Effect of selenium supplementation on CD4+ T-cell recovery, viral suppression and morbidity of HIV-infected patients in Rwanda: a randomized controlled trial

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Objective: To examine the effect of selenium supplementation on CD4+ T-cell counts, viral suppression, and time to antiretroviral therapy (ART) initiation in ART-naive HIV-infected patients in Rwanda.

Methods: A multicenter, double-blinded, placebo-controlled, randomized clinical trial was conducted. Eligible patients were HIV-infected adults ($\geq$21 years) who had a CD4+ cell count between 400 and 650 cells/μl (ART eligibility was $\leq$350 cells/μl throughout the trial), and were willing to practice barrier methods of birth control. Patients were randomized to receive once-daily 200 μg selenium tablets or identical placebo. They were followed for 24 months with assessments every 6 months. Declines in CD4+ cell counts were modeled using linear regressions with generalized estimating equations and effect modification, and the composite outcome (ART eligible or ART initiation) using Cox proportional-hazards regression, both conducted with intention to treat.

Results: Of the 300 participants, 149 received selenium, 202 (67%) were women, and median age was 33.5 years. The rate of CD4+ depletion was reduced by 43.8% [95% confidence interval (CI) 7.8–79.8% decrease] in the treatment arm – from mean 3.97 cells/μl per month to mean 2.23 cells/μl per month. We observed 96 composite outcome events – 45 (47%) in the treatment arm. We found no treatment effect for the composite outcome (hazard ratio 1.00, 95% CI 0.66–1.54) or viral suppression (odds ratio 1.18, 95% CI 0.71–1.94). The trial was underpowered for the composite outcome due to a lower-than-anticipated event rate. Adverse events were comparable throughout.

Conclusions: This randomized clinical trial demonstrated that 24-month selenium supplementation significantly reduces the rate of CD4+ cell count decline among ART-naive patients.   Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

HIV infection compromises the nutritional status of the infected individuals, and poor nutritional status can accelerate progression of the disease [1]. The relationship between immune function and nutritional supplementation has been well described [2–5]. Studies have reported high rates of nutrient deficiencies early in the course of HIV infection [6–8].

Among HIV-infected persons not receiving antiretroviral therapy (ART), observational studies have shown that low or deficient serum concentrations of several micronutrients, including the trace mineral selenium, are associated with low CD4$^+$ T-cell counts, advanced HIV-related diseases, faster disease progression, or HIV-related mortality [9–23]. Selenium is incorporated into a number of biologically active selenoproteins and is an essential element of glutathione peroxide (GPX), which plays an important role in endogenous antioxidant defense [24,25]. Selenium is also an essential factor in maintaining host immune competence, and it has been shown that optimal levels decrease the host's susceptibility to viral pathogenesis [26–30]. Selenium deficiency, as indicated by low plasma selenium concentrations, is common in HIV-infected individuals [31,32], particularly in areas of the world with low selenium levels in the soil, as is true in many regions of sub-Saharan Africa [33–35].

Evidence of the effect of selenium supplementation from clinical trials is limited in both developed and developing countries. Three randomized clinical trials (RCTs) have been conducted to assess the individual association of selenium supplementation on HIV viral load and CD4$^+$ T-cell count [36–38]. The first trial, conducted in Miami by Hurwitz *et al.* [37], found that selenium supplementation of 200 μg daily significantly suppressed the progression of HIV viral burden and indirectly improved CD4$^+$ T-cell count after 9 months of treatment. The second trial, conducted in Tanzania by Kupka *et al.* [38], found that selenium supplementation of 200 μg daily provided to HIV-infected pregnant women before and after pregnancy (between 12 and 27 weeks of gestation and 6 months after birth) had no significant effect on HIV viral load or CD4$^+$ T-cell count, but did significantly lower the risk of infant death. A third trial, a factorial design by Baum *et al.* [36] in Botswana, found no significant effect of selenium supplementation on the rate of depletion to 200 cells/μl among patients starting with a CD4$^+$ T-cell count above 350 cells/μl. The authors did, however, find a significant event rate decrease when combined with multivitamins [36].

It is important to recognize the inherent differences in their design, setting, and populations of these trials when drawing inferences. Additional evidence from other settings and populations is required to more accurately determine the effect of selenium supplementation on HIV progression in HIV-infected individuals. Therefore, we conducted an RCT to examine the effect of selenium supplementation on CD4$^+$ T-cell counts, viral suppression, and time to ART initiation in HIV-infected patients who are not yet on ART. We conducted our trial in Rwanda.

