Original Research Article

*Moringa oleifera*supplement for HIV/AIDS patients is safe but does not influence the CD4+ and CD8+ t-cell patterns

ABSTRACT

Aims:HIV is managed by expensive ARVs that cause side effects and lead to drug resistance and drug failure. The use of nutritional remedies in the treatment of HIV/AIDS is becoming popular. *Moringa oleifera* is a nutrient-dense plant with medicinal properties that results from its wide range of phytochemicals. This study investigated the effect of *Moringa oleifera* supplementation on CD4+ and CD8+ T cell patterns, and also renal functions of HIV/AIDS patients receiving antiretroviral drugs (ARV).

Study Design: A quasi-experimental study of regression discontinuity type was carried out at the Comprehensive Care Center ofMbagathi County Hospital, Nairobi, Kenya.

Methodology: One hundred and seventy-three HIV seropositive participants treated with ARV and attending the regular clinic were assigned to the intervention group supplemented with *Moringa oleifera* leaf powder or the control group that was not supplemented. The evaluationof CD4+ and CD8+ T cell counts wasperformedbefore supplementation, then monthly for 6 months during supplementation.

Results: No significant differences in CD4+ (p= 0.523) and CD8+ T cell counts (p= 0.908),werefound between the supplemented and non-supplemented groups. However, *M. oleifera*supplementation did not havean effect on renal function.

Conclusion: The study concluded that*M. oleifera*supplementation does not have an effect onimmune functions in HIV/AIDS patients.

Keywords*: Moringa oleifera*, *Supplementation, CD4, CD8, HIV, ARVs, Kenya*.

1. INTRODUCTION

Human immunodeficiency virus (HIV) affects the cells of the immune system such as CD4+ T cells, CD8+ T cells, macrophages, and dendritic cells[1]. CD4+ and CD8+ Tcells are important in controlling HIV infection [2]. Depletion of CD8+ Tcell responses is associated with infection; while regeneration restores control of viral replication [3].

The reduction in CD4+ Tcell counts signifies immune deficiency[4].It may becaused by the death of uninfected bystander cells by apoptosis and the killing of infected CD4+ Tcells by cytolytic lymphocytes [5]. This phenomenon indicatesthe progression of HIV in infected individuals [6]. A count below 200 CD4+ cells/µl leads to the development of various complications in HIV/AIDS. Patients with a CD4+ Tcell count of 500 are healthy[7]. The progression of the disease and an adequate response to ART therapy for most patients is associated with an increase in CD4 count in the range of (50-150) cells/mm^3 during the first year of treatment [8]. Any measures that can restore immune functions can help reduce pain, mortality, and morbidity, due to HIV/AIDS infection.

The use of ART results in decreased Tcell death and depletion, as well as a restoration of their numbers [9].Therapy helps prolong the lives of HIV and AIDS patients, but it is accompanied by drawbacks, such as challenges in adhesion, drug resistance, and other health complications [10].In search of an alternative, traditional remedies have become common among patients. These natural products, especially those of plant origin, are an excellent source of new anti-HIV drugs [11,12] that exhibit modulatory effects on HIV-1 replication[13]. Clinical tests of some of these compounds have produced remarkable results[14,15].

Moringa oleifera (Moringaceae) is a multipurpose tree with nutritional and medicinal properties that can be used as an alternative remedy to various health conditions in HIV/AIDS. The plant is packed with nutrients such as proteins, minerals, vitamins, fats, and fiber[16]; and minerals such as magnesium, copper, sulfur, sodium, phosphorus, zinc, and potassium [17]. This study used *Moringa oleifera,* which is a cheap, readily available, nutrient-rich, plant-based supplement that can bridge the gap of malnutrition, especially for patients who cannot afford a proper diet.

The results of this study are useful in understanding the effect of *M. oleifera* consumption onCD4+ T cells and CD8+ T cellpatterns of patients with HIV and AIDS.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a quasi-experimental study of regression discontinuity type involving HIV-positive male and female adults aged 18 to 55 years, all on ARV and attending the Comprehensive Care Center (CCC), Mbagathi County Hospital in Nairobi, Kenya. Patients with gastrointestinal infections and liver, cardiovascular, renal, or endocrine disorders thatcould interfere with the observations, as well as expectant, and breastfeeding mothers,were excluded. We screened 261 patients for eligibility,of which 173 participantswere recruited into the study. Then these were placed into two groups: the treatment group receiving *M. oleifera* supplementation and the control group not. CD4+ T cell counts and CD8+ T cell counts were monitored monthly over six months. The Kenyatta University Ethics Review Board and the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU) approved the study. Before participating in the study, all participants gave their informed consent.

