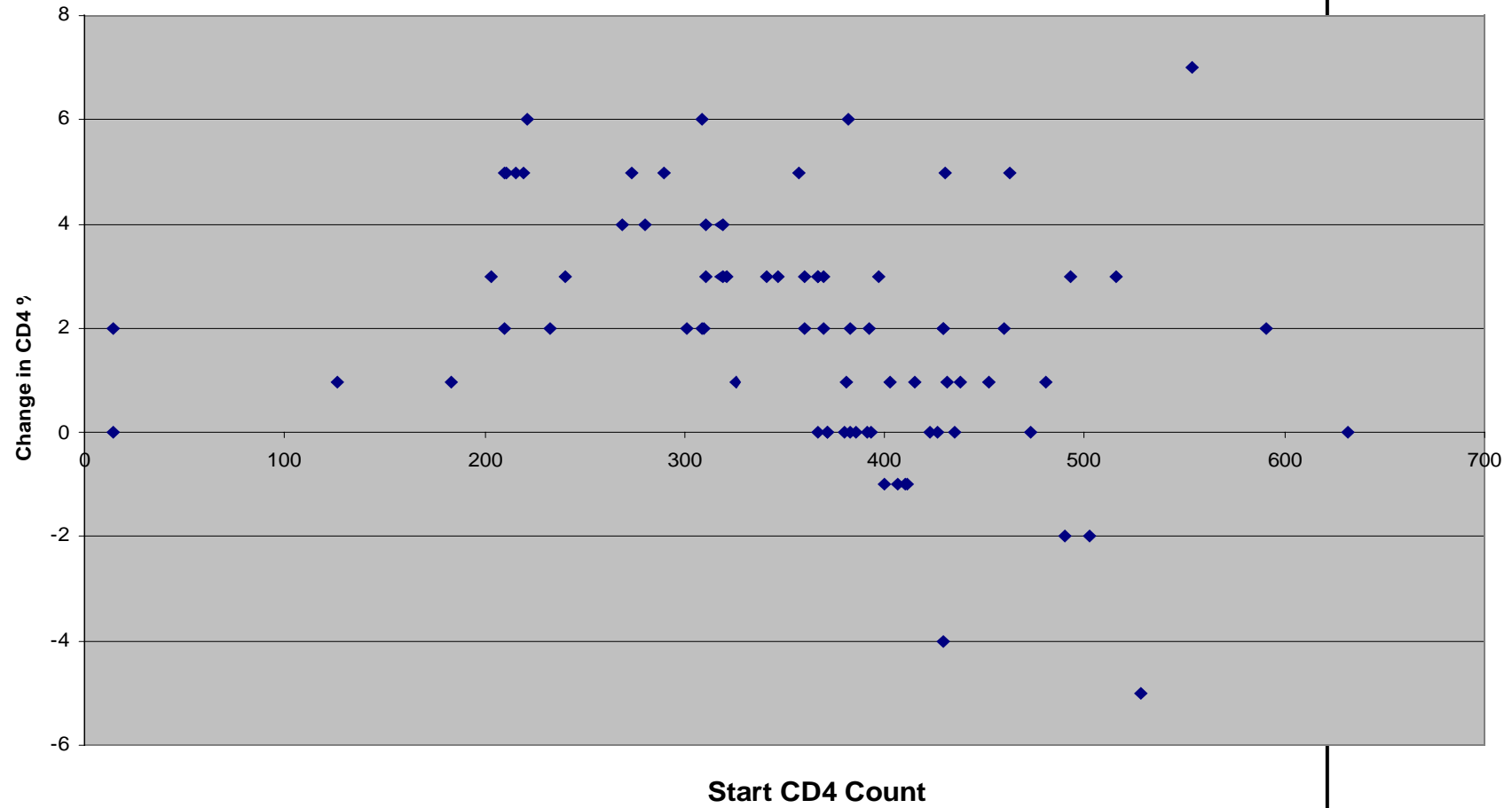
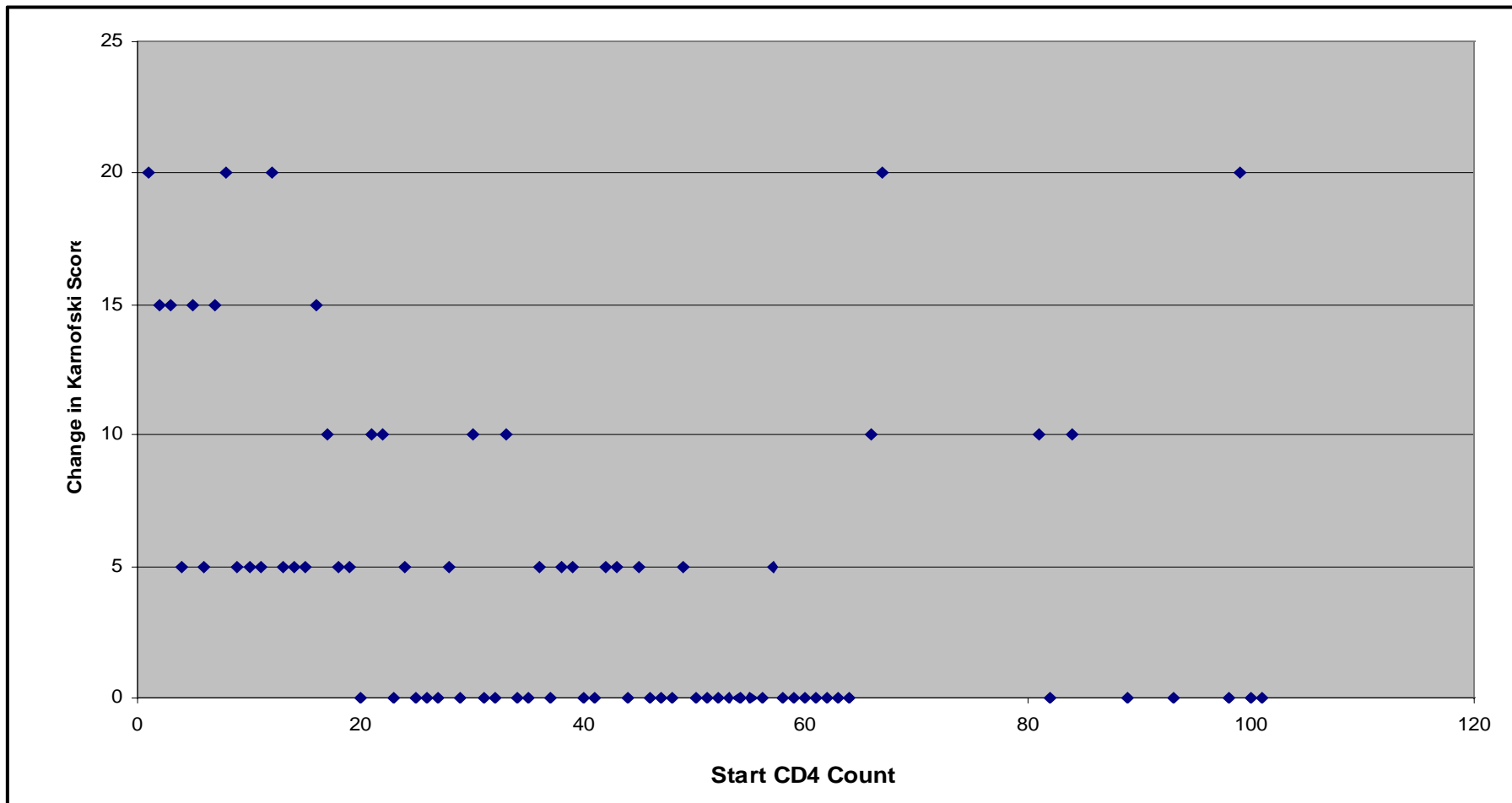


Figure 3.5: Change in CD4% in 24 weeks



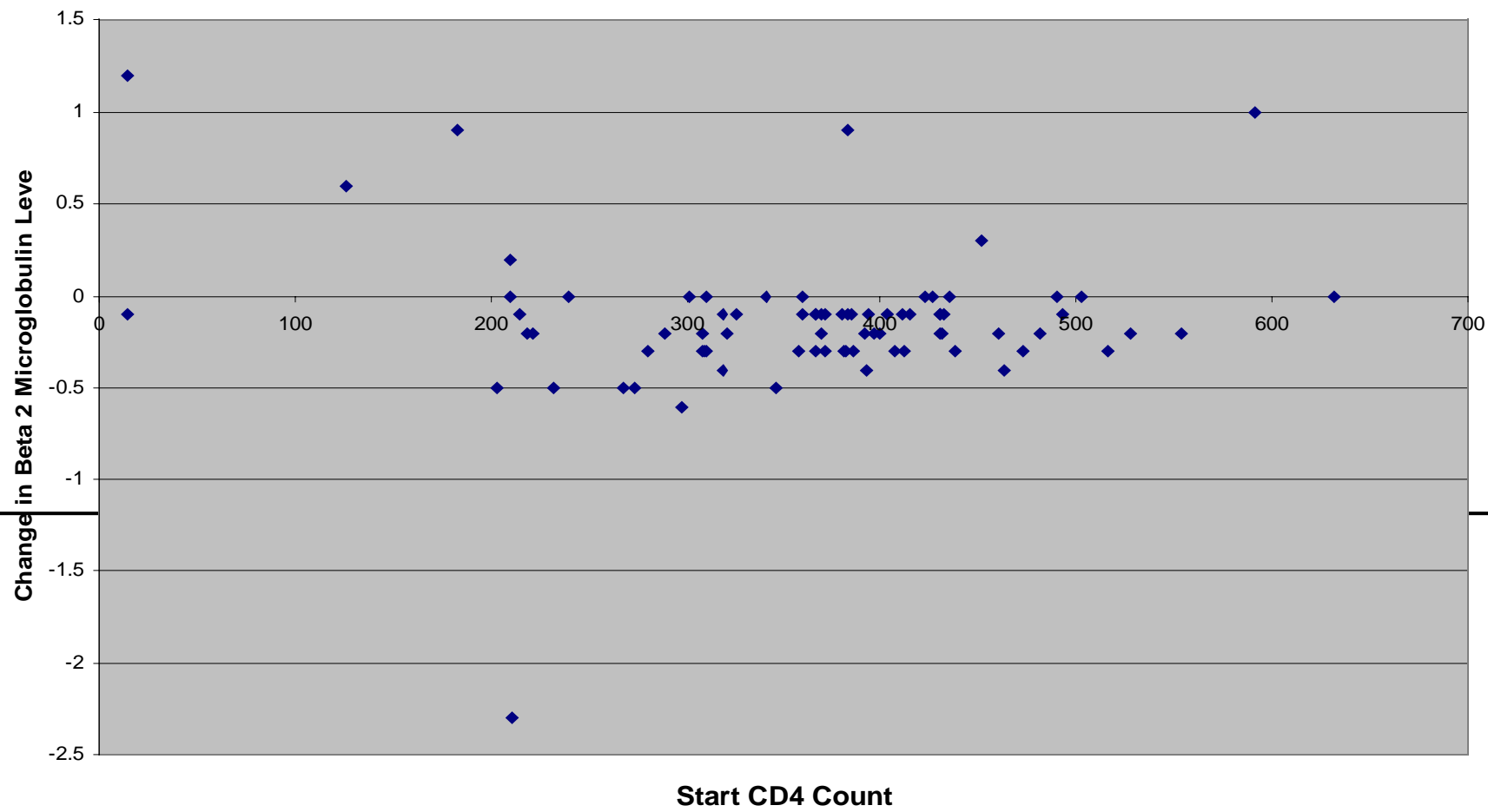


Figure 3.6: Change in Karnofski score in 24 weeks

Figure 3.7: Change in β 2 microglobulin levels in 24 weeks

3.2.4 DISCUSSION

A definite rise in CD4 count, as well as in the CD4%, was observed in all groups. This proved to be statistically highly significant ($p < 0.0001$). The clinical significance of an increase of this nature has to be investigated further by means of larger double-blind, placebo-controlled, follow-up studies. There was, however, also an associated improvement in the Karnofsky score and subjective sense of wellbeing, indicating some clinical improvement, with a decrease in the β -2 microglobulins.

The study concentrated on patients with a CD4 count at the onset of between 200 and 500. The main increase in CD4 and CD4% values occurred within the first 12 to 16 weeks, whereafter it was sustained up to week 24. Follow-up trials will, however, be needed to ascertain the full effect of these increases and determine the clinical value of a supplementation of this nature, specifically also looking at safety.

For further evaluation, the patients were retrospectively divided into two groups: those who received no other antiretroviral therapy, thus only receiving the L-methionine supplement ($n=29$) and those who received the L-methionine combination supplementary to their usual antiretroviral therapy ($n=74$). No significant differences could be found between the increases seen in the two groups within the first 12 weeks. The supportive

effect of L-methionine combination thus does not seem to be dependent, nor inhibited by the combined use of other antiretroviral drugs. In the longer term, 12 to 24 weeks, the increase in the CD4 count seemed to be slightly better sustained in the combined treatment group. The statistical significance of this was not conclusive. No significant differences could be seen between males and females and different race groups in this study. This will, however, have to be confirmed by larger follow-up trials, as the numbers in this study remained relatively small.

