Selenium and prevention of prostate cancer in high-risk men: the Negative Biopsy Study

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Epidemiological and clinical studies suggesting a significant inverse relationship between intake of dietary selenium and overall cancer risk have led to initiation of a randomized, placebo-controlled, phase III clinical trial testing the safety and efficacy of selenized yeast as a chemopreventive agent for prostate cancer. Participants eligible for the 'Negative Biopsy Study', which was initiated in August 1999, are men considered to be at high risk for prostate cancer because of at least one negative sextant prostate biopsy, which was clinically indicated within 1 year of enrollment to the study. After a 30-day run-in period to ensure protocol compliance, participants are randomized to receive either 200 or 400 μg selenized yeast or matched placebo once daily. Primary study endpoints include development of prostate cancer and prostate-specific antigen (PSA) velocity. Secondary biochemical endpoints include change in chromagranin A and alkaline phosphatase. As of 1 June 2003, 514 eligible participants had been enrolled. Randomization schema was effective for selected parameters including age, body mass index, smoking status, baseline PSA and baseline plasma selenium level. Various data, including medical history, family history, and urological symptoms and specimens (including blood and subsequent prostate biopsy samples) had been collected at baseline, and throughout both the intervention and follow-up stages of the protocol. The goal for accrual is 700 evaluable participants. *Anti-Cancer Drugs* 14:589–594 © 2003 Lippincott Williams & Wilkins.

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Introduction

Epidemiological and observational studies have suggested that intake of dietary selenium is inversely related to overall cancer risk. Studies conducted by Willett *et al.* measured peripheral selenium levels in 111 subjects with cancer, compared to 210 cancer-free, age-matched controls [1]. Analyses of these data suggested that risk for subjects in the lowest quintile of serum selenium was twice that of subjects in the highest quintile. The effect was most pronounced in gastrointestinal and prostate cancers. Salonen *et al.* also demonstrated an inverse association between peripheral selenium level and cancer risk [2]. In addition to the epidemiological data that have been collected over the past several decades, *in vivo* studies have demonstrated that dietary selenium supplementation can reduce cancer incidence in animal models of melanoma and cancers of the colon [3,4], breast [5], liver [6], esophagus [7], head and neck [8], kidney [9], and lung [10,11]. The most compelling findings in humans were from the Nutritional Prevention of Cancer (NPC) study conducted at the University of Arizona, where a greater than 60% reduction in the incidence of prostate cancer was observed in participants randomized to 200 μg selenium/day compared to a placebo group [12,13]. The protective effect was most significant in men with baseline prostate-specific antigen (PSA) ≤ 4 and baseline peripheral selenium level in the lowest tertile (below 123.2 ng/ml). The principal conclusion of the NPC trial was the need to conduct independent confirmatory studies prior to making public health recommendations regarding selenium supplementation.

The Negative Biopsy Study was initiated at the Arizona Cancer Center in August 1999. This is a 5-year, randomized, double-blind, placebo-controlled multi-center phase III clinical intervention trial that was designed to determine whether daily supplementation with two different doses of selenium can decrease prostate cancer incidence and have an inhibitory effect on PSA velocity (rate of rise over time) in men considered at high risk for prostate cancer. Eligible participants have had a prostate biopsy that was negative for cancer or high-grade prostatic intraepithelial neoplasia (HGPIN). All laboratory and immunohistochemical analyses, data collection, and participant monitoring are coordinated at the University of Arizona, Arizona Cancer Center (Tucson, Arizona). Participants are recruited from urology clinical sites throughout the US, including the University of Arizona;

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Arizona Cancer Center Extension (Phoenix, AZ), Southwest Veteran's Administration Healthcare Service (Tucson, AZ), Midwest Prostate/Urology (Chicago, IL), Stanford University (Palo Alto, CA), University of North Carolina (Chapel Hill, NC), University of New Mexico (Albuquerque, NM), University of Arkansas (Little Rock, AK), Louisiana State University (Shreveport, LA), Veterans Administration Medical Center (Shreveport, LA) as well as from clinics in Annapolis, MD; Columbia, SC and Roswell Park Cancer Institute (Buffalo, NY).

In an ancillary study, participants are also recruited from Auckland Hospital (Auckland, New Zealand) and Waikato Urology Clinic (Waikato, New Zealand). New Zealand is of particular interest due to the relatively low levels of selenium present in the local environment and, therefore, in the diet of New Zealanders [14,15].

