Vitamin D3 Supplementation for Low-Risk Prostate Cancer: A Randomized Trial

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Protocol

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A. SPECIFIC AIMS

Prostate cancer is the most prevalent malignancy in men and has been responsible for more than 33,000 deaths in 2011 (1). However, most prostate cancers have a relatively slow rate of growth and progression, and are ideal candidates for non-toxic pharmacological or nutritional interventions that could reduce the incidence of clinically relevant disease, delay cancer progression, and modify a malignant process into a chronic, manageable disease. This is particularly important for avoiding overtreatment of indolent disease, because of the considerable side effects, such as erectile dysfunction and incontinence, often associated with definitive treatment of prostate cancer by surgery and radiation therapy.

Vitamin D promotes the differentiation of prostate cancer cells and maintains the differentiated phenotype of prostate epithelial cells, raising the possibility that vitamin D deficiency over time contributes to the progression from subclinical prostate cancer to clinical disease (2).

The central hypothesis of this grant application is that vitamin D_3 (cholecalciferol) supplementation will benefit subjects diagnosed with early-stage, low-risk prostate cancer, who elect to have their disease monitored through active surveillance. Specifically, we hypothesize that subjects who take vitamin D_3 at a daily dose of 4000 international units (IU) for a minimum of one year (intervention group) will show an improvement in the number of positive cores and in Gleason score at repeat biopsy, and a decreased likelihood of undergoing additional treatment (hormone therapy, prostatectomy, or radiation therapy), compared to subjects taking placebo (control group).

Prostate cancer disproportionately affects African American men in terms of incidence, morbidity and mortality (70). We need to determine whether this is due to (a) patient knowledge deficiency about prostate cancer, (b) inadequate informed decision making concerning procedures for diagnosis and treatment, and (c) psychological and physiological stressors for patients involved in prostate cancer screening and treatment.

To test this hypothesis, we propose the following Specific Aims:

1) To determine whether vitamin D_3 (4,000 IU per day for at least one year) will result in a significant improvement of the pathology status at repeat biopsy in subjects taking vitamin D_3, compared to subjects taking placebo. This hypothesis will be tested through a randomized clinical trial, which will enroll 136 subjects (68 participants per arm), diagnosed with early-stage prostate cancer (Gleason score ≤6, PSA ≤10, clinical stage T1C or T2a). The pathology status will be measured by the change in Gleason score and the number of positive cores in prostate needle biopsy specimens between baseline and the end of the study. *Pre- and post-study biopsies will be performed as part of the standard medical care for diagnosis and active surveillance.*
2) To determine whether vitamin D_3 supplementation, compared to placebo, will result in a significant decrease in the number of subjects who will undergo additional treatment (hormone therapy, prostatectomy, or radiation therapy), following the outcome of repeat biopsy.
3) To analyze changes in the serum levels of cholecalciferol, 25(OH)D, $1,25(OH)_2D$, and prostate-specific antigen (PSA) at baseline and at the end of the study, and to estimate the associations between changes in these measures and pathology outcomes (Gleason score and number of positive cores).
4) To compare the expression of molecular biomarkers, which are prognostically relevant to prostate cancer progression, in pre- and post-treatment biopsy tissue specimens. Paraffin-embedded sections will be processed to assess by immunohistochemical techniques the

expression of the following biomarkers: vitamin D receptor (VDR), 25-Hydroxyvitamin D_3 1-alpha-hydroxylase (CYP27B1), p21, tumor growth factor β (TGFβ), cyclooxygenase 2 (COX-2), and NFκB. All of these protein products impact growth control and chronic inflammation in prostate cancer progression and are specifically affected by vitamin D status.

5) To determine psychological and physiological stress response for patients involved in prostate cancer screening and treatment options by administering a survey about the patients' prostate biopsy decision making experience and obtaining measurements (Allostatic load parameters) of stress response at the beginning and end of the study visits.

Implementation of the proposed studies would demonstrate that vitamin D_3 supplementation provides a welcome addition to active surveillance, since patients who respond to vitamin D_3 supplementation (as indicated by a decrease in Gleason score or number of positive cores at repeat biopsy) can safely continue active surveillance and would not need definitive treatment. In turn, this would result in a decreased likelihood of overtreatment. On the other hand, subjects who progress after vitamin D_3 supplementation, as indicated by an increase in Gleason score or number of positive cores at repeat biopsy, may have more aggressive disease and may need to consider additional treatment. Therefore, both groups of patients (responders as well as non-responders) would benefit from vitamin D_3 supplementation, an intervention strategy that is extremely cost-effective and easy to implement.

B. BACKGROUND AND SIGNIFICANCE

Prostate Cancer Screening and Treatment: Digital rectal examination and measurement of the serum level of prostate-specific antigen (PSA) remain the standard of care for prostate cancer screening, despite some shortcomings (3). There is general agreement among clinicians that the PSA test has served us reasonably well: US death rates from prostate cancer have fallen about 4% per year since 1992, five years after the introduction of PSA testing (4), although the results of two large randomized trials suggest that there may be a very small mortality benefit to patients from PSA screening (5, 6). Nonetheless, the latest report from the Surveillance, Epidemiology, and End Results (SEER) program reveals a very favorable 10-year outcome in the PSA era, following conservative management of early-stage, well-differentiated prostate cancer (7).There is also some disagreement as to what level of PSA should prompt a prostate biopsy. The use of a higher PSA threshold could lead to missing some of the clinically important cancers, for which more aggressive treatment regimens are required. A lower threshold would increase the number of unnecessary biopsies, while also potentially detecting clinically insignificant disease, which is unlikely to be detrimental to patients (8, 9). Therefore, the chance of overtreatment is very real with low-risk prostate cancer.

The use of age-specific PSA values for men over the age of 50 years has been adopted by many clinicians as striking a reasonable balance between high and low thresholds. Still, the Prostate Cancer Prevention Trial demonstrated a surprisingly high incidence of prostate cancer in American men 55 years of age or older, who consented to routine prostate biopsies over a period of seven years. In this large prospective study involving men with PSA levels of <4.0 ng/ml, up to 27% of subjects with PSA levels between 3.1 and 4.0 ng/ml had histologically proven prostate cancer (3, 10). Albertsen et al. have reported on the outcome of patients with untreated prostate cancer (11). Twenty-year follow-up of this group of patients revealed that the risk of dying from prostate cancer after twenty years was only 5% with a Gleason score of 4, and up to only 30% with a Gleason score of 6 (11). Therefore, a considerable number of patients diagnosed with low-grade, low-risk prostate cancer may not require aggressive treatment (surgery, radiation, or a combination thereof), especially considering the likelihood of side effects such as urinary incontinence and bother, rectal irritation, and erectile dysfunction. Thus, the indolent nature of prostate cancer and the risk of side effects from therapy have led to active surveillance as a therapeutic option, by which definitive therapy, such as radical prostatectomy, radiation therapy, or cryotherapy, is deferred to a later date or even indefinitely. The studies proposed in this VA Merit Award application will yield results that may be of significant value to the considerable number of Veterans diagnosed with early-stage prostate cancer, who opt for active surveillance.