The 'combined treatment' included a variety of anti-retroviral drugs, such as didanosine, lamivudine, etc., but mainly zidovudine (AZT). As the 'L-methionine treatment only' group was relatively small, a follow-up study needs to concentrate on this subsection of patients to elucidate the effect of the supplement fully, independent of any other anti-retroviral treatments.

CHAPTER 4

THE ROLE OF L-METHIONINE IN DISEASE – CLINICAL STUDY ON HIV+ PATIENTS

4.1 INTRODUCTION

Based on the success of the previous two pilot studies, it was decided to continue with a larger double-blind, placebo-controlled study. In the previous two studies some of the patients could be seen to be selected and some patients, in the case of the second study, were also on anti-retroviral treatment. Both previous studies were also open, meaning the placebo effect could not be fully excluded. More safety and clinical parameters also have to be taken into consideration. In view of the success achieved, the dosage was kept the same as in the previous two studies.

4.2 METHODS

4.2.1 GOOD CLINICAL PRACTICE

The study was conducted in accordance with good clinical practice (GCP) as stipulated in the guidelines “Good Clinical Practice for Trials on Medical Products in the European

Community” (GPMP). Compliance with these requirements also constitutes conformity with the ethical principles of the Declaration of Helsinki. The trial master file containing essential documents for this study has been established and archived.

4.2.2 ETHICS

4.2.2.1 Independent ethics committee/Institutional review board

The clinical study protocol, informed consent document(s), and any other appropriate study-related documents were reviewed and approved by the MASA Research Ethics Committee, Clindepharm International Pharma-Ethics ethical committee, as well as the Research Protocol and Ethics Committee of Pretoria Academic Hospital and Tshwane University of Technology Ethics Committee.

4.2.2.2 Subject information and informed consent

Informed consent was obtained prior to conducting any study-related procedures. A sample of the informed consent document is found in the addendum (page 132).

4.2.3 STUDY OBJECTIVE

The main objective was the evaluation of potential clinical, biochemical and immunological benefits of Equimmune (L-

methionine in combination with vitamin B₆, vitamin B₁₂, folic acid and magnesium), and to investigate the potential of this combination to act as a possible immune-supportive treatment in HIV+ patients.

4.2.4 INVESTIGATIONAL PLAN

4.2.4.1 Study design

The trial was a randomised, double blind, placebo-controlled study. All patients had to have laboratory-confirmed HIV infection with CD4 cell counts < 500 cmm and > 200 cmm. An independent trial monitoring and evaluation committee was appointed, comprising a statistician from the University of the Orange Free State, a clinician from the UOFS and a representative from the National Institute of Virology (which later became National Institute for Communicable Diseases), knowledgeable on AIDS. These committee members were required not to be involved with the trial or the company supplying the medication and sponsoring the trial, viz. Biomox Pharmaceuticals.

There was a 'washout period' of six weeks; thus for six weeks for all participating patients. This means that for six weeks before the start of the trial the patients did not receive any other antiviral or HIV treatment. The data were to be

analysed at six months, 12 months, 24 months, 36 months and 48 months. If at any of these intervals it should be found, by the discretion of the independent monitoring committee, that there was a definite and significant difference between the two groups, they would be allowed to break the code. It would then be determined which was the active and which the placebo group.

If the active treatment group showed a significant improvement in relation to the placebo group, the placebo group would fall away. This would result in the placebo patients going onto the Equimmune treatment for the remainder of the trial. If the opposite should occur, namely the placebo group show a significant improvement in relation to the active treatment group, the study would be terminated immediately. For the purposes of this specific study, a “clinically significant difference” was predefined in the protocol as a change in CD4 count of more than 30 cmm, thus approximately 10% difference.

4.2.4.2 Selection of patients

The inclusion criteria were:

- Age > 18 years

- Laboratory confirmation of HIV positivity
- CD4 count at entry < 500 cmm and > 200 cmm
- Prophylactic treatment of any possible secondary problem
- Patients giving their written consent and agreeing to the described protocol.

The exclusion criteria were:

- Pregnancy
- Women who refuse to use contraception
- Underlying/concomitant renal disease
- Concomitant significant hepatic disease (liver enzymes > 3 times upper limit of normal)
- Life expectancy on clinical grounds < 6 months
- Known inborn error(s) of metabolism
- Concomitant use of other NAC or GSH products
- Haemoglobin < 8 g/dl, WCC < 750/ml at entry
- Concomitant use of corticosteroids or other potentially immunosuppressive agents, e.g. adriamycin, atoposide, doxorubicin, cyclophosphamide
- Concomitant use of interferon, or any other immunological agent
- Any patient who, on clinical grounds, needed or would need other antiviral treatment within the next six months.

4.2.4.3 Number of patients

The planned, total population to be used in the trial was 400 HIV+ patients with a CD4 count of between 200 and 500 cmm. Two hundred and fifty-three patients (129 patients in the Equimmune treatment group and 124 in the placebo treatment group) were available for analysis. All (253) patients were from the Gauteng area and included both private and state patients. All patients were older than 18 years and the selection of patients was independent of race and sex.

4.2.4.4 Drop-outs

Any patient had the right to withdraw from the trial at any stage without giving any reasons, or if they at any stage felt that they were experiencing side effects, which warranted discontinuation. Furthermore a patient would automatically disqualify him/herself from further participation in the trial if they:

- Did not comply with the conditions as set out in the protocol
- Stopped the treatment for longer than one week
- Started taking any other antiviral or HIV treatment within the first six months from the start of the trial, except with permission (which would not be withheld unreasonably) of

the medical expert of the trial

- Started taking any other immune-stimulant/suppressing agents

4.2.4.5 Criteria for withdrawal

- Trial endpoint; 48 months
- Voluntary withdrawal by the patient
- Disqualification of a patient for not adhering to the treatment regime
- Death or serious disease
- Exclusion criteria
- Technical problems
- Women who wished to become pregnant.

Withdrawal took place on recommendation of the investigator involved, in collaboration with the trial monitor. The trial monitor informed FARMOVS of the withdrawal in writing within one week of the withdrawal. All data were also screened by FARMOVS for exclusion criteria within the first four weeks of the start of the trial. If the withdrawal took place within the first six weeks of the trial, a suitable replacement could be selected by the investigator involved. The trial monitor informed FARMOVS of the replacement within one week of the replacement. In the case of

withdrawal, the reason/s had to be carefully assessed and noted on the case report form (CRF).

4.2.5 STUDY TREATMENTS

All 253 patients included in this analysis received three capsules (colourless capsule, filled with fine, cream or pale yellow powder) and one tablet (cherry red, biconvex, round, film-coated tablet) twice daily on an empty stomach, i.e. every morning and evening for 48 months. The product was packed as a small box, containing two securitainers of 90 capsules each and one amber glass bottle containing 60 tablets, i.e. one month's supply. One hundred and twenty-four of the patients received only a placebo for the first 12 months of the study period. This product was produced and packed to look the same as the test treatment (Equimmune), but contained no active ingredient; instead, an applicable non-active filling agent was used. However, this group of patients switched to the Equimmune treatment after 12 months for the remainder of the trial. The other 129 patients received the active treatment (Equimmune), with a daily dose of six capsules and two tablets, as described.

Placebo: Starch 450 mg

Equimmune:	Capsule containing:	
	L-methionine	467.5 mg
	Vitamin B ₆	1.8 mg
	Vitamin B ₁₂	0.036 mg
	Folic acid	1.44 mg
	And Tablet containing:	
	Magnesium chloride	535 mg

4.2.6 DATA QUALITY ASSURANCE

The clinical study protocol, the case record forms and the study procedures were discussed with the investigator and the co-investigators.

Case record forms were used for data collection. Data entry into the database was done by the data management section of the Division of Biometry, FARMOVS Research Centre, Bloemfontein, South Africa. The coding tables used for data entry were the International Classification of Diseases, 9th Revision, Clinical Modification (ICD9CM) for diseases, the Hoechst Adverse Reaction Terminology System for adverse events, and the World Health Organisation drug reference list for drugs.

Data validation was performed using computerised and manual checks.

4.2.7 MONITORING AND AUDITING

The study site was monitored by Biomox monitors according to the International Conference on Harmonisation GCP guidelines as laid out in the guideline GPMP. CRF's were reviewed against source data during monitoring.

4.2.8 STATISTICAL PROCEDURES

4.2.8.1 Demographic data

Demographic and background variables were evaluated separately for the two treatment groups. Descriptive statistics, including means and standard deviations, were calculated for fixed variables and contingency tables were compiled for categorical variables.

4.2.8.2 Efficacy data

The two treatment groups were compared with regard to the change from baseline to endpoint (six weeks, three months, six months, 12 months). From 12 months onwards, the placebo group was put on active treatment, whereafter values at 12 months were used as baseline for the former placebo group and 18 months, 24 months, 36 months and 48 months as endpoints for both groups. Therefore, different baselines were used for the two groups from 12 months onwards.

The variables were:

The primary variable:

- CD4 cell count

The secondary variables:

1. Total lymphocyte count
2. CD4 cell percentage
3. CD8 cell count
4. CD8 cell percentage
5. CD4 + CD8 cell ratio
6. Erythrocyte count
7. Haemoglobin
8. Platelet count
9. Leucocyte count
10. HIV levels (viral loads)
11. MOS health rating
12. Body weight

The individual values of all efficacy variables, as well as the change from baseline (entry), were tabulated for each patient and assessed, with descriptive statistics for each assessment. The following descriptive statistics were calculated: mean, SD, coefficient of variation (CV%), minimum (Min), maximum (Max) and median values, as well

as the number of observations (n).

For all continuous variables, the change from baseline (entry) to six weeks, three months, six months and 12 months were subjected to analysis of variance (ANOVA) with treatment as main effect. Conventional 95% confidence intervals for the difference in mean change from baseline (Equimmune – placebo) were calculated for six weeks, three months and 12 months. CD4 cell counts were also subjected to analysis of covariance (ANCOVA), with treatment as main effect and baseline as covariate. Additional 'changes from baseline' for the CD4 cell count were also calculated for the following subgroups:

- 200 < CD4 cell count < 300 cmm
- 300 < CD4 cell count < 400 cmm
- 400 < CD4 cell count < 500 cmm
- Male patients
- Female patients
- White patients
- Black patients
- Patients of Centre 1
- Patients of Centre 2
- Patients of Centre 3
- Patients of Centre 4.

The analyses were done using LOCF, as well as no LOCF.

4.2.8.3 Safety data

Adverse events were listed on an individual basis and summarised by body system. This was done for all adverse events, regardless of causal relationship to study medication, as well as those considered to be at least possibly related to study medication.

Safety data, including laboratory findings, were tabulated. Laboratory values were compared with the investigator's normal ranges and were tabulated as normal or abnormal for each treatment group.

All nominal and ordinal variables were presented in frequency tables, grouped by treatment and visit. Continuous variables were summarised through descriptive statistics for each treatment and visit, where applicable.

The following definitions were used when screening all laboratory data to identify potentially important individual laboratory values:

- Abnormal value. Any value outside the normal range defined.
- Predefined change abnormal (PCA).

- A PCA increase is a laboratory value occurring on treatment that is abnormally high (i.e. above the upper limit of normal) and is an increase from baseline of at least a predefined amount.
- A PCA decrease is a laboratory value occurring on treatment that is abnormally low (i.e. below the lower limit of normal) and is a decrease from baseline of at least a predefined amount.
- Last evaluation, predefined change abnormal (LPCA). An LPCA is a PCA occurring at the patient's final evaluation on treatment (endpoint).

All laboratory data have been converted into Standard International units for the analysis. The investigator's normal ranges were used for all PCA and LPCA evaluations.

In general, the PC values have been fixed as the delta limits observed in a population of over 20 000 patients, of whom 1% had a change of at least the delta limit while under placebo treatment.

For some of the laboratory variables, changes in only one direction are relevant to indicate a possible problem. Only increases of at least the specified amount have been considered for liver enzymes and for creatinine, urea and total

bilirubin. For all other variables, changes in both directions were of interest.

4.2.8.4 Data-handling procedures

The following coding tables were used in the evaluation of findings:

ICD : International Classification of Diseases

* for concurrent diseases

* for past medical and surgical history

HARTS: Hoechst Adverse Reaction Terminology System

* for adverse events

DRUG 88: WHO 1988 Drug Reference List

* for concomitant medication

4.3 RESULTS

4.3.1 SUBJECT ACCOUNTING

It was planned to recruit a total of 400 patients at four centres for this double-blind, placebo-controlled randomised study. Two primary groups of patients were to be enrolled; eventually 200 would have received Equimmune and 200 the corresponding placebo. All the patients should have had laboratory-confirmed HIV infection with a CD4 cell count between 200 and 500 cmm. One hundred and twenty-six patients dropped out as a result of the above-mentioned inclusion criterion, with a CD4 cell count too low or too high. Only 253 patients, randomised into the two treatment groups, continued with the study after three months.

Finally, the data of 253 patients (129 patients in the Equimmune treatment group and 124 in the placebo treatment group, with CD4 cell counts between 200 and 500 cmm) were available for this 48-month analysis. There was a washout period of six weeks; thus for six weeks before the start of the trial the patients should not have received any other antiviral or HIV treatment.

