Randomized Placebo-Controlled, Double-Blind Study Of Cholecalciferol Replacement in Patients on Expectant Management for Localized Prostate Cancer

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Principal Investigator:
James Mohler, MD

Roswell Park Cancer Institute
Elm & Carlton Streets
Buffalo, NY 14263

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Abbreviations used in order of appearance

PSA	prostate specific antigen
PSAV	prostate specific antigen velocity
PSADT	prostate specific antigen doubling time
D3-25 hydroxy vitamin D	vitamin D3
SNP	single nucleotide polymorphism
ECOG	eastern cooperative group
AST-ALT	alanine aminotransferase (ALT/SGPT)
BUN	blood urea nitrogen
LDH	lactate dehydrogenase
Ht	height
Wt	weight
BP	blood pressure
TPR	temperature, pulse, respiration
DRE	digital rectal examination
PTH	parathormone
DBBR	data bank and biorepository
NCCN	National Comprehensive Cancer Network
AE	adverse event
SAE	serious adverse event

Study Summary

Title: Randomized Placebo-Controlled, Double-Blind Study Of Cholecalciferol Replacement in Patients on Expectant Management for Localized Prostate Cancer

Objectives:

Primary Objective

1) To determine PSA response with oral high dose vitamin D3 supplementation in patients with localized, histologically proven adenocarcinoma of the prostate that have not received any treatment for prostate cancer ever and have chosen expectant management.

Secondary Objectives

1) To examine the pattern of response of PSA dynamics as well as the absolute change in PSA following vitamin D3 supplementation.
2) Assess the toxicity of vitamin D3 supplementation in men with prostate cancer.

Exploratory Objectives

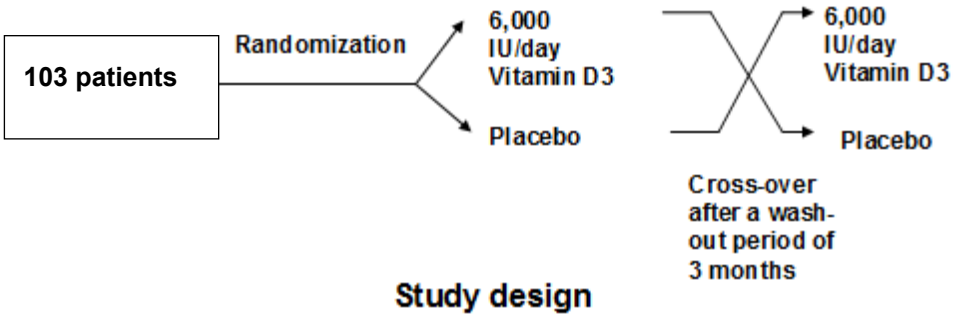
1) Track occurrence of infections, deep venous thrombosis, vascular events and falls in the study population.
2) To evaluate relationship between CYP24, 27B1, SNPs and serum 25(OH) vitamin D response to oral D3 supplementation.

Study Design: Double-blinded, parallel, placebo controlled randomized 2-group complete cross-over design with 12 week washout period. 103 patients are expected to enroll over 11 year period.

Treatment Plan: Eligible men will be randomly assigned to two groups in 1:1 ratio.
Group A will receive 6,000 IU daily of vitamin D3 provided as 3 capsules of 2000 IU each.
Group B will receive a placebo provided as 3 capsules of a rice based placebo.

Treatment Duration: Participants will be followed for 36 weeks and then crossed over after a wash out period of 12 weeks and followed for another 36 weeks.

Number of Participants: Each group will be comprised of approximately 50 men. Those who are not able to complete the study will be replaced.



Study design

Dose Modification:

1). If a participant's 25(OH) D3 level reaches > 200ng/ml with 6000 IU or 3 capsules a day, or any ≥grade 3 toxicity possibly related to cholecalciferol develops, 25(OH) D3 or placebo will be discontinued for 4 weeks. Treatment will then be resumed at lower dose of 4000 IU or 2 capsules a day. If a participant misses some period of time off the study drug, the treatment would be resumed such that the participant completes a total of 36 weeks on the study drug.

2). If a participant's 25(OH) D3 level reaches > 200ng/ml with the lower dose modification of 4000 IU or 2 capsules a day, or any grade 3 toxicity develops, 25(OH) D3 or placebo will be discontinued for 4 weeks. Treatment will then be resumed at lower dose of 2000 IU or 1 capsule a day.

3). If a participant's 25(OH) D3 level reaches > 200ng/ml with the lower dose modification of 2000 IU or 1 capsule a day, or any grade 3 toxicity develops, the participant will then be taken off study.

4). Participants that drop out after the first treatment period (36 weeks) will be included in the analysis but will require enrolling additional participants such that we have evaluable data on 100 participant-periods. An intention to treat analysis will be done.

5). If participant misses a daily dose of vitamin D3, or placebo, he should skip it and continue to take it as scheduled the following day. The participant should write down the day of the missed dose in the diary and should bring the diary with him on the day of physical. If participant misses more than 1/3 rd of the doses in 12 week interval, the participant will be taken out of the study and will be replaced by another participant.

6). At the end of study, participants may choose to continue taking vitamin D3 replacement per personal preferences.