Vitamin D Synthesis and Metabolism: Exposure of skin to sunlight in the ultraviolet B (UVB) range of the spectrum (290 to 315 nm) results in the photolytic conversion of 7-dehydrocholesterol (7-DHC) to previtamin D_3, which is transformed to vitamin D_3 by thermally induced isomerization (12, 13). Vitamin D_3 can be obtained from the diet; however, it is distributed very poorly in natural foodstuffs. It is found primarily in fish oils, egg yolk, butter and liver. Because of its extremely low abundance in foods, milk is fortified with low concentrations of vitamin D_3. The initial step in the metabolic activation of vitamin D_3 is the enzyme-catalyzed insertion of an OH group at carbon 25. This oxidation is primarily a hepatic microsomal function (14) and produces 25(OH)D, the most stable and abundant form of circulating vitamin D (15). 25(OH)D undergoes a second hydroxylation at position 1 in a number of organs, to produce $1,25(OH)_2D$, the hormonal, most potent form of vitamin D. The $t_{½}$ of 25(OH)D in the circulation is about 2-3 weeks in normal individuals (16). Because of its relatively long $t_{½}$ as compared with vitamin D_3 (1-2 days) and $1,25(OH)_2D$ (12-24 hrs), circulating 25(OH)D is the best indicator of vitamin D status (17). The kidney is the major site of $1,25(OH)_2D$ synthesis and expression of 1-α-hydroxylase (18, 19), and is the only organ with endocrine function that can release $1,25(OH)_2D$ into the bloodstream, thereby controlling the plasma levels of calcium and phosphorus (20). Vitamin D's non-calcemic functions are exercised within other organs (such as skin, prostate, brain, and cells of the immune system), which also express the 1-α-hydroxylase necessary to accomplish the last step in the synthesis of the hormonal form of vitamin D (21). In these tissues, $1,25(OH)_2D$ acts in a paracrine/autocrine fashion (i.e., it is not released into the bloodstream). The recent emphasis on non-calcemic functions of vitamin D has to do with the realization that vitamin D deficiency has major implications for human health (22).

Vitamin D Insufficiency and Deficiency: The results of clinical studies, in which circulating parathyroid hormone (PTH) and 25(OH)D were measured, indicate that secondary hyperparathyroidism occurs when serum 25(OH)D values fall below 20 ng/mL (23-25), considered the cutoff value for vitamin D deficiency. It was also shown (26) that maximal suppression of PTH by circulating 25(OH)D occurs at >80 nmol (32 ng/mL), which is considered the cutoff value for vitamin D insufficiency. The results of additional retrospective and interventional studies suggest that circulating 25(OH)D needs to exceed 80 nmol (32 ng/mL) to maximize skeletal integrity (27, 28). Furthermore, increased circulating 25(OH)D has been linked to improved glucose handling and beta-cell function (29). More importantly, the role of vitamin D and the innate immune system has now been described (30). Some of these data, as well as additional studies, have been summarized in a recent review regarding the optimization of circulating 25(OH)D levels (31). *Thus, the current reference ranges for circulating 25(OH)D are set too low, while the desirable range of values for serum 25(OH)D in a sun-rich environment is 54-90 ng/mL (32, 33).*

Oral Supplementation of Vitamin D_3: Several studies suggest that intakes of 1,000 IU per day raise serum 25(OH)D values only to slightly above 24 ng/mL (34-36). In a landmark study, Vieth, et al. (37) examined the efficacy and safety of relatively high intakes of vitamin D_3 by assessing the effects of 1,000 and 4,000 IU/day in 61 adults for up to five months. They found that vitamin D_3 at a dose of 4,000 IU/day was effective in elevating the serum 25(OH)D concentration to optimal values (≥40 ng/mL). It is important to note that in this study a steady-state of circulating 25(OH)D was achieved approximately 90 days following initiation of supplementation at the 4,000 IU/day level. Until recently, higher dose vitamin D_3 oral supplementation was not viewed as a viable treatment modality due to concerns about potential toxicity. Our own clinical experience with prolonged supplementation with 4,000 IU/day for up to twelve months, however, has demonstrated the safety of this regimen. We have observed that 4,000 IU/day are extremely effective at raising circulating 25(OH)D across racial groups (38 and Preliminary Studies below), to levels measured in athletes during summer training (39). In view of these data and our own clinical observations, we conclude that oral supplementation with 4,000 IU/day of vitamin D_3 would greatly benefit most subjects, especially African-Americans.

Vitamin D and Prostate Cancer: It is well established that human prostate cells express the vitamin D receptor (VDR) (40). Normal prostate cells also synthesize $1,25(OH)_2$ D (calcitriol) from 25(OH)D

(calcidiol) (41, 42), as prostate cells express CYP27A1, a vitamin D 25-hydroxylase, and the 25(OH)D 1α-hydroxylase (43). Furthermore, neoplastic progression in prostate tissue appears to be associated with loss of 25(OH)D 1α-hydroxylase activity (44). Several mechanisms of vitamin D-mediated anti-cancer action have been identified (45). Hereby will be discussed those mechanisms of action that are more relevant to this application. Calcitriol induces cell cycle arrest through the expression of insulin growth factor binding protein-3 (IGFBP-3), which increases the levels of the cell-cycle inhibitor p21 in LNCaP cells (46); calcitriol activates expression of IGFBP-3 by direct transcriptional stimulation through a vitamin D response element (VDRE) located in its promoter sequence (47). VDRE sequences have also been demonstrated in the promoter of the TGFβ-2 gene (48), and treatment of PC-3 prostate cancer cells with TGFβ leads to enhanced expression of IGFBP-3 and subsequent growth arrest and apoptosis (49). Additional studies have suggested a link between chronic inflammation and prostate cancer (50). Vitamin D suppresses the expression of COX-2, the key enzyme for the synthesis of prostaglandins, mediators of inflammation and thought to be important for cancer progression (51); COX-2 expression in biopsy cores and prostate cancer surgical specimen is an independent predictor of recurrence (52). Calcitriol also induces the transcription of mitogen-activated protein kinase phosphatase 5 (MKP5), which inhibits the subsequent phosphorylation and activation of the stress-activated protein kinase p38, an inducer of the pro-inflammatory cytokine interleukin 6, implicated in the initiation and progression of prostate cancer (53). Furthermore, there is considerable evidence that calcitriol inhibits NFκB signaling and decreases the levels of the angiogenic and pro-inflammatory cytokine IL-8 in prostate cancer cells (54). NKκB is a transcription factor that plays a central role in the control of inflammation and is expressed at high levels in prostate cancers with high Gleason scores (55). Calcitriol interferes with angiogenesis also by reducing the expression of VEGF in prostate cancer cells, through the repression of the transcription factor HIF-1 (56). Finally, calcitriol decreases matrix metalloproteinases and cathepsin activities, while increasing the activities of their counterparts, tissue inhibitors of metalloproteinase-1 and cathepsin inhibitors (57). This is only a partial list of the many molecular pathways and mechanisms affected by vitamin D, as it is now well established that VDR may recognize cognate VDRE present within the regulatory sequences of hundreds of human genes, implicating vitamin D in a vast network of gene regulation, and underlying its broad physiological actions (58, 59).