4.3.2 PROTOCOL DEVIATIONS

There were ten investigations/visits, i.e. at entry, 48 hours, six weeks, three months, six months, 12 months, 18 months, 24 months, 36 months and 48 months. The following table summarises the reasons for early withdrawal:

Table 4.1: Reasons for early withdrawal

Reason for withdrawal	Total		Equimmune		Placebo	
	n	%	n	%	n	%
Missing	9	5.56	4	5.71	5	5.43
Adverse event	1	0.62	-	-	1	1.09
Patient non-compliance	20	12.35	11	15.71	9	9.78
Consent withdrawn	13	8.02	6	8.57	7	7.61
Patient lost to follow-up	98	60.49	41	58.57	57	61.96
Death	3	1.85	2	2.86	1	1.09
Protocol violation	2	1.23	1	1.43	1	1.09
Other	16	9.88	5	7.14	11	11.96
Total	162	100.0	70	100.0	92	100.0

A total of 162 patients (70 in the Equimmune treatment group and 92 in the placebo treatment group) withdrew before the end of the 48-month study period. The majority of the patients [60.49%: 41 (58.57%) in the Equimmune treatment group and 57 (61.96%) in the placebo treatment group] withdrew as a result of neglecting to follow up by not keeping scheduled appointments. One patient withdrew because of an adverse event and two patients fell pregnant. One other patient was also one of the protocol violators. Three patients died during the 48-month study period: One patient (Equimmune treatment group) died of respiratory failure, and the second patient (placebo) died of pneumocystic pneumonia and the reason for the third patient is unknown.

4.3.3 ADMINISTRATION OF STUDY MEDICATION

4.3.3.1 Dosage and duration

The 253 patients who were included in the study were randomised into two treatment groups: 129 received Equimmune and 124 received placebo for the first 12 months. After 12 months, the placebo patients switched to the Equimmune treatment.

All patients received three capsules (colourless capsule, filled with fine, cream or pale yellow powder) and one tablet (cherry

red, biconvex, round, film-coated tablet) twice daily on an empty stomach. The patients in the Equimmune treatment group received the active daily dose and the patients in the placebo treatment group received the placebo, with no active ingredient. This report reflects the results after the final treatment period of 48 months.

The outcome of the treatment was assessed at regular examinations over 48 months. Examinations were carried out at entry, 48 hours, six weeks, three months, six months, 12 months, 18 months, 24 months, 36 months and 48 months.

4.3.3.2 Compliance

The medication was supplied at a maximum of three months' supply at a time. The importance of taking the medication without any interruptions was emphasised to the patients and they were supplied with a form to fill in each time they took the medication, for their own use to remind them. Patients were encouraged to inform the investigator if they forgot, or for any reason, missed a dosage. This was documented on the report form. From the non-missing compliance records it was seen that the majority of patients took 80 to 100% of the supplied capsules/tablets, except for the six weeks visit, where only 3.6% of the patients took 80 to 100% of their supply. The percentage of patients with compliance less than

80% decreased from about 29.2% at three months to 8.3% at six months and 1.6% at 12 months. However, there was a slight increase in the percentage of patients with compliance less than 80% from 12 months to 18 months (9.5%) and 24 months (13.8%).

4.3.3.3 Drug accountability

For the patients included in the interim analysis, all study materials (completed, partially completed and blank CRFs, study medication, etc.) were returned to Biomox.

4.3.4 STUDY POPULATIONS ANALYSED

Men and women, older than 18 years, with laboratory-confirmed HIV and a CD4 cell count between 200 and 500 cmm were enrolled in the study. Two hundred and fifty-three patients, randomised into two treatment groups, i.e. Equimmune (129 patients) and placebo (124 patients), continued with the study after three months. All the patients were evaluated for efficacy and safety in this 48-month analysis.

4.3.5 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Two hundred and fifty-three patients, randomised into two treatment groups, i.e. Equimmune (70 male, 59 female; total

129) and placebo (72 male and 52 female; total 124), participated in the study. Of the 253 patients, 73 were white patients, 174 were black patients and an ethnic group of six patients was described as 'Other'.

The two groups of patients compared well at baseline with regard to demographic characteristics.

Table 4.2: Demographic details of patients

	Equimmune (n = 129)	Placebo (n = 124)	Total (n = 253)
Mean age (years)	34.5	34.4	34.5
Age range (years)	19.8 – 72.8	19.0 – 56.5	19.0 – 72.8
Mean height (cm)	169.5 ^a	170.1 ^b	169.8^c
Height range (cm)	150.0 – 189.0	140.0 – 198.0	140.0–198.0
Mean weight (kg)	67.9 ^d	69.6 ^e	68.7^f
Weight range (kg)	40.0 – 117.0	38.0 – 142.0	38.0 – 142.0
Mean BMI (kg/m ²)	23.6 [*]	24.1 ^{**}	23.8[#]
BMI range (kg/m ²)	15.4 – 43.0	16.9 – 43.3	15.4 – 43.3

a n = 128

d n = 129

* n = 128

b n = 119

e n = 121

** n = 118

c n = 247

f n = 250

n = 246

4.3.6 PRIMARY DISEASE

Male and female patients with laboratory-confirmed HIV infection, with CD4 cell counts of < 500 cmm and > 200 cmm, were included in this study.

4.3.7 CONCOMITANT ILLNESSES

A total of 139 (54.9%) patients reported 229 diseases; 71 (55.0%; 116 mentions) patients in the Equimmune treatment group and 68 (54.8%; 113 mentions) patients in the placebo treatment group.

The most frequent system organ classes reported in the medical and surgical history were the following: infections and infestations, followed by surgical and medical procedures, gastrointestinal disorders, blood and lymphatic system disorders, psychiatric disorders, vascular disorders and other disorders. The two treatment groups compared well with regard to concomitant illnesses.

4.3.8 CONCOMITANT MEDICATION

Concomitant medication was taken by 193 (76.3%; 2 965 mentions) of the patients during the study: 106 (82.2%; 1 622

mentions) in the Equimmune treatment group and 87 (70.2%; 1 343 mentions) in the placebo treatment group. Vitamins were the most frequently reported additional therapy category and were taken by 76 patients (30.0%; 386 mentions): 42 (32.6%; 230 mentions) in the Equimmune treatment group and 34 (27.4%; 156 mentions) in the placebo treatment group. Medication in the category "All Other Therapeutic Products" was taken by 75 patients (29.6%; 418 mentions), of which 43 (33.3%; 223 mentions) were in the Equimmune treatment group and 32 (25.8%; 195 mentions) in the placebo treatment group. The category "Antibacterials for Systemic Use" was the third most frequently taken group of medicines and was taken by 72 patients (28.5%; 213 mentions); 43 (33.3%; 119 mentions) in the Equimmune treatment group and 29 (23.4%; 94 mentions) in the placebo treatment group. Ophthalmologicals was taken by 51 (20.2%, 117 mentions) patients in total and Antivirals for Systemic Use, representing "Direct Acting Antivirals" only, was taken by 48 patients (19.0%; 333 mentions); 25 (19.4%; 139 mentions) in the Equimmune treatment group and 23 (18.5%; 194 mentions) in the placebo treatment group. Analgesics (15.8%) and Topical Products for Joint Muscular Pain (14.6%) were also taken frequently.

4.3.9 EFFICACY

The efficacy variables measured in this study were:

The primary variable was:

- CD4 cell count
- The secondary variables were:
- Total lymphocyte count
- CD4 cell percentage
- CD8 cell count
- CD8 cell percentage
- CD4 + CD8 cell ratio
- Erythrocyte count
- Haemoglobin
- Platelet count
- Leukocyte count
- HIV levels (Viral loads)
- MOS health rating
- Body weight

Except where otherwise stipulated, the no LOCF principal was applied with respect to statistical evaluation.

4.3.9.1 Primary variable – CD4 cell count

With regard to the CD4 cell count, the two treatment groups compared well at baseline (entry), with mean CD4 cell counts of 340.3 and 342.6 cmm for Equimmune and placebo groups respectively. Clinically and statistically significant differences were already found after six months in at least two subgroups. Following the protocol, the decision to switch the placebo group to Equimmune treatment at the 12-month stage was based on the following results of females at six months and the results of Centre 1 at six months.

Although there was a decrease in CD4 count of females in both groups at six months, the decrease in the Equimmune groups was statistically significantly less than the decrease in the placebo group ($p = 0.0027$). The difference in change (Equimmune – placebo) of 59.43 cmm was of clinical importance (95% CI from 21.39 to 97.46) (Table 4.9). Although not statistically significant using LOCF, the results had a tendency in the same direction ($p = 0.0554$, 95% CI from -0.74 to 63.82).

After six months, patients of Centre 1 who were on Equimmune treatment also showed a statistically significant ($p = 0.0377$) smaller decrease in the CD4 count than those on placebo, with the difference in change (Equimmune –

placebo) of 45.08 cmm with 95% CI from 2.72 to 87.43) (Table 4.10). This difference was also of clinical importance. Although not significant, a trend in the same direction was observed using LOCF ($p = 0.2255$, 95% CI from -16.62 to 68.76). Graphic presentation of difference in change from baseline of CD4 mean values of the women can be found in Figure 4.1. It must be noted that entry was used as baseline in all figures.

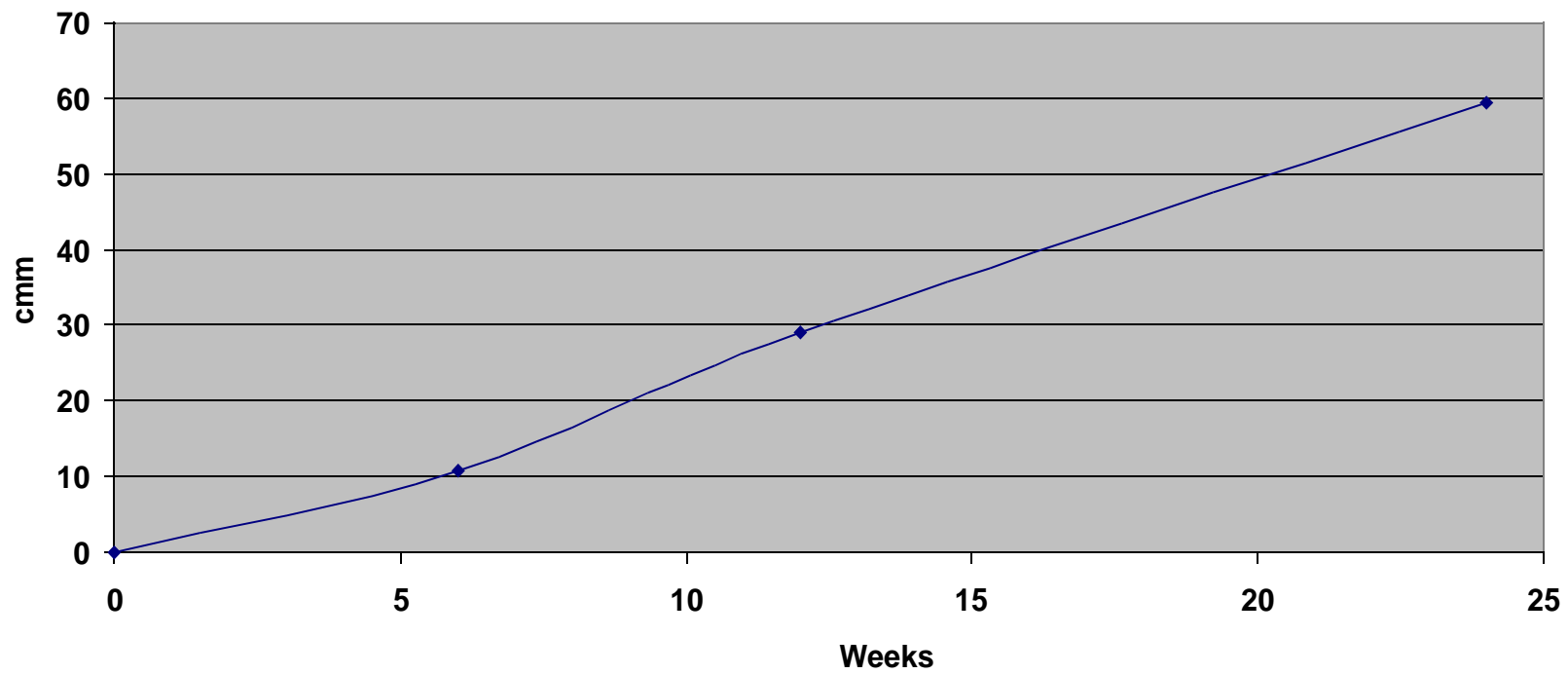


Figure 4.1: Difference in change of CD4 count from baseline (Equimmune – placebo) in the female group

The mean of the total Equimmune treatment group at 12 months was 346.3 cmm and the mean of the placebo treatment group was 336.8 cmm. Relative to the baseline (entry), there was an increase in the CD4 cell count of the Equimmune treatment group, but a decrease in the CD4 cell count of the placebo treatment group. The difference in change from baseline (Equimmune – placebo) is 13.25 cmm (ANCOVA: 13.1 cmm), with the 95% CI from –13.98 to 40.49 cmm (ANCOVA: -14.18 to 40.38 cmm), showing a trend towards higher values in the Equimmune than in the placebo treatment group. Even though this did not constitute a clinically or statistically significant difference, it still served to confirm previous results.

