Effects of Selenium Supplementation for Cancer Prevention in Patients With Carcinoma of the Skin

A Randomized Controlled Trial

Larry C. Clark, MPH, PhD; Gerald F. Combs, Jr, PhD; Bruce W. Turnbull, PhD; Elizabeth H. Slate, PhD; Dan K. Chalker, MD; James Chow, MD; Loretta S. Davis, MD; Renee A. Glover, MD; Gloria F. Graham, MD; Earl G. Gross, MD; Arnon Krongrad, MD; Jack L. Lesher, Jr, MD; H. Kim Park, MD; Beverly B. Sanders, Jr, MD; Cameron L. Smith, MD; J. Richard Taylor, MD; for the Nutritional Prevention of Cancer Study Group

Objective.—To determine whether a nutritional supplement of selenium will decrease the incidence of cancer.

Design.—A multicenter, double-blind, randomized, placebo-controlled cancer prevention trial.

Setting.—Seven dermatology clinics in the eastern United States.

Patients.—A total of 1312 patients (mean age, 63 years; range, 18-80 years) with a history of basal cell or squamous cell carcinomas of the skin were randomized from 1983 through 1991. Patients were treated for a mean (SD) of 4.5 (2.8) years and had a total follow-up of 6.4 (2.0) years.

Interventions.—Oral administration of 200 μg of selenium per day or placebo.

Main Outcome Measures.—The primary end points for the trial were the incidences of basal and squamous cell carcinomas of the skin. The secondary end points, established in 1990, were all-cause mortality and total cancer mortality, total cancer incidence, and the incidences of lung, prostate, and colorectal cancers.

Results.—After a total follow-up of 8271 person-years, selenium treatment did not significantly affect the incidence of basal cell or squamous cell skin cancer. There were 377 new cases of basal cell skin cancer among patients in the selenium group and 350 cases among the control group (relative risk [RR], 1.10; 95% confidence interval [CI], 0.95-1.28), and 218 new squamous cell skin cancers in the selenium group and 190 cases among the controls (RR, 1.14; 95% CI, 0.93-1.39). Analysis of secondary end points revealed that, compared with controls, patients treated with selenium had a nonsignificant reduction in all-cause mortality (108 deaths in the selenium group and 129 deaths in the control group [RR, 0.83; 95% CI, 0.63-1.08]) and significant reductions in total cancer mortality (29 deaths in the selenium treatment group and 57 deaths in controls [RR, 0.50; 95% CI, 0.31-0.80]), total cancer incidence (77 cancers in the selenium group and 119 in controls [RR, 0.63; 95% CI, 0.47-0.85]), and incidences of lung, colorectal, and prostate cancers. Primarily because of the apparent reductions in total cancer mortality and total cancer incidence in the selenium group, the blinded phase of the trial was stopped early. No cases of selenium toxicity occurred.

Conclusions.—Selenium treatment did not protect against development of basal or squamous cell carcinomas of the skin. However, results from secondary end-point analyses support the hypothesis that supplemental selenium may reduce the incidence of, and mortality from, carcinomas of several sites. These effects of selenium require confirmation in an independent trial of appropriate design before new public health recommendations regarding selenium supplementation can be made.

JAMA. 1996;276:1957-1963

THE NUTRITIONALLY essential trace element selenium was first associated with cancer risk in the late 1960s.[1-3] Since then, a substantial body of research has elucidated functions of selenium in normal metabolism,[2-6] supported the establishment of a recommended daily allowance,[7] and documented the cancer prevention potential of selenium supplementation in animals. Selenium compounds have been shown to have antitumorigenic activities in animal models when the drug is administered at levels greater than those associated with nutritional needs.[8] Several hypotheses have been proposed to explain the inhibition of tumorigenesis by supplemental selenium,[9-12] including protection against oxidative damage involving the function of selenium as an essential component of the antioxidant enzyme glutathione peroxidase; alterations in carcinogen metabolism; effects on the endocrine and immune systems; production of cytotoxic selenium metabolites; inhibition of protein synthesis; inhibition of specific enzymes; and stimulation of apoptosis.

For editorial comment see p 1984.

Geographical studies suggest an inverse relationship between selenium status and cancer incidence.[13] In a study of the ecological relationship of environmental selenium levels (forage crop selenium) and county levels of cancer mortality in the United States,[14] cancer mortality rates were significantly lower for total cancer and cancers of the lung, colon and rectum, bladder, esophagus, pancreas, breast, ovary, and cervix in counties with intermediate selenium or high selenium levels compared with low-selenium counties.

Affiliations for the named authors and a complete list of the Nutritional Prevention of Cancer Study Group appear at the end of this article.

Corresponding author: Larry C. Clark, MPH, PhD, Arizona Cancer Center, 2504 E Elm St, Tucson, AZ 85716-3417 (e-mail: selenium@ccit-arizona.edu).

Table 1.—Baseline Characteristics of Study Population

Characteristic	Selenium Group	Placebo Group
Patients randomized, No.	653	659
Age, mean (SD), y	63.4 (10.2)	63.0 (10.0)
Plasma selenium, mean (SD), ng/mL	114.4 (22.5)	114.0 (21.2)
Clinical sun damage, mean (SD)*	4.8 (1.7)	4.7 (1.7)
Male, %	73.8	75.6
Smoking status, %		
Never smoked	34.3	31.0
Ex-smoker	39.1	39.3
Current smoker	26.6	29.7
Sun sensitivity		
Always burns, %	49.5	45.2
Never tans, %	12.1	10.2
Prostate-specific antigen >4 ng/mL, %	10.4	9.1
Prior squamous cell carcinomas, mean No.	0.92	1.07
Prior basal cell carcinomas, mean No.	3.06	2.64
Patients reporting other prior cancers, %†	3.8	5.8

*Average determination of degree of sun damage at the left and right temples and dorsal surface of the hands on a scale of 0-9.

†Excludes squamous and basal cell carcinomas.

Epidemiologic studies that use prediagnostic tissue to determine selenium levels for investigating the epidemiology of selenium and cancer demonstrate the plausibility of this hypothesis in humans.[15] While such case-control studies nested in cohort studies have yielded both significant[16-25] and nonsignificant[26-32] associations between selenium and cancer, no study has excluded the possibility of an inverse association. Among these studies are our previous case-control[33] and cohort[34] studies in patients with histories of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) skin cancers. These observations provided the rationale for the primary study end points of this trial.