Participants in the intervention group were supplemented with *Moringa oleifera* leaf powder obtained from East Africa Nutraceutical Company, Nairobi. Each study participant in the experimental group received a monthly supply of 300 grams of *Moringa oleifera* leaf powder in teabags weighing 2 grams each. Participants were advised to use four tea bags (about 8 grams) daily [18] by adding them to hot water in a standard 200 ml mug and steeping for ten minutes(2 tea bags in the morning and 2 tea bags in the evening).

2.2 Determination of CD4+and CD8+ Tcell counts

CD4+ and CD8+ Tcell counts were determined by flow cytometry according to the manufacturer's instructions (BD FACSCaliburanalyzer, BD Biosciences, USA) at baseline, in the third and sixth month of the study.Briefly, fifty microliters of whole blood were added to the CD4 and CD8 reagent tubes and incubated. Fifty microliters of 5% formaldehyde were added and processed in the BD FACSCalibur™analyzer. The tests were carried out with washing cycles between the assays, and the results were acquired by computer printing.

2.3Evaluation of toxicity

To determine whether*M. oleifera* had toxic effects on participants,creatinine tests were performed on each study subject at the beginning of the study, in the third and sixth months of the study.The Cock Croft Gault formula (CGF) [19]was used to calculate the glomerular filtration rate (GFT). The age was in years, the weight in kilograms, and the creatinine (Cr)in mg/Dl.

$$GFR = \frac{(140 - age) \times weight}{Cr\ (Creatinine) \times 85}$$

Low GFRs indicate a decrease in renal functional capacity. Creatinine levels were measured using HumaLyzer Primus Chemistry Analyzer equipmentaccording to the manufacturer's instructions (Wiesbaden, Germany).

2.4 Data analysis

Analysis of variance (ANOVA) followed by Tukey's post hoc tests compared the means of CD4+ Tcell and CD8+ T cell counts, as well as the creatinine levels of the two study groups. The results were significantly different if $p < 0.05$.

3. RESULTS

3.1 Social demographic characteristics ofstudy participants

Table 1 shows the demographic characteristics of the study participants. There were 62 males (35.8%) and 111 females (64.2%). The females were more than the males in both study groups. The age subset 46 – 55 years had the most participants (54.3%), while the age subset 18 – 25 years, had the least (2.3%).

Table 1: Social demographic characteristics of study participants

Characteristic	N	(%)
Age Groups		
18-25	4	2.3
26-35	23	13.3
36-45	52	30.1
46-55	94	54.3
Sex		
Male	62	35.8
Female	111	64.2

3.2 Study process

Figure 1 shows the CONSORT flow chart for the study. Of the two hundred and sixty-one (261) patients screened for eligibility, only one hundred and seventy-three participated in the study. Twenty-two (22) participants did not complete the study; three were lost to follow-up, fourteen (14) refused follow-up, three (3) were discontinued due to health problems, and two (2) succumbed to cancer detected during supplementation.

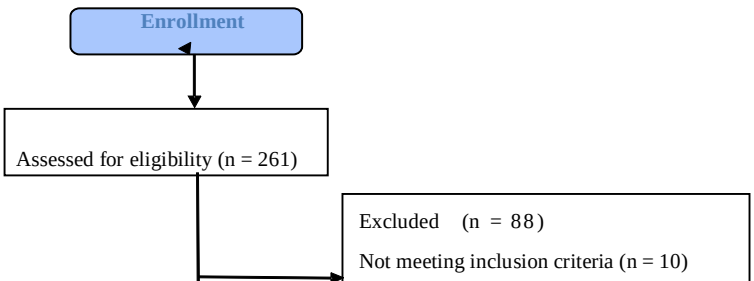

Figure 1: Participant flow diagram (CONSORT FLOW 2010)

3.3 Changes in CD4+and CD8+ Tcell counts

Table 2 shows the analysis of variance (ANOVA) comparingthe meancounts of CD4+ Tcells and CD8+Tcellsof the two study groups from baseline, months three, and month six. The CD4+ T cell count of the intervention group increased significantly from a baseline value of (521.60 ±215.40) to (656.23 ± 274.8) at month six after the start of the intervention (p< 0.005). The CD4+ Tcell count of the control group also increased significantly from a baseline value of (449.24 ± 214.82) to (624.53 ± 299.32) at month six after the start of the study (p <0.005). There was no significant change in the baseline CD8+ Tcell counts of the intervention group (F - 0.0065; p = 0.994) and the control group (F = 0.0466; p = 0.954) during the follow–up period.

