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Phase II Open Label, Multi-Center Clinical Trial of Modulation of Intermediate Endpoint Biomarkers by 1 α -Hydroxyvitamin D2 in Patients With Clinically Localized Prostate Cancer and High Grade Pin

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Abstract

BACKGROUND—Prostate cancer is the most common malignancy and second leading cause of cancer related deaths in American men supporting the study of prostate cancer chemoprevention. Major risk factors for this disease have been associated with low serum levels of vitamin D. Here, we evaluate the biologic activity of a less calcemic vitamin D analog 1 α -hydroxyvitamin D2 [1 α -OH-D2] (Bone Care International, Inc.) in patients with prostate cancer and high grade prostatic intraepithelial neoplasia (HG PIN).

METHODS—Patients with clinically organ-confined prostate cancer and HG PIN were randomized to 1 α -OH-D2 versus placebo for 28 days prior to radical prostatectomy. Intermediate endpoint biomarkers included serum vitamin D metabolites, TGF β 1/2, free/total PSA, IGF-1, IGFBP-3, bFGF, and VEGF. Tissue endpoints included histology, MIB-1 and TUNEL staining, microvessel density and factor VIII staining, androgen receptor and PSA, vitamin D receptor expression and nuclear morphometry.

RESULTS—The 1 α -OH-D2 vitamin D analog was well tolerated and could be safely administered with good compliance and no evidence of hypercalcemia over 28 days. While serum vitamin D metabolite levels only slightly increased, evidence of biologic activity was observed with significant reductions in serum PTH levels. TGF- β 2 was the only biomarker significantly altered by vitamin D supplementation. Whether reduced TGF- β 2 levels in our study is an early indicator of response to vitamin D remains unclear.

CONCLUSIONS—While further investigation of vitamin D may be warranted based on preclinical studies, results of the present trial do not appear to justify evaluation of 1 α -OH-D2 in larger clinical prostate cancer prevention studies.

Keywords

prostate cancer; vitamin D; chemoprevention

INTRODUCTION

Prostate cancer is the most common malignancy and second leading cause of cancer related deaths in American men. Recent yearly estimates report >241,740 men diagnosed with and approximately 28,170 men dying from prostate cancer in 2012 [1]. The high incidence and lethality of the disease and the morbidity associated with curative therapy strongly support the need for studying prostate cancer chemoprevention [2]. There are specific groups of patients who are at high risk of being diagnosed with prostate cancer and may benefit from chemopreventive agents as a primary cancer prevention strategy. For instance, patients with high grade prostatic intraepithelial neoplasia (HG PIN) or a strong family history of prostate cancer are at significant risk for subsequent diagnosis of this disease [3,4]. There is also a role for secondary prevention of prostate cancer in patients who have been treated for localized prostate cancer and are at risk for tumor recurrence [5]. As of this time only two Phase III trials for prostate cancer prevention have been completed, both of which feature 5- α reductase inhibitors. The Prostate Cancer Prevention Trial (PCPT) with finasteride and the REDUCE trial (dutasteride) were found to confer respectively a 25% and 23% reduction in the incidence of biopsy-proven prostate cancer [6,7].

Interest in the relation of vitamin D to prostate cancer risk was raised by observations by Schwartz and Hulka who noted that the major risk factors for the development of clinical prostate cancer (old age, black race, and residence in northern latitudes) are associated with low serum levels of vitamin D [8,9]. They hypothesized that vitamin D helps to maintain the differentiated state of prostate cells and that in the presence of low levels of vitamin D, subclinical cancers can progress to clinical disease. Corder et al. [10], in 1993, published results of a cohort study showing an inverse relationship between pre-diagnostic vitamin D levels in samples of stored sera and subsequent development of prostate cancer. More recently, Ahonen et al. [11], showed that men in Finland with greater serum concentrations of vitamin D were at reduced risk of prostate cancer, whereas a subsequent larger study by this group [12] carried out in Finland, Norway, and Sweden also revealed increased risks for men at the lowest concentrations as well as at the highest concentrations of serum vitamin D.