One pair of intervention trials,[35,36] conducted in Linxian, China, used dietary supplements containing selenium in combination with other nutrients to prevent esophageal cancer. One trial[36] was conducted among patients with esophageal dysplasia and compared 4 treatment arms, one of which combined selenium, as a high-selenium yeast (50 μg), with α-tocopherol and beta carotene. This treatment arm suggested a modest protective effect against total mortality, total cancer mortality, and stomach cancer mortality. The other trial[35] used inorganic selenium in combination with 26 other vitamins and minerals in the general population and did not detect a significant protective health effect of selenium.

The purpose of the present study was to test the hypothesis that increased selenium status can reduce the risk of BCC and SCC of the skin. We conducted this trial in dermatologic practices to facilitate the collection of incident skin cancers and other patient health data, which allowed evaluation of both beneficial and adverse health effects. To our knowledge, this is the first double-blind, placebo-controlled cancer prevention trial to test the hypothesis that a nutritional supplement of selenium can reduce the risk of cancer.

METHODS

Protocol

This study was a randomized, double-blind, placebo-controlled cancer prevention trial using a nutritional dose of selenium in patients with histories of SCC and BCC skin cancers. The randomization procedure was blocked on time and stratified on clinic. Patients were eligible for randomization if they had (1) a history of 2 or more BCCs or 1 SCC of the skin, with 1 of these carcinomas occurring within the prior year; (2) a 5-year life expectancy; and (3) no internal malignancies treated within the previous 5 years. Patients were ineligible if they reported histories of significant liver or kidney disease. Recruitment was sex neutral. All patients signed informed consent forms approved by the institutional review board of each participating institution.

Assignment

Seven dermatology clinics located in the following cities in low-selenium areas of the United States (Augusta, Ga; Macon, Ga; Columbia, SC; Miami, Fla; Wilson, NC; Greenville, NC; Newington, Conn)[12] recruited patients for the trial, including 3 academic units associated with Veterans Affairs medical centers and 4 private practices. Recruitment commenced on September 15, 1983, with successive cohorts of patients randomized each year through 1991. Each clinic recruited to a target sample size from an open population of eligible patients.

Blinding

The intervention agent was 200 μg of selenium supplied as a 0.5-g high-selenium brewer's yeast tablet (Nutrition 21, La Jolla, Calif). Each pill was coated with titanium oxide to ensure the identical appearance and smell of the selenium and placebo pills. At the time of randomization, each patient was interviewed for eligibility, medical history, and selected personal characteristics and was assigned a unique sequential treatment number. Treatment group assignment was made centrally using sealed pill bottles distributed at the clinic. The coordinating center held all treatment information in blinded form.

Patient Evaluation

The baseline examination included items related to sun exposure and sensitivity (Table 1). A dermatologic examination included the assessment of the degree of sun damage for each temple and the dorsum of the hands using a 9-point scale. The index of clinical sun damage was the average of these 4 assessments. A chart review yielded the number of histologically confirmed BCCs and SCCs prior to randomization, and the health history interview identified persons who reported a diagnosis of cancer (other than BCC or SCC), cardiovascular disease, and other major medical conditions.

Patients were scheduled to return to the clinic every 6 months to be examined for new dermatologic problems and potential signs of selenium toxicity. Patients visited the clinic more frequently if needed. At each semiannual study visit, each patient was given a 6-month supply of pills and was queried as to use of the previous supply. Patients who missed clinic visits were contacted, and visits rescheduled. Interviews to identify new illnesses and medications occurred at each regularly scheduled clinic visit. A sample (14 mL) of whole blood was collected using trace element–free glass tubes (Vacutainer, Becton-Dickinson and Co, London, England) treated with heparin sodium.

Laboratory Measurements

The selenium contents of each batch of pills was determined (G.F.C.) and checked (Ivan S. Palmer, PhD, South Dakota State University, Brookings), using the diaminonaphthalene-fluorimetric procedure after nitric-perchloric acid digestion.[37] For each plasma sample, the selenium concentration was determined by automated electrothermal atomic absorption spectrophotometry (Perkin-Elmer 3030, Perkin-Elmer Corp, Norwalk, Conn) equipped with an electrodeless discharge lamp and automatic Zeeman-effect background correction. Quality control included multiple aliquots of human plasma as external control samples; a coefficient of variation of less than 7% (for duplicate analyses) was the criterion for acceptance.[38] Prostate-specific antigen levels were determined using available frozen archival plasma samples with the Abbott Diagnostics IMx PSA assay (Abbott Park, Ill).

Outcome Measures

The primary end points were skin BCC and SCC. Safety end points included known signs of frank selenosis (pathologic nail changes, brittle hair, garlic breath), and other illnesses. In 1990, secondary end points were identified when funding for long-term follow-up became available. These end points included total mortality and cancer mortality, as well as the incidences of lung, colorectal, and prostate cancers, the most frequently occurring cancers in the cohort. The addition of secondary end points was approved by the Executive Committee and the external

Safety Monitoring and Advisory Committee (SMAC), which were blinded at that time to the treatment group identities.

End-Point Ascertainment

Incident BCC and SCC were diagnosed by biopsy and confirmed by board-certified dermatopathologists. Recurrent and re-treated skin tumors, and clinically diagnosed skin tumors without biopsy confirmation were excluded from analyses.

The study used individualized, computer-generated patient questionnaires throughout. These forms displayed previously collected information on the patient's illnesses, medication use, dermatologic diagnoses, and treatments. This approach simplified the collection of new information and prompted the review of previously collected information. Patient medical records from each dermatologist were reviewed periodically to ascertain information from both study and nonstudy visits to ensure the completeness and accuracy of the information. In addition to the semiannual clinic visits and the follow-up by the clinical coordinators, annual contact was attempted with all randomized patients to confirm vital status and identify diagnoses of new illnesses.

The identification of secondary end points in 1990 caused no change in illness-ascertainment procedures at the clinic because information on new illnesses and medication use had been collected routinely since the beginning of the trial. However, at that time, the patient coordination center began an active program to acquire medical records for all significant illnesses that were identified either from patient interviews, medical histories, or death certificates for both the period to 1990 and the post-1990 period. The patient coordinating center began active follow-up of all patients who had discontinued participation in the trial. The National Death Index (NDI) was searched each year (1983-1993) for patients for whom vital status could not be ascertained. In 1994 and 1995, the cancer registry for each clinic state was given a list of study patients and NDI identifiers so that the 10-year period could be searched for diagnosed cases of cancer; these searches yielded no additional cases.