Table 2: Analysis of variance of mean values of CD4+ and cd8+ T cell counts during follow-up

	Parameter	Baseline	Month 3	Month 6	ANOVA F - value	*P* – value
Intervention Group	**CD4 cells/µl**	521.60 ± 215.40	559.52 ± 214.43	656.23 ± 274.8	6.2863	0.002*

	CD8 cells/µl	1075.71 ± 485.55	1071.08 ± 412.1	1079.47 ± 437.69	0.0065	0.994
Control Group	**CD4 cells/µl**	449.24 ± 215.82	581.26 ± 297.92	624.53 ± 299.32	6.8949	0.001*
	CD8 cells/µl	1058.92 ± 442.25	1084.68 ± 513.78	1070.58 ± 451.99	0.0466	0.954

Table 3 shows a comparison of mean CD4+ and CD8+ Tcell counts in month 3 and month 6 of the intervention and control groups; using unpaired t-tests. There was no significant difference in the means of CD4+ T cell counts and CD8+ T cell counts in month three,as well asCD4+ T cell counts and CD8+ T cell counts inmonth six between the two groups (p<0.005).However, the mean CD4+ T cell count of the intervention group was higher (656.23 ± 274.8)than that of the control group (624.53 ± 299.32) in the sixth month of the study, although not significant.

Table 3: Unpaired t-tests for the means of months 3 and 6 CD4+ and CD8+ T cell counts of the intervention and control groups

Time point	**Parameter**	**Intervention group (n = 83)**	**Control group (n = 68)**	**Difference (*P* – value)**
Month 3	CD4 cells/µl	559.52 ± 214.43	581.26 ± 297.92	0.633
	CD8 cells/µl	1071.08 ± 412.1	1084.68 ± 513.78	0.867
Month 6	CD4 cells/µl	656.23 ± 274.8	624.53 ± 299.32	0.523
	CD8 cells/µl	1079.47 ± 437.69	1070.58 ± 451.99	0.908

3.4 Toxicity

The toxicity of the kidneys was evaluated by changes in kidney glomerular filtration rates calculated from creatinine levels. At the beginning of the study, the mean GFR of the control group (84.35 ± 22.97) was significantly higher (p = 0.002) than that of the intervention group (72.83 ± 22.57).It remained significantly higher (108.72 ± 32.09) (p = 0.004) thanthat of the intervention group (94.21 ± 27.71) at the end of the study(Figure 2).