Major Entry Criteria:

1) Any patient with clinically localized, histologically proven adenocarcinoma of prostate who has not received any treatment for prostate cancer ever and has chosen active surveillance. Treatment for prostate cancer is defined as prostatectomy, androgen deprivation, brachytherapy or a full course of external beam irradiation.
2) Age ≥ 18 years
3) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
4) Willingness to comply with study guidelines
5) Willingness and ability to consent
6) 25(OH) D3 level less than 40ng/ml within 3 months of initiation of study. Most recent 25 hydroxy D level within last 3months will be used.

Exclusion Criteria:

1) History of malabsorption syndrome e.g. pancreatic insufficiency, celiac disease, tropical sprue
2) Creatinine > 2.0mg/dl
3) Corrected serum calcium level >10.5 mg/dL
(Serum Corrected Calcium= Serum Calcium+0.8(4-Serum Albumin)
4) Most recent PSA value more than 18 months ago
5) Prior or current therapy for prostate cancer
6) Documented history of nephrolithiasis within the past 5 years

7) Patients receiving finasteride (Proscar) or dutasteride (Avodart) or men who have received either agent within 90 days of entry are ineligible.
8) Patients cannot take any additional vitamin D supplementation during study treatment. Patients taking >2000IU per day prior to treatment will be ineligible.

Plan of the Study:

Pretreatment Evaluation

1) Signed written informed consent
2) Complete medical history, including diagnosis of prostate cancer, concurrent illness, concomitant medications, and allergy history
3) Physical examination, including weight, height, ECOG performance status, temperature, vital signs (blood pressure, pulse and respiration rate), and digital rectal examination by patient's urologist
4) Serum alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), total serum bilirubin, BUN, serum creatinine, electrolytes, calcium, phosphorous, total protein, albumin, glucose and LDH; Tumor biomarkers: PSA
5) Imaging studies for potential evaluable disease sites as clinically indicated and at clinician's discretion.
6) Baseline toxicity evaluation to document residual toxicity and baseline symptoms
7) 25(OH) D3 level
8) 24 hour urinary calcium and creatinine
9) Research labs (PTH, serum/DNA/Plasma samples)
10) All pretreatment PSA values done up to 2 years prior to enrollment will be recorded

Toxicity assessment will be done after 4 weeks of enrollment via a telephone assessment, thereafter will be done every 12 weeks at a clinic visit. Relevant laboratory data will be repeated every 12 weeks (please see the table 6.1). Investigational biomarkers will also be done with these laboratory tests every 12 weeks.

Patients will be continued on a treatment group for 36 weeks and then will have a 12 week washout period where they will be off any study drug or placebo. After this 12 week washout period, patients will be crossed-over with follow up every 12 weeks for another 36 weeks (total = 84 weeks) with assessments similar to those done at 36 week visit.

Study Duration: 11 years

Study Medication

Oral Vitamin D3 supplementation will be provided as capsules of 2000 IU each (manufacturer-Vital Nutrients). Patients will be advised to take 3 capsules once a day to provide 6,000 IU of Vitamin D3.
Cholecalciferol is to be taken orally as a single AM dose.

An identical placebo containing rice powder will be provided by the manufacturer.

1.0 Rationale /Background

1.1 Prostate Cancer and Role of Expectant Management

Prostate cancer is the most common visceral malignancy in American males and is the second most common cause of cancer related deaths. It is estimated that 218,890 men will be diagnosed with and 27,050 men will die of prostate cancer in 2007[1] . Many patients are diagnosed with prostate cancer based on a screening prostate specific antigen (PSA) test.[2] In some patients, an abnormal PSA is the only indication of prostate cancer (stage T1c). Localized prostate cancer is usually treated with either surgery or radiation.[3] [4] The necessity of definitive therapies in all men with prostate cancer is unclear and certain individuals choose a "watchful waiting" approach for their localized disease due to the morbidity sometimes associated with irradiation or prostatectomy. There is limited evidence for improved quality or quantity of survival in certain population (e.g. elderly men, men with substantial comorbidities).[5] Expectant management (observation, active surveillance, watchful waiting) is a reasonable option in many situations.[6] [7] [8] [9] [10] [11]

1.1.1 NCCN Guidelines for Expectant Management

Expectant management involves actively monitoring the course of disease with the expectation to intervene if the cancer progresses. Patients with clinically localized cancers who are candidates for definitive treatment and choose expectant management should have regular follow up as suggested by 2007 updated NCCN guidelines with DRE and PSA every 6 months for life expectancy greater than or equal to 10 years and every 6-12 mo for life expectancy < 10 years. Needle biopsy of the prostate may be repeated within 6 months of diagnosis if initial biopsy was < 10 cores or assessment discordant (e.g. palpable tumor contralateral to side of positive biopsy). Needle biopsy may be performed within 18 months if > 10 cores obtained initially, then periodically.
Cancer progression may have occurred if any of the following is noted: Gleason grade 4 or 5 cancer is found upon repeat prostate biopsy; prostate cancer is found in a greater number of prostate biopsies or occupies a greater extent of prostate biopsies; PSA doubling time < 3 y or PSA velocity is > 0.75; a repeat prostate biopsy is indicated for signs of disease progression by exam or PSA.