When patients reported illnesses or medical procedures related to the primary, secondary, or safety end points of the trial, research nurses requested medical, surgical, and pathology records from physicians and hospitals to document the illnesses. An oncologist or appropriate medical specialist reviewed each record and made the final diagnosis. Particular care was taken by the oncologic reviewers to ensure that carcinomas of multiple sites were distinguished from metastatic tumors. Death certificates were independently coded by an experienced nosologist. Review and coding of all records were conducted blindly.

Patient Safety Oversight

The SMAC held its first annual meeting in 1985. The secondary end points for the trial were established in 1990 when cancer screening protocols were implemented with approval of the National Cancer Institute (NCI). The SMAC recommended in December 1994 that the trial be unblinded and the results be published when the data for the 10-year period were complete and confirmed. Three senior NCI scientists and 2 data auditors conducted a trial audit in May 1995. With NCI approval, the blinded phase of patient treatment and follow-up ended in January 1996.

Statistical Analyses

The incidence and mortality data were evaluated using Kaplan-Meier estimates and log rank tests. Supporting analyses included the Cox proportional hazards model, which allowed adjustment for covariates and permitted a check of the robustness of treatment-effect inferences based on the log rank test. The covariates used for this adjustment were identified by a step-wise procedure from the baseline characteristics shown in Table 1. Unless otherwise noted, P values are from log rank tests, and the relative risks (RRs) are calculated using the ratio of the incidence density for the treatment groups. Consistency of treatment effect across subgroups was evaluated using the sign test. The P values are reported as usually computed and not adjusted for multiplicity. These techniques were implemented using STATA 4.0 (Stata Corp, College Station, Tex).

Each analysis is limited to the diagnosis of the first category-specific, postrandomization cancer. Patients with multiple cancers at different sites are counted only once in the analysis of total cancer and total carcinoma, and once in each site-specific analysis in which an incident cancer was diagnosed. The primary analyses of SCC and BCC end points were conducted separately from those of other cancers and included a mixed-effects Poisson regression model[39] to examine the treatment effect on the occurrence of multiple tumors and treatment lag on the occurrence of multiple tumors per patient.[40]

RESULTS

Patient Population

Table 1 presents the baseline characteristics of the study population, which consisted of 1312 randomized patients, ranging in age from 18 to 80 years. There are no significant differences between the treatment and placebo groups for these factors.

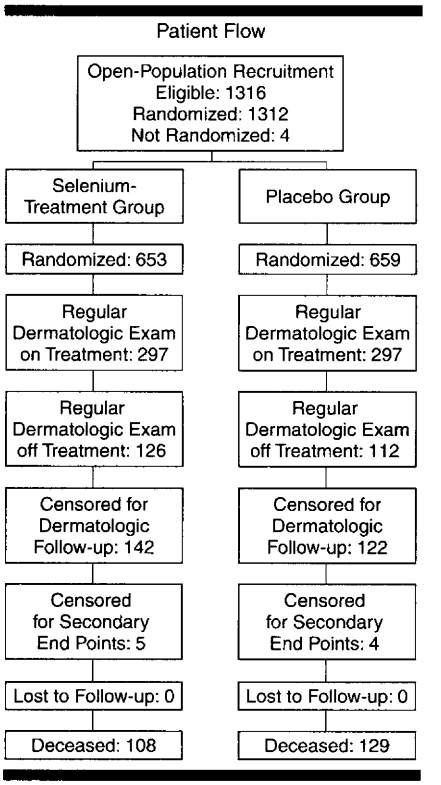

Figure 1.—Enrollment, progress, and outcome of patients in the Nutritional Prevention of Cancer study as of December 31, 1993.

At the end of the study period on December 31, 1993, 43.6% of patients were still on treatment, 18.0% were off treatment but were still having routine dermatologic examinations, 20.1% of patients were censored for dermatologic end points but not other end points, and 18.3% had died (Figure 1). After a total of 8271 person-years of follow-up, no patients were lost to vital follow-up and only 9 patients (5 in the selenium group and 4 in the placebo group) declined to provide additional illness information for a period of 48 person-years of follow-up (0.6% of the total potential follow-up for the trial). The range of active patient treatment was 0.0 to 10.3 years.

The patient-reported compliance indicated that 82% of patients in both the selenium and placebo groups had missed taking a pill less than twice a month. For both the selenium and placebo groups, the number of years on treatment (mean [SD]) (4.5 [2.8] vs 4.3 [2.8]) the length of dermatologic follow-up (5.6 [2.6] vs 5.4 [2.6]), and total follow-up (6.4 [2.0] vs 6.2 [2.1]) were not significantly different.

Mean plasma selenium concentration at the time of randomization was 114 ng/mL (SD, 23 ng/mL) (Figure 2), which is in the lower range of normal plasma levels reported in the United States. Plasma selenium concentration of the placebo

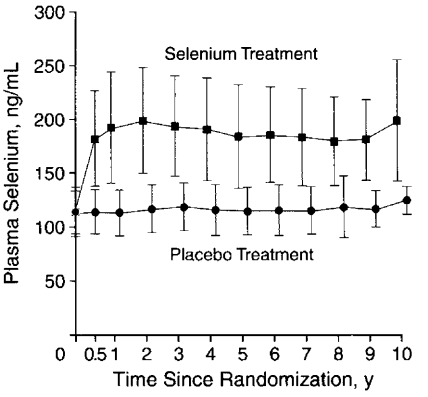

Figure 2.—Plasma selenium concentrations among patients receiving selenium (squares) and those receiving placebo (circles). Values are means and error bars indicate ±1 SD. The placebo-treatment lines are offset to allow for examination of overlapping areas.

group remained constant throughout the trial, whereas patients in the selenium-treatment group increased selenium levels by approximately 67% to 190 ng/mL within 6 to 9 months of supplementation. No patient had plasma selenium levels indicative of selenium deficiency (ie, <30 ng/mL). There was a small decline in selenium concentration associated with the number of years on trial in the selenium-treatment group, but no significant calendar trends in plasma selenium concentration were observed.

Patient Safety

No dermatologic signs of selenium toxicity were observed. A total of 35 patients complained of adverse effects (most involved gastrointestinal upset) which resulted in their withdrawing from treatment use, 21 in the selenium group and 14 in the placebo group. Within each group, patients reporting adverse effects did not have significantly different plasma selenium concentrations from those not reporting such effects.