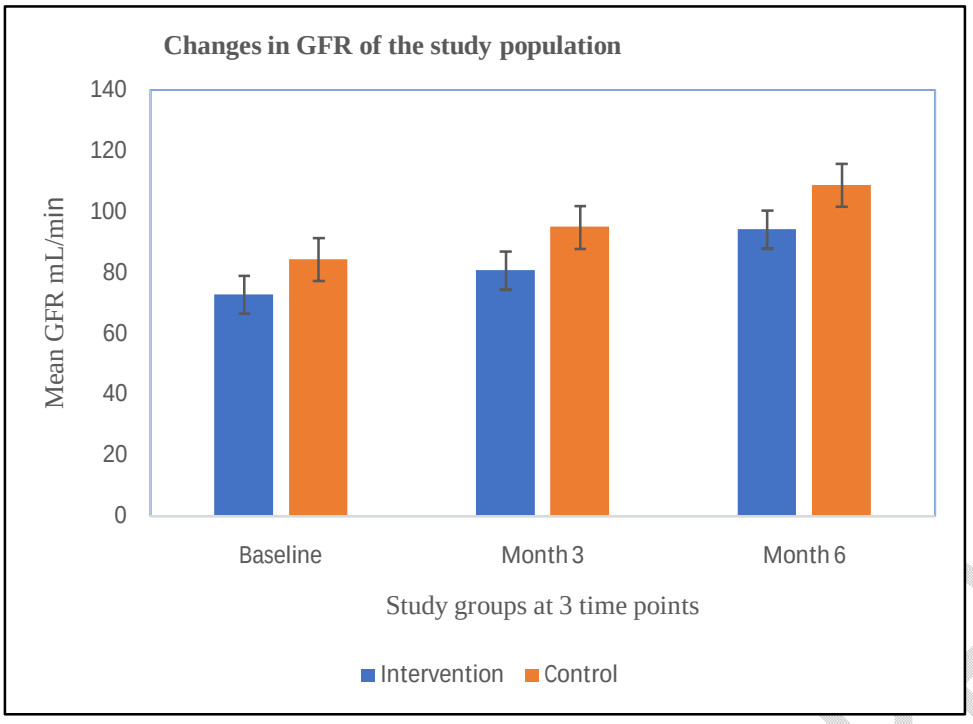

Figure 2: Changes in the glomerular filtration rate (GFR)

4.DISCUSSION

The results of the current study indicate that the mean CD4+ Tcell count of the intervention group was higher than that of the control group at the end of the sixth month, but the difference was not significant. However, an increase in baseline CD4+ Tcell count was observed over time within the two groups, suggesting that the two groups had improved immunity.

The effect of *M. oleifera* on CD4+ Tcell counts has been investigated *in vitro*, in animal models, and humans. The results of these studies are varied; some suggest up-regulation, no change, or dose-dependent suppression of CD4+ Tcell counts. A team of scientists observed an increased CD4+ Tcell activity in diabetic mice[20]. An *in vitro* study at the University of Brawijaya, Malang, Indonesia, observed immunomodulatory activity through active *Moringa* compounds such as flavonoids and saponins,which stimulated CD4+ Tcell counts and other immune cells. Low doses were effective and high doses caused immunosuppression [21]. A dose-dependent decrease in CD4+ Tcell counts and their respective pro-inflammatory cytokines was observed in diabetic-induced Balb/C mice[22]. The results obtained from these animal models may not be duplicated in humans.

In a study conducted at Dr. Rubini Mempawah Hospital, Indonesia, in patients with HIV/AIDS and also using ARV, the study team observed an increase in CD4+ Tcell count that was dose-dependent and duration-dependent [23]. Another study conducted in southeast Nigeria demonstrated that *M. oleifera* improved the CD4 + Tcell count of HIV-positive patients on HAART[24]. These studies used a sample size less than the recommended sample size for thenormal distribution.Furthermore, the mean increase in CD4+ Tcell count of the intervention group of our study (690.07 ± 292.60) was much higher than those achieved by other studies. The findings of a recent study conducted in Kano, Nigeria, found no differences in mean CD4+ Tcell counts throughout the study period, except for the sixth month, a difference influenced by some sociodemographic characteristics of the participants[25]. Another study conducted in Kinshasa in the Democratic Republic of Congo (DRC) also did not observe anysignificant change in CD4+ Tcell counts[26].

These variations may have been influenced by differences in the amounts and formulations of *M. oleifera*.The lack of a significant effect of *M. oleifera* on CD4+ Tcell count in the current study may have resulted from several factors that future research can address. In environments of food insecurity, an intervention using *Moringa* alone may not affectpeople who are malnourished. It is also possible that the doses administered were too low to influence the results.Although these differences are small, they are a signalof the possibility that *M. oleifera* consumed by participants in the intervention group may increase CD4+ Tcell counts in HIV/AIDS patients.

CD8+ Tcells are a critical component of the cellular immune response during viral infection, resulting in decreased viremia [5]. Cytolysis due to CD8+ Tcells is high during the asymptomatic phase of HIV infection [27] but decreases as the patient progresses to AIDS [28].

This study indicates no changes in CD8+ Tcell counts during the follow-up period. The findings of other authors indicate that *M. oleifera* stimulates CD8+ Tcell activities. In a study conducted in Malang, Indonesia [21],it was observed that low doses of leaf extract stimulated CD8+ Tcells and other immune cells in mice. Another study conducted in Cairo, Egypt, made similar observations [20]. However, a dose-dependent decrease in CD8+ Tcell activity was observed in diabetic Balb/C mice[22]. These findings are from animal models and may not be duplicated in humans. Little information is available on the effects of *M. oleifera* on CD8+ Tcell counts in humans.