With regard to human studies, it has been reported that low serum levels of 25(OH)D_3 (<50 nmol/L equivalent to <20ng/mL) strongly correlate with death from prostate cancer (60), raising the possibility that vitamin D deficiency over time would favor the progression of subclinical prostate cancer to clinical disease (2). However, the latest recommendations from the Institute of Medicine (IOM) concerning dietary reference intake for calcium and vitamin D (61), emphasize that the role of vitamin D in non bone-related health issues such as cancer remains unresolved because of the conflicting nature of the available evidence. These doubts have been reaffirmed in a recent article (62). Therefore, clinical studies that involve robust and sustained, but non-toxic, vitamin D_3 supplementation would bring some clarity to this important issue. To date, we have results from three randomized clinical trials employing vitamin D_3 supplementation. 1) Trivedi et al (63) administered an oral dose of 100,000 IU vitamin D_3 on a quarterly basis for 5 years which proved to be effective on skeletal homeostasis but was ineffective on cancer prevention. 2) The Women's Health Initiative (WHI) study, which involved thousands of subjects, was positive for colorectal cancer versus circulating 25(OH)D levels based on their nested case-control data (64). However, the interventional arm of the study was negative because of insufficient supplementation: in this study, 400 IU/day of vitamin D_3 for 7 years as a treatment would have been unable to raise systemic levels of 25(OH)D. In fact, the actual vitamin D intake in this large study was approximately 280 IU/day, if their reported compliance rate is taken into account. Furthermore, this study did not perform any post-treatment 25(OH)D analyses on the subjects and implied that a 280 IU/d vitamin D supplement would be an effective dose, which is not likely (65). 3) The third randomized clinical trial (66) was a vitamin D-skeletal study. Although this study was a bone-focused trial, a secondary analysis of the data demonstrated a strong preventive effect on cancer. It should be noted, however, that none of these published randomized clinical trials had to do with low-

risk prostate cancer.
The observed latency in prostate cancer development provides a long window of opportunity for intervention by chemopreventive agents that would reverse, suppress, or prevent the carcinogenic process (45), and early-stage, low-risk prostate cancer provides an excellent model in which to study the effects of enhancing vitamin D status and related changes in tumor progression over an extended period of time.

Relevance to Veterans' Health: Veterans diagnosed with early-stage prostate cancer are justifiably reluctant to undergo definitive treatment such as surgery (radical prostatectomy) or radiation therapy (external beam or seed implants), because of the potentially serious side effects associated with these interventions (e.g., impotence and incontinence). The likelihood of these side effects has to be weighed against the indolent nature of low-risk prostate cancer, and the generally low probability that it will progress to lethal disease. Thus, it is not surprising that a large number of patients diagnosed with early-stage low-risk prostate cancer elect to be monitored through active surveillance, a medical management approach also aimed at reducing over-treatment of primary disease. The use of serum values of PSA for screening and monitoring prostate cancer has had a beneficial impact on prostate cancer mortality statistics; however, active surveillance is essentially a monitoring regimen that does not specifically address the issue of how to treat early-stage disease, and does not entail specific criteria to identify the type of cancer that will progress to invasive or metastatic disease. Below we discuss the results of our clinical studies, which provide strong evidence that daily supplementation with 4000 IU of vitamin D_3 for one year is safe and is associated with a decrease in the number of positive cores at repeat biopsy in supplemented subjects compared to control subjects. These results are particularly relevant to Veterans' health in light of a recent report linking medical costs to vitamin D testing patterns in southeastern Veterans Medical Centers (67). This retrospective study looked at a sample of 15,340 Veterans from VISN 9 and concluded that vitamin D deficiency and lack of monitoring correlated with increased inpatient health care costs at all sites.

C. PRELIMINARY STUDIES

Our research team has completed an open-label clinical study aimed at assessing the safety and potential efficacy of vitamin D_3 supplementation at 4000 IU/day for one year in patients diagnosed with early-stage, low-risk prostate cancer. Eligible subjects were enrolled through the Departments of Radiation Oncology and Urology at MUSC, and through the Urology Clinic of the Ralph H. Johnson VA Medical Center. They had histologically confirmed diagnosis of adenocarcinoma of the prostate (Gleason score ≤6, PSA ≤10, clinical stage T1c or T2a) and had elected to be managed through active surveillance. For the purpose of eligibility, these additional criteria were verified: serum creatinine $\leq$ 2.0mg/dL, serum phosphate (measured as phosphorus) >2.3 and <4.8 mg/dL, and serum calcium > 8.5 and < 10.5 mg/dL. Exclusion criteria were the following: any concurrent malignancy, except non-melanoma skin cancer; history of sarcoidosis; vitamin D_3 supplementation at ≥1,000 IU per day; history of hypercalcemia; use of lithium as a medication. This clinical trial was approved by the Institutional Review Board (IRB) of MUSC and VAMC (ClinicalTrials.gov Identifier: NCT01045109). Study objectives were: 1) to determine whether vitamin D_3 (4000 IU per day for one year) would be safe and result in a measurable decrease of serum PSA levels in a significant number of enrolled subject; and 2) to determine whether this supplementation regimen would result in a stabilization or improvement of their disease, assessed through histological examination of repeat biopsy specimens (Gleason score and number of positive cores), obtained at the end of the study as part of the standard medical care for active surveillance. The results of this open-label trial have recently been published (68).

Study Design: The open-label study enrolled 52 eligible subjects. Forty eight subjects completed the study and were included in the safety analysis; these subjects had complete PSA lab results for inclusion in evaluation of changes in PSA. Forty four subjects had both baseline and repeat biopsy (as part of their standard of care) to compare the number of positive cores and Gleason score to baseline,

after completing vitamin D_3 supplementation. All subjects had study visits every two months for one year, to measure serum levels of 25(OH)D, PSA, phosphorus, and PTH, plus complete blood count, basic metabolic panel, and urinary calcium:creatinine ratio (to rule out any potential toxicities from vitamin D_3 supplementation). In addition, circulating levels of vitamin D_3 (cholecalciferol) and $1,25(OH)_2D$ (calcitriol) were measured at baseline and exit in 19 subjects for whom extra serum samples were available.

Historical Control Subjects: From an institutional (MUSC) database of >700 patients diagnosed with prostate cancer within the last five years, we identified 19 control subjects who were under active surveillance, underwent repeat biopsies, and met all eligibility criteria of the open-label clinical trial, except that they received no vitamin D_3 supplementation. We selected only patients who had their follow-up biopsy at 10 months or later after their initial positive biopsy, to ensure comparability to the timing of the follow-up visit of the patients in the open-label trial. All subjects that met eligibility criteria were included. From these control subjects, we abstracted information on their serum PSA levels and assessment of tissue biopsy specimens.

Safety and Efficacy: Supplementation with vitamin D_3 at 4000 IU per day for one year appeared to correct all cases of vitamin D deficiency, although the magnitude of the response varied among subjects. No adverse events linked to vitamin D_3 supplementation at 4000 IU per day were observed. Serum PSA levels (expressed as median ± SD in ng/mL) remained relatively constant and changes do not appear to be related to the baseline PSA level (entry: 4.1±1.8 vs. exit: 4.5±2.7; p=0.27). Furthermore, serum PSA levels did not seem to correlate with the repeat biopsy outcome. To determine whether individual subjects improved or progressed, we compared the number of positive cores and Gleason grade at baseline and repeat biopsy. By these criteria, 34% of these subjects progressed; five subjects (11%) showed no change; however, more than half of the subjects (55%) showed improvement, defined as a decrease in positive cores and no increase in Gleason score at repeat biopsy. We also compared the number of positive cores and Gleason grade at baseline and repeat biopsy in the supplemented group and the control group. Using the same criteria, 63% of the subjects in the control group progressed (because of an increase in the number of positive cores or in Gleason score), compared to 34% in the supplementation group (p=0.05). Furthermore, in the historical control group, four patients (21%) had improvements in biopsy results, three showed no differences (16%), and twelve (63%) progressed. Comparing the proportions who responded in each group, a significant difference was found (p=0.025) (68).