Primary End Points

There were no statistically significant differences in the incidence of BCC or SCC between the 2 groups, although there were more cases of SCC (RR, 1.14; 95% CI, 0.93-1.39; $P=.15$) and BCC (RR, 1.10; 95% CI, 0.95-1.28; $P=.20$) in the selenium group than the placebo group (Table 2). The log rank test and the Cox regression models for time to first skin tumor occurrence, after adjustment for the covariates in Table 1, indicated no statistically significant treatment effects. In addition, a treatment lag effects model and a mixed-effects Poisson model that considered times to subsequent development of multiple tumors[37] also showed no significant associations. The trial had 80% power to detect a 25% change in the incidence of SCC and a 19% change in the incidence of BCC at significance level of 0.05.

Table 2.—Numbers of Patients With New Squamous Cell and Basal Cell Carcinomas*

Tumor Type	Selenium Group, No.	Incidence	Placebo Group, No.	Incidence	RR (95% CI)	P Value†
Squamous cell carcinoma	218	0.07	190	0.06	1.14 (0.93-1.39)	.15
Basal cell carcinoma	377	0.16	350	0.15	1.10 (0.95-1.28)	.20

*RR indicates relative risk; and CI, confidence interval.
†*P* values derived from log rank tests.

Table 3.—Cancer Incidence by Treatment Group

Cancer Sites, No.	Selenium	Placebo	RR (95% CI)*	P Value	HR (95% CI)†	P Value
Lung‡	17	31	0.54 (0.30-0.98)	.04	0.56 (0.31-1.01)	.05
Prostate‡	13	35	0.37 (0.18-0.71)	.002	0.35 (0.18-0.65)	.001
Colorectal‡	8	19	0.42 (0.18-0.95)	.03	0.39 (0.17-0.90)	.03
Head and neck	6	8	0.74 (0.21-2.43)	.58	0.77 (0.27-2.24)	.64
Bladder	8	6	1.32 (0.40-4.61)	.62	1.27 (0.44-3.67)	.66
Esophageal	2	6	0.33 (0.03-1.84)	.15	0.30 (0.06-1.49)	.14
Breast	9	3	2.88 (0.72-16.5)	.09	2.95 (0.80-10.9)	.11
Other specific carcinomas	5	9	0.55 (0.14-1.82)	.27	0.54 (0.18-1.62)	.27
Total carcinomas‡§	59	104	0.55 (0.40-0.77)	<.001	0.54 (0.39-0.75)	<.001
Melanomas	8	8	0.97 (0.32-2.96)	.91	0.92 (0.34-2.45)	.87
Leukemia/lymphomas	8	5	1.58 (0.46-6.14)	.41	1.50 (0.49-4.60)	.48
Other specific noncarcinomas	3	3	0.99 (0.13-7.37)	.98	0.99 (0.20-4.94)	.99
Total noncarcinomas	19	16	1.17 (0.57-2.44)	.65	1.16 (0.60-2.27)	.65
Total cancer‡§	77	119	0.63 (0.47-0.85)	.001	0.61 (0.46-0.82)	<.001

*RR indicates relative risk; and CI, confidence interval. *P* values derived from log rank tests.
†HR indicates hazard ratio. *P* values derived from the Cox proportional hazard model adjusted for age, sex, and smoking status at randomization.
‡These cancer sites were secondary end points.
§Total carcinoma and total cancer columns do not sum because multiple cancers were counted only once.

Secondary End Points

Total cancer incidence was lower (RR, 0.63; 95% CI, 0.47-0.85; $P=.001$) in the selenium group than in the placebo group (Table 3). The selenium group also had fewer total carcinomas (RR, 0.55; 95% CI, 0.40-0.77; $P<.001$). For site-specific cancers, the selenium group had fewer prostate cancers (RR, 0.37; 95% CI, 0.18-0.71; $P=.002$), fewer colorectal cancers (RR, 0.42; 95% CI, 0.18-0.95; $P=.03$), and fewer lung cancers (RR, 0.54; 95% CI, 0.30-0.98; $P=.04$) than the placebo group. Hazard ratios adjusted for sex, age, and smoking status (Table 4) make little difference in the risk estimates. For other sites, the small numbers of cases implied little statistical power to detect any treatment effects. Only breast cancer, bladder cancer, and leukemia-lymphoma had more events in the selenium group than in the placebo group, but none of these differences was statistically significant. A pathology report was available to confirm 76% of the cancer diagnoses.

There were no significant differences in unadjusted all-cause mortality (RR, 0.83; 95% CI, 0.63-1.08; $P=.14$) in the selenium-treatment group and in the placebo group (Table 4). The Cox proportional hazards model, with adjustment for sex, current smoking, and age, indicated that the selenium group experienced a 21% reduction in all-cause mortality vs the placebo group (RR, 0.79; 95% CI, 0.61-1.02; $P=.07$), with this difference largely due to 50% lower total cancer mortality (RR, 0.50; 95% CI, 0.31-0.80; $P=.002$) in the selenium group (Figure 3). Lung cancer deaths (Table 4), which comprised approximately half of all cancer deaths, were also lower in the selenium-treatment group than in the placebo group (RR, 0.47; 95% CI, 0.22-0.98; $P=.03$). The numbers of deaths for other specific types of cancer were insufficient for meaningful statistical analysis. Rates of mortality from cardiovascular disease and cerebrovascular disease and other causes were not significantly different between the 2 groups.

Consistency of Treatment Effect

The consistency of the treatment effect was examined according to prior history of cancer, time on study, clinic site, and incidence before and after the definition of the secondary end points. There were more subjects with a history of cancer prior to randomization in the placebo group than in the treatment group (38 vs 25, respectively); however, a stratified analysis indicated that the treatment effect was comparable in both groups (with prior cancers: RR, 0.38; 95% CI, 0.04-2.00; $P=.16$; without prior cancers: RR, 0.64; 95% CI, 0.47-0.87; $P=.003$). Six of the 7 clinics had lower rates of cancer incidence and cancer mortality in the selenium group ($P=.06$ by the sign test) whereas 5 had lower total mortality (data not shown). During the 10 years of follow-up the selenium group also had lower rates of total mortality for 9 years ($P=.01$)