Advantages of expectant management:

- Avoid possible side effects of definitive therapy that may be unnecessary
- Quality of life/normal activities retained
- Risk of unnecessary treatment of small, indolent cancers is reduced

Disadvantages of expectant management:

- Chance of missed opportunity for cure
- Risk of progression and/or metastases
- Subsequent treatment may be more intense with increased side effects
- Increased anxiety
- Requires frequent medical exams and periodic biopsies
- Uncertain long term natural history of prostate cancer

1.2 PSA Velocity and Slope

PSA is a useful marker to identify progressive disease in patients with prostate cancer.[12] Studies have shown that serum PSA levels correlate with tumor volume and pathologic stage. [13] [14] [15] In addition, a rapid PSA change (expressed as PSA velocity [PSAV] or PSA doubling time [PSADT]) was shown to correlate with the presence of cancer and metastatic disease. PSAV is defined as rate of change in PSA over a predefined period of time.[16] PSA dynamics provides an insight into the biological behavior of the tumor and tumor kinetics. There is recent evidence to suggest that PSA dynamics is not only useful in prostate cancer detection but also in predicting treatment outcomes. Schmid et al. studied serial measurements of PSA in men during at least 1 year of expectant management, and they found that PSADT was related to tumor stage and grade.[17] McLaren et al. found that PSADT was the most powerful indicator of clinical progression in 113 men observed expectantly; whereas, T classification, histologic grade, and initial levels of PSA were not important.[18] D'Amico et al reported that a PSAV greater than 2 ng/ml per year was associated with greater prostate cancer-specific mortality following radical prostatectomy and external beam radiation therapy.[19] [20]

PSA slope is defined as the slope of the linear line of $\log_2$ (PSA) versus time. The advantage to this transformation is the inverse of the slope is the time to doubling the PSA value. [21] [22] Following PSA dynamics can help guide treatment strategy in patients who choose expectant management.

1.3 Prostate Cancer and Vitamin D

Multiple studies demonstrate links between vitamin D deficiency and prostate cancer.[23] [24] [25] [26] [27] [28] [29] .

1.3.1 Vitamin D analogues and in vivo and in vitro growth of prostate cancer:

In preclinical studies, 1, 25 dihydroxyvitaminD3 [calcitriol; $1\alpha,25(OH)_2D_3$] has been shown to be effective in killing prostate cancer cells [30] [31] [32] [33] [34] [35] [36]. $1\alpha,25(OH)_2D_3$-induces cell-cycle arrest of LNCaP cell in the G0–G1 phase involves decreased phosphorylation of the retinoblastoma gene-encoded protein (Rb). This is followed by a reduction in the activity of the transcription factor, which leads to increased activity of $p21^{waf1}$, and decreased CDK2 activity. Blutt *et al.* observed apoptosis after treating LNCaP cells with $1\alpha,25(OH)_2D_3$ accompanied by down regulation of two anti-apoptotic proteins, Bcl2 and $BclX_L$, and is prevented by overexpression of the gene that encodes Bcl2. Other anti-apoptotic proteins (Mcl-1, BAG1L, XIAP, cIAP1 and cIAP2) are also down regulated by 1α, $25(OH)_2D_3$ in LNCaP cells but proapoptotic Bax and Bak are unaltered . This down regulation leads to the activation of caspase-3 and caspase-9, the apical proteases in the mitochondrial pathway for apoptosis. Thus, both growth arrest and apoptosis are involved in growth regulation of LNCaP cells in response to 1α, $25(OH)_2D_3$. Studies have also implicated the increased expression of insulin-like growth factor binding protein-3 (IGFBP-3) in the growth inhibition induced by calcitriol which in turn increases $p21^{Waf/Cip1}$ expression. Other mechanisms of calcitriol actions in PCa cells include the stimulation of differentiation, modulation of growth factor actions and the inhibition of invasion, metastasis and angiogenesis.
Similar anti-proliferative effects are seen in vivo in many human, mouse and rat models.[37]

1.3.2. Epidemiological relationship between Vitamin D levels and prostate cancer:

There appears to be an inverse correlation between the mortality rate of prostate cancer and exposure to ultraviolet radiation (UVR) in the US population. An association between vitamin D deficiency and prostate cancer was reported by Ahonen *et al.* in a 13-year follow-up of 19 000 middle-aged men in the Helsinki Heart Study. [27] In this study, 149 cases of prostate cancer were identified and matched to 566 sample controls. The study showed that low circulating levels of 25(OH) D3 (<40 nmol l^{-1} or 16 ng ml^{-1}) were associated with an increased risk of subsequent earlier onset and more aggressive progression of prostate cancer, especially before the age of 52.

UVR exposure has a significant protective effect in prostate cancer.[38] Luscombe et al showed that cancer patients with the lowest quartile of sun exposure developed cancer at a median age of 67.7 years compared with 72.1 years in patients in other quartiles.[39] Although the mechanism of this association is unclear, it is possible that increased cutaneous synthesis of vitamin D_3 increases the circulating levels of 25(OH) D3 and the subsequent formation of 1α, $25(OH)_2D_3$ in the prostate by prostatic 1α-OHase (20). 1α, $25(OH)_2D_3$ then interacts with VDR in the prostate and induces cell-cycle arrest and apoptosis-suppressing cancer development .[40] [41] [42] Populations in whom vitamin D levels are estimated to be low have a greater risk of prostate cancer and other cancers.[43] The greater risk of prostate cancer in Afro-Caribbean men indicates that one factor contributing to the genesis of prostate cancer might be vitamin D insufficiency.[44]