The Negative Biopsy Study is the first phase III, randomized clinical study testing selenized yeast as a prostate cancer chemopreventive agent as a primary objective in this population. The endpoints for this trial include the incidence of biopsy-proven prostate cancer and the effect of selenium supplementation on PSA velocity, which is measured semiannually. Two additional serum markers will also be measured on a semiannual basis as evidence of biochemical progression of prostate carcinogenesis—alkaline phosphatase is a biochemical marker that can be indicative of prostate cancer progression involving invasion and metastases to bone tissue [16–18], and chromogranin A is a marker of neuroendocrine differentiation. Increased chromogranin A expression has been shown to be associated with progressive prostate cancer compared to peripheral expression in men with benign prostatic conditions or early prostate cancer [19–21]. This article will summarize the study design of the Negative Biopsy Study and baseline characteristics of participants at the time of enrollment.

Patients and methods

Study design and participants

This is a 5-year, randomized, double-blind, placebo-controlled, multi-center, phase III, clinical intervention trial designed to determine whether daily supplementation with two different doses of selenium can decrease prostate cancer incidence and inhibit early stages of prostate carcinogenesis. Participants must be less than 80 years of age and have clinical indicators consistent with the community standards of medical care that would justify a biopsy of the prostate for the diagnosis of cancer. Men with a prostate biopsy negative for cancer within 12 months of enrollment into the trial are being recruited from clinical sites in the US and two sites in New Zealand. Eligibility criteria are outlined in Table 1. Study procedures are summarized in Figure 1.

The protocol and Informed Consent Form are approved by the University of Arizona Institutional Review Board (IRB) and the IRB or equivalent committee at each respective clinical site. A Data Safety Monitoring Board (DSMB), comprised of individuals with expertise in the areas of basic science, medicine and biostatistics, has been established to serve as an external review committee to monitor the progress of the study including accrual and toxicities.

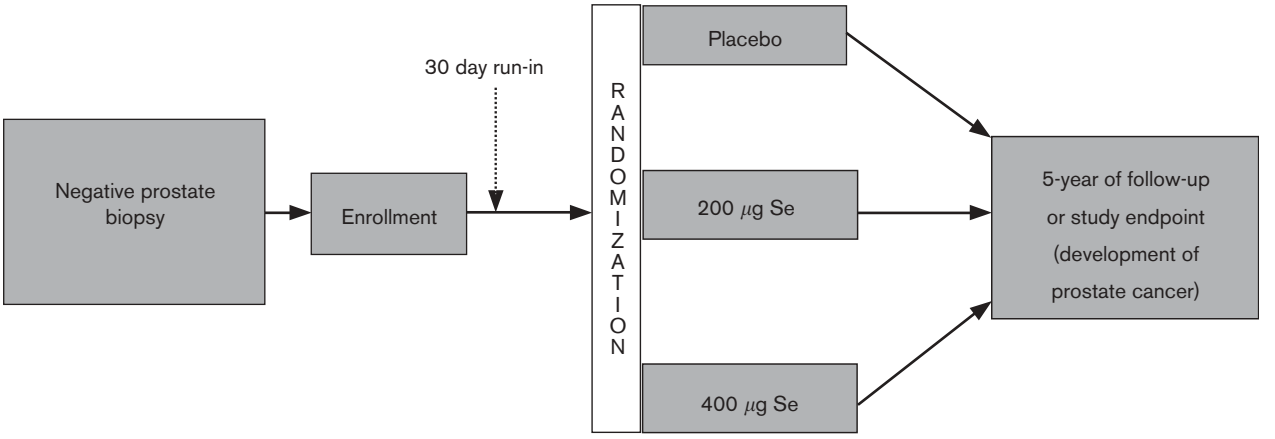
Recruitment and enrollment

Recruitment for the Negative Biopsy Trial began in August 1999 and is presently ongoing. As of 1 June 2003, 514 participants had been enrolled and 444 had been randomized. Fifty-seven participants dropped out before randomization, 49 withdrew within the first year after randomization, 21 withdrew between 1 and 2 years after randomization, and 122 have remained on study for 3 years or more. Twenty-two participants reached study endpoint and developed biopsy proven prostate cancer. As of 1 June 2003, 341 participants were