Table 4.—Total and Cause-Specific Mortality by Treatment Group

Cause, No.	Selenium	Placebo	RR (95% CI)*	P Value	HR (95% CI)†	P Value
Total cancer‡	29	57	0.50 (0.31-0.80)	.002	0.48 (0.31-0.76)	.001
Lung cancer	12	25	0.47 (0.22-0.98)	.03	0.47 (0.23-0.93)	.03
Other carcinoma	15	25	0.59 (0.29-1.17)	.10	0.56 (0.30-1.07)	.08
Noncarcinoma	2	7	0.28 (0.03-1.48)	.09	0.26 (0.05-1.27)	.10
Cardiovascular and cerebrovascular diseases	47	46	1.00 (0.66-1.55)	.96	0.96 (0.64-1.44)	.83
All other causes§	32	26	1.22 (0.70-2.12)	.47	1.16 (0.69-1.95)	.57
Respiratory disease	14	11	1.26 (0.53-3.06)	.57	1.26 (0.57-2.77)	.57
All causes‡	108	129	0.83 (0.63-1.08)	.14	0.79 (0.61-1.02)	.07

*RR indicates relative risk; and CI, confidence interval. *P* values from the log rank test.
†HR indicates hazard ratio. *P* value from the Cox proportional hazard model adjusted for age, sex, and smoking status at randomization.
‡These mortality causes are secondary end points.
§Respiratory disease is the only cause of death that contributed more than 5 deaths to either group.

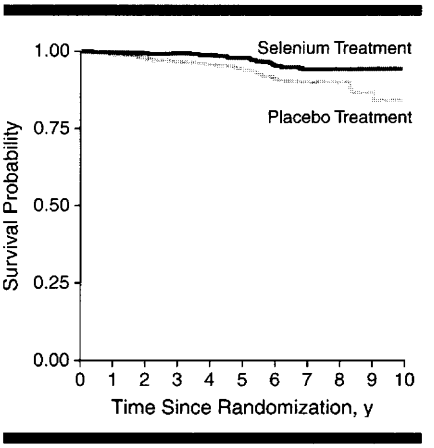

Figure 3.—Kaplan-Meier curve of total cancer mortality.

and lower rates of total cancer incidence and mortality for 8 years (P=.06).

Exclusion of SCC of the skin diagnosed in the first 2 years of the trial results in a change in the direction of the treatment effect for the primary outcome from a nonsignificant excess risk (RR, 1.14; 95% CI, 0.94-1.39; P=.15) to a nonsignificant decreased risk (RR, 0.95; 95% CI, 0.75-1.19; P=.79). Exclusion of cases of BCC from the first 2 years did not materially change the treatment effect. The treatment effect for total cancer incidence and all-cause mortality were unaffected by a treatment lag of 2 years, whereas total cancer mortality was weakened slightly. The treatment effect was enhanced in the 2-year lag analysis for prostate and colon cancer and weakened slightly for lung cancer (data not shown).

The treatment effect on secondary end points was further evaluated by considering only those events that occurred subsequent to their formal definition. The treatment effects for this time period were statistically significant (Table 5) for total cancer mortality, total cancer incidence, and cancers of the colon-rectum and the prostate. For lung cancer, the post-1990 results were consistent with the overall 10-year lung cancer results, but the treatment effect was not statistically significant.

COMMENT

The patient population in this trial was recruited from the eastern coastal plain of the United States, a region characterized by relatively low selenium levels in soils and crops,[12,41] and by high rates of SCC and BCC of the skin and cancer mortality.[42] Basal cell carcinoma and SCC were selected as primary end points because plasma selenium levels had been associated with these skin cancers in previous case-control and cohort studies. Each randomized patient had a history of BCC or SCC with at least 1 cancer within the prior 12 months and was a patient of a clinical investigator.

The primary purpose of this randomized, double-blind, placebo-controlled trial was to test the hypothesis that a nutritional supplement of selenium can reduce the incidences of BCC and SCC of the skin. The results do not support that hypothesis. The primary end points, BCC and SCC of the skin, were not reduced by selenium treatment. One possible explanation for this observation is that the length of treatment may have been too short for prevention of SCC and BCC because ultraviolet (UV) radiation increases the risk of SCC and BCC at both the initiation and promotion-progression stages of carcinogenesis.

Ultraviolet radiation can induce damage to the *p53* gene which inhibits the transformed cells from undergoing apoptosis. While this is often a late event in many carcinomas, it is apparently an early event for SCC of the skin with 69% of premalignant actinic keratosis and more than 90% of SCC expressing UV-induced mutations of *p53*.[43] In BCC, *p53* mutations are less common and occur in 40% to 60% of all lesions.[44] The UV radiation also appears to promote the progression of *p53*-mutated cells by inducing apoptosis of sunburn cells in the epidermis—thereby facilitating the clonal expansion of the transformed cells. If the primary cancer preventive effect of selenium supplementation is the enhancement of apoptosis,[45] then SCC and BCC of the skin would require a substaintually longer treatment period before the manifestation of a protective treatment effect than would other types of cancer in which *p53* mutations are a late stage event. This would occur because a significant proportion of the premalignant skin lesions already contain *p53* mutations.

The safety of the intervention agent was an important consideration in the design and conduct of the trial. The selenium dose of 200 μg/day is within the normal range of dietary intake of Americans, provides approximately twice the projected typical dietary intake of these patients, and is 3 to 4 times the recommended daily allowance. The absence of the dermatologic signs of selenosis[46] reinforces the safety of selenium supplementation. Plasma selenium concentrations remained below the no adverse effect level (1000 ng/mL in whole blood) established by the Environmental Protection Agency.[6]

The results point to the possibility of protective effects on the secondary end points (total cancer mortality, total cancer incidence, incidences of lung, prostate, and colorectal cancers). Supplementation with selenium inhibits tumor growth and stimulates apoptosis in cultured tumor cells.[47] These observations support the hypothesis that selenium supplementation inhibits the late stage promotion and progression of tumors. Accordingly, selenium treatment might be expected to manifest cancer-protective effects within a relatively short time frame.

It appears that regulation of apoptosis is an important determinant of cancer risk in humans as well as in experimental systems. In a cohort of elderly subjects, the use of calcium channel blockers that inhibit apoptosis increased the risk of cancer in a dose-dependent manner.[48] Thus, the enhancement of apoptosis with selenium supplementation may decrease cancer risk particularly in individuals with suboptimal selenium status. In addition, geographic studies[1,12] suggest that, while it is plausible that selenium might protect against cancer mortality in humans, such effects should not be expected at all sites. The present results are consistent with the geographic study[12] that indicated no association with noncarcinomas.