Methods

The present study is a 24-month, multicenter, patient and provider-blinded, randomized, placebo-controlled clinical trial, involving 300 pre-ART HIV-infected patients in Rwanda. We *a priori* calculated our sample size by assuming a 20% reduction in CD4$^+$ T-cell count depletion. This trial has been registered with Clinical-Trials.gov under the registration number NCT01327755.

Patients were recruited at two health facilities that offer care and treatment for HIV/AIDS patients in Rwanda. These facilities were chosen due to the feasibility of recruiting all patients within a short period and the feasibility of coordination. Patient eligibility was restricted to: HIV-infected adults (21 years of age and older at study enrollment), who were not yet ART-eligible based on Rwanda guidelines for ART initiation, had a CD4$^+$ T-cell count between 400 and 650 cells/μl, were willing to practice barrier methods of birth control at all times, and be able to provide written informed consent. The CD4$^+$ cell count at baseline was considerably above the Rwanda guidelines for initiation of ART (≥350 cells/μl as of 2012). Eligible patients were identified from pre-ART registers. Participants were enrolled and followed for 2 years. Study assessments occurred at baseline, 6, 12, 18, and 24 months.

Patients were excluded if they intended on transferring out of the clinic catchment area before the study ended and/or if they were scheduled to start ART. Patients with psychiatric health concerns and pregnant women were also excluded.

Randomization

The randomization flowchart is shown in Fig. 1. Participants were randomized using a simple randomized block design to receive either selenium or an identically appearing placebo to be taken once daily for 24 months. The research department of the treatment supplier prepared the randomization schedule. Study participants were identified by unique study identification numbers and were assigned a specific allocation number. An unblinded list was provided to the treatment provider and to the independent statistician for the drug safety monitoring board.

Intervention

The trial intervention consisted of once-daily tablets containing 200 μg of selenium in the form of seleno-
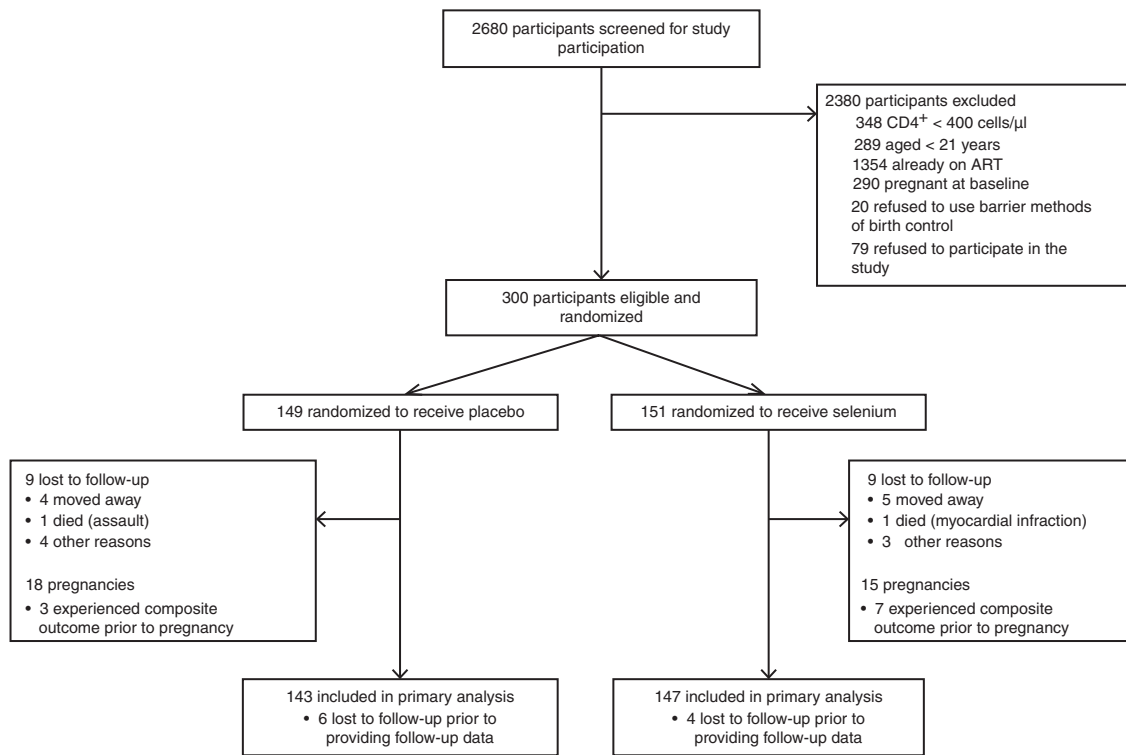
Fig. 1. Randomization flowchart.

methionine containing selenium yeast. The control arm received an identical placebo. To ensure optimal adherence, participants received adherence counseling at baseline and when picking up refills on a monthly basis. Additional adherence counseling was provided to patients who had sub-optimal adherence.