Table 1 **Eligibility criteria**

- Participants must be less than 80 years of age.
- Participants will have the clinical indicators consistent with the community standards of medical care that would justify a biopsy of the prostate for the diagnosis of cancer. These documented indications would include *one or more* of the following:
  - A PSA level above the absolute value of 4 ng/ml or above a published age/ethnic-adjusted PSA level appropriate for the community.
  - A rising PSA that would represent a clinically significant PSA velocity such as an estimated annual change in the PSA velocity of 0.75 ng/ml or more.
  - An abnormal digital rectal examination of the prostate, which identifies a clinically significant change in the prostate such as a prostate nodule or a change in the firmness of the prostate.
  - The documentation of the clinical assessment of the study patient that justified the prostate biopsy that will allow classification of the subject to high-risk groups.
- Participants must have had a prostate biopsy negative for cancer within 12 months of enrollment into the trial.
- Participants must have a prostate biopsy negative for high-grade PIN (if PIN is present, must be grade 1).
- Participants must have liver and kidney enzyme activity $<2\times$ upper limit of normal.
- Participants must not have a history of a prior malignancy except for the following: adequately treated basal cell or squamous cell carcinoma, adequately treated (complete surgical removal with negative margins) stage I cancer from which the participant is currently in complete remission or any other cancer from which the participant has been disease-free for 5-years. No prior systemic chemotherapy or radiation therapy is allowed.
- Participants are not taking more than 50 μg selenium/day as a dietary supplement, including multivitamin supplements (if candidate has recently or is now taking more than 50 μg/day selenium, enrollment will be delayed until 90 days after the selenium has been stopped).
- Are not concurrently participating or have participated in any other clinical trial involving a medical, surgical, nutritional or life-style intervention (dietary modifications, exercise) within 30 days of registration.

Fig. 1



Negative Biopsy Study schema. Participants go through a screening and run-in period to assess eligibility and compliance. Then, they are randomized to receive 0, 200 or 400 μg selenized yeast daily. Participants are followed for up to 5 years or until study endpoint is reached.

actively on study drug and 35 were off study drug, but on active follow-up.

At the enrollment visit, an Informed Consent Form is signed by the participant, and blood draw is obtained to assess baseline plasma selenium and PSA levels. A comprehensive metabolic panel that includes alkaline phosphatase, SGPT, SGOT, creatinine, and bilirubin and chromogranin A is also performed. Participants are not considered eligible for the study if their baseline creatinine, bilirubin, SGOT or SGPT is $> 2\times$ upper limit of normal.

At the time of enrollment, participants enter the run-in phase of the protocol. Placebo run-in caplets are issued, and questionnaires assessing a medical history and urological symptoms are distributed. During this phase, eligibility criteria are verified including obtaining a copy of the biopsy pathology report to confirm that cancer and HGPIN are not present. Participants are informed that the tablets contain only placebo and are instructed to take one pill per day for 30 consecutive days at which time they return to the clinic for the randomization visit. Remaining run-in caplets are counted to ascertain protocol adherence. Participants who demonstrate compliance of 80% or above are eligible for randomization.

Randomization procedures

After collection of blood samples and completion of the initial questionnaires, participants are randomized to one of the three treatment groups (0, 200 or 400 μg selenized yeast/day). Participants are assigned the next available randomization number based on the stratified randomization. The randomization codes are held by designated, unblinded personnel, and are used only for assigning the randomization number to the appropriate study drug and for the safety monitoring meeting reports issued to the DSMB.

Following randomization, the appropriate study caplets are labeled with the subject identification information and are either distributed directly to the participant or they are forwarded to the appropriate urology clinic for disbursement to the participant. This procedure maintains blinding for all urology clinic personnel.

Participant follow-up

Study participants are supplied with study drug and followed on a semi-annual basis for a period of up to 5 years. The follow-up phase entails blood draws, study drug and questionnaire distribution, and monitoring for adverse events. Study questionnaires capture the onset of new illnesses and symptoms, including potential selenium-related toxicities.

Study participants are supplied with study drug and followed on a semi-annual basis for a period of up to 5 years. The follow-up phase entails blood draw, distribution of study drug and questionnaires, and monitoring for adverse events. In addition, mid-point telephone calls will be conducted 3 months following a clinic visit. Study questionnaires and mid-point calls capture the onset of new illnesses and symptoms, including potential selenium-related toxicities.

Laboratory tests include semi-annual serum PSA, plasma selenium analyses, annual complete metabolic panels, alkaline phosphatase levels and chromogranin A levels. For participants who consent to having whole blood collected and stored for future testing, which may include genetic testing relevant to prostate cancer, a sample of

whole blood is collected at the follow-up visit occurring 12 months after the randomization visit.