The secondary results were consistent across both clinic and time. The apparently protective effect for total cancer incidence and cancer mortality occurred in 6 of the 7 clinics. These effects also were observed in 8 of 10 years of follow-up for total cancer incidence and mortality. The lower number of cancer deaths in

Table 5.—Secondary End-Point Analysis Before and After Definition

	1983-1989*				1990-1993†			
	Events, No.				Events, No.			
End Point	Selenium Group	Placebo Group	RR (95% CI)	*P* Value	Selenium Group	Placebo Group	RR (95% CI)	*P* Value
Lung cancer incidence	7	16	0.44 (0.18-1.06)	.06	10	15	0.66 (0.30-1.46)	.30
Prostate cancer incidence	4	8	0.50 (0.15-1.67)	.25	9	27	0.33 (0.15-0.70)	.002
Colorectal cancer incidence	6	5	1.20 (0.37-3.95)	.76	2	14	0.14 (0.32-0.62)	.002
Total cancer incidence	27	42	0.64 (0.39-1.03)	.07	50	77	0.63 (0.44-0.90)	.01
Total cancer mortality	7	16	0.44 (0.18-1.07)	.06	22	41	0.53 (0.31-0.89)	.01
All-cause mortality	36	40	0.90 (0.57-1.41)	.63	72	89	0.80 (0.58-1.08)	.15

*Events prior to secondary end point definition. RR indicates relative risk; and CI, confidence interval. *P* values derived from the log rank test.
†Events after secondary end point definition.

the selenium group was not counterbalanced by an increased risk of death from other causes, as has been observed in cardiovascular prevention trials.[49,50] In addition, the proportion of patients with elevated prostate-specific antigen levels was slightly greater in the selenium group; thus, the treatment result for prostate cancer cannot be explained by a favorable distribution of prostate-specific antigen levels at randomization.

While secondary end points were not formally defined at the beginning of the study, the ascertainment of these end points with patient interviews did not change during the course of the study. Confirmation of secondary end points with medical record review began in 1987. Approximately 76% of patients with cancer had their diagnosis confirmed with pathology reports. The uniform searches of the NDI and of state cancer registries for deaths and cancer cases decreased the possibility of ascertainment bias explaining the results.

Comstock et al[51] reviewed 10 cohort studies that obtained prediagnostic serum samples and measured beta carotene, retinol, vitamin E, or selenium. All 4 nutrients showed a relatively consistent pattern of increased cancer risk associated with lower nutrient levels. For selenium, a majority of these studies showed case subjects had lower levels than control subjects, although most studies showed a case-control difference of less than 10%. Individual studies of the epidemiology of selenium and cancer are complex and difficult to interpret because of methodologic problems. These problems include the large degree of misclassification of selenium status with only a single measurement of blood levels; the relative homogeneity of selenium levels within specific populations that decrease the statistical power of studies; the relatively low power to test hypotheses for individual sites of cancer because of small study populations; and low incidence of cancer in most of these cohorts defined to study cardiovascular disease.

After considering the results of this trial, the SMAC recommended unblinding the trial in advance of its planned termination in 1998. This decision was based on a number of issues, including lack of effect on the primary end points and on reductions in cancer mortality and cancer incidence in the selenium group. It should be noted that, as is typical in large clinical and epidemiologic studies, a large number of statistical tests on the data were performed and reported herein. There is no generally agreed upon method for adjusting *P* values for the multiplicity of end points considered.[52-55] Furthermore, this trial was subject to interim monitoring by the SMAC. In the decision to stop or to continue the trial at each stage, the SMAC entertained a variety of quantitative and qualitative considerations. Informal stopping guidelines were adopted early on: *z* values of 3.0 for primary end points,[56] 3.5 for secondary end points, and 2.0 for mortality end points. It was agreed, however, that these guidelines would not be interpreted as rigid stopping rules.

The reported *P* values should be interpreted with great care, since there is no generally accepted method for adjusting *P* values when the early stopping is not based on the primary end point.[57,58] However, the significant reductions in cancer incidence and mortality found in this study cannot easily be dismissed as chance observations resulting from multiplicity of end-point testing. The strength of the associations found and their consistencies across clinic, across time, and across all the major cancer sites for which there were enough events to allow statistical tests of adequate power all weigh against the role of chance in these findings.

The magnitude of the apparent effects on cancer incidence and cancer mortality were unanticipated and should be interpreted with caution considering confounding, multiplicity, and generalizability. That there may be important confounding not yet considered is unlikely. The stratified randomization design by clinic should provide balance in potential confounders within each clinic for the 2 treatment groups. For confounding to have produced these effects in secondary end points, any imbalances that may have occurred would have to have been replicated across most or all of the clinics; for this, we have no evidence. The stratified randomization by clinic provides balance for such potential confounders as type of clinic, and local screening and diagnostic methods. In addition, the baseline characteristics listed in Table 1 were not imbalanced, and adjustment for them did not materially affect the results. That this cohort consisted of subjects at high risk of BCC, SCC, or both, who showed relatively high cancer rates, may limit the generalizability of these findings to other populations. Confirmation of the apparently protective effects of selenium supplementation shown in the study remains necessary. Therefore, additional studies should be conducted to determine whether selenium supplementation can protect against cancer in other populations and among particular subgroups of individuals with known cancer risk factors.

CONCLUSION

The results of this randomized controlled trial do not support the hypothesis that selenium supplementation reduces the risk of BCC or SCC of the skin, showing no statistically significant treatment effect on their incidence. However, selenium supplementation was found to be associated with significant reductions in secondary end points of total cancer incidence (all-sites combined), lung, colorectal and prostate cancer incidences, and lung cancer mortality. These apparent beneficial effects of selenium supplementation require confirmation in independent trials of appropriate design before public health recommendations regarding selenium supplementation can be made.

From the Arizona Cancer Center, College of Medicine, University of Arizona, Tucson (Dr Clark); the Division of Nutritional Sciences (Dr Combs) and the Statistics Center (Drs Turnbull and Slate), Cornell University, Ithaca, NY; the Georgia Dermatology and Skin Cancer Clinic, Macon (Drs Chalker and Sanders); Columbia Skin Clinic, Columbia, SC (Dr Chow); the Medical College of Georgia, Augusta (Drs Davis and Lesher); the Wilson Dermatology Clinic, Wilson, NC (Drs Glover and Graham); the Division of Dermatology, School of Medicine, University of

North Carolina at Chapel Hill (Dr Graham); the Division of Dermatology, University of Connecticut School of Medicine, Farmington (Dr Gross); School of Medicine and the Veterans Administration Medical Center (Drs Kongrad and Taylor), University of Miami, Fla; Department of Pathology (Dr Park) and the Department of Medicine, Division of Dermatology (Dr Smith), School of Medicine, East Carolina University, Greenville, NC; Eastern Dermatology, Greenville, NC (Drs Park and Smith).