1.3.3 Clinical Data:

Limited but intriguing clinical data suggest 1α,$25(OH)_2D_3$ alone or in combination with other agents may slow PSA rise in men with either androgen stimulated or castration resistant prostate cancer patients. [45] [46] [47] [48] [49] [50] [51] [52] [53] NCCN guideline--

- We have reported an approximately 25% response rate (PSA) in men with castration resistant prostate cancer using a regimen of 12mcg calcitriol 3X/week [MTW] + dexamethasone [4mg SMTW].[54]
- Gross et al using calcitriol and Vieth et al using cholecalciferol showed slowing of PSA increase in patients with androgen stimulated prostate cancer. [46] [55]
- Vieth et al noted that the rate of rise in PSA is slower during the spring-summer than during the rest of the year, in men who had been followed on an expectant management protocol with selective delayed intervention for clinically localized, low-to-intermediate grade prostate cancer.[56]

These trials demonstrated that the rate of increase in PSA velocity may be altered by high doses of calcitriol. Well tolerated, safe agents are needed that might slow PSA increase. Based on above data, it is rational to study the effect of oral calcitriol supplementation on PSA dynamics in patients with prostate cancer who have chosen expectant management for clinically localized prostate cancer.

1.4 Vitamin D Supplementation and Toxicity

Supplementation with vitamin D has been studied in normal individuals and those with vitamin D deficiency. Recommended daily allowance of vitamin D is 400 IU. Considerable data suggests this may be inadequate to bring and maintain serum vitamin D levels in normal range.[Hollis et al] The currently accepted normal range of 25(OH) D3 is 32-100ng/mL [ng/ml X 2.5 ≈ nmol/L]. Supplementation with 10 µg (400 IU) of vitamin D raises 25(OH) D by only ≈0.45 nmol/(L µg day).

- Various studies report that one minimal erythema dose(Exposure of the body in a bathing suit to sunlight that causes a minimum redness to the skin) of UV-B radiation to the whole body produces between 10,000 and 25,000 IU (250 and 625 µg) of cholecalciferol and is safe. [57] Indeed, a continuing daily oral intake of 10,000 IU produces a mean serum 25OHD value approximating what is seen naturally after extensive summer sun exposure at mid latitudes.[58]
- All instances of vitamin D toxicity have involved serum 25(OH) D concentrations more than 250nmol/L, a value that would be produced only at continuing oral intakes in excess of 10,000IU (250microg)/day.[59]
- To attain a steady state of equilibrium in serum Vitamin D level, at least 3 months are required post a change in vitamin D supplementation dose.[60] The graph below depicts the same with varying doses of oral vitamin D supplementation. [61]
- The serum 25 (OH) D concentrations is maintained within a narrow range ≈75–220 nmol/L across a wide range of D3 intake: 20 µg (800 IU) to 500 µg (20000 IU)/d. The following graph clearly demonstrates that it is very safe to treat patients with vitamin D up to dosage of 10,000 IU-especially if 25(OH)D3 levels are monitored. [56] The serum levels appear to plateau between 120-130 nmol/L. Several studies have demonstrated the essential safety of continuous daily dosing with 4000, 5000 and 10,000 IU/day (100, 125 and 250 µg/day) for up to 16 weeks, and of 50,000 IU (1250 µg)/day for 8 weeks, all without evidence of toxicity. [62] [63] [64]

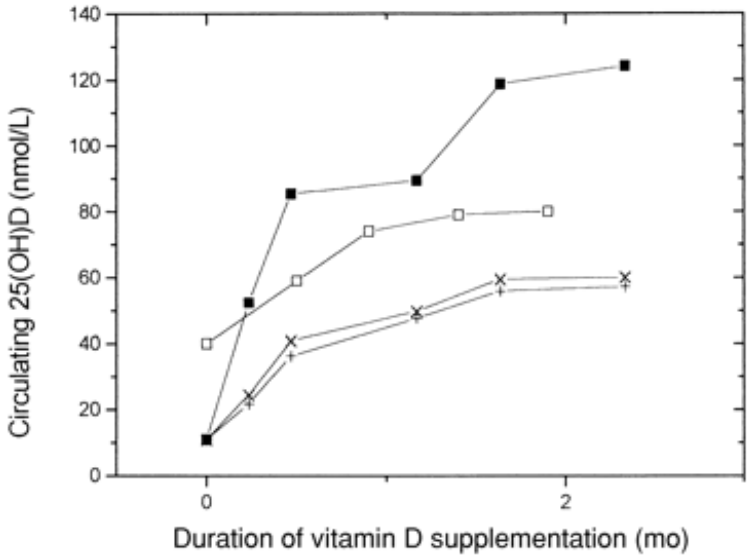


Figure 4. Effect of the duration of vitamin D supplementation on the mean serum 25-hydroxyvitamin D [25(OH)D] concentration achieved. Vitamin D intakes for the groups were as follows: 10 µg (400 IU)/d (+; 28), 25 µg (1000 IU)/d (X; 28), 50 µg (2000 IU)/d (□; 64), 250 µg (10000 IU)/d (28).

2.0 Primary Objective

1) To determine the PSA response with oral high dose vitamin D3 supplementation in patients with localized, histologically proven adenocarcinoma of the prostate who have not received any treatment for prostate cancer ever and have chosen expectant management.