The use of positive cores as an endpoint for pathology outcomes of active surveillance has been reported by the Johns Hopkins group (69). In this study, stipulated adverse pathological features at repeat biopsy included a Gleason score ≥7, or a Gleason pattern grade >4, or >2 positive cores, or >50% cancer involvement of any 1 core. PSA changes did not trigger curative intervention in this program. Consistent with this approach, the results of our open-label clinical trial suggest that serial measurements of PSA serum levels (every two months for one year) are a relatively poor predictor of progression in patients with low-risk prostate cancer. However, the combination of active surveillance and vitamin D_3 supplementation at 4000 IU per day resulted in a decreased number of positive cores at repeat biopsy in more than half of patients diagnosed with low-risk prostate cancer. These subjects (responders) are all eligible to remain on active surveillance and do not require additional treatment (hormone therapy, surgery, or radiation therapy). Therefore, this regimen may decrease the chances of overtreatment for patients with low-risk prostate cancer who, based on the results of the repeat biopsy, respond to the combination and remain stable or improve. Conversely, vitamin D_3 supplementation did not benefit 35-40% of subjects (non-responders), for reasons yet to be investigated. Nevertheless, combination of active surveillance and vitamin D_3 supplementation may also help identify those patients (non-responders) who are more likely to need definitive treatment.

Comparison of the outcomes of repeat biopsy, between supplemented subjects and historical controls, suggests that supplementation with vitamin D_3 at 4000 IU per day may benefit patients with early-stage,

low-risk prostate cancer on active surveillance. However, we realize that there are many limitations to the use of historical controls for comparison purposes. The historical control cohort analyzed in this study was small and there may be additional differences in this group, leading to potential biases. Furthermore, we could not measure levels of circulating vitamin D in these control subjects. Therefore, it is essential to validate the effectiveness of vitamin D_3 supplementation in active surveillance by conducting a randomized clinical trial.

D. RESEARCH DESIGN AND METHODS

The central hypothesis of this grant application is that vitamin D_3 supplementation at 4,000 IU per day for a minimum of one year will benefit subjects diagnosed with early-stage, low-risk prostate cancer, who elect to have their disease monitored through active surveillance. Specifically, we hypothesize that subjects who take vitamin D_3 (intervention arm) will show a decrease in the number of positive cores and Gleason score at repeat biopsy, compared to subjects in the control group who take placebo (control arm). We also hypothesize that vitamin D_3 supplementation at 4,000 IU per day for at least one year (compared to placebo) will result in a decrease in the number of subjects who will undergo additional treatment (hormone therapy, prostatectomy, or radiation therapy), following the outcome of the repeat biopsy.

One of the objectives of this project is to investigate any changes in the clinical parameters associated with the hypothalamic-pituitary-adrenal (HPA) axis, known to play a central role in regulating the physiological stress response. A new specific aim is to determine whether the HPA axis is affected in subjects enrolled in the study, by measuring allostatic load parameters twice on each subject, and comparing the corresponding numerical values by race and supplementation groups.
Allostatic Load Studies: Allostatic load defines a set of clinical and biochemical parameters that can be easily measured in a basic clinical setting. These parameters can be analyzed to gauge the level of stress experienced by each subject. These values reflect chronic, steady state levels of stress as well as failure to shut-off responses to acute stressors. The results of these analyses will be compared across racial and supplementation groups.

Allostatic load parameters will be measured twice: at enrollment and at study exit. Allostatic load parameters selected for this study are the following:

- Systolic and diastolic blood pressure (indices of cardiovascular health)
- Waist-hip ratio (index of adipose tissue deposition associated with increased glucocorticoid activity)
- Serum high-density lipoproteins (HDL) and total cholesterol (index of atherosclerosis)
- Blood plasma levels of glycosylated hemoglobin (HbA1C, index of glucose metabolism over time)
- Serum dihydroepiandrosterone sulfate (DHEA-S) a functional HPA axis antagonist.

For each of the chosen parameters, subjects will be classified into quartiles, based on the distribution of scores. Allostatic load will be measured by summing the number of parameters for which the subject will fall into the highest risk quartile, with the exception of HDL and DHEA-s values, for which the lowest quartile corresponds to the highest risk (http://www.macses.ucsf.edu/research/allostatic/allostatic.php).
We anticipate that we will observe significant differences in allostatic load across races. It will be also important to determine whether one year of sustained vitamin D_3 supplementation at 4,000 IU per day will affect differences in allostatic load across racial groups.

1) Recruitment: This randomized clinical trial will enroll 136 subjects (68 subjects per study arm), recently diagnosed with Early-Stage prostate cancer (histologically documented adenocarcinoma of the prostate, Gleason score ≤6, PSA ≤10, clinical stage T1C or T2a), who elect active surveillance as their treatment option. Eligible subjects will be subjects older than 18 years of age, and will be recruited by Dr. Stephen Savage, Chief of Urology at the Ralph H. Johnson VA Medical Center in Charleston and Professor of Urology at MUSC and Dr. David Marshall, Professor of Radiation Oncology at MUSC. Eligible subjects will be enrolled into the study by the Clinical Research Coordinators (CRC). All research personnel have successfully completed all research compliance requirements for human studies and for obtaining informed consent from eligible subjects.
After Dr. Savage or Dr. Marshall has explained the general purpose of the study to an eligible subject and the potential subject states he will consider participating in the study, the CRC will meet the subject, and review the background, purpose, procedures and objectives of the study. Adequate time will be given to the subject for reading the informed consent document (ICD) and the Health Insurance Portability and Accountability Act (HIPAA) document, discuss the documents with his significant other(s), ask questions and then determine if he wishes to participate in the study. Only when the subject states that he wishes to participate, will the ICD and the HIPAA be signed. If a potential subject calls the CRC after receiving a verbal referral from Dr. Savage or Dr. Marshall, the background, purpose, procedures and objectives of the study will be explained to him. A copy of the ICD will be mailed to the subject to be read thoroughly and discussed with family members before his initial visit. At his first visit, the ICD will be reviewed, and questions encouraged and answered before the ICD is signed. Time for the subject to read the HIPAA and ask questions will be allotted before it is signed. The ICD/HIPAA will be signed after the subject states he wishes to participate and before any study procedures are initiated. Only subjects who are capable of giving consent may enroll in the study. All aspects of the recruiting and consent process will be documented in Progress Notes maintained as part of the subject's Source Documents.