The Nutritional Prevention of Cancer Study Group: David S. Alberts, MD, Bruce L. Dalkin, MD, Arizona Cancer Center, College of Medicine, University of Arizona, Tuscon; Richard J. Allison Jr, MD, College of Medicine, University of South Carolina, Columbia; James C. Bradshaw, DO, Warner Robins, Ga; David Curtis, MD, Medical College of Georgia, Augusta; Davey R. Deal, MD, Division of Dermatology, Coliseum Hospital, Macon, Ga; Mark Dellasega, MD, Quadrangle Medical Specialists, Greenville, NC; John D. Hendrix, MD, Department of Medicine, Division of Dermatology, East Carolina University, Greenville, NC; James H. Herlong, MD, Columbia Urological Specialists, PA, Columbia, SC; Lee J. Hixson, MD, Dixie Medical Center, St George, Utah; Fred Kight, MD, Augusta, Ga; James Moore, MD, South Suburban Gastroenterology, South Weymouth, Mass; Joseph S. Rice, MD, Columbia Gastrointestinal Endoscopy Center Inc, Columbia, SC; Arvey I. Rogers, MD, University of Miami Veterans Affairs Medical Center, Miami, Fla; Bernard Schuman, MD, Medical College of Georgia, Augusta; Edward H. Smith Jr, MD, Medical College of Georgia, Augusta; and Jerry C. Woodard, MD, Wilson Gastroenterology, Wilson, NC.

The Safety Monitoring and Advisory Committee: Tim E. Byers, MD, MPH; Harvey Cohen, MD, PhD; Stephen L. George, PhD; E. Robert Greenberg, MD; and Philip R. Taylor, MD, DSc.

The Executive Committee: Gerald F. Combs Jr, PhD, and Bruce W. Turnbull, PhD.

This study was funded in part by grants from the American Institute of Cancer Research (84B01R87A), the American Cancer Society (PDT-298B), the National Institutes of Health (R01-CA42334 and R01-CA49764), and Nutrition 21, La Jolla, Calif.

The authors gratefully acknowledge the important contributions of the following people: Doug Albreski, DPM, Margery Bates, Maria Bishop, MD, Teresa Bogue, John Bray, PhD, Lynne Deuschle, Lisa Higgins, MPH, Anne Hodgson, Margie Knox, Thomas Kramer, Frances Lee-Lin, MSN, Chemaine McCray, Tamara Montaño, Ann Pursley, Jean Shelton, Regina Spivey, Donna Sugerman, Cynthia Tie, MD, Edward Wittke, and Rebecca Williams.

We especially thank the Nutritional Prevention of Cancer project participants for their years of dedication and perseverance.