Additional analyses were done on the CD4 cell count for the following subgroups:

- Patients with $200 < \text{CD4 cell count} < 300$ cmm
- Patients with $300 < \text{CD4 cell count} < 400$ cmm
- Patients with $400 < \text{CD4 cell count} < 500$ cmm
- White patients
- Black patients
- Male patients
- Female patients
- Patients of Centre 1
- Patients of Centre 2

- Patients of Centre 3
- Patients of Centre 4

The results can be interpreted in a similar manner as for the CD4 cell count of all patients. Detailed results on ANOVA on these subgroups can be found in Table 4.3 to Table 4.13 using no LOCF.

4.3.9.2 Secondary variable – Total lymphocyte count

The mean of the Equimmune treatment group (using LOCF), regarding the total lymphocyte count at 12 months was 1 972.2 cmm and the mean of the placebo treatment group 1 794.6 cmm. Relative to baseline (entry), there was an increase in the total lymphocyte count of the Equimmune treatment group and a slight decrease in the total lymphocyte count of the placebo treatment group. The difference in change from baseline (Equimmune – placebo) was 165.0 cmm ($p = 0.0362$; 95%CI [10.74 cmm; 319.45 cmm]), indicating a strong statistically significant difference in the change between the two groups (Table 4.14). Using no LOCF yielded an increase in lymphocyte count in both groups compared to baseline, but no significant difference in change.

At this stage the placebo group switched to Equimmune and comparing the groups from this point onwards is no longer relevant.

4.3.9.3 Secondary variable – HIV levels

There was an increase in the mean HIV levels of both treatment groups at 12 months (using LOCF) relative to baseline (entry), with the mean of the Equimmune treatment group being

57 463.4 copies/ml and that of the placebo treatment group being 54 587.5 copies/ml. The difference in change from baseline (Equimmune – placebo) at 12 months is 9 619.7 copies/ml with the 95% CI of –21 123.0 copies/ml to 40 362.0 copies/ml. The changes seen in HIV levels were unfortunately not clinically or statistically significant.

Further details on the HIV levels with the natural logarithm of the HIV levels in Table 4.15.

4.3.9.4 Secondary variable – MOS health rating

Keeping in mind the phase of their treatment, patients were asked the question that elicited response “Much better than one year ago”. This response was evaluated as follows:

At entry, less than 6.2% of patients on Equimmune treatment and 8.1% on placebo felt much better than a year before.

At 12 months, 27.6% of the patients in the Equimmune group and only 18.3% in the placebo group responded favourably to the same question. The placebo group received treatment from this time onwards and after being on Equimmune for 12 months, 31% of patients in this group felt much better than the year before, comparing well with the Equimmune groups at 12 months.

After being on treatment for 24 months, 38.5% of patients in the Equimmune group and 34.8% in the former placebo group were feeling much better than one year before. The trend continued until the final time point.

Table 4.3: Total CD4 cell count between 200 and 300 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	49	29	21	37	27	27	16	15	9	11	5
	Mean	249.3	250.7	-7.3	245.5	-5.3	251.2	-10.7	254.3	-4.3	265.8	12.7
	Median	247.0	256.0	-3.0	241.0	-12.0	251.0	-14.3	257.0	-3.5	269.0	14.5
	SD	26.0	24.5	21.0	30.4	32.7	27.5	30.6	29.2	26.6	19.2	31.5
	CV%	10.4	9.8	-286.0	12.4	-612.4	11.0	-285.5	11.5	-622.0	7.2	247.9
	Min	205.0	206.0	-72.0	206.0	-63.0	202.0	-67.0	202.0	-55.0	231.0	-28.0
	Max	297.0	288.0	22.0	295.0	82.0	298.0	53.0	295.0	39.0	296.0	52.0
*PI acebo	N	55	33	23	29	18	23	11	9	6	11	8
	Mean	248.4	246.9	6.0	258.4	5.6	249.2	-3.6	270.4	18.8	251.5	0.3
	Median	249.0	248.0	14.0	261.0	6.8	252.0	7.0	270.0	21.3	253.0	3.0
	SD	26.4	26.8	32.8	28.1	39.0	28.1	30.2	28.9	49.9	25.1	40.3
	CV%	10.6	10.9	550.9	10.9	695.1	11.3	-839.8	10.7	264.7	10.0	16132.3
	Min	204.0	207.0	-63.0	210.0	-83.0	204.0	-55.0	223.0	-60.5	216.0	-70.5
	Max	298.0	295.0	67.0	293.0	70.0	291.0	38.0	298.0	68.0	295.0	67.0
p-value				0.1209		0.3140		0.5551		0.2609		0.5706
Diff				-13.29		-10.94		-7.13		-23.11		12.45
95% Conf				-30.23 to 3.65		-32.61 to 10.72		-31.67 to 17.42		-65.58 to 19.36		-34.42 to 59.32
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	11	7	8	4	4	1					
	Mean	252.5	11.5	246.3	-1.3	244.5	42.0					
	Median	248.0	8.0	243.0	0.0	242.0	42.0					
	SD	18.0	17.2	21.5	27.4	44.2						
	CV%	7.1	149.9	8.7	-2192.2	18.1						
	Min	233.0	-10.0	223.0	-36.0	205.0	42.0					
	Max	285.0	43.5	280.0	31.0	289.0	42.0					
*PI acebo	N	9	7	5	3	2	1					
	Mean	240.6	-12.3	251.0	3.7	281.5	27.0					
	Median	238.0	-6.0	251.0	-21.0	281.5	27.0					
	SD	22.1	28.1	39.8	48.9	13.4						
	CV%	9.2	-228.3	15.9	1333.9	4.8						
	Min	215.0	-51.5	201.0	-28.0	272.0	27.0					
	Max	275.0	25.0	293.0	60.0	291.0	27.0					
p-value				0.0802		0.8705						
Diff				23.79		-4.92						
95% Conf				-3.33 to 50.90		-78.57 to 68.74						

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.4: Total CD4 cell count between 300 and 400 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	61	46	33	37	28	33	22	12	10	10	5
	Mean	345.9	351.2	4.3	347.4	-6.5	347.1	10.7	362.7	19.2	358.4	20.7
	Median	347.0	352.0	-1.0	345.0	-10.5	343.0	-3.0	361.5	32.0	365.0	16.0
	SD	24.8	27.7	28.7	30.3	37.1	29.3	34.4	30.0	39.2	33.1	44.8
	CV%	7.2	7.9	669.4	8.7	-572.0	8.4	320.8	8.3	204.6	9.2	216.5
	Min	306.0	302.0	-49.0	301.0	-84.5	301.0	-41.0	306.0	-44.0	308.0	-42.0
	Max	397.0	395.0	83.5	398.0	54.0	397.0	81.0	399.0	59.0	396.0	80.0
*Placebo	N	59	31	19	31	22	29	16	17	9	10	5
	Mean	349.1	347.1	-9.0	340.2	-19.6	354.9	8.2	351.6	10.1	356.5	-16.6
	Median	348.0	346.0	-9.5	329.0	-24.0	359.0	13.0	348.0	-4.0	349.0	-11.0
	SD	27.7	25.1	32.6	31.4	36.8	25.3	23.9	27.6	29.9	27.6	33.6
	CV%	7.9	7.2	-362.3	9.2	-188.1	7.1	293.4	7.8	295.3	7.8	-202.7
	Min	301.0	301.0	-77.0	302.0	-84.0	311.0	-34.0	302.0	-24.0	316.0	-70.0
	Max	399.0	395.0	62.0	392.0	72.0	396.0	46.0	398.0	69.0	399.0	20.0
p-value				0.1324		0.2200		0.7989		0.5825		0.1749
Diff				13.29		13.09		2.57		9.04		37.30
95% Conf				-4.16 to 30.74		-8.08 to 34.26		-17.74 to 22.89		-24.99 to 43.07		-20.48 to 95.08
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	15	6	5	1	3	1					
	Mean	352.5	1.7	352.2	29.0	347.7	28.0					
	Median	343.0	9.0	349.0	29.0	348.0	28.0					
	SD	26.3	23.6	33.6		44.5						
	CV%	7.5	1416.6	9.5		12.8						
	Min	305.0	-40.0	309.0	29.0	303.0	28.0					
	Max	397.0	28.0	386.0	29.0	392.0	28.0					
*Placebo	N	9	6	2	1	2	1					
	Mean	348.8	-12.6	367.5	80.0	356.0	34.5					
	Median	343.0	-2.5	367.5	80.0	356.0	34.5					
	SD	20.2	31.3	30.4		55.2						
	CV%	5.8	-248.6	8.3		15.5						
	Min	324.0	-72.0	346.0	80.0	317.0	34.5					
	Max	377.0	13.0	389.0	80.0	395.0	34.5					
p-value				0.3941								
Diff				14.25								
95% Conf				-21.40 to 49.90								

p-value (ANCOVA)

Difference in change from baseline (Equimmune – Placebo)

95% Confidence Interval

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.5: Total CD4 cell count between 400 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	47	18	13	20	12	19	14	17	10	9	5
	Mean	444.5	447.5	6.7	451.0	12.0	438.3	-7.6	446.1	16.2	423.8	-13.5
	Median	441.0	453.5	13.0	453.0	19.5	428.0	-4.0	439.0	24.8	420.0	-19.0
	SD	29.1	23.6	29.0	32.3	42.8	27.3	33.5	24.9	32.0	16.4	39.0
	CV%	6.6	5.3	433.2	7.2	355.5	6.2	-442.8	5.6	198.2	3.9	-288.9
	Min	404.0	406.0	-62.0	404.0	-59.5	406.0	-53.5	412.0	-34.0	404.0	-61.0
	Max	496.0	482.0	46.0	498.0	85.0	487.0	51.0	496.0	63.5	449.0	40.5
*PI acebo	N	43	14	10	15	9	8	4	16	8	7	4
	Mean	443.8	452.5	0.3	445.7	8.0	433.4	-8.1	434.8	2.1	450.0	25.9
	Median	440.0	450.0	1.0	444.0	-8.0	421.5	-24.8	431.0	1.5	449.0	31.0
	SD	24.0	31.5	50.8	30.8	36.2	31.3	64.8	31.5	34.4	27.7	37.9
	CV%	5.4	7.0	20319.5	6.9	453.0	7.2	-798.0	7.2	1666.6	6.2	146.5
	Min	401.0	406.0	-91.0	401.0	-22.0	401.0	-66.0	402.0	-58.0	416.0	-21.0
	Max	497.0	497.0	96.0	493.0	87.0	484.0	83.0	496.0	50.0	496.0	62.5
p-value			0.7044		0.8220		0.9814		0.3824		0.1716	
Diff			6.44		4.04		0.55		14.09		-39.38	
95% Conf			-28.40 to 41.28		-33.04 to 41.12		-49.02 to 50.13		-19.16 to 47.34		-100.5 to 21.76	
Equimmune	N		5	3	3	1	4	3				
	Mean		451.2	-17.5	452.7	74.0	436.5	2.0				
	Median		444.0	-13.0	467.0	74.0	419.0	4.0				
	SD		17.6	11.9	44.3		40.1	81.0				
	CV%		3.9	-68.0	9.8		9.2	4050.9				
	Min		434.0	-31.0	403.0	74.0	412.0	-80.0				
	Max		477.0	-8.5	488.0	74.0	496.0	82.0				
*PI acebo	N		10	6	4	2	2	1				
	Mean		446.0	20.6	447.5	27.5	464.5	34.0				
	Median		433.5	29.3	442.0	27.5	464.5	34.0				
	SD		32.2	51.2	28.6	35.4	6.4					
	CV%		7.2	248.6	6.4	128.6	1.4					
	Min		401.0	-50.0	420.0	2.5	460.0	34.0				
	Max		496.0	74.0	486.0	52.5	469.0	34.0				
p-value			0.2577		0.4773		0.7649					
Diff			-38.08		46.50		32.00					
95% Conf			-111.2 to 35.00		-503.7 to 596.69		-434.5 to 370.52					

p-value (ANCOVA)

Difference in change from baseline (Equimmune – Placebo)

95% Confidence Interval

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.6: White patients with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	35	35	35	35	35	32	32	23	23	19	19
	Mean	342.2	327.4	-14.7	341.9	-0.3	316.3	-18.2	314.5	-10.0	301.1	-11.7
	Median	349.5	346.0	-16.0	330.0	-11.0	304.0	-21.0	295.0	-28.5	271.0	-19.0
	SD	82.4	100.4	70.2	112.4	84.5	97.7	71.6	133.1	105.9	162.9	141.1
	CV%	24.1	30.7	-476.1	32.9	-26875	30.9	-393.1	42.3	-1056.8	54.1	-1207.8
	Min	180.0	150.0	-158.5	157.0	-172.5	129.0	-160.5	122.0	-274.0	58.0	-355.0
	Max	512.5	528.0	138.0	606.0	224.0	504.0	125.0	514.0	185.5	531.0	221.0
*Placebo	N	38	35	35	34	34	30	30	25	25	21	21
	Mean	325.7	318.5	-5.5	300.6	-22.3	307.2	-23.2	345.4	16.9	357.7	33.8
	Median	326.5	335.0	-9.0	292.5	-26.5	334.5	-34.0	377.0	37.5	345.0	25.0
	SD	76.6	96.4	63.3	103.6	72.7	98.1	80.9	125.9	102.1	136.5	115.8
	CV%	23.5	30.3	-1160.1	34.5	-325.4	31.9	-348.6	36.5	605.8	38.2	342.3
	Min	191.5	106.0	-149.5	72.0	-211.5	57.0	-202.5	56.0	-164.0	20.0	-171.5
	Max	490.5	500.0	105.0	578.0	138.0	484.0	151.0	570.0	175.0	603.0	343.0
p-value				0.5630		0.2504		0.7980		0.3754		0.2700
Diff				-9.29		22.02		4.98		-26.88		-45.52
95% Conf				-41.17 to 22.59		-15.89 to 59.94		-33.77 to 43.73		-87.34 to 33.58		-127.8 to 36.80
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N		16	16	9	9	10	10				
	Mean		270.3	-48.3	269.8	-68.4	239.9	-71.4				
	Median		255.0	-83.8	269.0	-108.0	185.5	-105.3				
	SD		163.9	166.0	178.1	177.3	176.8	135.0				
	CV%		60.6	-343.9	66.0	-259.3	73.7	-189.1				
	Min		33.0	-380.0	11.0	-247.0	8.0	-226.0				
	Max		765.0	437.0	515.0	303.0	552.0	213.0				
*Placebo	N		19	19	12	12	6	6				
	Mean		354.7	18.8	447.3	133.4	613.3	272.4				
	Median		361.0	-10.0	442.0	56.3	694.0	286.3				
	SD		128.1	109.7	243.8	226.9	211.7	201.2				
	CV%		36.1	584.0	54.5	170.1	34.5	73.9				
	Min		148.0	-114.5	196.0	-137.5	317.0	34.5				
	Max		581.0	321.0	1026.0	650.5	795.0	535.0				
p-value				0.1620		0.0400		0.0011				
Diff				-67.07		-201.8		-343.8				
95% Conf				-162.5 to 28.33		-393.3 to -10.27		-523.0 to -164.6				