2) Study Design: Using a double-blinded randomized trial design [intervention (4,000 IU of vitamin D_3 taken daily for at least twelve months) vs. control (placebo)], the effects of supplementation will be determined on subjects diagnosed with early-stage, low-risk prostate cancer (Gleason score ≤6, PSA ≤10, clinical stage T1C or T2a). This trial will enroll 136 subjects (68 participants per arm). Eligible study participants will be recruited through the Ralph H. Johnson VAMC and MUSC, both in Charleston, SC. Inclusion and exclusion criteria will be identical to those of the open-label study: histologically confirmed diagnosis of adenocarcinoma of the prostate (Gleason score ≤6, PSA ≤10, clinical stage T1c or T2a) and decision to be managed through active surveillance. For the purpose of eligibility, these additional criteria will be verified: serum creatinine $\leq$ 2.0mg/dL, serum phosphate (measured as phosphorus) >2.3 and <4.8 mg/dL, and serum calcium > 8.5 and < 10.5 mg/dL. Exclusion criteria will be the following: any concurrent malignancy, except non-melanoma skin cancer; history of sarcoidosis; vitamin D_3 supplementation at >2,000 IU per day; history of hypercalcemia; or use of lithium as a medication All of these criteria have been validated by the FDA.
Eligible subjects who agree to participate in the study will be enrolled and assigned to the supplementation or placebo group according to the randomization schema developed by the biostatistician. All subjects will be dispensed vitamin D_3 or placebo pills and remain on the study until the outcome of the repeat biopsy is established, and after each subject has been classified as stable, improving, or progressing. After knowing the outcome of the repeat biopsy, a decision will be made by the subject and his Doctor regarding possible additional prostate cancer treatment (hormone therapy, surgery, or radiation therapy).

3) Study Medication: FDA IND# 77,839 was originally granted to the PI in 2007 for an open-label clinical study (see Preliminary Studies) designed to assess the effect of vitamin D_3 on subjects with early-stage prostate cancer. This randomized clinical trial will be conducted under the same IND. The vitamin D_3 (cholecalciferol) is manufactured by JR Carlson Laboratories, Inc., 15 College Ave.,

Arlington Heights, IL 60004-1985. JR Carlson Laboratories will manufacture soft gel capsules containing 4000 IU of vitamin D_3 for the intervention arm of the study. JR Carlson Laboratories will also manufacture placebo capsules to be used in the control arm of the study. The study medications will be shipped to the pharmacy of the Ralph H. Johnson VA Medical Center and the PI's office (for the MUSC site) in Charleston, where it will be stored and dispensed according to the randomization schema developed by the biostatistician.
A multivitamin containing the Institute of Medicine (IOM) recommended cholecalciferol supplementation of 400 IU daily will be provided for all subjects.

4) Procedures and Study Calendar: At Visit #1 (Screening Visit), past medical history, concomitant medications/supplements and inclusion/exclusion criteria will be reviewed and verified. Blood (45mL) will be drawn for Safety labs [Basic Metabolic Panel (BMP), including serum levels of calcium and creatinine; phosphorus, albumin; and Complete Blood Count (CBC)], plus serum levels of PSA; cholecalciferol, 25(OH)D, $1,25(OH)_2D$, biomarkers and parathyroid hormone (PTH), all measured as described (68). Allostatic load parameters: serum (HDL, total cholesterol, HbA1c and DHEA-S) and waist/hip ratio and blood pressure measurements will be collected. Urine for calcium/creatinine ratio will also be collected as a safety lab. Five ml of urine, if available, will be collected for biomarkers. A survey will be completed by the subject concerning his decision making about his prostate biopsy. At Visit #2 (Enrollment Visit) baseline lab results, inclusion/exclusion criteria and concomitant medications and supplements will be reviewed. If the subject qualifies for enrollment, he will be randomized, stratified by baseline 25(OH)D level, to one of the two study arms. All subjects will receive a blinded study medication bottle with 130 soft gel capsules (sufficient for 16 weeks of supplementation/placebo before the next scheduled visit) and a supply of a daily multivitamin. They will be instructed to: a) take one soft gel per day for the duration of the study (at least one year); b) return the study medication bottle at the next study visit (after 16 weeks); c) make a notation and report any missed days taking the study medication; d) notify the CRC as soon as possible if the study medication supply is damaged or lost ; and e) take the multivitamin daily.
Subsequent visits will be scheduled every 16 weeks. During these visits, bloodwork will be performed for the same tests as at baseline, except for cholecalciferol, $1,25(OH)_2D$, HDL, total cholesterol, HbA1C, DHEA-S and PTH. Urine for calcium/creatinine ratio and biomarkers will be collected. The waist/hip ratio and survey will not be completed. Concomitant medications and supplements will be reviewed as will any reports of adverse events. The CRC will collect the returned study medication bottle, assess compliance (pill count), and dispense a new study medication bottle. At the appropriate time (12-18 months from the previous biopsy and/or study enrollment) a Urology Clinic visit will be scheduled for repeat prostate biopsy as per standard of care for active surveillance. After the outcome of the repeat biopsy has been established and each subject has been classified as stable, improving, or progressing, the final visit will be scheduled,(ideally within 16 weeks of the previous study visit). At the final visit: a) bloodwork will be performed for the same tests as baseline, including cholecalciferol, $1,25(OH)_2D$, HDL, total cholesterol, HbA1C, DHEA-S and PTH; b) a urine sample will be collected ;c) concomitant medications/supplements will be reviewed; d) reports of adverse events will be reviewed; and e) study medication bottle will be returned and assessed for compliance (pill count), f) the survey concerning the subjects' decision making about his second prostate biopsy will be completed, and g) waist/hip ratio and blood pressure will be measured. Each subject's response will be assessed by the Clinical Co-Investigator based on the repeat biopsy outcome. The outcome, whether stable disease, improvement or progression, will then be discussed with the subject at study exit , in order to determine whether to continue with Active Surveillance or plan additional treatment (hormone therapy, surgery, or radiation therapy).
For the purpose of safety data monitoring, this proposed clinical trial will be under the purview of the Medical/Surgical Data Monitoring Committee at the Hines VAMC (Hines, IL), currently chaired by Dr. James Kaufman.

Study Visit Flowchart

	Visit 1	**Visit 2**	**Visit 3**	**Visit 4** **Visit 5**	**Repeat Biopsy [6]**	**Visit 6** **Study Exit**
Phase	Screening	Enrollment	Treatment	Treatment	Treatment	Exit
Time frame	-1 to -14 days	**0** Baseline	+16 weeks	+every 16 weeks (+32,+48)	Urology Clinic schedule	within 16 weeks of previous study visit
(Visit Window)			± 14 days	± 14 days		± 14 days
Informed Consent	X					
BP/HR	X		X	X		X
Past Medical History	X					
Inclusion/exclusion criteria	X	X				
Randomization		X				
Labwork:						
*BMP[1], *phosphorus, * albumin *CBC[2]	X		X	X		X
PSA[3]	X		X	X		X
25(OH)D[4] and biomarkers	X		X	X		X
Cholecalciferol, 1,25$(OH)_2$D, PTH[5]	X					X
HDL, total Cholesterol, HbA1C and DHEA-S	X					X
*Urine calcium/creatinine ratio	X		X	X		X
Urine biomarkers	X		X	X		X
Study VIsit Procedures:						
Prostate Biopsy Survey	X					X
Waist/hip ratio	X					X
Concomitant meds	X	X	X	X		X
Adverse Event Review		X	X	X		X
Collect study medication Medication compliance			X	X		X
Dispense study medication		X	X	X		
Dispense Multivitamin		X				
Follow-up: Assessment of repeat biopsy outcome and determination to continue active surveillance vs. definitive treatment						X

*Safety lab to monitor hypercalcemia; [1]BMP= Basic Metabolic Panel (Ca, Creat, BUN, glucose, Na, K, CO_2); [2]CBC=Complete Blood Count; [3]PSA= Prostate Specific Antigen; [4]25(OH)D=circulating vitamin D (calcidiol); [5]PTH=parathyroid hormone; [6]Repeat prostate biopsy: to be scheduled by subject and Urologist 12-18 months after previous biopsy, as per Standard of Care/Active Surveillance recommendations.

Expression of VDR (C-20 rabbit polyclonal, Santa Cruz biotechnology), p21 (CP74 monoclonal antibody, SIGMA), TGFβ (HPA027923 rabbit polyclonal, SIGMA), COX-2 (H-62, rabbit polyclonal, Santa Cruz biotechnology), and NFκB (sc-372 rabbit polyclonal, Santa Cruz biotechnology) will be measured by immunohistochemistry on de-identified sections of pre- and post-study biopsy specimens of enrolled subjects. De-identified paraffin sections of pre- and post-study prostate tissue biopsy specimens will be deparaffinized by serial treatment with xylene, decreasing concentrations of ethanol followed by rehydration with distilled water. Sections will then be pretreated with 0.1% Triton-X100-TBS buffer for 5 min and incubated in 3% H_2O_2–TBS buffer for 30 min at room temperature to eliminate endogenous peroxidase activity. Following 1hr blocking with 5.0% serum (horse or goat), the sections will be incubated overnight with primary antibodies. The next day, sections will be washed three times (5min/wash) with 0.1% Triton-X100/TBS buffer to remove excess primary antibody. Thereafter, primary antibodies will be detected using HRP-conjugated mouse or rat IgG VECTASTAIN ABC kit and DAB/substrate reagents (Burlingame, CA) according to the manufacturer's instructions. Assessment of the intensity of the immunohistochemical staining (from negative to 4+) will be performed by the

collaborating Pathologist, Dr. Smith, who will be blinded to the randomization. It must be noted that only core biopsies containing tumor tissue (positive cores) can be informative, and that the paucity of tissue in each core biopsy may allow the testing of a limited number of biomarkers. It is also possible that all biopsy specimens from a particular subject will be needed for diagnostic purposes, and none will be available for research purposes. In any case, we will make every effort to measure the expression of as many relevant biomarkers as the availability of biopsy tissue specimens will allow.
Lab results, when feasible, will be reviewed by a member of the research team within 24 hours. Abnormal lab results will be reported to the PI and to the Clinical Co-Investigator within 72 hours (allowance for final results and communication over weekends). Critical lab values will be reported to the Clinical Investigator(s) immediately. Subjects and their Primary Care Provider will be notified of any unusual lab results for follow-up as determined by the Clinical Co-Investigator. Laboratory testing will be repeated if necessary. If a subject's serum calcium exceeds 10.5 mg/dl, confirmed via a second test, the patient will be removed from the study, his Primary Care Provider notified and a follow-up appointment scheduled.
The CRC, PI and Co-Investigators will update and maintain the Regulatory Documents: protocol and amendments, personnel CV's, licenses and training, IRB, VA R&D and FDA correspondence; review and document the safety and research laboratory results; document the study medication receipt, dispensing, return and subject compliance; and maintain subject confidentiality with an assigned de-identified number for source documents and electronic data entry.

5. Measurement of Primary Outcome: Assessment of tissue biopsy specimens will be performed as part of the standard of care for each subject by the VAMC and MUSC Pathologist, (who will be blinded to the randomization), according to the criteria listed in Table 1.

TABLE 1: DEFINITION OF RESPONSE (R), STABLE DISEASE (SD) AND PROGRESSION (P) OF PATHOLOGY BASED ON CHANGE IN GLEASON SCORE AND NUMBER OF POSITIVE CORES (OUT OF 12) AT BASELINE AND REPEAT BIOPSY.

		GLEASON SCORE		
		LOWER	SAME	HIGHER
NUMBER OF POSITIVE CORES	LOWER	R	R	P
	SAME	R	SD	P
	HIGHER	P	P	P

Assessment of definitive treatment after repeat biopsy and final study visit will be performed by following subjects who return to their Urology clinic for standard of care visits.
Serum levels of vitamin D_3 (cholecalciferol) will be determined by high-performance liquid chromatography (HPLC); 25(OH)D, $1,25(OH)_2D$, and parathyroid hormone (PTH) will be determined by radioimmunoassay (RIA), as previously described (38, 68). Serum levels of PSA will be measured in the VAMC Clinical Laboratories. Expression of VDR, p21, TGFβ , COX-2, and NFκB will be measured by immunohistochemistry on sections of pre- and post-study biopsy specimens of enrolled subjects. Dr. M. Timothy Smith, Professor of Pathology and a Consultant for this study, will be responsible for determining the intensity of immunostaining on de-identified prostate biopsy sections from subjects enrolled in the study. Assessment of the intensity of the immunohistochemical staining (from negative to 4+) will be performed by the collaborating Pathologist, Dr. Smith, who oversees Pathology Residents rotating through the Pathology Services of the Ralph H. Johnson VA Medical Center and MUSC and will be blinded to the supplementation status of the subjects enrolled in the study.

6. Statistical Considerations

a) Analysis Plan: The first aim of this proposal is to determine whether vitamin D_3 supplementation, compared to placebo, is more effective in maintaining stable or lowered number of positive cores and Gleason score, assessed by the pathologist as per standard of care. Baseline and end-of-study biopsy will be compared in each patient by calculating the change in number of positive cores and the change in Gleason score. These will be described using summary statistics of the ordinal changes and graphical displays. Each patient will also be categorized using three binary indicators: (1) stable Gleason (better or the same score as baseline) vs. worse Gleason (higher Gleason score); (2) stable number of cores (fewer or the same number of positive cores) vs. higher number of cores (more positive cores than at baseline; (3) stable pathology, as defined in Table 1. In each arm of the study and for each outcome, the proportions will be estimated with 95% confidence intervals. Arms will be compared using Fisher's exact test with a 1-sided alpha of 0.05. We will focus on stable number of cores and stable pathology as we expect that there will be less significant changes in Gleason scores. The second aim focuses on additional treatment after the end-of-study biopsy. We will estimate the proportion of men in each group who go on to hormone therapy, prostatectomy, or radiation therapy along with their 95% confidence intervals. We will also compare the proportions across groups using a Fisher's exact test with 2-sided alpha of 0.05.
For aim 3, serum levels of PSA and 25(OH)D will be measured at baseline and every 16 weeks until the end of the study; cholecalciferol, $1,25(OH)_2D$, and PTH will be measured at baseline and at the end of the study. Graphical displays will be created to show the trends by patient over time in each group. Linear longitudinal modeling will be used to estimate trajectories over time in each group and to estimate changes from baseline to each relevant time point. Slopes will be estimated and tested for differences from zero in each group. We also plan to compare slopes across groups. Associations between changes in each of these levels with changes in Gleason, number of positive cores, and need for definitive surgery will be estimated using logistic regression models (with pathology outcomes defined above as the outcome measures).
For aim 4, the expression of VDR, CYP27B1, p21, TGFβ, COX-2, and NFκB will be assessed by immunohistochemistry in pre- and post-treatment biopsy tissue specimens. As stated above, assessment of the intensity of the immunohistochemical staining (from negative to 4+) will be performed by the collaborating Pathologist, Dr. Smith, who will be blinded to the randomization.

b) Sample Size Justification: The sample size was determined based on the ability to detect significant differences in the proportion of subjects with stable number of cores and stable pathology outcomes. Our assumptions regarding null and alternative hypothesis are based on our preliminary data from our single arm study of men receiving an intervention of vitamin D_3 alone with very similar inclusion/exclusion criteria. Our null hypothesis is that both arms will have 40% of men with stable number of cores, and our alternative is that the vitamin D_3 arm will have 70% of men with stable cores (and 40% in the placebo arm). Using a 2-sided alpha of 0.05, we have 87% power to detect this difference with 58 men per arm. Based on our preliminary data there are relatively few men who have an increase in Gleason without an increase in the number of positive cores, so we use the same sample size calculation to power the comparison of proportions of number of men with stable pathology. Examining trends in historical data and our previous study, we would expect 15% of men in the treatment arm to have additional treatment after repeat biopsy, compared to 40% in the placebo arm. This difference can be detected with 81% power - alpha of 0.05. From our earlier studies, we anticipate that some patients will drop out of the study, making them inevaluable for the primary endpoints which require end-of-study biopsy results. As such, we will enroll 68 patients per arm to ensure at least 58 evaluable patients per arm (i.e, allowing 15% dropout). This is a conservative estimate but consistent with previous experience. Aims 3 and 4 are secondary and, as such, power is not provided. Hypothesis testing across groups is not planned for these aims.

***Concluding Remarks*:** The focus of this clinical trial is to test a therapeutic modality that is non-toxic and, therefore, ideal for men with early-stage prostate cancer, who select active surveillance as a treatment option. Furthermore, the randomized clinical trial design will allow us to confirm the beneficial effects of vitamin D_3 supplementation, as we anticipate that the number of subjects showing improvement in the intervention arm will be significantly higher than in the control arm of the trial. One of the overarching challenges in managing early-stage prostate cancer is the ability to distinguish between lethal and indolent disease. Based on the results of our preliminary studies, we anticipate that subjects who respond to our proposed supplementation regimen have indolent disease, while subjects who progress, are affected by aggressive disease that may evolve to invasive and metastatic prostate cancer. Vitamin D_3 supplementation may not only provide a significant addition to active surveillance, but also help us avoid the overtreatment of low-risk disease in subjects who respond to an intervention strategy that is extremely cost-effective and easy to implement. At the same time, this non-toxic regimen would help us identify those patients (non-responders) who are affected by potentially aggressive disease and should consider definitive treatment. Implementation of our proposed randomized clinical trial will provide us with definitive information that would have a major impact towards better management of low-risk disease, while also reducing or eliminating the risk of over-treating indolent prostate cancer..

E. PROTECTION OF HUMAN SUBJECTS

1. Risk to Subjects

a. Human Subjects Involvement and Characteristics:

1) Characteristics of Subject Population
   - 136 subjects (68 intervention group vs. 68 control group)
   - Males
   - 19 - 90 years of age
   - Diagnosis of low-grade prostate cancer (biopsy <12 months before screening)
   - Decision to manage prostate cancer with Active Surveillance

2) Inclusion Criteria
   - Male 19 – 90 years old
   - Low-grade prostate cancer
   - Clinical Stage T1C or T2a
   - Serum PSA ≤ 10.0 ng/ml
   - Gleason Score ≤ 6 (either architectural pattern ≤ 3)
   - Decision to manage prostate cancer with Active Surveillance
   - Serum creatinine ≤ 2.0 mg/dL
   - Serum phosphorus ≥ 2.3 and < 4.8 mg/dL
   - Serum calcium > 8.5 and < 10.5 mg/dL
   - Must be capable of giving consent to participate in the study

3) Exclusion Criteria
   - Any concurrent malignancy, except non-melanoma skin cancer
   - History of sarcoidosis
   - History of hypercalcemia
   - Vitamin D supplementation > 2,000 IU daily
   - Primary Hyperparathyroidism (serum Ca > 10.5 mg/dL and PTH > 72 pg/ml)
   - Lithium medication

Targeted/Planned Enrollment : 136 Subjects

Ethnic Category	Females	**Males**	**Total**
Hispanic or Latino	0	8	8
Not Hispanic or Latino	0	128	128
Ethnic Category: Total of All Subjects	0	136	136

Racial Categories	Females	**Males**	**Total**
American Indian/Alaska Native	0	0	0
Asian	0	2	2
Native Hawaiian or Other Pacific Islander	0	2	2
Black or African American	0	50	50
White	0	82	82
Racial Categories: Total of All Subjects	0	136	136

b. Sources of Material

1) Research material obtained from living human subjects:
   - Specimens: blood and urine at study visits #1, #3, and subsequent visits (every 16 weeks) until the exit visit; prostate tissue specimens (paraffin blocks) from pre-study biopsy and repeat biopsy prior to study exit.
   - Records: date of birth; race; pre-study prostate biopsy results (Gleason score; number of positive cores and percent involvement of positive cores); Progress Note from Urologist verifying patient's decision to be on active surveillance; pre-study PSA (prostate specific antigen) levels; serum calcium levels; past medical history (inclusion/exclusion criteria).
   - Data: vitamin D levels; safety lab; PSA, HDL, total cholesterol, HbA1C, DHEA-S and PTH results; waist/hip ratio and Blood pressure measurements; decision making about prostate biopsy survey results; post-study pathology results (Gleason score, number of positive cores and percent involvement of positive cores); definitive prostate cancer treatment (including Active Surveillance) following repeat prostate biopsy; specimen biomarkers (blood, urine or prostate tissue).
2) Linkages to Subjects
   - Name and SSN [(last 4 digits) at VAMC] or Medical Record Number [(MRN) at MUSC] to identify laboratory specimens and results as per policy and to review medical records
   - The Clinical Research Coordinator (CRC) will maintain a Master Enrollment Log which will include name, SSN (at VAMC) or MRN (at MUSC), date of birth, address, and telephone number with the corresponding de-identified number assigned to each subject. This will be a singular list accessed only by the CRC and maintained in secured electronic files on the assigned office computer.
   - All data collected for analysis will be with the assigned de-identified number.
   - The Investigators and CRC will have access to subject identities while performing their study visit procedures
3) Collection of specimens, records and data
   - All data will be collected for the specific purpose of this research project

c. *Potential Risks*

1) The risk from drawing blood (venipuncture) include temporary discomfort (likely side effect), bruising (less likely side effect), and infection (rare side effect). Fainting may also occur (rare side effect).
2) Loss of Confidentiality of protected health information (unlikely side effect).
3) Vitamin D_3 intake of 4,000 I.U. daily (very rare side effects: hypercalcemia, flank pain due to kidney stones, constipation, muscular twitches).
4) Alternative treatment is not to participate in the study and continue with an Active Surveillance regimen.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

1) Informed consent document (ICD) will be reviewed and approved by the MUSC IRB – IRB of record for the Ralph H. Johnson VAMC. No children will be involved in this study.
2) Eligible subjects will be recruited by Dr. Stephen Savage, Chief of Urology at the Charleston VAMC and Professor of Urology at MUSC, and Dr. David Marshall, Professor of Radiation Oncology at MUSC and enrolled into the study by the CRCs.
3) All research personnel have successfully completed all research compliance requirements for human studies and for obtaining informed consent from eligible subjects.