The Rwandan Ministry of Health recommends the use of cotrimoxazole – a sulfonamide antibiotic combination of trimethoprium and sulfamethoxazole used for the treatment of a variety of bacterial infections – for all HIV-infected patients. Therefore, all participants also received cotrimoxazole, irrespective of experimental assignment. Participants who did not return to the clinic as scheduled were followed up at home and received optimal adherence counseling.

Outcomes and study measures
The primary outcome measures for this study was change in $CD4^+$ T-cell counts, and a composite of $CD4^+$ T-cell depletion to 350 cells/μl (as confirmed by two consecutive measures), or start of ART, or the emergence of a documented CDC^+-defined AIDS-defining illness. For analyses of the $CD4^+$ T-cell count changes, patients were censored after ART initiation. Women who initiated ART through prevention of mother-to-child transmission programs prior to reaching other endpoints the composite outcome were censored at time of pregnancy because pregnancy is a mechanism by which ART is initiated independent of immunological failure.

Secondary outcomes included: viral suppression; mortality; and adverse events. This study used the standard level of expedited adverse event reporting as defined in the Division of AIDS (DAIDS) of the National Institute of Allergy and Infectious Diseases (NIAID) Manual for Expedited Reporting of Adverse Events (Version 2.0, January 2010). Adverse event follow-up was reported on a standardized form during the protocol-defined reporting period. After the end of the protocol-defined adverse event-reporting period, sites were asked to report serious, unexpected, clinical suspected adverse drug reactions or if the study site staff becomes aware of the event on a passive basis (e.g. from publicly available information).

Trained nurses used structured questionnaires to collect data on patient demographics at baseline. Additionally, at baseline and at each follow-up visit, a questionnaire was used to collect information on psychosocial factors, access to care and treatment, attitudes towards and experiences with the supplementation, quality of life, self-efficacy, nutrition, opportunistic infections, and adherence to the study protocol. The questionnaire was available in both English and Kinyarwanda. Nurses also collected clinical data at baseline and at each follow-up visit, including information on height, weight, and blood.

Analysis
Baseline demographic and clinical characteristics were tabulated and compared using Fisher's exact test for

dichotomous outcomes and Wilcoxon's rank-sum test for continuous outcomes. Statistical significance was assessed at the two-sided 5% level unless otherwise indicated. All analyses were based on the intention-to-treat (ITT) approach using the randomized treatment assignment. All available data were used and missing data were removed.

To examine the effect of selenium supplementation on CD4$^+$ T-cell decay, we used linear regression to model change from baseline (i.e. all patients were assigned a change of 0 at baseline). The model included both time in study and treatment group, with an effect modification on time-by-treatment group. Generalized estimating equations (GEEs) were used to account for the repeated-measures nature of the data. Assumptions for linear regression, including homogeneity and normality of residuals, were assessed graphically and met.

Both time-to-event analyses and simpler contingency table analyses were used to determine whether selenium supplementation could delay the initiation of ART. Survival analysis was carried out by way of Cox proportional-hazards and Kaplan–Meier curves. Only two models were considered. The first was a simple model by which the only explanatory variable was treatment, and the second was an explanatory model with the addition of self-reported adherence to treatment (measured as all pills taken). As self-reported adherence was measured over time, we used it as a time-varying covariate. Moreover, we used baseline CD4$^+$, age, sex, viral load, and other measures of adherence to generate multiple imputations to overcome the missingness in the adherence. These analyses were then repeated for unadjusted events (a single CD4$^+$ cell count event). All conditions for survival analysis were verified using tests based on the Schoenfeld residuals and all assumptions were met.