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.7: Black patients with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	92	78	78	75	75	62	62	51	51	35	35
	Mean	339.8	332.2	-7.7	328.4	-11.6	326.0	-1.4	360.7	31.3	364.8	30.2
	Median	331.8	324.0	-6.8	315.0	-19.0	331.0	-12.8	389.0	30.0	386.0	12.0
	SD	104.9	107.5	63.6	108.4	69.5	115.7	67.4	156.6	99.3	157.9	119.3
	CV%	30.9	32.4	-825.3	33.0	-596.7	35.5	-4803.0	43.4	317.3	43.3	394.7
	Min	150.0	150.0	-143.0	114.0	-172.0	109.0	-125.0	50.0	-135.5	94.0	-169.5
	Max	713.0	696.0	287.5	598.0	169.0	735.0	272.0	729.0	307.0	794.0	385.5
	*PI acebo	N	82	60	60	53	53	40	40	31	31	23
Mean	350.0	340.0	-14.8	332.0	-26.0	336.4	-27.3	370.5	3.3	455.5	65.9	
Median	345.3	318.5	-31.8	316.0	-34.0	328.5	-38.8	373.0	2.0	419.0	41.5	
SD	97.7	134.4	93.0	108.8	70.5	142.6	132.7	140.3	107.1	224.0	161.6	
CV%	27.9	39.5	-627.3	32.8	-271.4	42.4	-486.7	37.9	3270.2	49.2	245.2	
Min	197.5	142.0	-179.5	151.0	-188.0	108.0	-220.0	88.0	-233.5	101.0	-199.5	
Max	599.5	722.0	244.5	615.0	207.0	936.0	543.5	691.0	200.5	1001.0	401.5	
p-value				0.5950		0.2556		0.1970		0.2326		0.3377
Diff				7.11		14.33		25.86		28.02		-35.68
95% Conf				-19.29 to 33.52		-10.50 to 39.15		-13.64 to 65.36		-18.34 to 74.38		-109.6 to 38.24
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	34	34	34	18	18	11	11				
	Mean	312.3	312.3	-20.3	358.4	31.3	370.0	23.9				
	Median	317.5	317.5	-19.0	294.5	9.0	348.0	4.0				
	SD	144.2	144.2	97.7	191.4	183.7	163.6	166.2				
	CV%	46.2	46.2	-480.6	53.4	586.8	44.2	696.6				
	Min	59.0	59.0	-257.5	116.0	-226.0	174.0	-187.0				
	Max	595.0	595.0	213.0	725.0	442.0	680.0	316.5				
*PI acebo	N	21	21	21	10	10	7	7				
	Mean	382.1	382.1	7.9	474.4	93.7	482.9	125.9				
	Median	428.0	428.0	6.5	462.5	78.0	469.0	55.5				
	SD	152.3	152.3	97.7	190.8	158.1	184.6	186.5				
	CV%	39.9	39.9	1236.2	40.2	168.8	38.2	148.2				
	Min	103.0	103.0	-133.5	167.0	-99.5	272.0	-98.5				
	Max	668.0	668.0	187.0	800.0	417.5	826.0	443.5				
p-value				0.3026		0.3754		0.2432				
Diff				-28.23		-62.34		-102.0				
95% Conf				-82.62 to 26.16		-204.4 to 79.74		-280.4 to 76.45				

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.8: Male patients with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	70	61	61	61	61	51	51	40	40	35	35
	Mean	341.5	333.1	-8.9	332.6	-8.3	325.5	-5.6	335.9	9.9	341.4	15.8
	Median	339.5	335.0	-4.5	317.0	-12.5	332.0	-13.5	334.5	14.0	335.0	6.5
	SD	89.2	93.7	63.9	107.3	77.2	108.1	72.3	134.0	102.3	156.2	128.6
	CV%	26.1	28.1	-718.1	32.3	-935.4	33.2	-1284.9	39.9	1033.1	45.8	813.0
	Min	174.5	150.0	-158.5	156.0	-172.5	149.0	-153.5	139.0	-274.0	58.0	-355.0
	Max	544.0	523.0	138.0	606.0	169.0	735.0	272.0	652.0	237.0	703.0	240.0
	*Placebo	N	72	60	60	56	56	49	49	38	38	30
Mean	330.4	327.8	-7.9	315.8	-15.6	329.2	-7.9	350.0	16.9	363.8	30.8	
Median	323.8	330.5	-9.3	304.0	-26.5	333.0	-19.5	349.0	20.0	346.0	13.0	
SD	84.0	114.4	80.7	107.3	74.2	127.4	115.1	103.6	91.6	135.7	108.1	
CV%	25.4	34.9	-1016.8	34.0	-476.3	38.7	-1461.5	29.6	543.4	37.3	351.3	
Min	191.5	106.0	-175.0	72.0	-190.5	57.0	-206.0	56.0	-200.0	20.0	-171.5	
Max	498.0	722.0	244.5	613.0	207.0	936.0	543.5	553.0	175.0	603.0	343.0	
p-value				0.9422		0.6025		0.9067		0.7530		0.6167
Diff				-0.96		7.33		2.25		-6.96		-14.97
95% Conf				-27.13 to 25.21		-20.46 to 35.11		-35.74 to 40.24		-50.82 to 36.91		-74.44 to 44.50
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	33	33	33	18	18	16	16				
	Mean	279.7	-43.7	326.2	-8.3	286.9	-45.6					
	Median	265.0	-60.5	289.0	-85.5	248.5	-70.3					
	SD	149.4	128.8	186.3	201.9	151.0	129.5					
	CV%	53.4	-294.4	57.1	-2447.2	52.6	-284.0					
	Min	33.0	-380.0	11.0	-247.0	8.0	-226.0					
	Max	765.0	437.0	725.0	442.0	552.0	213.0					
	*Placebo	N	28	28	16	16	9	9				
Created by stalssem	Mean	371.6	29.7	441.8	124.8	571.7	236.4					
Median	363.0	8.0	442.0	68.0	536.0	190.0						
SD	128.0	103.6	231.1	206.2	179.9	178.3						
CV%	34.4	348.7	52.3	165.2	31.5	75.4						
Min	148.0	-114.5	167.0	-137.5	317.0	34.0						
Max	581.0	321.0	1026.0	650.5	795.0	535.0						
p-value				0.0184		0.0666		0.0001				
Diff				-73.44		-133.0		-282.0				
95% Conf				-134.1 to -12.82		-275.7 to 9.68		-409.9 to -154.2				

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.9: Female patients with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	59	54	54	51	51	45	45	35	35	20	20
	Mean	338.9	329.7	-8.8	333.3	-6.8	319.7	-8.5	355.6	27.6	340.4	14.9
	Median	325.5	323.5	-11.3	324.0	-21.5	318.0	-17.5	389.0	4.0	302.0	-6.0
	SD	109.4	115.8	69.3	110.6	72.6	109.8	65.1	167.3	102.0	170.4	126.1
	CV%	32.3	35.1	-784.2	33.2	-1073.5	34.3	-764.9	47.0	369.4	50.1	849.1
	Min	150.0	150.0	-143.0	114.0	-172.0	109.0	-160.5	50.0	-135.5	91.0	-169.5
	Max	713.0	696.0	287.5	603.0	224.0	573.0	125.0	729.0	307.0	794.0	385.5
*PI acebo	N	52	37	37	34	34	24	24	19	19	15	15
	Mean	359.6	338.2	-19.5	329.0	-35.7	304.8	-67.9	374.5	-6.6	486.3	84.7
	Median	367.3	313.0	-34.5	312.0	-31.8	280.5	-69.8	413.0	-39.0	520.0	95.0
	SD	99.3	131.0	87.0	108.6	62.3	117.5	91.9	179.3	124.7	258.0	187.6
	CV%	27.6	38.7	-445.6	33.0	-174.3	38.5	-135.3	47.9	-1887.6	53.1	221.3
	Min	207.0	142.0	-179.5	151.0	-211.5	108.0	-220.0	88.0	-233.5	101.0	-199.5
	Max	599.5	649.0	214.0	615.0	90.0	559.0	169.5	691.0	200.5	1001.0	401.5
p-value				0.5166		0.0604		0.0027		0.2817		0.1964
Diff				10.69		28.96		59.43		34.21		-69.88
95% Conf				-21.93 to 43.32		-1.29 to 59.20		21.39 to 97.46		-28.90 to 97.31		-177.7 to 37.95
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	18	18	18	9	9	5	5				
	Mean	339.6	339.6	6.8	334.2	10.7	375.8	55.6				
	Median	352.5	352.5	-0.3	280.0	1.0	303.0	60.0				
	SD	145.8	145.8	110.0	204.0	154.0	257.6	222.1				
	CV%	42.9	42.9	1622.8	61.0	1436.2	68.6	399.5				
	Min	59.0	59.0	-257.5	26.0	-154.0	37.0	-187.0				
	Max	595.0	595.0	213.0	696.0	269.5	680.0	316.5				
*PI acebo	N	12	12	12	6	6	4	4				
	Mean	363.3	363.3	-25.8	507.2	90.1	478.8	96.9				
	Median	402.5	402.5	-46.3	471.0	58.5	408.5	21.3				
	SD	171.5	171.5	92.1	182.2	177.4	258.7	239.5				
	CV%	47.2	47.2	-357.5	35.9	196.9	54.0	247.2				
	Min	103.0	103.0	-133.5	293.0	-96.5	272.0	-98.5				
	Max	668.0	668.0	129.0	800.0	417.5	826.0	443.5				
p-value				0.4054		0.3735		0.7965				
Diff				32.53		-79.36		-41.28				
95% Conf				-46.34 to 111.40		-265.4 to 106.65		-405.7 to 323.13				

p-value (ANCOVA)

Difference in change from baseline (Equimmune – Placebo)

95% Confidence Interval

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.10: Patients of centre 1 with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	29	29	29	24	24	18	18	16	16	13	13
	Mean	343.0	325.2	-17.8	333.6	-15.7	298.8	-13.7	351.5	46.0	318.3	20.1
	Median	351.0	324.0	-16.0	349.5	-31.0	320.5	-19.5	379.5	17.8	278.0	6.5
	SD	123.2	110.4	60.8	99.8	78.1	104.3	41.2	172.9	94.1	128.4	106.8
	CV%	35.9	34.0	-340.9	29.9	-497.5	34.9	-301.7	49.2	204.4	40.3	531.2
	Min	150.0	158.0	-126.0	156.0	-172.0	138.0	-78.5	91.0	-59.0	154.0	-169.5
	Max	713.0	587.0	125.5	541.0	130.5	487.0	57.5	729.0	280.5	514.0	221.0
*Placebo	N	28	19	19	20	20	16	16	12	12	10	10
	Mean	378.5	397.2	15.2	345.2	-27.8	305.6	-58.8	377.8	1.9	393.3	12.1
	Median	394.0	370.0	9.5	311.0	-38.0	310.5	-48.5	366.0	19.3	296.0	-22.3
	SD	92.6	159.4	106.7	129.3	83.5	118.2	76.8	147.6	69.0	255.9	174.3
	CV%	24.5	40.1	700.4	37.5	-300.5	38.7	-130.6	39.1	3677.9	65.1	1440.8
	Min	216.5	143.0	-120.5	169.0	-211.5	108.0	-202.5	144.0	-107.5	174.0	-199.5
	Max	599.5	722.0	244.5	615.0	138.0	562.0	84.5	691.0	91.5	1001.0	401.5
p-value				0.1779		0.6230		0.0377		0.1822		0.8929
Diff				-33.06		12.09		45.08		44.16		8.02
95% Conf				-81.71 to 15.58		-37.17 to 61.36		2.72 to 87.45		-22.07 to 110.38		-114.3 to 130.32
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N	11	11	11	0	0	3	3				
	Mean	283.5	-23.9				246.0	-62.7				
	Median	272.0	-30.5				166.0	-69.5				
	SD	95.0	99.0				143.8	63.5				
	CV%	33.5	-415.0				58.5	-101.4				
	Min	144.0	-257.5				160.0	-122.5				
	Max	397.0	142.5				412.0	4.0				
*Placebo	N	10	10	10	0	0	3	3				
	Mean	324.8	-52.7				451.3	87.5				
	Median	298.5	-61.8				469.0	55.5				
	SD	143.1	61.0				171.2	74.8				
	CV%	44.1	-115.9				37.9	85.5				
	Min	116.0	-133.5				272.0	34.0				
	Max	581.0	70.0				613.0	173.0				
p-value				0.4383				0.0570				
Diff				28.79				-150.2				
95% Conf				-47.32 to 104.90				-307.5 to 7.17				