The findings of our study show that the creatinine levels and GFR of both the intervention and control groups were within normal ranges. Several animal studies suggest that various preparations of *M*. *oleifera* leaves may be very safe at the amounts and doses commonly used [17]. So far, there has been no documented effect of *M. oleifera* on renal function in humans. It can be concluded that the use of *M. oleifera* is not toxic.

5. CONCLUSION

In conclusion, *M. oleifera*consumptiondoes not increase CD4+ T cell counts in HIV/AIDS patients at the dose used in this study but may be effective at higher doses.It is still unclear what dose of *M. oleifera* leaf powder may improve patientoutcomes. The different studies reviewed have used different doses. We recommend thatthe same experiment be repeated using varying doses of *M. oleifera* to establish any dose-dependent impact on the immune system.

Availability of data

The data used to arrive at the conclusions of this work will be provided upon request.

REFERENCES

1. Cunningham, H.D., Andrew, N.H., Kim, M., and Turville, S. G. (2010). Manipulation of dendritic cell function by viruses. ELSEVIER, 13(4): 524–529.
2. Rosenberg, E.S., Altfeld, M., Poon, S.H., Phillips, M.N., Wilkes, B.M., Eldridge, R.L., Robbins, G.K., D'Aquila, R.T., Goulder, P.J., and Walker, B.D. (2000). Immune control of HIV-1 after early treatment of acute infection. Nature, 407(6803): 523-526.
3. Schmitz, J.E., Kuroda, M.J., Santra, S., Sasseville V.J., Simon, M.A., Lifton, M.A., Racz, P., Tenner-Racz, K., Dalesandro, M., Scallon, B.J., Ghrayeb, J., Forman, M.A., Montefiori, D.C., Rieber, E.P., Letvin, N.L., and Reimann, K. A. (1999). Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science, 283(5403): 857–860.
4. Brenchley, J., and Douek, D. (2008). HIV infection and the gastrointestinal immune system. Mucosal Immunology. 1, 23–30.