For this secondary outcome of viral load, we considered observed suppression as a measured viral load less than 20 copies/μl. Missing viral loads were considered 'not observed as suppressed' (missing equals failure). Viral loads were measured at three time points: baseline, 12 months, and 24 months. Given that there were only two follow-up points, logistic regression was favored over survival analysis. As such, we used GEEs to account for repeated measurements on the same individual. Finally, we removed observation following ART initiation, as this would clearly interfere with the effects of selenium supplementation on viral suppression. Two models were fit – one with only treatment as a predictor and the other with time of measurement.

All analyses were conducted in SAS 9.3 (Cary, North Carolina, USA) and in R version 3.0.2 (Vienna, Austria).

Ethics
The present trial received approval from the institutional review boards of the Canadian College of Naturopathic Medicine and Wilfred Laurier University in Canada, and the National Ethics Committee (NEC) in Rwanda.

Results

Between September 2010 and January 2011, 300 patients were identified as eligible and randomized in this study. The study was conducted between January 2011 and June 2014 (24 months follow-up). Figure 1 shows the randomization, loss to follow-up, and censoring due to pregnancy over the study period. Selenium supplements were provided to 151 participants. Over the 24-month study period, 18 patients were lost to follow-up – nine within 6 months of study initiation, four more between 6 and 12 months into the study, three more in the next 6-month period, and finally two in the final 6 months. Reasons for loss to follow-up included accidental death, moving outside the study area, and unknown. The loss to follow-up counts did not include women who became pregnant and initiated ART through prevention of mother-to-child transmission programs. Pregnancy was nonetheless used as a censoring mechanism. Only two deaths were experienced, one in each arm, and neither appeared to be treatment-related.

Table 1 presents baseline characteristics of the study participants. There were 202 (67%) women, and the median age among participants was 33.5 years. The median baseline CD4$^+$ cell count was 540 cells/μl with an interquartile range (IQR) from 468 to 627 cells/μl. The base 10 logarithm of viral load at baseline was 3.87 (IQR 3.15–4.45). In total, 84 (28%) patients reported not using barrier methods of birth control, which explains the 33 pregnancies observed over the 24-month study period. The duration of follow-up was 24 months for all but 19 patients.

Changes in CD4$^+$ T-cell counts were heterogeneous, with a SD of 120.3 cells/μl. Table 2 summarizes the linear regression with GEEs used to model change in CD4$^+$ according to the treatment group. The average rate of CD4$^+$ decline among patients using placebo was 3.97 cells/μl per month [95% confidence interval (CI) 3.03, 4.91]. The rate of CD4$^+$ decline was reduced by 43.8% (95% CI 7.8–79.8) among patients using selenium supplementation. Figure 2 presents the estimated regression lines for each treatment group and suggests a difference of approximately 40 cells in decline at the end of the study period in favor of the treatment group over the placebo group.

For the composite endpoint, a total of 86 events were observed after censoring for pregnancy (Table 3). None of the outcomes were an AIDS-defining illness. Most were CD4$^+$ cell count depletion to below 350 cells/μl. Slightly more events occurred in the treatment group

Table 1. Baseline demographics and risk factors.

Variable	Values	Total	Active count (%) or median (IQR)	Placebo count (%) or median (IQR)	P value
Sex	Male	98	44 (29.1%)	54 (36.2%)	0.151
	Female	202	107 (70.9%)	95 (63.8%)	
Age		300	33.0 (28.0–39.0)	35.0 (28.0–41.0)	0.418
Marital status	Married or living with partner	180	90 (60.8%)	90 (60.4%)	0.916
	Single	26	15 (10.1%)	11 (7.4%)	
	Widowed	32	15 (10.1%)	17 (11.4%)	
	Separated	41	19 (12.8%)	22 (14.8%)	
	Divorced	18	9 (6.1%)	9 (6%)	
Employment	No	132	65 (43.3%)	67 (45.3%)	0.585
	Yes	165	84 (56%)	81 (54.7%)	
	Refused	1	1 (0.7%)	0 (0%)	
BMI at baseline		266	21.5 (19.8–23.7)	21.6 (20.0–24.3)	0.379
$CD4^+$ at baseline		300	552 (470–636)	527 (465–610)	0.126
Viral load (log of)		268	3.8 (3.0–4.5)	3.9 (3.3–4.4)	0.324
Has had sex in past month	No	110	50 (33.3%)	60 (40.3%)	0.214
	Yes	189	100 (66.7%)	89 (59.7%)	
Number of partners in past 30 days	NA (skipped)	110	50 (33.3%)	60 (40.5%)	0.390
	1	185	98 (65.3%)	87 (58.8%)	
	2	3	2 (1.3%)	1 (0.7%)	
Condom use in past 30 days	NA (skipped)	110	50 (33.3%)	60 (40.5%)	0.233
	Always	104	59 (39.3%)	45 (30.4%)	
	Sometimes	30	12 (8%)	18 (12.2%)	
	Never	54	29 (19.3%)	25 (16.9%)	