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.11: Patients of centre 2 with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	45	43	43	42	42	38	38	32	32	29	29
	Mean	347.4	347.6	0.8	336.4	-8.2	326.3	-16.3	356.6	11.1	370.1	28.8
	Median	345.0	348.0	-4.5	318.0	-15.5	295.0	-19.5	371.0	-25.5	358.0	8.5
	SD	90.3	111.2	69.8	118.5	77.5	124.9	79.4	147.0	93.6	174.4	127.2
	CV%	26.0	32.0	8704.0	35.2	-947.2	38.3	-487.7	41.2	846.5	47.1	441.3
	Min	176.0	150.0	-126.0	157.0	-172.5	129.0	-153.5	122.0	-134.0	91.0	-140.5
	Max	544.0	696.0	287.5	606.0	167.0	735.0	272.0	652.0	237.0	794.0	385.5
*Placebo	N	43	37	37	35	35	30	30	26	26	25	25
	Mean	356.5	332.7	-12.5	321.9	-20.8	323.4	-24.0	385.6	32.3	420.6	73.1
	Median	360.5	330.0	-22.5	317.0	-34.0	334.5	-29.3	395.5	33.8	393.0	41.5
	SD	96.1	110.9	77.6	97.5	63.0	100.7	98.2	111.9	105.5	174.5	133.8
	CV%	27.0	33.3	-618.8	30.3	-303.0	31.1	-409.7	29.0	326.5	41.5	182.9
	Min	212.0	106.0	-179.5	140.0	-155.0	158.0	-220.0	133.0	-200.0	101.0	-119.0
	Max	539.0	586.0	122.0	579.0	207.0	559.0	169.5	583.0	200.5	880.0	395.5
p-value				0.4208		0.4416		0.7221		0.4200		0.2183
Diff				13.34		12.62		7.69		-21.26		-44.31
95% Conf				-19.48 to 46.16		-19.88 to 45.12		-35.29 to 50.67		-73.70 to 31.17		-115.7 to 27.05
	Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base				
Equimmune	N		27	27	20	20	16	16				
	Mean		331.0	-11.3	354.6	25.8	314.3	-11.0				
	Median		330.0	-8.5	340.5	-17.5	289.5	-57.5				
	SD		152.7	120.0	209.9	200.9	195.4	176.6				
	CV%		46.1	-1060.4	59.2	778.9	62.2	-1610.4				
	Min		95.0	-165.0	11.0	-226.0	8.0	-226.0				
	Max		765.0	437.0	725.0	442.0	680.0	316.5				
*Placebo	N		22	22	20	20	10	10				
	Mean		393.5	47.8	465.2	121.0	570.6	225.3				
	Median		412.0	33.0	468.5	68.0	531.0	229.8				
	SD		152.0	110.5	227.0	204.2	208.7	217.4				
	CV%		38.6	231.3	48.8	168.7	36.6	96.5				
	Min		103.0	-117.0	167.0	-137.5	291.0	-98.5				
	Max		668.0	321.0	1026.0	650.5	826.0	535.0				
p-value				0.0822		0.1455		0.0057				
Diff				-59.09		-95.20		-236.3				
95% Conf				-126.0 to 7.85		-224.9 to 34.48		-396.8 to -75.74				

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.12: Patients of centre 3 with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	14	14	14	14	14	11	11	6	6	1	1
	Mean	356.1	353.7	-2.4	366.7	10.6	360.0	22.4	339.3	9.8	131.0	-84.0
	Median	362.3	353.5	5.0	334.0	-10.0	346.0	26.5	342.0	21.0	131.0	-84.0
	SD	76.3	86.1	72.8	112.2	77.5	80.3	67.1	92.8	32.1		
	CV%	21.4	24.3	-3043.3	30.6	730.3	22.3	299.3	27.4	329.2		
	Min	215.0	212.0	-158.5	225.0	-79.5	246.0	-101.5	202.0	-43.0	131.0	-84.0
	Max	512.5	473.0	138.0	603.0	224.0	504.0	125.0	461.0	46.0	131.0	-84.0
*PI acebo	N	17	12	12	13	13	10	10	7	7	1	1
	Mean	313.1	320.3	-0.8	306.7	-15.2	325.6	-7.4	357.6	25.2	537.0	139.0
	Median	318.5	318.0	1.0	304.0	-8.5	314.0	23.0	343.0	10.0	537.0	139.0
	SD	58.3	84.1	54.6	92.2	76.5	73.9	71.5	116.2	103.6		
	CV%	18.6	26.3	-6892.2	30.1	-504.9	22.7	-972.8	32.5	410.7		
	Min	212.0	212.0	-112.5	134.0	-190.5	226.0	-149.5	223.0	-160.5	537.0	139.0
	Max	429.5	497.0	99.0	488.0	90.0	484.0	86.0	570.0	172.0	537.0	139.0
p-value				0.9507		0.3934		0.3374		0.7332		
Diff				-1.60		25.76		29.76		-15.46		
95% Conf				-54.45 to 51.25		-35.33 to 86.85		-33.52 to 93.04		-112.8 to 81.87		
Equimmune	N											
	Mean											
	Median											
	SD											
	CV%											
	Min											
	Max											
*PI acebo	N											
	Mean											
	Median											
	SD											
	CV%											
	Min											
	Max											
p-value												
Diff												
95% Conf												

p-value (ANCOVA)

Difference in change from baseline (Equimmune – Placebo)

95% Confidence Interval

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.13: Patients of centre 4 with CD4 cell count between 200 and 500 cmm (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	41	29	29	32	32	29	29	21	21	12	12
	Mean	325.2	303.2	-17.4	313.0	-8.6	318.9	-1.8	324.2	10.1	313.0	-13.6
	Median	312.0	316.0	-12.0	299.5	-3.8	341.0	-8.0	338.0	29.5	294.0	1.3
	SD	95.9	92.0	63.5	99.3	69.7	96.9	67.3	155.4	130.7	149.8	150.3
	CV%	29.5	30.3	-365.7	31.7	-806.8	30.4	-3751.6	47.9	1288.8	47.9	-1106.5
	Min	169.5	150.0	-143.0	114.0	-124.0	109.0	-160.5	50.0	-274.0	58.0	-355.0
	Max	508.5	528.0	103.5	497.0	169.0	506.0	124.0	689.0	307.0	567.0	185.0
	*PI acebo	N	36	29	29	22	22	17	17	12	12	9
Mean	312.0	292.5	-35.0	305.0	-27.5	329.4	-16.7	279.4	-43.7	358.3	11.8	
Median	307.5	276.0	-50.0	294.5	-20.5	324.0	-48.5	325.0	-42.3	416.0	0.0	
SD	86.7	101.4	78.9	112.0	68.9	185.1	168.2	151.2	117.6	177.2	116.6	
CV%	27.8	34.7	-225.4	36.7	-250.0	56.2	-1006.8	54.1	-269.0	49.5	989.9	
Min	191.5	112.0	-166.0	72.0	-188.0	57.0	-206.0	56.0	-233.5	20.0	-171.5	
Max	497.0	519.0	114.0	493.0	70.0	936.0	543.5	461.0	128.5	560.0	177.0	
p-value				0.3527		0.3297		0.6726		0.2473		0.6796
Diff				17.62		18.90		14.91		53.85		-25.36
95% Conf				-20.04 to 55.28		-19.65 to 57.46		-55.73 to 85.55		-39.30 to 147.00		-151.9 to 101.19

Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base
Equimmune	N	12	12	7	7	2	2
	Mean	268.8	-49.8	255.4	-81.1	351.0	-44.0
	Median	262.0	-51.8	253.0	-85.0	351.0	-44.0
	SD	171.2	154.9	76.8	98.6	87.7	62.2
	CV%	63.7	-311.4	30.1	-121.5	25.0	-141.4
	Min	33.0	-380.0	166.0	-247.0	289.0	-88.0
	Max	595.0	213.0	403.0	51.5	413.0	0.0
	*PI acebo	N	7	7	2	2	0
Mean	354.6	2.8	404.5	58.5	404.5	58.5	
Median	338.0	-10.0	404.5	58.5	404.5	58.5	
SD	102.6	88.8	21.9	30.4	52.0	37.0	
CV%	28.9	3189.2	5.4	52.0	37.0	37.0	
Min	224.0	-97.5	389.0	37.0	37.0	37.0	
Max	496.0	120.0	420.0	80.0	80.0	80.0	
p-value			0.4256		0.1001		
Diff			-52.54		-139.6		
95% Conf			-188.3 to 83.25		-314.0 to 34.71		

p-value (ANCOVA)

Difference in change from baseline (Equimmune – Placebo)

95% Confidence Interval

*Patients on Placebo received active treatment (Equimme) after 12 months

Table 4.14: Total lymphocyte count (no LOCF)

Variable	Stats	Base	6 Weeks	Change 6 Weeks-Base	3 Months	Change 3 Months-Base	6 Months	Change 6 Months-Base	12 Months	Change 12 Months-Base	18 Months	Change 18 Months-Base
Equimmune	N	129	115	115	119	119	119	119	119	119	119	119
	Mean	1789.5	1780.0	-30.3	1850.7	40.2	1838.6	28.1	1972.2	161.7	2001.8	191.2
	Median	1634.0	1692.0	-75.5	1735.0	-8.0	1728.0	29.5	1862.0	143.5	1894.0	167.5
	SD	632.4	653.1	434.3	661.1	405.9	680.1	427.9	773.8	575.7	732.6	600.5
	CV%	35.3	36.7	-1433.5	35.7	1010.1	37.0	1525.0	39.2	356.1	36.6	314.0
	Min	774.5	722.0	-1471.0	712.0	-874.0	210.0	-1932.0	210.0	-1932.0	210.0	-1932.0
	Max	5022.0	4660.0	1249.0	4660.0	1622.5	4531.0	1135.0	4623.0	1461.5	4531.0	2341.5
*Placebo	N	124	97	97	102	102	102	102	102	102	102	102
	Mean	1782.2	1706.4	-111.4	1685.3	-112.8	1654.7	-143.3	1794.6	-3.4	1845.0	47.0
	Median	1726.3	1595.0	-57.0	1583.0	-125.0	1551.5	-130.0	1710.5	17.5	1781.0	84.0
	SD	545.5	560.0	459.2	608.9	516.5	522.7	523.0	530.7	585.9	592.6	612.1
	CV%	30.6	32.8	-412.1	36.1	-458.0	31.6	-364.9	29.6	-17148	32.1	1302.5
	Min	813.0	112.0	-1349.0	353.0	-2797.0	353.0	-2797.0	353.0	-2797.0	353.0	-2797.0
	Max	3718.0	3438.0	840.0	4956.0	1502.0	3350.0	913.0	3108.0	1062.0	3744.0	1268.0
p-value				0.1883		0.0145		0.0079		0.0362		0.0791
Difference				81.14		152.96		171.40		165.10		144.23
95% Conf				-40.04 to 202.31		30.57 to 275.36		45.31 to 297.49		10.74 to 319.45		-16.89 to 305.35

Variable	Stats	24 Months	Change 24 Months-Base	36 Months	Change 36 Months-Base	48 Months	Change 48 Months-Base
Equimmune	N	119	119	119	119	119	119
	Mean	1951.1	140.6	1950.9	140.3	1918.0	107.5
	Median	1924.0	134.5	1924.0	134.5	1900.0	134.5
	SD	731.3	580.6	740.1	586.5	775.7	669.8
	CV%	37.5	413.0	37.9	418.0	40.4	623.3
	Min	210.0	-1932.0	210.0	-1932.0	3.9	-3177.9
	Max	4531.0	2341.5	4531.0	2341.5	4531.0	2341.5
*Placebo	N	102	102	102	102	102	102
	Mean	1807.0	9.0	1812.5	14.5	1786.6	-11.4
	Median	1752.5	35.8	1733.5	47.5	1713.0	35.8
	SD	609.2	610.6	611.9	612.9	636.8	664.1
	CV%	33.7	6803.2	33.8	4239.5	35.6	-5821.8
	Min	353.0	-2797.0	353.0	-2797.0	5.9	-2797.0
	Max	4452.0	1555.0	4452.0	1555.0	4452.0	1555.0
p-value			0.1024		0.1207		0.1881
Difference			131.62		125.86		118.87
95% Conf			-26.51 to 289.76		-33.38 to 285.09		-58.56 to 296.31