2.1 Secondary Objectives

1) To examine the pattern of response of PSA dynamics as well as the absolute change in PSA following vitamin D3 supplementation.
2) Assess the toxicity of vitamin D3 supplementation in men with prostate cancer.

2.2 Exploratory Objectives

1) Track occurrence of infections, deep venous thrombosis, vascular events and falls in the study population.
2) To evaluate relationship between CYP24, 27B1, SNPs and serum 25(OH) vitamin D response to oral D3 supplementation.

3.0 Study Population

Patients with prostate cancer who have chosen to undergo expectant management for their disease will be eligible. Patients who are not able to complete the study will be replaced.

4.0 Inclusion Criteria

1) Any patient with clinically localized, histologically proven adenocarcinoma of prostate who has not received any treatment for prostate cancer ever and has chosen active surveillance. Treatment for prostate cancer is defined as prostatectomy, androgen deprivation, brachytherapy or a full course of external beam irradiation
2) Age ≥ 18 years
3) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
4) Willingness to comply with study guidelines
5) Willingness and ability to consent
6) 25(OH) D3 level less than 40ng/ml within 3 months of initiation of study. Most recent 25 hydroxy D level within last 3 months would be used.

4.1 Exclusion Criteria

1) History of malabsorption syndrome e.g. pancreatic insufficiency, celiac disease, tropical sprue
2) Creatinine > 2.0mg/dl
3) Corrected serum calcium level of >10.5 mg/dL
(Serum Corrected Calcium = Serum Calcium + 0.8(4-Serum Albumin)
4) Most recent PSA value more than 18 months ago
5) Prior or current therapy for prostate cancer
6) Documented history of nephrolithiasis within the past 5 years
7) Patients receiving finasteride (Proscar) or dutasteride (Avodart) or men who have received either agent within 90 days of entry are ineligible.
8) Patients cannot take any additional vitamin D supplementation during study treatment. Patients taking >2000IU per day prior to treatment will be ineligible.

5.0 Pre-treatment evaluation

All evaluations should be done within 4 weeks of registration unless otherwise noted.

1) Signed written informed consent. All patients will have the option to participate in the DBBR/Vitamin D trial.
2) Complete medical history, including diagnosis of prostate cancer, concurrent illness and concomitant medications.
3) Physical examination, including weight, height, ECOG performance status, temperature, vital signs (blood pressure, pulse and respiration rate), and digital rectal examination by patient's urologist
4) Serum alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), total serum bilirubin, BUN, serum creatinine, electrolytes, calcium, phosphorous, total protein, albumin, glucose and LDH; Tumor biomarkers: PSA
5) Imaging studies for potential evaluable disease sites as clinically indicated
6) Baseline toxicity evaluation to document baseline symptoms
7) 25(OH) D3 level
8) 24 hour urinary calcium and creatinine
9) Research labs (PTH, serum/DNA/Plasma samples)
10) All pretreatment PSA values done up to 2 years prior to enrollment will be recorded.

5.1 Registration

Following evaluation and informed consent the patient will be registered. At the time of registration, the following information will be recorded:

1. Patient's name and medical record number
2. Patient's date of birth
3. Date of commencement of the protocol
4. Stage and Gleason score of prostate cancer
5. To which treatment arm patient is assigned

Eligibility criteria will be verified at the time of registration. A case number will be assigned following registration.

6.0 Study design

Study design
Double-blinded, parallel, placebo controlled randomized 2-group complete cross-over design with 12 week washout period.

Participant Assignment
Eligible patients will be randomly assigned to two groups in 1:1 ratio.
Group A will receive 6,000 IU daily of vitamin D3 (3 capsules of 2000 IU pills).
Group B will receive an identical placebo.

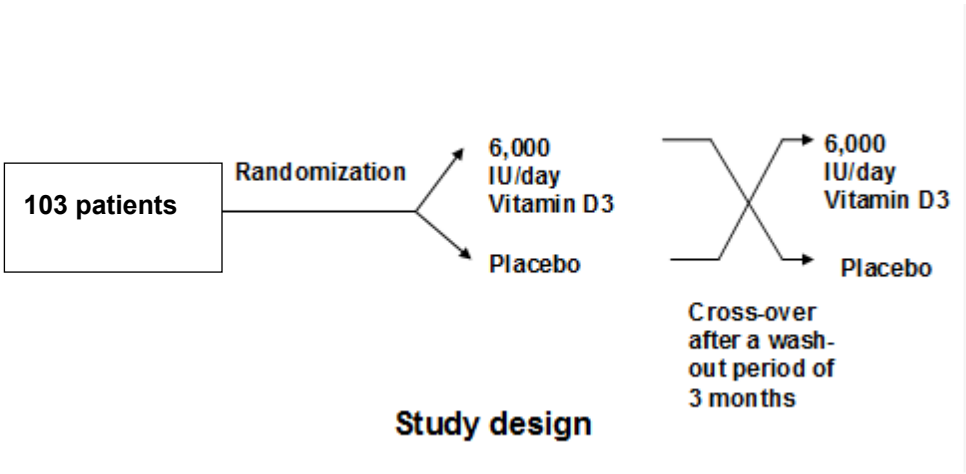
Each group will be comprised of approximately 50 patients. Patients who are not able to complete the study will be replaced.