Follow-up after supplementation

Participants who have reached study endpoint and developed prostate cancer or discontinue study supplement for any reason and have been randomized at least 3 months have the option to continue follow-up by means of questionnaires only. Follow-up commences 6 months after discontinuation of study supplement and continues on a bi-annual basis until participant withdraws consent or completion of the trial. Questionnaires will capture time to development of prostate cancer and subsequent biopsies. All relevant medical documentation is obtained. A safety blood draw and a follow-up questionnaire are obtained 30 days from the drop date at the participant's discretion.

Study drug

The study agent used in this study is high-selenium yeast (IND #66,698) provided by Cypress Systems (Fresno, CA). Cypress Systems was the supplier of the selenized yeast and placebo for the NPC project since the inception of that project in 1983. This product is known to be stable for up to 20 years stored in opaque containers at ambient temperature of 70°F or below. Furthermore, stability testing of the raw material and caplets is performed annually.

The study agent is supplied as placebo, 200 and 400 μg selenized yeast caplets. All selenium and placebo caplets are coated with titanium oxide to ensure identical appearance, taste and smell of the placebo and selenium caplets. In addition, placebo caplets and both dose levels of selenized yeast caplets are matched with regard to quantity and weight.

Once a participant is randomized, a 6-month supply of experimental agent is dispensed at the randomization study visit. Participants are instructed to take one caplet per day, and bring unused caplets and empty bottles back at the next study visit (after 6 months). Experimental agent is then dispensed at 6-month intervals during the participant's scheduled study visit. Returned caplets are counted and protocol adherence is ascertained at each biannual study visit.

Statistical considerations and data analyses

The primary endpoint of the Negative Biopsy Study is development of biopsy-proven prostate cancer. Biopsies are performed when clinically indicated. The secondary endpoint is the rate of rise in serum PSA (PSA velocity). Serum PSA levels are measured on a semiannual basis. A log + 1 variance stabilizing transformation of the PSA level will be used to calculate the rate of rise.

The sample size estimate for this trial was based on a three-arm design using information on the expected incidence of prostate cancer in this high-risk group of participants within 3 years (at least 25%) to estimate the percent reduction in the median time to diagnosis [22,23] for the treatment groups compared to placebo. The sample size of 700 participants will allow for detection of at least a 50% treatment effect with 90% power, an α of 0.05, a drop out rate of about 5% per year, and a censoring rate of 19% from the first biopsy, 7% from the second and 3% for subsequent biopsies.

The statistical analyses of the trial results will employ the intention-to-treat paradigm. Standard survival techniques will be used for the analysis of the primary endpoint, the incidence of prostate cancer. This statistical method was used for data analyses for the NPC trial [12]. The analyses of the velocity of PSA will be based on a non-linear mixed-effects regression model with the dependent variable being the velocity of PSA log (PSA + 1).

Logistic regression analyses will be used to evaluate the differences between the occurrences of each of the other serum biomarkers with binary distributions. This model will also be used for adjustment of covariates. Analyses of the proportions will employ least-squares regression analysis with appropriate adjustments for important covariates. The Lan and DeMets approach with an O'Brien and Fleming [24] boundary spending function will be used to provide guidelines for possible early stopping of the trial because of discrepant efficacy or adverse event rates between the three arms.

Baseline characteristics

Baseline characteristics are summarized in Table 2. Participants are men under the age of 80 who have had a negative prostate biopsy within 12 months of enrollment. The mean ages in each group are 67.07, 66.04 and 65.81. Participants are primarily Caucasian. Relatively equal distribution of potentially confounding factors was achieved including smoking status, body mass index

Table 2 Baseline characteristics of randomized participants in the Negative Biopsy Trial by treatment group

Variable	Group 1	Group 2	Group 3
Group number (*n*)	162	162	161
Age [years, mean (SD)]	66.6 (7.2)	66.2 (7.5)	65.6 (7.7)
Race [*n* (%)]			
Caucasian	133 (82.1)	136 (84)	135 (83.9)
African-American	6 (3.7)	4 (2.5)	6 (4.3)
Asian	1 (0.6)	1(0.6)	3 (1.9)
Hispanic	12(7.4)	14(8.6)	12(7.5)
Native American	2 (1.2)	1 (0.6)	0
BMI [kg/m^2, mean (SD)]	26.8 (3.4)	28 (4.9)	27.7 (4.6)
Baseline PSA [ng/ml, mean (SD)]	7.79 (4.9)	7.66 (7.9)	7.60 (6.4)
Baseline selenium [ng/ml, mean (SD)]	121.7 (20.7)	122.5 (24.5)	123.3 (29.5)
Current smoking status [*n* (%)]	15 (9.3)	12 (7.4)	13 (8.1)

There are no significant differences in baseline characteristics between treatment groups.