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimmune) after 12 months

Table 4.15: Natural logarithm of HIV levels

Variable	Stats	Change			Change			Change			Change		
		Base	6 Weeks	6 Weeks-Base	3 Months	3 Months-Base	6 Months	6 Months-Base	12 Months	12 Months-Base	18 Months	18 Months-Base	
Equimmune	N	127	114	112	119	117	119	119	117	119	117	119	117
	Mean	9.3	9.5	0.2	9.6	0.4	9.2	9.2	-0.0	9.4	0.1	9.4	0.1
	Median	9.5	9.8	0.1	9.8	0.3	9.0	9.0	0.1	9.3	0.2	9.3	0.2
	SD	1.7	1.7	1.1	1.6	1.1	1.8	1.8	1.2	1.8	1.5	1.8	1.5
	CV%	18.8	17.6	600.9	17.0	301.6	19.2	19.2	-3473.5	19.5	1076.4	19.5	1076.4
	Min	2.6	6.2	-3.3	6.2	-2.6	6.2	6.2	-4.1	4.5	-4.5	4.5	-4.5
	Max	13.0	13.3	5.5	13.3	6.2	13.3	13.3	5.5	13.6	6.6	13.6	6.6
*Placebo	N	122	97	95	102	100	102	100	102	100	102	100	100
	Mean	9.3	9.5	0.3	9.6	0.3	9.3	9.3	0.0	9.2	-0.0	9.2	-0.0
	Median	9.5	9.3	0.3	9.6	0.2	9.2	9.2	0.0	9.4	0.1	9.4	0.1
	SD	1.8	2.0	1.3	1.9	1.3	1.8	1.8	1.4	2.0	1.7	2.0	1.7
	CV%	18.9	21.1	440.2	19.4	392.1	19.4	19.4	4640.5	21.2	-10555	21.2	-10555
	Min	6.2	6.2	-2.9	6.2	-3.1	6.2	6.2	-3.3	3.9	-5.7	3.9	-5.7
	Max	13.4	13.6	4.8	13.5	5.2	13.5	13.5	6.2	13.9	5.6	13.9	5.6
p-value			0.4959		0.9087			0.6766		0.4707		0.4707	
Difference			-0.11		0.02			-0.07		0.15		0.15	
95% Conf			-0.43 to 0.21		-0.28 to 0.32			-0.40 to 0.26		-0.25 to 0.54		-0.25 to 0.54	
Equimmune	N		119	117	119	117	119	117	119	117	119	117	
	Mean		8.8	-0.5	8.6	-0.7	8.7	-0.7	8.7	-0.6	8.7	-0.6	
	Median		9.0	-0.3	9.0	-0.2	9.0	-0.2	9.0	-0.2	9.0	-0.2	
	SD		2.2	2.2	2.4	2.4	2.4	2.4	2.4	2.5	2.4	2.5	
	CV%		25.1	-419.6	28.0	-365.5	27.3	-365.5	27.3	-434.5	27.3	-434.5	
	Min		3.9	-7.4	3.9	-7.4	3.9	-7.4	3.9	-7.9	3.9	-7.9	
	Max		13.3	6.6	13.3	6.6	13.3	6.6	13.3	6.6	13.3	6.6	
*Placebo	N		102	100	102	100	102	100	102	100	102	100	
	Mean		8.9	-0.3	8.7	-0.6	8.6	-0.6	8.6	-0.7	8.6	-0.7	
	Median		9.4	0.0	9.1	0.0	9.0	0.0	9.0	-0.0	9.0	-0.0	
	SD		2.3	2.2	2.5	2.3	2.6	2.3	2.6	2.5	2.6	2.5	
	CV%		25.2	-639.9	28.2	-410.4	30.5	-410.4	30.5	-350.9	30.5	-350.9	
	Min		3.9	-8.0	3.9	-7.1	3.9	-7.1	3.9	-7.2	3.9	-7.2	
	Max		13.9	5.6	13.9	5.6	13.9	5.6	13.9	5.6	13.9	5.6	
p-value			0.4909		0.7216			0.6943		0.6943		0.6943	
Difference			-0.19		-0.11			0.13		0.13		0.13	

p-value (ANCOVA)

95% Confidence Interval

Difference in change from baseline (Equimmune – Placebo)

*Patients on Placebo received active treatment (Equimme) after 12 months

4.3.9.5 Other secondary variables

The following results were obtained for the other secondary efficacy variables at 12 months.

Table 4.16: Other secondary variables at 12 months (no LOCF)

	EQUIMMUNE			PLACEBO		
	Before (Entry)	After (12 months)	Change 'After' – 'Before'	Before (Entry)	After (12 months)	Change 'After' – 'Before'
CD4 cell percentage (%)	20.6	19.5	-0.9	20.5	19.2	-1.3
CD8 cell count (cmm)	1035.8	1179.4	126.7	1047.0	1075.1	15.3
CD8 cell percentage (%)	59.4	56.8	-3.0	58.3	57.0	-2.2
CD4 + CD8 cell ratio	0.4	0.4	-0.1	0.4	0.4	0.0
Erythrocyte count (/pl)	5.0	4.6	-0.4	4.9	4.6	-0.3
Haemoglobin (g/dl)	14.4	13.5	-1.0	14.3	13.5	0.2
Platelet count (/nl)	222.9	220.8	-0.9	218.9	215.9	-2.8
Leukocyte count (/nl)	4.6	4.8	0.2	4.7	4.9	0.4
Weight (kg)	67.9	69.1	0.8	69.8	70.4	0.7

There was no statistically significant difference in change in CD4 cell percentage at 12 months between the groups, whether LOCF or no LOCF was used.

A statistically significant difference in change from baseline for CD8 cell count between the two groups was found at 12 months, using LOCF. By using no LOCF, no significant difference in change was found.

No significant difference in change in CD8 cell percentage at the 12-month stage was found between the two groups, whether LOCF or no LOCF was used.

The CD4CD8 cell ratio, erythrocyte count, platelet count and leucocyte count also showed no statistically significant difference in change between the groups at any time up to 12 months. Change in total haemoglobin count differed significantly at six weeks, but at no other time. The conclusions were the same for the above variables, whether LOCF or no LOCF was used.

The following results were obtained for the other secondary efficacy variables at 48 months:

Table 4.17: Other secondary variables for 48 months (no LOCF)

	EQUIMMUNE			PLACEBO		
	Before (Entry)	After (48 months)	Change 'After' – 'Before'	Before (Entry)	After (48 months)	Change 'After' – 'Before'
CD4 cell percentage (%)	20.6	17.5	-2.9	20.5	19.6	-0.9
CD8 cell count (cmm)	1035.8	1222.6	169.9	1047.0	1100.7	40.9
CD8 cell percentage (%)	59.4	57.6	-2.3	58.3	56.4	-2.7
CD4 + CD8 cell ratio	0.4	0.3	-0.1	0.4	0.4	0.0
Erythrocyte count (/pl)	5.0	4.5	-0.5	4.9	4.5	-0.5
Haemoglobin (g/dl)	14.4	13.6	-1.0	14.3	13.5	0.2
Platelet count (/nl)	222.9	223.3	2.4	218.9	217.9	-0.8
Leukocyte count (/nl)	4.6	5.2	0.6	4.7	5.0	0.4
Weight (kg)	67.9	69.4	1.2	69.8	69.6	-0.1

4.3.10 SAFETY

A total of 906 adverse events occurred in both treatment groups. One hundred and sixty-seven (66.0%) patients reported adverse events, irrespective of the relationship to the treatment: 95 (73.6%; 556 events) patients in the Equimmune treatment group and 72 (58.1%; 350 events) in the placebo treatment group, of which 30 patients mentioned 94 events after the switch to Equimmune.

Ninety (35.6%) patients reported 383 adverse events at least 'possibly' related to the study medication: 55 (42.6%; 255 events) in the Equimmune treatment group and 35 (28.2%; 128 events) in the placebo treatment group. Of the 128 mentioned by the placebo group, 18 events were reported by 10 patients after the switch to Equimmune. The system organ class most affected by the adverse events at least 'possibly' related to study medication was Infections and Infestations, with 129 events in 49 (19.4%) patients; Skin and Subcutaneous Tissue Disorders (39 events in 19 patients) and Gastrointestinal Disorders (26 events in 12 patients) were the most frequently reported events.

The majority of adverse events were mild to moderate in intensity. Twenty-nine patients (18 patients in the Equimmune treatment

group and 11 patients in the placebo treatment group) experienced 50 severe adverse events (30 events were reported in the Equimmune treatment group and 20 in the placebo treatment group). Twelve of the events (nine patients) were reported by placebo patients after they started using Equimmune at 12 months.

No possible causal relationship to the treatment was reported in any of the above-mentioned cases. The majority of adverse events improved or had been resolved by the end of the 48 months. Some of the adverse events were unchanged and the minority were insufficiently followed up.

Thirteen serious adverse events were reported (six events in six patients in the Equimmune treatment group and seven events in five patients in the placebo treatment group). Six of the serious adverse events reported by the placebo patients (five patients), were reported after they started using Equimmune at 12 months.

Five of the 13 serious adverse events were classified as severe; three of them in the Equimmune treatment group, and two events were reported by one patient after switching to Equimmune at 12 months. Two patients, one patient (appendicitis) and the other patient (pneumonia), required or prolonged hospitalisation, while the study medication of a third patient (tracheostomy) was

discontinued and restarted. No action was taken in the case of another patient (cytomegalovirus). No causal relationship to the treatment was reported in any of the serious adverse events.

Three patients (two in the Equimmune treatment group) died during the study period. In the Equimmune treatment group Patient 2044 died due to respiratory failure and the reason for the death of Patient 2006 was unknown. Patient 3029 (placebo) died due to pneumocystic pneumonia.

There were no overt differences between the Equimmune treatment group and the placebo treatment group with regard to the safety variables. In general, there were no relevant changes in the majority of the clinical chemistry variables. There were 117 LPCAs: 64 in the Equimmune treatment group and 53 in the placebo treatment group.

4.4 DISCUSSION AND OVERALL CONCLUSIONS

The main objective of this double blind study was to test Equimmune, a recently formulated nutritional supplement, in HIV infected and AIDS patients.

It was planned to recruit a total of 400 patients at four centres for this study. Because of too low or high CD4 cell counts at entry, only 253 patients continued with the study after three months, the data of whom were available for the final analysis. All evaluable patients were included in all analyses of safety and efficacy.

After comparing well at baseline with regard to CD4 count, a trend towards higher values in the Equimmune group was observed. Already after six months clinically and statistically significant differences could be seen in two subgroups. At this time the decision to switch the placebo group to Equimmune was made, in accordance with the protocol. This decision was based on the fact that female patients who were on treatment had a statistically and clinically significantly smaller decrease in CD4 count at the six-month phase than the patients on placebo. Patients of Centre 1 on treatment also showed a statistically significant smaller decrease in CD4 count than those on placebo.

Over 12 months there was an increase in the CD4 cell count of the total Equimmune treatment group, but a decrease in the CD4 cell count of the placebo treatment group.

The mean increase in lymphocyte count was statistically significantly higher at 12 months in the Equimmune group than in

the placebo group. The changes seen in HIV levels were, however, not clinically or statistically significant.

No causal relationship to the treatment was reported in any of the serious adverse events.

No overt differences were observed between the Equimmune treatment group and the placebo treatment group with regard to the safety variables. In general, there were no relevant changes in the clinical chemistry variables. One hundred and seventeen LPCAs occurred at 48 months, 64 in the Equimmune treatment group and 53 in the placebo treatment group.

The Equimmune treatment thus proved to be a safe combination with relatively few side effects when compared to the placebo group. At the same time, the formula proved to be effective, passing the most difficult challenge medically possible with respect to the immune system, namely showing a clinically and statistically significant difference in the immune system of HIV+ patients.

Although certain follow-up studies are still required, these exciting results present the HIV+ patients with new alternatives. This does not have an impact on only the HIV+ patient, but as has already

been shown, any immune-compromised condition in, theoretically,
any person.

CHAPTER 5

SUMMARY AND EVALUATION OF THE STUDY

The aim of this study was to investigate the essential amino acid L-methionine as a possible immune-supportive supplement, specifically by means of a clinical study.

Up to now supplementation with L-methionine was only used in the treatment of conditions such as depression and to a lesser extent processes supporting liver function etc. (Chapter 1). This was the first time that L-methionine was considered for its possible immune-supportive function.

Mechanistic studies, for the first time done in a South African HIV+ subgroup, confirmed the results of previous studies done in other populations, that GSH levels in HIV+ patients are decreased (Buhl *et al.*, 1989, Buhl, 1994; Rodriguez *et al.*, 1998). Not only GSH levels were decreased, but also the methionine precursors, for example CYS and HCYS. These results served to confirm the hypothesis that L-methionine could play an important and positive role in these patients' immune system by increasing the body's methylation capacity (SAH to SAM ratio) and by increasing GSH levels.

The protective and supportive role that co-factors such as vitamin B₆, vitamin B₁₂, folic acid and magnesium play in this reaction, was also further elucidated by means of the second study, comparing L-methionine to the combination (L-methionine together with vitamin B₆, vitamin B₁₂, folic acid and magnesium). The results have emphasised the importance of keeping safety in mind, even when working with so-called natural or relatively safe substances. Although no significant differences in the bioavailability of L-methionine could be shown between the two groups, this should be followed up by means of a more comprehensive study. The methionine excretion levels need to be measured over a longer period, as large individual variations in L-methionine absorption could have masked a possible difference between the two groups. A larger study population should be used. The possible role of HCYS as a risk factor in heart disease prompted the researcher to continue all further investigations using only the combination.