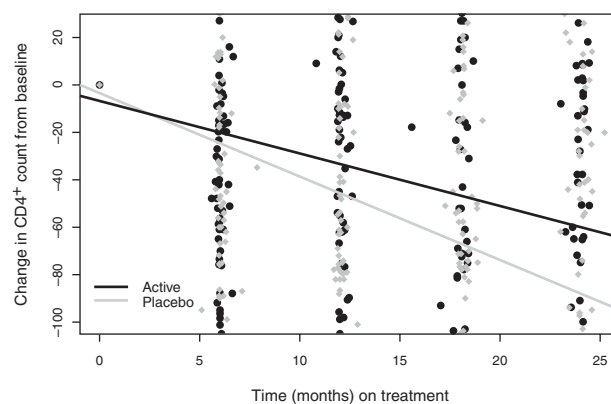IQR, interquartile range; NA, not available.

(51.2%), leading to a Fisher's exact *P* value of 0.899. When the definition of the composite outcome was relaxed to single measurement below 350 cells/µl, to account for missing second $CD4^+$ measurements, a total of 115 composite outcomes were observed and 52 (45.2%) occurred in the selenium group. However, this comparison was also not statistically significant, with a Fisher's exact *P* value of 0.192. Figure 3 shows the Kaplan–Meier estimates for reaching the composite endpoint. Table 4 shows the results of the Cox proportional-hazards regressions. In the analysis using the two consecutive low $CD4^+$ measurements in the composite outcome, the hazard ratio comparing the treatment group to the placebo group was 1.00 (95% CI 0.66–1.53). The result was consistent when accounting for adherence. The sensitivity analysis using a relaxed definition for the $CD4^+$ event led to similar results, but with a lower estimate hazard ratio (0.93) and a larger hazard ratio for being nonadherent.

For the secondary outcome of viral suppression, the effect of treatment in the unadjusted model was an odds ratio

(OR) of 1.18 (95% CI 0.71–1.93) in favor of selenium. During the trial, the only serious adverse event (SAE) reported was a myocardial infarction, resulting in death in the treatment group. There was no evidence to suggest that this SAE was directly related to the treatment. With respect to the self-reported adverse events collected at every 6 months, there was minimal evidence of statistical differences between the treatment groups. The comparisons across treatment groups are presented in Table A1 (Supplemental Digital Content 1, http://links.lww.com/QAD/A679) of the 'Web Appendix'. Patients taking the placebo were more likely to be anxious ($P = 0.035$). Patients taking selenium supplementation were more likely to report that sleep symptoms bothered them a lot, but less likely to report that it bothered them a

Table 2. Linear regression with generalized estimating equations.

Variable	Average $CD4^+$ change (95% confidence interval)	P value
Treatment		
Active vs. placebo	−4.37 (−13.78, 5.04)	0.363
Time (per month)	−3.97 (−4.91, −3.03)	<0.001
Time adjustment		
Active vs. placebo	1.74 (0.31, 3.17)	0.017

Fig. 2. Rate of $CD4^+$ T-cell count decline across treatment groups.

Table 3. Contingency tables for composite outcome.

| Treatment	Total	No	Yes	P value
Composite outcome				
Active	151	107 (50%)	44 (51.2%)	0.899
Placebo	149	107 (50%)	42 (48.8%)	
Total (n)	300	214	86	
Use only a single CD4$^+$ measurement below 350 as event				
Active	151	99 (53.5%)	52 (45.2%)	0.192
Placebo	149	86 (46.5%)	63 (54.8%)	
Total (n)	300	185	115	

little ($P = 0.011$). Both groups reported having no symptoms, with approximately equal probability. When adjusting for multiple testing, none of these results were statistically significant at the 0.05 significance level. Overall, selenium and placebo supplements were very well tolerated, helping explain the high percentage of study completers.

Discussion

The present RCT on selenium supplementation in pre-ART HIV-positive patients in Rwanda provides evidence of the benefits of selenium supplementation with respect to reduced rates of CD4$^+$ declines. Our study found a significant decrease in CD4$^+$ depletion rates, but not on the combined composite events, including reaching a CD4$^+$ point of less than 350 cells/μl, AIDS-defining illness, or death.