(BMI), baseline plasma selenium and baseline PSA. Participants must not be taking more than 50 μg of supplementary selenium/day as a component of a multivitamin. Participants will be eligible if they discontinue the supplement for a 3-month wash-out period and agree to take less than 50 μg/day of supplemental selenium for the duration of participation in the study.

Analyses of tissue

One of the aims of the Negative Biopsy Study is to perform immunohistochemical analyses of biomarkers that may be modified by selenium supplementation and that can be used to evaluate development of prostate carcinoma. Paraffin-embedded prostate biopsies will be available from the qualifying biopsy, any previous biopsies and from subsequent additional clinically indicated biopsies during the course of the trial. However, repeat biopsies are not a requirement in this protocol. Proposed biomarkers include the survival gene, *bcl-2*, which is thought to play a role in prostate cancer progression [25], the tumor suppressor gene, *p53*, and selenoprotein P, which is involved in cellular redox function as is known to be downregulated in cancer [26,27].

Discussion

The most common strategy for reducing prostate cancer morbidity and mortality is periodic screening of peripheral PSA, but this screening method still remains controversial [28–30]. Prevalence of indolent prostate cancer found during autopsies can be greater than 40% for men over the age of 60 [31]. This emphasizes the need for agents that can prevent initiation of carcinogenesis or impede early stages of prostate carcinogenesis. Men who have had a negative biopsy and continue to have a sustained elevation in PSA are at relatively high risk for developing prostate cancer within 1–2 years of the initial biopsy. Keetch and Catalona followed a cohort of 551 men who had a negative biopsy and PSA level above 4.0 ng/ml. Within the 3-year follow-up, 23% of the men were diagnosed with prostate cancer on subsequent biopsy. Upon further examination of the data, Smith and Catalona reported that 17% of the men on this cohort were diagnosed with prostate cancer at a second biopsy performed within 1 year of the initial biopsy. Seven percent were diagnosed at the third biopsy and an additional 7% were diagnosed in the fourth biopsy [32]. Development of successful agents with inhibitory activity at the early stages of prostate carcinogenesis may have a significant impact on healthcare and healthcare economics in the US.

In 1983, the NPC trial was initiated to test the chemopreventive efficacy of selenium as selenized yeast in a population at high-risk for non-melanoma skin cancer. Secondary endpoints included overall cancer mortality, and incidence of cancers of the colon, lung and prostate. When this study was unblinded in 1996, one of the central findings was a greater than 60% reduction in the incidence of prostate cancer in participants randomized to 200 μg selenium/day versus placebo [12]. These findings led to the development of the 'Negative Biopsy Study', which was designed to test whether selenium supplementation with two doses of selenized yeast can decrease the incidence of prostate cancer and whether or not selenium supplementation can inhibit PSA velocity in a high-risk population of men who have had at least one negative prostate biopsy.

Selenized yeast was selected for use in this trial because of its availability and well-characterized safety profile. The most common selenium-related adverse effects observed in participants randomized to the treatment arm on the NPC study were brittle nails, brittle hair and garlic breath [12]. The Negative Biopsy Study is progressing in terms of patient recruitment and the estimated time to reach the accrual goal of 700 participants is approximately 2 years (June 2005). No new safety concerns have yet been raised. An extended follow-up period will be pursued in order for all participants to be active on the trial for a period of at least 4 years. Results of this trial could confirm the results of the NPC study, and enhance our understanding of the process of early carcinogenesis of the prostate and lead to selenium-based strategies for prevention of prostate caner.

Future directions

Selenium has shown promise as a cancer chemopreventive agent in epidemiologic, preclinical and clinical studies. Several possible mechanisms of action have been proposed by *in vitro* and animal studies, including modulation of apoptosis, cell growth and tumor invasive properties. Completion of ongoing clinical studies and additional mechanistic studies will be required to determine the efficacy and elucidate the mechanism(s) of action for selenium for cancer chemoprevention.

It is of interest that the NPC study results showed a decrease in prostate cancer incidence in men in the lowest tertile baseline selenium and low baseline PSA. The Negative Biopsy Study will provide confirmatory data and perhaps identify a population for whom selenium supplementation would be most efficacious. Additional randomized studies in populations of men with low baseline selenium and low PSA may be of great benefit to further our understanding of selenium and prostate cancer chemoprevention.

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