5. Vidya Vijayan, K.K., Karthigeyan, K.P., Tripathi, S.P., and Hanna, L.E. (2017). Pathophysiology of CD4+ T-cell depletion in HIV-1 and HIV-2 infections. Frontiers in Immunology, 23(8): 580.
6. Hogg, R.S., Yip, B., Chan, K.J., Wood, E., Craib, K.J., O'Shaughnessy, M.V., and Montaner, J. S. (2001). Rates of disease progression by baseline CD4 cell counts and viral load initiating triple-drug therapy. Journal of the American Medical Association, 286: 2568–2577.
7. Pebody, R. (2021). CD4 Cell Counts. Aidsmap May 2021 https://www.aidsmap.com/about-hiv/cd4-cell-counts
8. Kaufmann, G.R., Perrin, L., Pantaleo, G., Opravil, M., Furrer, H., Telenti, A., Hirschel, B., Ledergerber, B., Vernazza, P., Bernasconi, E., Rickenbach, M., Egger, M., and Battegay, M. (2003). CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Archives of Internal Medicine, 163(18): 2187–2195.
9. Li, T.S., Tubiana, R., Katlama, C., Calvez, V., Mohand, H.A., and Autran, B. (1998). Long-lasting recovery in CD4 T cell function and viral load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. Lancet, 351(9117): 1682–1686.
10. HIV.gov. (2018). HIV viral suppression and undetectable viral load. February 2023. https://www.hiv.gov/hiv-basics/staying-in-hiv-care/hiv-treatment/viral-suppression/
11. Esimone, C.O., Eck, G., Nworu, C.S., Hoffman, D., Uberla, K., and Proksh, P. (2010). Dammarenolic acid, a secodammarane triterpenoid from Anglaia sp. Shows potent antiretroviral activity in vitro. Phytomedicine, 17(7): 540–547.
12. Sabde, S., Bodiwala, H.S., Karmase, A., Deshpande, P.J., Kaur, A., Ahmed, N., Chauthe, S.K., Brahmbhatt, K.G., Phadk, R.U., Mitra, D., Bhutani, K.K., and Singh, I.P. (2011). Anti-HIV activity of Indian Medicinal plants. Journal of Natural Medicines, 65(3-40: 662-669.
13. Cos, P., Maes, L., Vanden, B.D., Hermans, N., Pieters, L., and Vlietink, A. (2004). Plant Substances as Anti-HIV Agents Selected According to their Putative Mechanism of Action. Journal of Natural Products, 67 (20): 284-293.
14. Vo, T. S. and Kim, S.K. (2010). Potential anti-HIV agents from marine resources: An overview. Marine Drugs, 8:2871-289[2].
15. Filho, J.R.; de Sousa Falcon, H.; Batista, L.M.; Filho, J.M. and Piuvezam, M.R. (2010). Effects of plant extracts on HIV-1 protease. Current HIV Research, 8:531-544.
16. Rockwood, J.L., Anderson, B.G., and Casamatta, D. A. (2013). Potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by Moringa oleifera seed and leaf extracts using crude extraction techniques available to under-served indigenous populations. International Journal of Phytotherapy Research, 3: 61–71.
17. Asiedu-Gyekye, I.J., Frimpong-Manso, S., and Awortwe, C. (2014). Micro- and macro-elemental composition and safety evaluation of the nutraceutical Moringa oleifera leaves. Journal of Toxicology, 2014, 786979.
18. Kumari, D. J. (2010). Hypoglycemic effects of M. oleifera and Azadirachta Indica in type 2 diabetes. Biosca., 5: 211–214.
19. Cockcroft, D.W. and Gault, M.H. (1976). Prediction of creatinine clearance from serum creatinine. Nephron. 16(1):31-41.
20. Moubarak, J.T., Abdel-Aziz, A.Z., and Enas, A.A. (2018). How Moringa oleifera supplementation affects T-cell subsets and circulating angiogenic, myeloid, and endothelial cells in mice with alloxan-induced diabetes. Immunology, Endocrine, and Metabolic Agents in Medical Chemistry. 18(1): 55-67.
21. Richmawati, I., and Rifai, M. (2014). Immunomodulatory activity of aqueous extract of Moringa oleifera Lam. Leaf to the CD4+, CD8+, and B220+ cells in Mus musculus. Journal of Experimental Life Science, 4: 1.
22. Suheer K.A., Noviana D., Sri Rahayu, and Muhaimin R. (2019). The effect of Moringa oleifera leaves and VipAlbumin on the immune system of diabetes mellitus Balb/C mice model. Journal of Experimental Life Science, 9(3) 170-175.
23. Novitra, J., Wasita, B., Sugiarto. (2020). The effect of Moringa oleifera cookies on CD4+ T cell number and hemoglobin levels in HIV patients: A randomized controlled trial. Arabian Journal of Medical Sciences. 4(1): 3-8.
24. Ogbuagu, E.N., Ufearo, S., Ogbuagu, C.N., and Okonkwo, R. (2016). CD4 pattern in HIV positive patients on HAART exposed to Moringa oleifera leaf powder in Southeast Nigeria. International Journal of Infectious Diseases, 45(1): 267.
25. Gambo, A., Moodley, I., Babashani, M., Babalola, T. K., and Gqaleni, N. (2021). A double-blind, randomized controlled trial to examine the effect of Moringa oleifera leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting. National Library of Medicine, 16(12): e0261935.
26. Tshingani, K., Donnen, P., Mukumb, H., Duez, P., and Dramaix-Wilmet, M. (2017). Impact of Moringa oleifera Lam. leaf powder supplementation versus nutritional counseling on the body mass index and immune responses of HIV patients on antiretroviral therapy: a single-blind randomized control trial. BMC Complementary and Alternative Medicine, 17(1): 420.

27. Addo, M. M., Altfeld, M., Rathod, A., Yu, M., Yu, X.G., Goulder, P. J. R., Rosenberg, E. S., and Walker, B.D. (2002). HIV-1 Vpu represents a minor target for cytotoxic T lymphocytes (CTL) in HIV-1 infection. AIDS 16: 1071-1073.
28. Klein, M.R., van Baalen, C.A., Holwerda, A.M., Kerkhof, Garde, S.R., Bende, R.J., Keet, I.P., Eeftinck-Schattenkerk, J.K., Osterhaus, A.D., Schuitemaker, H., and Miedema, F. (1995). Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatic. Journal of Experimental Medicine, 181:1365–1372.