References

1. Shamberger RJ, Frost DV. Possible protective effect of selenium against human cancer. *Can Med Assoc J.* 1969;100:682.
2. Shamberger RJ, Willis CE. Selenium distribution of human cancer mortality. *Crit Rev Clin Lab Sci.* 1971;2:211-221.
3. Schrauzer GN, Thead WJ. Interpretation of the methylene blue reduction test of human plasma and the possible cancer protecting effect of selenium. *Experientia.* 1971;27:1069-1071.
4. Combs GF Jr, Combs S. *The Role of Selenium in Nutrition.* New York, NY: Academic Press; 1986.
5. Burk RF, Hill KE. Regulation of selenoproteins. *Annu Rev Nutr.* 1993;13:65-81.
6. Combs GF Jr. Essentiality and toxicity of selenium: a critique of the recommended dietary allowance and the reference dose. In: Mertz W, Abernathy CO, Olin SS, eds. *Risk Assessment of Essential Elements.* Washington, DC: ILSI Press; 1994:167-183.
7. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences, National Research Council. *Recommended Dietary Allowances, Tenth Edition.* Washington, DC: National Academy Press; 1989.
8. Combs GF Jr. Selenium and cancer. In: Wang Z, ed. *Biochemistry and Molecular Biology of Selenium.* Beijing, China: Chinese Academy of Science; 1995.
9. Milner JA. Effect of selenium on virally induced and transplantable tumor models. *Fed Proc.* 1985; 44:2568-2572.
10. Ip C. The chemopreventive role of selenium in carcinogenesis. *J Am Coll Toxicol.* 1986;5:7-20
11. Ip C, Medina D. Current concept of selenium and mammary tumorigenesis. In: Medina D, Kidwell W, Heppner G, Anderson EP, eds. *Cellular and Molecular Biology of Breast Cancer.* New York, NY: Plenum Press; 1987:479.
12. El-Bayoumy K. The role of selenium in cancer prevention. In: DeVita VT, Hellman S, Rosenberg SS, eds. *Practice of Oncology.* 4th ed. Philadelphia, Pa: JB Lippincott; 1991:1-15.
13. Schrauzer GN, White DA, Schneider CJ. Cancer mortality correlation studies, III. *Bioinorganic Chem.* 1977;7:23-34.
14. Clark LC, Cantor KP, Allaway WH. Selenium in forage crops and cancer mortality in US counties. *Arch Environ Health.* 1991;46:37-42.
15. Clark LC. The epidemiology of selenium and cancer. *Fed Proc.* 1985;44:2584-2590.
16. Salonen JT, Alfthan G, Huttunen JK, Puska P. Association between serum selenium and the risk of cancer. *Am J Epidemiol.* 1984;120:342-349.
17. Salonen JT, Salonen R, Lappeteläinen R, Mäenpää PH, Alfthan G, Puska P. Risk of cancer in relation to serum concentrations of selenium and vitamins A and E. *BMJ.* 1985;290:417-420.
18. Willett W, Polk B, Morris S, et al. Prediagnostic serum selenium and risk of cancer. *Lancet.* 1983;2: 130-134.
19. Kok FJ, De Bruijn AM, Hofman A, Vermeeren R, Valkenburg HA. Is serum selenium a risk factor for cancer in men only? *Am J Epidemiol.* 1987;125:12-16.
20. Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK, Karvonen MJ. Serum selenium and risk of cancer. *Cancer.* 1987;60:145-148.
21. van den Brandt P, Goldbohm R, van't Veer P, et al. A prospective cohort study of toenail selenium levels and risk of gastrointestinal cancer. *J Natl Cancer Inst.* 1993;85:224-229.
22. Peleg I, Morris S, Hames CG. Is serum selenium a risk factor for cancer? *Med Oncol Tumor Pharmacother.* 1985;2:157-163.
23. Knekt P, Aromaa A, Maatela J, et al. Serum selenium and subsequent risk of cancer among Finnish men and women. *J Natl Cancer Inst.* 1990;82:864-868.
24. Glattre E, Thomassen Y, Thoresen SO, et al. Prediagnostic serum selenium in a case-control study of thyroid cancer. *Int J Epidemiol.* 1989;18:45-49.
25. Fex G, Pettersson B, Akesson B. Low plasma selenium as a risk factor for cancer death in middle-aged men. *Nutr Cancer.* 1987;10:221-229.
26. Menkes MS, Comstock GW, Vuilleumier JP, Helsing KJ, Rider AA, Brookmeyer RP. Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. *N Engl J Med.* 1986;315:1250-1254.
27. Garland M, Morris JS, Stampfer MJ, et al. Prospective study of toenail selenium and cancer among women. *J Natl Cancer Inst.* 1995;87:497-505.
28. Schober SE, Comstock GW, Helsing KJ, et al. Serologic precursors of cancer. *Am J Epidemiol.* 1987;126:1033-1041.
29. Nomura A, Heilbrun LK, Morris JS, Stemmermann GN. Serum selenium and the risk of cancer, by specific sites. *J Natl Cancer Inst.* 1987;79:103-108.
30. Knekt P, Aromaa A, Maatela J, et al. Serum vitamin E, serum selenium and the risk of gastrointestinal cancer. *Int J Cancer.* 1988;42:846-850.
31. Ringstad J, Jacobsen BK, Tretli S, Thomassen Y. Serum selenium concentration associated with risk of cancer. *J Clin Pathol.* 1988;41:454-457.
32. Coates RJ, Weiss NS, Daling JR, Morris JS, Labbe RF. Serum levels of selenium and retinol and the subsequent risk of cancer. *Am J Epidemiol.* 1988; 128:515-523.
33. Clark LC, Graham GF, Crouse RG, Grimson R, Hulka B, Shy CM. Plasma selenium and skin neoplasms. *Nutr Cancer.* 1984;6:13-21.
34. Clark LC, Graham GF, Turnbull BW, Bray J, Hulka B, Shy CM. Non-melanoma skin cancer and plasma selenium: a prospective cohort study. In: Combs GF Jr, Spallholz JE, Levander OA, Oldfield JE, eds. *The Third International Symposium on Selenium in Biology and Medicine.* Westport, Conn: AVI Publishing Co; 1986:1122-1135.
35. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China. *J Natl Cancer Inst.* 1993;85:1483-1492.
36. Li JY, Taylor PR, Li B. Nutrition intervention trials in Linxian, China. *J Natl Cancer Inst.* 1993; 85:1492-1498.
37. Olson OE, Palmer IS, Cary EE. Modification of the official fluorometric method for selenium in plants. *J Assoc Off Anal Chem.* 1975;58:117-126.
38. McShane LM, Clark LC, Combs GF Jr, Turnbull BW. Reporting the accuracy of biochemical measurements for epidemiologic and nutrition studies. *Am J Clin Nutr.* 1991;53:1354-1360.
39. Abu-Libdeh H, Turnbull BW, Clark LC. Analysis of multi-type recurrent events in longitudinal studies. *Biometrics.* 1990;46:1017-1023.
40. Luo X, Turnbull BW, Cai H, Clark LC. Regression for censored survival data with lag effects. *Commun Stat.* 1994;23:3417-3438.
41. Allaway WH, Kubota J, Losee F, Roth M. Selenium, molybdenum and vanadium in human blood. *Arch Environ Health.* 1968;16:342-348.
42. National Cancer Institute, State Cancer Control and Data Program. *Cancer Mortality by Pentad 1953-1957 to 1983-1987.* Bethesda, Md: National Institutes of Health; 1992.
43. Nelson MA, Einspahr JG, Alberts DS, et al. Analysis of the *p53* gene in human precancerous actinic keratosis lesions and squamous cell cancers. *Cancer Lett.* 1994;85:23-29.
44. Gailani MR, Leffell DJ, Ziegler A, Gross EG, Brash DE, Bale AE. Relationship between sunlight exposure and a key genetic alteration in basal cell carcinoma. *J Natl Cancer Inst.* 1996;88:349-354.
45. Lanfear J, Fleming J, Wu L, Webster G, Harrison PR. The selenium metabolite selenodiglutathione induces *p53* and apoptosis. *Carcinogenesis.* 1994; 15:1378-1392.
46. Yang GQ, Yin S, Zhou R. Studies of safe maximal daily dietary Se-intake in a seleniferous area of China. *J Trace Elem Electrolytes Health Dis.* 1989;3:123-130.
47. Thompson JH, Wilson A, Lu J, et al. Comparison of the effects of an organic and inorganic form of selenium on a mammary carcinoma cell line. *Carcinogenesis.* 1994;15:183-186.
48. Pahor M, Gurainik JM, Ferrucci L, et al. Calcium-channel blockade and incidence of cancer in aged populations. *Lancet.* 1996;348:493-497.
49. The LRC Study Group. The Lipid Research Clinics Coronary Primary Prevention Trial results. *JAMA.* 1984;251:351-364.
50. Frick MH, Elo O, Haapa K, et al. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. *N Engl J Med.* 1987;317:1237-1245.
51. Comstock GW, Bush TL, Helzlsouer K. Serum retinol, beta-carotene, vitamin E and selenium as related to subsequent cancer of specific sites. *Am J Epidemiol.* 1992;135:115-121.
52. Pocock SJ, Geller NL, Tsiatis AA. The analysis of multiple endpoints in clinical trials. *Biometrics.* 1987;43:487-498.
53. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology.* 1990;1:43-46.
54. Tannock IF. False-positive results in clinical trials. *J Natl Cancer Inst.* 1996;88:206-207.
55. Cook RJ, Farewell VT. Multiplicity considerations in the design and analysis of clinical trials. *J R Stat Soc A.* 1996;159:93-110.
56. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient, I: introduction and design. *Br J Cancer.* 1976;34:585-612.
57. Cox DR. In Discussion: Jennison C, Turnbull BW. Interim analyses. *J R Stat Soc B.* 1989;51:338.
58. Jennison C, Turnbull BW. Interim analyses. *J R Stat Soc B.* 1989;51:305-361.