Cross-over will be done after a period of 36 weeks followed by 12 weeks washout period. After 12 week washout period, patients on either arm will be crossed over to receive the drug on the other arm (patients who were on vitamin D arm will get crossed-over to receive placebo, while patients on placebo arm will be crossed-over to receive vitamin D).

They will be stratified randomization based on the Gleason score (less than or equal to 6, OR greater than 6) as well as the season of entry (winter/spring versus summer/fall). Seasonal stratification assignment will be based on the date of randomization:

> If randomized between 12/22 and 6/21, it will be considered the winter/spring group and if randomized between 6/22 and 12/21 it will be the summer/fall group.

After completion of study, patient may choose to continue vitamin D if they desire.



Study design

Duration of the trial

Patients will be followed for 36 weeks after randomization followed by a washout period of 12 weeks and then be crossed over and followed for an additional 36 weeks (total 84 weeks). 103 patients are expected to enroll over an 11 year period.

Dose Modification:

1). If a patient's 25(OH) D3 level reaches > 200ng/ml with 6000 IU or 3 capsules a day, or any ≥grade 3 toxicity possibly related to cholecalciferol develops, 25(OH) D3 or placebo will be discontinued for 4 weeks. Treatment will then be resumed at lower dose of 4000 IU or 2 capsules a day. If a patient misses some period of time off the study drug, the treatment will be resumed such that the patient completes a total of 36 weeks on the study drug.

2). If a patient's 25(OH) D3 level reaches > 200ng/ml with the lower dose modification of 4000 IU or 2 capsules a day, or any grade 3 toxicity develops, 25(OH) D3 or placebo will be discontinued for 4 weeks. Treatment will then be resumed at lower dose of 2000 IU or 1 capsule a day.

3). If a patient's 25(OH) D3 level reaches > 200ng/ml with the lower dose modification of 2000 IU or 1 capsule a day, or any grade 3 toxicity develops, the patient will then be taken off study.

4). Patients that drop out after the first treatment period (36 weeks) will be included in the analysis but will require enrolling additional patients such that we have evaluable data on 100 patient-periods. An intention to treat analysis will be done.

5). If patient misses a daily dose of vitamin D3, or placebo, he should skip it and continue to take it as scheduled the following day. The patient should write down the day of the missed dose in the diary and should bring the diary with him on the day of physical. If patient misses more than 1/3 rd of the doses in a 12 week period, the patient will be taken out of the study and will be replaced by another patient.

6). At the end of study, patients may choose to continue taking vitamin D3 replacement per personal preferences.

6.1 Study Schedule: Pre-treatment evaluations are described in section 5.0 and are repeated here for sake of convenience.

Parameters	**Pre-study[e]**	**4 weeks** (+/- 7 days)	**12 weeks** (+/- 7 days)	**24 weeks** (+/- 7 days)	**36 weeks** (+/- 7 days)	**4 weeks f/u[a]** (+/- 7 days)
Physical Exam, Medical History, Vital Signs and ECOG(Wt, temperature, BP, pulse and respiration rate)	**X**			**X**		
Height	**X**					
Toxicity assessment	**X**	**X[a]**	**X**	**X**	**X**	**X[a]**
Serum alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), total serum bilirubin, BUN, serum creatinine, electrolytes, calcium, phosphorous, total protein, albumin, glucose and LDH	**X**		**X**	**X**	**X**	
Serum 25(OH)D3	**X**		**X**	**X**	**X**	
PTH[b]	**X**		**X**	**X**	**X**	
PSA	**X**		**X**	**X**	**X**	
24 hr urinary calcium and creatinine	**X**		**X[g]**	**X[g]**	**X**	
Assessment of infection, fall, fracture, thrombosis	**X**		**X**	**X**	**X**	
Investigational Biomarker samples [b, c] and DBBR Blood Draw	**X**	**X**	**X**	**X**	**X**	
Radiographic study(Computed tomography and/ or bone scan)[f]	**X**		**X**	**X**	**X**	
Study drug compliance assessment		**X**	**X**	**X**	**X**	
Nurse visit: ECOG, Wt, temperature & BP			**X**		**X**	

a- Telephone assessment
b- Performed in the lab. See Sec. 6.1 "Monitoring"
c- DNA, serum, plasma isolated through ongoing Vitamin D assessment protocol (only for patients at Roswell Park).
d- After a wash-out period of 12 weeks, patients will be crossed over. Patients would be followed every 12 weeks with similar plan.
e- Pre study evaluations are to be done within 4 weeks of registration. Complete history to be done at pre study evaluation.
f- If clinically indicated. CT/Bone scan to be done at the clinician's discretion.
g- Needed only if serum Ca level increases and it is per clinician's discretion.

All evaluations should be done within 4 weeks of registration unless otherwise noted.

1. Signed written informed consent. Roswell Park patients will have the option to participate in DBBR/Vitamin D trial.
2. Complete medical history, including diagnosis of prostate cancer, concurrent illness and concomitant medications.
3. Physical examination, including weight, height, ECOG performance status, temperature, vital signs (blood pressure, pulse and respiration rate), and digital rectal examination by patient's urologist
4. Serum alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), total serum bilirubin, BUN, serum creatinine, electrolytes, calcium, phosphorous, total protein, albumin, glucose and LDH; Tumor biomarkers: PSA
5. Imaging studies for potential evaluable disease sites as clinically indicated
6. Baseline toxicity evaluation to document baseline symptoms
7. 25(OH) D3 level
8. 24 hour urinary calcium and creatinine
9. Research labs (PTH, serum/DNA/Plasma samples).
10. All pretreatment PSA values done up to 2 years prior to enrollment will be recorded.