4) After Dr. Savage or Dr. Marshall has explained the general purpose of the study to an eligible subject and the potential subject states he will consider participating in the study, the CRC will meet the subject, and review the background, purpose, procedures and objectives of the study. Adequate time will be given to the subject for reading the ICD and the Health Insurance Portability and Accountability Act (HIPAA) document, discuss the documents with his significant other(s), ask questions and then determine if he wishes to participate in the study. Only when the subject states that he wishes to participate, will the ICD and the HIPAA be signed.
5) If a potential subject calls the CRC after receiving a verbal referral or letter from Dr. Savage or Dr. Marshall, the background, purpose, procedures and objectives of the study will be reviewed with him. If the subject states he wishes to participate, a copy of the ICD will be mailed to the subject to be read thoroughly and discussed with family members before his initial visit. At his first visit, the ICD will be reviewed, and questions encouraged and answered before the ICD is signed. Time for the subject to read the HIPAA and ask questions will be allotted before it is signed. The ICD/HIPAA will be signed only after the subject states he wishes to participate and before any study procedures are initiated.
6) Only subjects who are capable of giving consent may enroll in the study.
7) All aspects of the recruiting and consent process will be documented in Progress Notes maintained as part of the subject's Source Documents.

b. <u>Protection Against Risk</u>

1) Venipuncture: Risk will be minimized by having licensed nursing personnel perform the procedure using hospital-approved techniques to minimize hematoma, infection and/or discomfort. Subjects will be sitting for the procedure and asked whether they feel dizzy before allowed to stand after their blood sample has been collected.
2) Loss of Confidentiality of protected health information
   - Data will be collected and maintained according to VAMC and MUSC guidelines.
   - Subjects will be de-identified with an assigned number that will not be related to any personal information.
   - The original signed ICD and HIPAA with subject signature/name will be filed in a locked cabinet and kept separate from the source documents maintained for each subject.
   - Source documents will be de-identified and will be transported and/or handled only by the CRC, during a scheduled study visit. At all other times, these documents will be stored in a locked cabinet.
   - Only the Master Subject List will contain subject identified information. It will be maintained in a secured file server that is accessed only by the CRC through the assigned office computer.
   - A database will be designed for each component of data collection. There will be no patient identifiable information on the outcome databases; linkage can be accomplished only by the unique de-identification code.
   - Electronic data will be maintained in the secured file server, not on an individual computer hard drive or laptop. The specific study files are limited to the CRC, Co-Investigators and the PI.
   - No names or identifying information will be used in any presentations and/or publications.
3) Vitamin D_3 intake of 4000 IU daily: the study drug dose is the safe upper limit according to the Institute of Medicine (IOM) report released in December 2010. The vitamin D_3 supplement will be purchased through JR Carlson Laboratories, Inc. This company is based and manufactures its products in the United States. It has provided the vitamin D_3 supplement for multiple studies at MUSC, involving hundreds of subjects, with no related adverse events.
4) This is a double-blind placebo controlled study, therefore to correct potential insufficient levels of circulating vitamin D_3 for those subjects who are assigned to the placebo group, all subjects will receive a daily multivitamin containing the IOM recommendation of cholecalciferol supplementation.

5) Subjects will have routine safety blood and urine tests at every clinic visit except #2 (enrollment). Lab results, when feasible, will be reviewed by a member of the research team within 24 hours. Abnormal lab results will be reported to the Clinical Investigator within 72 hours (allowance for final results and communication over weekends). Critical lab values will be reported to the Clinical Investigator(s) immediately. Subjects and their Primary Care Provider will be notified of any unusual lab results for follow-up as determined by the Clinical Investigator. Laboratory testing will be repeated if necessary.
6) Subjects will be closely monitored for symptoms of hypercalcemia they will be instructed to call for early evaluation, if they have any symptoms of hypercalcemia including new onset of bone pain, abdominal pain, flank pain, frequent urination, frequent thirst, nausea, vomiting, constipation, memory loss, depression, muscle weakness, or muscle twitches. Also, users of thiazides will be closely monitored for symptoms of hypercalcemia. When feasible, laboratory test results will be reviewed the day they are obtained. If results are suspect, the labs will be repeated, as per the Clinical Investigator recommendation. If results remain abnormal and indicate possible toxicity, study medication will be stopped immediately. If a subject's serum calcium continues to exceed 10.5 mg/dl, confirmed via a second test, the patient will be removed from the study and follow-up appointment scheduled with his Primary Care Provider.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Supplementation with vitamin D_3 routinely takes 3 months to reach a stable level in the body. The subjects may or may not receive direct benefit from participating in this study, whether they have been randomized to the intervention or the control group. However, during the study, they will be more closely monitored for possible progression of their prostate cancer than the standard of care regimen of Active Surveillance.

4. Importance of the Knowledge to be Gained

The risks to subjects associated with this clinical trial appear to be reasonable in relation to the importance of knowledge to be gained. Vitamin D_3 supplementation may not only provide an appealing additional component to Active Surveillance, but would also allow us to distinguish between potentially lethal vs. indolent disease and, therefore, decrease the likelihood of over treating low-risk disease in early-stage prostate cancer.

5. Subject Safety and Minimizing Risks

The therapeutic risks to subjects are considered minimal and therefore data monitoring will be for research purposes only.

a) Data monitoring will include:
- Study medication compliance
- Circulating vitamin D levels at baseline, visits #3, and then every 16 weeks through the exit visit
- PSA levels
- Safety lab results (blood and urine)
- Cholecalciferol, $1,25(OH)_2D$ and PTH at baseline and at final visit
- Allostatic load parameters (HDL, total cholesterol, HbA1C, DHEA-S; BP; waist/hip ratio)
- Decision Making about Prostate Biopsy survey results
- Pre-study prostate biopsy report results (Gleason score of positive cores; number of positive cores and percent involvement)
- Repeat prostate biopsy results (Gleason score of positive cores; number of positive cores and percent involvement)
- Prostate tissue biomarkers
- Blood and urine biomarkers

b) Frequency of monitoring:
- Baseline data (date of birth, race, PSA results, pre-study prostate biopsy results, etc. for inclusion/exclusion determination) will be obtained by the CRC at the screening visit.
- The safety labs and vitamin D levels will be obtained at baseline and every 16 weeks until the exit visit.
- Allostatic load parameters will be obtained at baseline and final study visits
- Decision Making about Prostate Biopsy survey will be completed at baseline and final visit
- Repeat prostate biopsy results and the prostate cancer treatment plan will be obtained by the CRC and entered in the de-identified data spreadsheets.
- Study endpoints will be evaluated by the PI and Co-Investigators who will be blinded to the subject randomization.

c) Adverse Event Reporting:
Internal Adverse Events and Serious Adverse Events related to the study medication will be reported to the corresponding VAMC/MUSC IRB, VA R&D and FDA, within the required time period, as soon as discovered and documented. The Clinical Investigator will communicate with the subject's Primary Care Provider (or team) describing the adverse event to insure continuity of care is initiated/scheduled.

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H. FACILITIES AVAILABLE

Subjects enrolled in this study will be seen in the Ralph H. Johnson VA Medical Center, MUSC Urology and Radiation Oncology Clinics and MUSC CTRC. The VAMC and MUSC will provide the necessary patient volume, personnel, and facilities for the proposed project.

The PI has access to 1,200 sf of VA-Leased laboratory space located in Room 514 of the Strom Thurmond Biomedical Research building. The laboratory is fully equipped to conduct biochemical research.

The PI and all Co-Investigators have adequate office and computer facilities for supporting and implementing the proposed study. In their offices and laboratories they have Apple, Dell or IBM computers with Pentium 4 processors or higher, as well as laser copier/printer/scanner/fax machines. Administrative support is available through the VAMC Research Office and the MUSC Department of Radiation Oncology.