Our study has strengths and limitations. The strengths include our recruitment of patients with higher CD4$^+$ levels than previous trials that provide evidence for supplementation at an earlier stage and prior to the use of
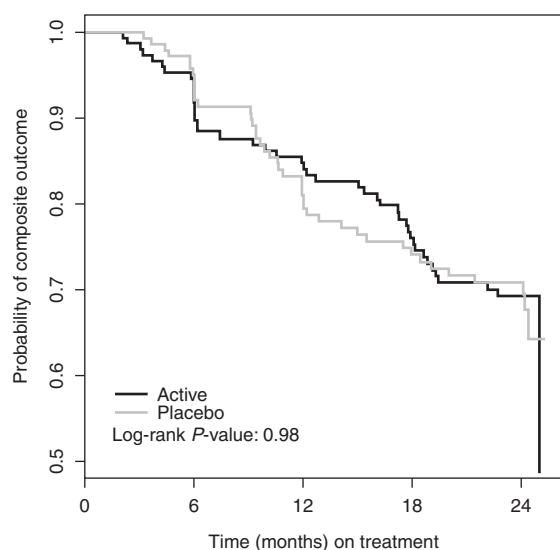
antiretrovirals. Moreover, we monitored adherence and high retention rate as a result of a strong commitment to the study on the part of the site nurses. Limitations include that the event rate for the composite outcome was much lower than the 60% used for our power calculations. This resulted in an underpowered analysis for this outcome. Moreover, only eight women were censored due to pregnancy in the placebo group compared to 15 in the treatment group. This unbalanced censoring may have biased the results in favor of the placebo treatment. This was the only observed factor by which the treatment groups differed following randomization. The different rates of censoring are not believed to have affected the CD4$^+$ and viral load analyses.

Although the Miami trial provided strong evidence for suppression of viral burden and an indirect improvement of CD4$^+$, Ross et al. [39] pointed out that these sub-analyses did not retain the original randomized treatment allocation. As such, these findings may be subject to bias not identified by Hurwitz et al. Particularly, analyses were performed in which participants in the selenium group who showed a large increase in serum selenium concentration ('selenium responders': defined as a change in serum selenium concentration between baseline and 9 months of above 3 SDs more than the mean change in the placebo group) were compared with 'selenium nonresponders' and with those allocated to placebo. Our findings were most closely observed in the Botswana trial, where selenium supplementation was shown to significantly reduce CD4$^+$ decline, but with no impact on viral burden.

The outcome of this study provides further evidence of the benefit of low-cost micronutrient supplementation to HIV-positive patients. This is particularly important for patients living in resource-limited settings such as much of Africa. Although assistance has been received from international funding agencies in order to make the antiretroviral drugs available for widespread distribution and treatment of HIV/AIDS patients, it is still a major financial burden to these countries. This financial burden is compounded by escalating healthcare costs associated with a rise in noncommunicable diseases [33,40]. In a period of worldwide financial uncertainty, any low-cost

Fig. 3. Kaplan–Meier plot of time to composite outcome results.

Table 4. Survival analysis for the composite outcome.

Variable	Protocol data hazard ratio (95% CI)	Unadjusted data[a] hazard ratio (95% CI)
Simple model		
Active vs. placebo	1.00 (0.66–1.53)	0.93 (0.66–1.31)
Adjusted model		
Active vs. placebo	1.00 (0.66–1.54)	0.94 (0.66–1.33)
Less-adherent vs. adherent	1.17 (0.70–1.96)	1.31 (0.85–2.02)

CI, confidence interval.
[a]This represents a sensitivity analysis by which a CD4$^+$ event only required a single CD4$^+$ below 350 cells/μl.

treatment that will slow the progression of the disease prior to the requirement for pharmacological intervention should be welcomed.

A growing concern in the treatment of HIV/AIDS in sub-Saharan Africa is the increase in drug resistance among those being treated for HIV/AIDS, particularly in regions where there had been an early roll out of ART [41]. A delay in progression of the disease brought about by low-cost micronutrient supplementation would be advantageous in modifying the rate of drug resistance at least at the level of the individual.

The HIV disease burden in sub-Saharan Africa is still important, in terms of incidence, social impact, and healthcare costs. On the basis of the outcome of this study, micronutrient supplementation with the trace mineral selenium may be something to consider in the overall treatment plan for HIV-positive patients in the pre-ART phase of care.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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