Toxicity assessment will be done after 4 weeks of enrollment via a telephone assessment, thereafter would be done on every 12 week basis at clinic visit. Doses remaining will be assessed at each clinic visit and compliance ensured by telephone check by clinical research Coordinator. Laboratory data (as pre-study evaluation) will be repeated every 12 weeks. Investigational biomarkers will also be done with these laboratory tests on every 12 weeks basis.

Patients will continue on treatment for 36 weeks and then will have a 12 week washout period where they will be off any study drug or placebo. After 12 week washout period, patients will be crossed-over with follow up every 12 weeks for another 36 weeks (total =84 weeks) with assessments similar to those done at 36 weeks follow-up visit.

Prior studies have used similar design and there are substantial data that indicate that 36 weeks is a time period over which modification of PSA slope can be assessed. [65, 66] In extensive vitamin D literature there are substantial data to indicate that "equilibrium" is achieved in 25(OH)D3 levels after a 12 weeks equilibration period. The literature indicates that 3 months is adequate "washout" to re-establish serum levels. [60, 61] The half-life of 25(OH)D3 in the circulation is reported as ≈1 mo in humans, the results for the submariners suggest a 2-mo half-life.[67, 68]. Conventional pharmacology indicates it should take 4 half-lives before a drug's equilibrium is achieved. Unlike a conventional drug, 25(OH)D3 is a metabolite whose concentration can be altered through balance between its production and clearance so that an equilibrium can be achieved earlier than would be expected from the half-life. [69,70]

Monitoring

Parameters noted in the above table will be assessed as per standard clinical laboratory procedures. 25(OH) D3 levels will be measured in the hospital chemistry laboratory and are the standard measure for determination of vitamin D health. PTH levels after basal level will be measured as a research test in the lab of Dr. Johnson.

Procedure for collecting serum samples for PTH measurements.

Collect 5 mL blood in a red top tube (no additives). It is recommended that all samples to be collected A.M (to avoid diurnal variation issues). Blood samples are spun in a refrigerated centrifuge at 2000 x g for 10 min. Divide serum into two aliquots of 1.0 mL each/ patient (this avoids repetitive freezing and thawing every time the assay is performed). Freeze immediately at -70°C in plastic tubes. Under these storage conditions PTH is stable for up to 11 months.

Please call 845-1257 to notify Dr. Johnson's lab of impending sample or if there are questions.

In addition all patients will be offered to enroll, after informed consent, on the DBBR-vitamin D repository trial.

PSA Measurement

Once enrolled on study, individual patients should get their PSA drawn at the same laboratory to ensure correct calculation of PSA dynamics for that individual.

Correlative/Special Studies

Blood samples will be collected and stored for future studies by the RPCI Data Bank and BioRepository (DBBR) for Roswell Park patients only, per Standard Operating Procedures for vitamin D trial banking. The DBBR is a shared resource (RPCI protocol I 03103), where participants are consented to donate blood for research (prior to and following treatments), to complete an epidemiologic questionnaire, and to give permission to have their blood specimens linked to the questionnaire and clinical data including diagnosis and laboratory test results for research. Collected biospecimens and/or data are provided to investigators with RPCI IRB approved research protocols.

7.0 Criteria for Discontinuation

1) Patient does not comply with study guidelines.
2) Patient wants to withdraw
3) Unacceptable side effects attributed to study treatments. e.g. development of genitourinary stones or grade 2 or higher hypercalcemia
4) Patient unable to tolerate the drug. e.g. persistent nausea and vomiting for more than 48hrs or any grade 3 or higher toxicity attributed to study drug.
5) Creatinine ≥ 2.5 X baseline.
6) Need for any active treatment for prostate cancer on clinician's discretion.

7.1 Criteria for initiation of more aggressive treatment

1) PSADT < 3year.
2) Tumor grade progression (e.g. Gleason = or > 7).
3) Clinical progression.
4) Patient choice.
5) Physician discretion.

8.0 Drug information

8.1 Vitamin D3 (Cholecalciferol)

Availability
Vitamin D3 is the standard replacement approach and would be utilized for the purpose of our study. Oral Vitamin D3 supplementation will be provided as capsules provided by Vital Nutrients. Patients will be advised to take 3 capsules once a day to provide 6,000 IU of Vitamin D3.

Administration
Cholecalciferol is to be taken orally as a single AM dose.

Side Effects of Cholecalciferol
Reported side effects are all related to hypercalcemia which can result in nausea, vomiting, constipation, polyuria, nocturia, polydipsia, hypercalcemia and genitourinary stones and rarely cardiac arrhythmias. Refer to product monograph for more details.

8.2 Placebo

Oral placebo will be available in rice powder based capsules which appear identical to vitamin D capsules. It will again be dispensed as 3 capsules a day as a single AM dose.

8.3 Drug Availability

Cholecalciferol (D3) and identical placebo are available as capsules provided by Vital Nutrients.

8.4 Drug ordering:

Cholecalciferol (D3) is commercially available but will be provided free of charge to patient enrolled in this clinical trial. It will be ordered by the Investigational Drug Service through the appropriate grant.