A limited study, testing the effect of this combination on healthy individuals, followed. Ultra-long distance athletes training for an endurance event, the Comrades marathon, were decided on as it is speculated that such ultra-long distance races compromise the immune system of such athletes to some extent, influencing especially their recovery period negatively. Although no statistically significant differences could be detected in their immunological parameters, some interesting trends were still observed, especially in respect to decreased cortisol levels in the treatment group. This could be regarded as being indicative

of a positive TH1 (cellular immune response), which is important especially in respect to viral infections. It should be emphasised that these were and remained healthy athletes and that these trends fluctuated within normal ranges. Dramatic differences were, however, observed in incidence of illness (treatment group 36% and placebo group 80%), as well as in days of training lost owing to illness (treatment group 19 days and placebo group 42 days). These results were positive enough to motivate further investigation of L-methionine in immune compromised patients. Also of importance was the positive results seen in respect to recovery.

An initial limited pilot study on HIV+ and full-blown AIDS patients yielded dramatic results, with all patients showing increases in their CD4 count, CD4%, as well as in their general feeling of well-being (Karnofsky scores). This was the first study of its kind ever to implicate methionine as immune-supportive supplement. This was, however, an open study with a relatively small number of patients (n=17). A more comprehensive study to confirm these results, also using more than one centre, was therefore required.

A second pilot study was therefore initiated with 103 HIV+ patients (CD4 count between 200 and 500). This was again an open, but multi-centre trial to try to confirm previous results. More than 90% of the patients again showed definite improvement, especially in respect of an increased CD4 count, CD4% and Karnofsky score values. No serious side effects were reported in any of the

patients. This was also of importance, as the safety of the patients, even though using a natural supplement, remained of paramount importance. A large percentage of the patients on this study (n=74), however, were also using other anti-retroviral therapy, which could have influenced, to some extent, the positive results seen.

Based on these positive results, a large double-blind, placebo-controlled study on HIV+ patients was initiated, not only to try to elucidate the full effect of L-methionine as immune-supportive supplement in HIV+ patients, but also to investigate all possible safety parameters in these patients fully, while on treatment. In the end 253 patients were randomly divided into two well defined groups, active and placebo, and regularly assessed, not only by means of clinical and safety parameters, as has been reported, but also by means of a full physical examination by a medical doctor and a full assessment of their quality of life and feeling of well-being by means of a MOS questionnaire.

Within six months of the commencement of study, clinically and statistically significant differences between the two groups were observed. This was especially so in the female group, which showed a statistically significant decreased level of decline in their CD4 counts and in the patients of Centre 1. As a result of this positive reaction, all patients were placed on active treatment (L-methionine combination) at the end of the 12-month period and followed up till the 48-month endpoint.

Although not all statistically significant, the same trends observed in these two subgroups could be seen in the group as a whole, thus presenting with a general improvement in CD4 count, total lymphocyte count, as well as general improvement in quality of life. After 12 months, the differences between the two groups, now both on active treatment, slowly diminished. This serves to confirm the positive results noticed during the first 12 months, because no further difference was expected once all patients were on active treatment. No serious side effects directly associated with treatment were observed and even though the active group showed a slightly higher incidence of adverse events compared to the placebo group, most of these were resolved within the 24-month period, or were shown not to be related to the treatment.

This study therefore clearly confirms the positive role of L-methionine in the supportive treatment of immune-compromised or deficient patients, for example HIV+ patients. The impact of this study could have dramatic effects not only locally, but worldwide, in the supportive treatment of HIV+ patients as it presents a relatively safe and affordable option in the treatment of these patients.

Further work still needs to be done investigating in more detail the mechanism of action of this combination. GSH levels in these patients should be further investigated, specifically the possible increase of these levels due to methionine treatment. Also regarding safety, HCYS levels should be controlled over the run of a long-term study. Further work also needs to be done, investigating the

possible positive effect of combining this treatment with other anti-retroviral treatment, to see whether or not an even greater response can be achieved. Increases observed in the second study, where a large percentage of the patients were also on anti-retroviral treatment, compared to the increase found in the main clinical study, might indicate possible beneficial effects of combined therapy. As the absorption of nutrients in these HIV+ patients is often affected and is usually compromised during the late stages of the disease, different routes of administration should also be investigated. This could explain the better results observed in some of the early-stage patients compared to patients in the later stage, although improvement in some individual patients even at this late stage was still dramatic.

The positive effect seen in the female group should be investigated further to determine whether this could be attributed to compliance or possible genetic differences.

The positive results achieved in this study, from healthy long-distance athletes to HIV+ patients, warrant investigation of the role that L-methionine could also play in other serious and less serious conditions. The possible role it could play in prevention of disease should also be investigated at the end of the 12-month period further.

This study indicates that positive effects can be achieved by identifying the

problems in the body on a biochemical or enzymatic level and supplying the body with what it needs to overcome the problem. It also proves the role of L-methionine as an immune-supportive supplement, giving new hope to immune-compromised patients worldwide.

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ADDENDUM A**PATIENT CONSENT FORM****EVALUATION OF POTENTIAL CLINICAL, BIOCHEMICAL AND
IMMUNOLOGICAL BENEFITS OF EQUIMMUNE (LMC)**

Clinical Trial No; EQUIMMUNE A/1/5/97, a multi-centre, double-blind, randomised, placebo-controlled trial in patients with HIV, to investigate the efficacy of Equimmune.

INTRODUCTION

You are invited to volunteer for a research study. You have been asked if you would be willing to take part in this trial. This information is to help you decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator. You should not agree to take part unless you are completely happy about all the procedures involved.

WHAT IS THE PURPOSE OF THIS TRIAL?

The purpose of this trial is to evaluate the potential clinical, biochemical and immunological benefits of Equimmune. Equimmune is a vitamin, mineral and amino acid combination formulated to act as a possible immune supportive

supplement. During the study you will receive either the active agent or a placebo. A placebo is an inactive vehicle, it does not contain any of the drug.

Equimmune supplements the essential amino acid; L-methionine, in combination with vitamin B6, vitamin B12, folic acid, as well as the mineral; magnesium. L-Methionine is believed to enhance the methylation capacity of the body, as well as the synthesis of glutathione, a peptide involved in the body intracellular defence mechanism. The possible advantages of this combination, which we will be monitoring during this study, include:

An increase in the immune system or a decrease in the deterioration rate of the immune system, as measured by parameters i.e. CD4 Count. A clinical improvement, thus improvement in the productivity and/or quality of life (feeling of well-being). A decrease in secondary problems and/or complications. A possible decrease in the actual virus level due to the general improvement of the immune system. It should be emphasised that the purpose of this trial is to test the potential benefits of this combination; Equimmune. These benefits have thus not yet been fully established and might still be proven wrong. As this combination exists of only natural substances, no disadvantages are expected other than the possible side-effects as are addressed under "What are the risks involved in this study?" in this document.

WHAT IS THE DURATION OF THIS TRIAL?

If you decide to take part you will be one of approximately 400 patients. The study will last for up to 48 months. You will be asked to visit the investigator nine times as per the following schedule. Time 0 (Start of trial), Week six, Month three, Month six, Month 12, Month 18, Month 24, Month 36, Month 48.

At each visit you will undergo the following:

Clinical examination

Productivity/feeling of well-being questionnaire

Questionnaire with regard to symptoms and/or possible side effects

Blood drawn for tests (10 to 15ml)

It is important that you let the investigator know of any medicine (both prescriptions or over-the-counter medicines), alcohol or other substances that you are currently taking. The trial will be monitored by an independent trial-monitoring committee of experts from different fields. If at any stage during the trial, it is judged by them that a clinical significant difference is proven between the group taking the active against the group taking the placebo, the permission of the South African Medicine Control Council will be requested, to allow the placebo group to fall away. This will result in the placebo patients going onto the active for the remainder of the trial.

HAS THE TRIAL RECEIVED ETHICAL APPROVAL?

This clinical trial protocol was submitted to the MASA Research Ethics Committee (MREC), Clindepharm International Pharma-Ethics ethical committee, as well as the Research Protocol & Ethics Committee of the Pretoria Academic Hospital and written approval has been granted by that committee. The study has been structured in accordance with the Declaration

of Helsinki, a copy of which may be obtained from the investigator should you wish to review it.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS TRIAL?

Your participation in this trial is entirely voluntary and you can refuse to participate or stop without stating any reason. Your withdrawal will not affect your access to other medical care. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest. If it is detected that you did not give an accurate history or did not follow the guidelines of the trial and the regulations of the trial facility, you may be withdrawn from the trial at any stage.

IS ALTERNATIVE TREATMENT AVAILABLE?

This is an unique internationally patented combination with nothing exactly the same on the market. If you decide not to take part in this study, then your doctor may treat you with other suitable alternatives, please consult with your doctor/investigator with regard to the possibilities.

MAY ANY OF THESE TRIAL PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

Drawing blood may normally result in a slight bruise at the puncture site or, less commonly, fainting, swelling of the vein, infection and bleeding from the site. Your protection is that experienced personnel perform the procedures under clean conditions.

WHAT ARE THE RISKS INVOLVED IN THIS TRIAL?

All medicine carries some risk, however small. In previous studies, some patients have reported side-effects which included: One patient developed a skin rash that necessitated dosage-reduction of Equimmune by 50%, whereafter the rash resolved. Dyspepsia occurred at a low frequency and was relieved by administration of Equimmune with meals, rather than prior to food. It must be emphasised that Equimmune only contains an essential amino acid, in combination with vitamins and a mineral. No side-effects occurred with Equimmune in the previous trial that were severe enough to warrant discontinuation of medication.

ARE THERE ANY WARNINGS OR RESTRICTIONS FOR MY ACTIVITY IN THIS TRIAL?

In females of child-bearing age, for the duration of the treatment you must take adequate precautions to ensure that you do not become pregnant.

DISCONTINUATION OF TRIAL TREATMENT?

To fully benefit from the treatment, the medication must be taken strictly as prescribed. Uncontrolled discontinuation of the medication is inadvisable. The importance of taking the medication without any interruptions must be emphasised, please inform the investigator if you forgot or for any reason missed a dosage.

INSURANCE AND FINANCIAL ARRANGEMENTS

The company; Biomox Pharmaceuticals, will provide payment for all trial procedures as determined by the company and the investigator. Neither you nor your medical aid will be expected to pay for these procedures. Biomox Pharmaceuticals (Pty) Ltd is covered by the guarantee of an insurance policy

for civil, professional and product liability with St Paul Insurance; policy no. ZPM1024. This policy covers Biomox Pharmaceuticals for all pecuniary consequences of the civil liability it may incur under the terms of common law for the damages which result from the clinical studies carried out on voluntary patients for approved experiments with new vitamin, mineral and amino acid combinations. Further detailed information on the payment of medical treatment and compensation due to injury can be obtained from the investigator should you desire to review it. You must notify the investigator immediately of any research or other related complications, side-effects and/or injuries during the trial. If a research-related injury occurs, you have not waived any of the legal rights which you otherwise would have as a participant in this trial by signing this form.

SOURCE OF ADDITIONAL INFORMATION

For the duration of the trial, you will be under the care of Dr

If at any time between your visits, you feel that any of your symptoms are causing you any problems, or you have any questions during the trial, please do not hesitate to contact him/her.

CONFIDENTIALITY

All information obtained during the course of this trial is strictly confidential. Data that may be reported in scientific journals will not include any information, which identifies you as a patient in this trial. In connection with this trial, it might be important for domestic and foreign regulatory authorities, the ethical committee, as well as your doctor to be able to review your

medical records pertaining to this trial. Therefore, you hereby authorise your investigator to release your medical records to Biomox Pharmaceuticals, its' employees or agents, domestic and foreign regulatory health authorities and ethical committee. You understand that these records will be utilised by them only in connection with carrying out their obligations relating to this clinical trial. Any information uncovered regarding your test results or state of health as a result of your participation in this trial will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this trial but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases in which a law exists compelling us to report individuals infected with communicable diseases. In this case, you will be informed of our intent to disclose such information to the authorised state agency.

AGREEMENT

I have been fully informed as to the purpose of the treatment. Possible advantages as well as possible adverse effects, which may occur as a result of the treatment have been explained to me. I have also read and understand the written information regarding the trial. I have had sufficient opportunity to ask questions. I am aware that the results including personal details regarding my sex, birth date and diagnosis will be anonymously processed into a trial report. I confirm that I have informed the supervising doctor of:

- Any illness or other medical condition; whether past or present.
- Any drugs that I have taken in the past six weeks or am planning to take whether prescribed or not.

In females of child-bearing age:

I understand that for the duration of the treatment I must take adequate precautions to ensure that I do not become pregnant.

I agree to co-operate fully with the supervising doctor and will report any unexpected or unusual symptoms to him without delay.

I understand that I am free to withdraw from the treatment at any time, without the need to justify my decision. Should I choose to discontinue the medication I understand that this will not in any way compromise or interfere with the provision of appropriate treatment.

NAME OF PATIENT:

SIGNATURE OF PATIENT:

DATE:

I, Dr. _____, herewith confirm that the above patient has been informed fully about the nature, conduct and risks of the above trial.

NAME OF INVESTIGATOR:

SIGNATURE OF INVESTIGATOR:

DATE:

WITNESS NAME:

SIGNATURE:

DATE:

WITNESS NAME:

SIGNATURE:

DATE: