Selenium and inhibition of disease progression in men diagnosed with prostate carcinoma: study design and baseline characteristics of the 'Watchful Waiting' Study

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Impediment of the promotion and progression stages of carcinogenesis of the prostate could have a profound impact on treatment choice and prognosis for prostate cancer. Efficacious chemopreventive agents that elicit their activity by slowing the processes of progression could make watchful waiting a viable alternative for a large population of men or could delay the necessity for surgery, radiation or other more invasive treatment modalities associated with frequent side effects. Reports from the Nutritional Prevention of Cancer (NPC) study reported that dietary supplementation with selenium significantly reduced the risk of developing prostate cancer. These data led to initiation of the Watchful Waiting Study, a phase II, multi-center, randomized, double-blind, placebo-controlled clinical intervention study testing the effects of two doses of selenized yeast on progression of prostate cancer. Participants are men with biopsy-proven prostate cancer who have elected to forgo therapy and be closely followed by 'watchful waiting' that includes quarterly prostate-specific antigen (PSA) screening. Subjects are randomized to receive 200 or 800 μg of selenized yeast or matched placebo daily. Endpoints include time to disease progression and PSA velocity. Secondary endpoints include time to initiation of therapy as well as biochemical markers of disease progression including chromagranin A and alkaline phosphatase. Immunohistochemical analyses for indicators of apoptosis, proliferation and differentiation will be performed on baseline and subsequent prostate biopsy specimens. This report summarizes the primary objectives, research methods and the randomized subjects in this important clinical trial. *Anti-Cancer Drugs* 14:595–600 © 2003 Lippincott Williams & Wilkins.

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Introduction

The Nutritional Prevention of Cancer (NPC) study was designed to test the effects of daily supplementation with 200 μg selenized yeast daily on recurrence of non-melanoma skin cancers in a high-risk population. Secondary endpoints included total cancer incidence, and mortality and development of primary cancers of the prostate, colon, lung and breast. Results from the blinded phase of the study showed a 63% decrease in incidence of primary prostate cancer [1]. However, secondary prevention of prostate cancer and the effects of selenium supplementation on rate of disease progression were not measured.

Prostate cancer research increasingly relies upon measurement of serum intermediate endpoint biomarkers of disease severity for the clinical monitoring of disease progression [2–4]. Development of metastatic disease and a rise in prostate-specific antigen (PSA) are considered valid markers of prostate cancer progression. Despite difficulties in using serum PSA levels as endpoints in chemotherapy trials, recent studies have shown that changes in serum PSA are associated with survival in patients with prostate cancer [5–8]. Time to initiation of therapy can also be used as a marker of disease progression. Changes in chromogranin A, a marker that indicates the presence of metastases to bone tissue [9–11], and alkaline phosphatase, a marker of neuroendocrine differentiation [12–14], have also been used as surrogate endpoints. In order to help elucidate the molecular mechanisms by which selenium elicits chemopreventive effects, tissue from the qualifying biopsy, any previous biopsies and clinically indicated subsequent biopsies will be analyzed for markers of apoptosis, cell proliferation and differentiation by immunohistochemical analyses.

The Watchful Waiting Study is the first randomized, multi-center, intervention clinical trial designed to test the effects of selenium supplementation on progression of prostate cancer in men with biopsy proven prostate cancer. This study will utilize several intermediate

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markers of prostate cancer progression and help to identify the molecular mechanisms associated with the chemopreventive activity of selenium.

Patients and methods

Study design

The Watchful Waiting Study is a randomized, double-blind, placebo-controlled, multi-center, phase II clinical intervention trial that will follow participants for up to 5 years. The study is designed to investigate the effects of two doses of selenized yeast compared to placebo on the prevention of the progression of clinical prostate cancer, as measured by decreased risk of subsequent prostate biopsies, decrease in the rate of rise of PSA and differences in other biochemical markers of prostate cancer progression, chromogranin A and alkaline phosphatase. Secondary endpoints include time elapsed before initiation of therapy, and modified expression of molecular intermediate biomarkers in baseline and subsequent biopsy tissue specimens. This study will also further establish the safety profile of selenized yeast in the experimental clinical setting. Study procedures are summarized in Figure 1.

The protocol and Informed Consent Form were 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. DSMB meetings are held twice yearly to focus on progress of accrual and study drug-related toxicities.

Subject recruitment, enrollment and randomization

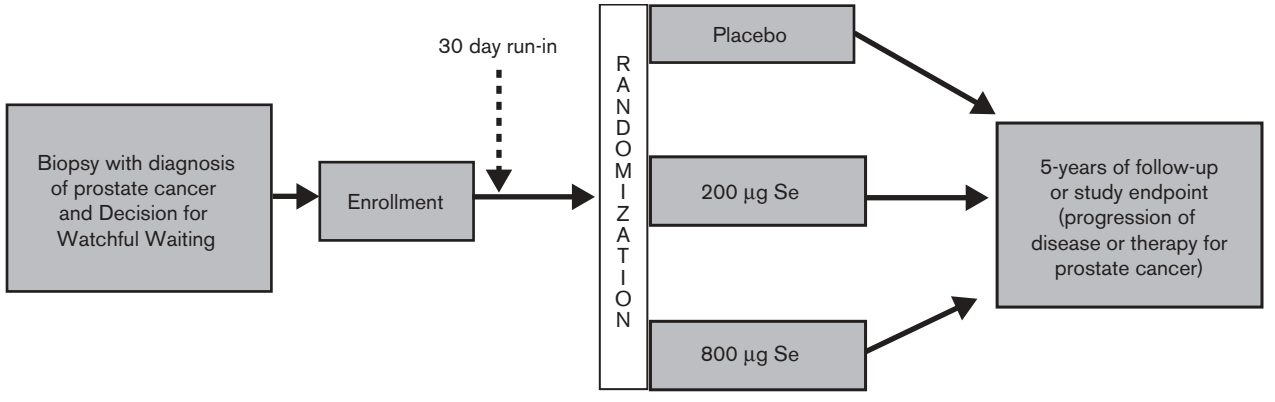
Participants of this study are men who have been diagnosed with prostate cancer within four years prior to enrollment into the study. The qualifying prostate biopsy must have a Gleason score of less than 8. Table 1 summarizes the eligibility and exclusion criteria for this trial.

The Watchful Waiting Study began randomizing participants in 1998, and is presently ongoing. As of 1 June 2003, 191 participants had been enrolled and 157 had been randomized. Twenty-seven participants dropped out prior to randomization. An Informed Consent Form is signed at the enrollment visit and a blood draw is obtained to assess baseline plasma selenium level and PSA. A comprehensive metabolic panel that includes alkaline phosphatase, SGPT, SGOT, creatinine, bilirubin and chromagranin A is also performed. Participants will not be considered eligible for the study if their baseline creatinine, bilirubin, SGOT, SGPT or alkaline phosphate is $> 1.5 \times$ 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 qualifying biopsy pathology report to confirm the diagnosis of prostate cancer and the Gleason score. Participants are instructed to take one pill per day for 30 consecutive days and return to the clinic to be randomized to one of the three treatment groups. Remaining run-in caplets are counted to ascertain protocol adherence. Participants with 80% or greater compliance to taking the pills are considered eligible for randomization.

Prior to randomization, blood samples are collected and the initial questionnaires are completed, including demographic information, past medical and medication

Fig. 1



Watchful Waiting 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 800 µg selenized yeast daily. Participants are followed for up to 5 years or until study endpoint (development of biopsy-proven prostate cancer) is reached.

Table 1 Eligibility and exclusion criteria

- Age <85 years at time of study entry
- Biopsy proven prostate cancer within 48 months
- PSA <50 ng/ml
- Have not received any therapy for prostate cancer including surgery, radiation, hormone or chemotherapy
- Have not been diagnosed with metastatic disease
- At least 3-year life expectancy
- No history of any type of malignancy within the past 5 years with the exception of non-melanoma skin cancer
- Liver and kidney function within 1.5 × upper range of normal
- Are not taking >50 μg selenium/day as a supplement
- Gleason score <8
- No participation in any interventional study within 30 days of enrollment

histories, and baseline symptoms characteristic of selenium toxicity (hair and nail brittleness and garlic breath). Participants are then randomized to one of the three treatment groups (0, 200 or 800 μg selenized yeast/day). Treatment group assignment is stratified based on Gleason score: low (4 or lower) or high (5–7) Gleason scores.

Following randomization, the study caplets are labeled with the subject identification information and are distributed directly to the participant.

Participant follow-up

Follow-up visits are scheduled for every 3 months. Participants are supplied with study drug every 6 months and are seen in the clinic on a quarterly basis for a period of up to 5 years. The follow-up phase entails blood collections, 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. Laboratory tests include serum PSA and plasma selenium analyses, annual complete metabolic panels, alkaline phosphatase, and chromogranin A.

Thirteen participants withdrew during the first year after randomization, 16 withdrew between 1 and 2 years after randomization, and 65 have remained on study for 3 years or more. To date, 23 participants have reached a study endpoint by developing progressive disease or electing to initiate cancer therapy. As of 1 June 2003, 96 participants were on active supplement.

Participants who have been diagnosed with disease progression or have elected to have prostate surgery or other types of cancer therapy even in the absence of disease progression, or who wish to discontinue study supplement and have been randomized for at least 3 months, have the option to continue follow-up by continuing to complete questionnaires. Follow-up commences 6 months following discontinuation of study supplement and continues on a semi-annual basis. Questionnaires capture information on prostate cancer treatment modalities and progression of the cancer. A safety blood draw and a follow-up questionnaire are obtained 30 days from the drop date at the participant's discretion. As of 1 June 2003, 26 participants had discontinued taking supplement, but are continuing follow-up.

Study drug and adherence

The study agent 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 [1]. The study agent is supplied as placebo, 200 and 800 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 caplets dispensed at the randomization study visit. Returned caplets are counted and protocol adherence is ascertained at 6-month time points.

Blood collection and analyses

Plasma samples are collected at baseline and at each 3-month clinical follow-up visit. Total selenium content is measured by automated electrothermal atomic absorption spectrophotometer (3030; Perkin-Elmer, Norwalk, CT).

Serum PSA levels are measured on a quarterly basis. A $\log + 1$ variance stabilizing transformation of the PSA level will be used to calculate the rate of rise. Total PSA will be measured using the Abbott tumor markers assay module on the IMX (Abbott, Abbott Park, IL). Total and free PSA will also be tested using the Tandem-MP PSA Immunoenzymetric Assay on the Tecan (Hybritech, San Diego, CA).

Statistical considerations and data analyses

The sample size estimate for the trial is based on a three-group design and uses information on the velocity rate of PSA obtained from prostate cancer cases in the placebo group from the NPC trial [1]. The mean rise in PSA for men whose initial PSA was less than 10 ng/ml is 0.25 ng/ml/year for the 4-year period prior to diagnosis of prostate cancer. The standardized τ for a 50% difference in the PSA velocities is 0.55, which produces a sample size of approximately 60 patients per group, or a total of 180 evaluable patients at the end of the trial, or 220 randomized patients. The sample size assumes a 50% treatment effect, 80% power, an α of 0.05 and a drop out or censoring rate of about 5% per year. The sample size of 220 patients randomized to the trial will, after 4 years on trial, yield an effective sample size of 180 or 60 per group.

This estimate of PSA velocity for the sample size is likely to be conservative since the NPC cases are more heterogeneous in composition than patients who are eligible for this study. Subjects in this trial may have less than a 10-year life expectancy and will be about 10 years older than the average patient in the NPC study. There is also evidence to suggest a change in the velocity of PSA approximately 2 years before diagnosis for the NPC cohort cases [15].

The statistical analysis of the trial results will use the intention-to-treat paradigm. Subjects will not be censored for study endpoints if they go off treatment temporarily. The analysis of the primary endpoint will be based on a non-linear mixed effects regression model with the dependent variable being the trajectory of PSA log (PSA + 1) [15,16]. Random mixed-effects and adjustment for age at diagnosis, treatment group dose, and grade of tumor are easily incorporated into this model. A Cox proportional hazards model will be used to investigate the treatment effect for the second endpoint, time to initiation of any therapy. Analysis of the third endpoint, time to documented metastatic disease, will also employ a Cox proportional hazards model which will adjust for age and tumor grade.

Logistic regression analysis will be used to evaluate the differences between the occurrence of each of the other biomarkers that have binary distributions and adjustment of co-variants. Analysis 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 [17] 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 treatment groups. Repeated confidence intervals described by Jennison and Turnbull [18,19] will be used to summarize trial endpoint experience at interim monitoring stages.

Immunohistochemical analyses

One of the goals of the Watchful Waiting Study is to identify intermediate endpoint biomarkers that may be modified by selenium supplementation and that can be used to evaluate disease progression. 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. One of the challenges of this study will be to conduct meaningful biomarker and mechanism analyses on these samples. Proposed biomarkers include the survival gene, *bcl-2*, which is thought to play a role in prostate cancer progression [20], the tumor suppressor gene, *p53*, and selenoprotein P, which is involved in cellular redox function as is known to be downregulated in cancer [21,22].

Automated immunohistochemistry will assure high-efficiency antigen retrieval and rapid processing of paraffin tissue specimens [23–25]. Antigen retrieval methods have been devised for each of the biomarkers of interest. From each embedded biopsy, 3–4 μm sections attached to poly-lysine-coated glass slides will be deparaffinized through xylene and graded alcohol. Immunostaining will be done using the automated Immunostainer 320 (Ventana, Tucson, AZ). All primary antibody dilutions will be optimized for the material using dilutions that have been predetermined on a training set of archived prostate cancer specimens. Secondary antibodies include biotinylated immunoglobulin. Immunohistochemical reactions will be graded using a scale of staining intensity [0 (negative) to 4 + (intense)] or by percent positive cells stained based on 200 cells.

Apoptotic index

Apoptotic index will be assessed using the terminal transferase TdT-mediated dUTP-biotin nick end-labeling (TUNEL) assay. Several studies have suggested that elevated apoptosis rates may be one of the mechanisms by which selenium compounds elicit an anticancer effect [26–28]. Tissue sections will be analyzed for *in situ* apoptotic DNA fragmentation using the TUNEL technique. The TUNEL assay will be validated by apoptotic index that will be determined in a subset of H & E sections by measuring the number of apoptotic cells using the criteria of Montironi *et al.* [29,30].

Baseline characteristics

Baseline characteristics by blinded treatment groups are summarized in Table 2. The mean ages in years in each group (1–3) are 74.0, 73.5 and 71.8, respectively. Participants are primarily Caucasian (85.4–87.1%), with body mass indexes (BMI) ranging from 25 to 27, with approximately 83–85% presenting with a Gleason score of > 4. There are no significant differences between treatment groups with respect to the distribution of baseline characteristics. The mean selenium level at baseline is 129.1, 137.9 and 124.8 ng/ml for groups 1–3, respectively, while the baseline PSA values ranged from 8.2 to 8.6 ng/ml.

Discussion

Current diagnostic technology does not allow differentiation between a slow- and fast-growing prostate cancer. Yet, while the majority of men diagnosed with prostate cancer elect to receive standard therapy, including hormonal therapy, nerve-sparing surgery or radiation, some men elect to be followed by watchful waiting. This type of observational treatment generally includes periodic physical exams and regular PSA screening.

Table 2 Baseline characteristics of enrolled and randomized subjects by blinded treatment group

Variable	Group 1	Group 2	Group 3
Group number (*n*)	45	43	42
Age [years, mean (SD)]	74.01 (5.9)	73.27 (5.79)	73.8 (6.44)
Race [*n* (%)]			
Caucasian	39 (86.7)	37 (86.0)	36 (85.7)
African-American	2 (4.4)	4 (9.3)	1 (2.4)
Asian	1 (2.2)	0	0
Hispanic	1 (2.2)	1 (2.3)	2 (4.8)
Native American	1 (2.2)	1 (2.3)	2 (4.8)
BMI [kg/m^2, mean (SD)	25.9 (2.87)	27.66 (4.25)	27.2 (4.46)
High Gleason strata [*n* (%)][a]	38 (84.4)	37 (86.0)	36 (85.7)
Baseline PSA [ng/ml, mean (SD)]	8.14 (6.75)	8.33 (5.67)	8.48 (5.56)
Baseline selenium [ng/ml, mean (SD)]	128.10 (17.21)	137.68 (49.21)	123.42 (16.02)
Current smoking status [*n* (%)]	2 (4.4)	4 (9.3)	5 (11.9)

There are no significant differences in baseline characteristics between treatment groups.
[a]Gleason score strata: low (≤ 4); high (5–8).

Identification of agents that elicit anticancer activity in the promotion and progression stages of prostate carcinogenesis would provide a safe and non-invasive alternative for men with cancer who have declined other treatments. In addition, development of these agents could have an impact on the prognosis of watchful waiting patients and other therapies for early stage prostate cancer.

High-selenium yeast has shown promise as a chemopreventive agent for prostate cancer in ecological studies and clinical trials [31,32]. Furthermore, *in vitro* studies and animal models have suggested numerous molecular mechanisms by which selenium compounds may elicit anticancer activity [33,34]. Potential mechanisms of action include regulation of selenoproteins [35], increased activity of enzymes involved in apoptosis and inhibition of cell cycle regulatory proteins [33], inhibition of transactivation activity of transcription factors, such as NF-κB [36,37] and activator protein-1 [38], known to regulate expression of genes that play a role in carcinogenesis and downregulation of matrix metalloproteinases [34,39,40], a family of proteins involved with invasion and metastases. The Watchful Waiting Study will examine the effect of two doses of high-selenium yeast on prostate cancer progression. Furthermore, immunohistochemical analyses of prostate biopsy specimens before and after treatment will allow the unique opportunity to examine the effects of selenium directly on prostate tissue in a controlled clinical trial setting.

Since the inception of the Watchful Waiting Study, new biomarkers of interest have been identified. In addition to the originally proposed biomarker studies, we plan to perform immunohistochemical analyses for some of these other relevant markers. One of the markers of interest is hepsin, a transmembrane serine-threonine kinase that is expressed in early-stage prostate cancer, but is lost in androgen-independent cancers, and is known to increase invasive and metastatic capacity of prostate cancer cells *in vitro* [41–43]. Racemase is another potential marker of prostate cancer progression [44–46]. This enzyme is involved in metabolism of branched-chain fatty acids within the peroxisome and has been shown to be overexpressed in prostate cancers. Integrin-linked kinase (ILK) is another protein of interest. ILK associated with the β_1 and β_3 subunits of integrin, and is known to be constitutively overexpressed in prostate cancer cells. Furthermore, Persad *et al.* have shown that inhibition of ILK induces cell cycle arrest and apoptosis in prostate cancer cells [47].

Selenized yeast was selected for use in this trial because of its availability and well-characterized safety profile. This study is progressing successfully in terms of patient recruitment and no safety concerns have 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 provide timely evidence that selenium may have anticancer activity in the promotion and progression stages of prostate carcinogenesis, and may elucidate the molecular mechanisms of action.

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