8.5 Agent Inventory records:

The IDS will maintain inventory and dispensing records on the NIH2564 DARF.

8.6 Administration:

Cholecalciferol (D3) and placebo will be taken orally.

9.0 Ethical Requirements

The participant must give a written, informed consent to participate in study. Participants may withdraw from the study at any time, for any reason, without prejudice to their present or future care. In outlining the study, the principal investigator will explain the treatment plan; the potential hazards and benefits to the patient and proposed visit schedule to evaluate the effects of the study. Informed consent will be signed by the participant and an investigator.

10.0 SAFETY EVALUATION

10.1 Adverse Events

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

10.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

10.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.
However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

10.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory

abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

10.1.4 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

10.2 Grading and Reporting Adverse Events

10.2.1 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 5.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 5.0 of the CTCAE is identified and located at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 5.0.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition,

therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

10.3 Reporting Adverse Events:

Routine AEs occurring between the start date of intervention until 30 days after the last intervention, or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

- **Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)**

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

- **Guidelines for Routine Adverse Event Reporting for Pilot, Phase 2, and Phase 3 Studies (Regardless of Expectedness)**

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

10.4 Serious Adverse Events

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

10.4.1 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The Roswell Park SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to **Section 10.6** for details on reporting Unanticipated Problems.

10.5 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.6 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    a. The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
    b. The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious per Section 17.4.

10.6.1 Reporting Unanticipated Problems:

The Reportable New Information (RNI) Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems to the IRB in accordance with their local institutional guidelines.

10.7 Data Safety and Monitoring

If any literature becomes available which suggests that conducting this trial is no longer ethical, the study will be terminated and the IRB will be notified of the new findings. The IRB will be notified of any change in the risk/benefit ratio that would affect whether the study should continue. All serious adverse events will be reported to the IRB according to the established guidelines. A cumulative summary of all adverse events occurring on this study will also be submitted to the IRB at six months review. Toxicity is reported, as required, to the FDA. All study data reviewed and discussed during these meetings will be kept confidential. Any breech in participant confidentiality will be reported to the IRB.

11. Statistical Analysis

Power analysis:

A 2x2 cross-over design with total sample size 100 achieves approximately 80% power to detect 0.5 effect size (the relative magnitude of the effect under the alternative) at significance level 0.05. In other words, assuming the Log_2(PSA) slope prior to therapy is 0.7 ng/ml/yr. with a standard deviation as 0.7 (based on statistical evaluation of data provided by Dr. Vieth from his trial of cholecalciferol replacement in men with PSA rising after local therapy. [55] **,** this design is able detect a 50% reduction of PSA doubling time. [71]

Statistical analysis plan:

This is 2 by 2 cross over trial. To address the research objectives, we want to pool information across periods by taking account of the correlation among repeated responses for each individual. The Log_2(PSA) will be analyzed by linear mixed model in which the fixed effects include treatment (1 for vitamin D3, 2 for control), period (0 for pre study, 1 for first 36 weeks and 2 for second 36 weeks), time (as a continuous variable), time and period interaction, and a variable indicating carry-over effect (1 if the previous treatment assignment is vitamin D3, 2 if the previous treatment assignment is control). The random effect includes random intercept and slope (and a quadratic term if necessary). The significance in the difference between PSA responses for vitamin D3 group and control groups will be assessed by approximate t tests. This will be done using SAS PROC MIXED (SAS 9.1.3., SAS Institute Inc., Cary, NC, USA). The pattern of response of PSA dynamics as well as the absolute change in PSA following vitamin D3 supplementation will be analyzed similarly using the mixed model approach.

In addition to the data analysis described above which analyze Log_2(PSA), for the men who have 3 or more PSA values from the same laboratory over a period of 18 months or more prior to enrollment, PSA velocity (PSAV) will be calculated and compared to a PSA velocity obtained by analysis of the PSA's during the 2 periods of investigation on the study (the 36 weeks on placebo and the 36 weeks on treatment). Specifically, PSAV can be analyzed as a summary statistics as follows: PSAV will be calculated for the pre-study period, the first 36 week study period, and the second 36 week study period for each participant by simple linear regression. As a preliminary step, summary statistics such as sample mean and standard deviations will be calculated for each group at each period (pre study, first 36 weeks, and second 36 week). Furthermore, the difference in the PSAV caused by vitamin D3 treatment will be tested using two-sample t test comparing the means of differences (first 36 week - second 36 week) in PSAV for the first group (vitamin D3 treatment then control sequence) and the second group (control then vitamin D3 treatment sequence).

The toxicity will be compared as difference in proportion with 95% confidence intervals.

Survival would be evaluated on an exploratory basis but no comparison would be made between groups. Relationship between CYP24, 27B1, SNPs and serum 25(OH)D3 vitamin D response to oral D3 supplementation will again be done as an exploratory analysis. We are developing assays to help in this regards and methods for these exploratory analysis would be added as these assays are formulated.

12. PARTICIPANT DIARY

Study Title: Randomized Placebo-Controlled, Double-Blind Study Of Cholecalciferol Replacement in Patients on Expectant Management for Localized Prostate Cancer

- **Take 3 pills daily in the morning**
- **Return pill bottles and diaries at your next clinic appointment**

DATE	Time AM Dose	Comments /Symptoms/ Missed Dose

Participant Signature

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