The primary circulating form of vitamin D is 25-hydroxyvitamin D₃ [25(OH)D₃], a prohormone that can be supplied from dietary sources and generated endogenously from sunlight exposure [13]. Prostate and renal cells can convert 25(OH)D₃ to 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the biologically active form which influences cellular differentiation, proliferation, and apoptosis in prostate cancer cell lines in vitro [14–20]. Skowronski et al. showed that treatment of the androgen responsive prostate cancer cell line, LNCaP, with 1,25(OH)₂D₃ results in inhibition of growth with an IC₅₀ of 1–10 nM [normal values for 1,25(OH)₂D₃ are 35–125 pM [21]] as well as evidence of differentiation as manifested by an increase in PSA production per cell [22]. The same group was able to demonstrate similar effects with a number of vitamin D analogues [23].

The clinical development of 1,25(OH)₂D₃ has been limited primarily by problems with hypercalcemia. This has led to interest in the development of vitamin D analogues with the goal of retaining the anti-proliferative and differentiating properties of the naturally occurring metabolites, but with less calcemic activity. The purpose of this study was to evaluate the biologic activity of a less calcemic vitamin D analog 1 α -hydroxyvitamin D₂

[1 α -OH-D₂] (Bone Care International, Inc) [24–26] in patients with HG PIN and clinically organ-confined prostate cancer undergoing radical prostatectomy. More specifically, the primary objective of this study was to determine if 1 α -OH-D₂ modulates intermediate endpoint biomarkers (IEB) of potential significance in the development of prostate cancer.

PATIENTS AND METHODS

This study was designed as a phase II open label, randomized, controlled trial of 1 α -OH-D₂ being conducted jointly at the University of Wisconsin Carbone Cancer Center, the Medical College of Wisconsin, and the University of Iowa. Patients diagnosed with either HG PIN and/or organ-confined prostate cancer who were scheduled for radical retropubic prostatectomy (RRP) were eligible for enrollment in this study. This study was designed with an accrual goal of 60 patients. Patients were randomized to 1 α -hydroxyvitamin D₂ 10 μ g po $\times$ 4 weeks (28 days) prior to surgery versus observation.

Eligibility

Patients who are candidates for a prostatectomy had to satisfy a set of inclusion and exclusion criteria to be eligible for the study. Inclusion criteria were as follows: histologic confirmation of adenocarcinoma of the prostate; prostate cancer confined to the prostate (clinical judgement of the surgeon); adequate organ function (marrow: WBC 4,000/mm³; platelets 100,000/mm³; hepatic: bilirubin 1.4 mg/day; AST 3 $\times$ normal; renal: creatinine 2.0 mg%; Metabolic: serum calcium 10.2 mg/dl). Patients were required to sign a consent form indicating the investigational nature of the treatment and its potential risks.

Exclusion criteria were as follows: prior hormone therapy, brachytherapy, or external radiation therapy for prostate cancer; concurrent hormone therapy, including LHRH agonist, antiandrogens, glucocorticoids, ketoconazole, finasteride, dutasteride, DES or progestins; prior therapy with vitamin D and/or calcium supplements was allowed, but was to be discontinued 7 days prior to enrollment; concurrent magnesium-containing antacids, thiazide diuretics, phenytoin, phenobarbital, glutethimide or digoxin; and idiopathic urinary calcium stone disease.

Safety Labs

Prior to the study, blood was drawn for baseline safety parameters (BUN, creatinine, electrolytes (sodium, potassium, chloride, and CO₂), bilirubin, AST, LDH, alkaline phosphatase, WBC, differential, platelets), testosterone, PTH, serum calcium, and serum phosphate. On day 8 $\pm$ 3 days, day 15 $\pm$ 3 days subjects receiving 1 α -OH-D₂ had safety labs drawn (serum calcium, phosphate, albumin, and creatinine). At the time of prostatectomy all pre-study studies were repeated (in all subjects). A 24-hr urine specimen was also collected for calcium, phosphate, and creatinine pre-study and at the time of surgery.

Serum Intermediate Endpoint Biomarkers

Serum biomarkers measured pre-study and again at the time of surgery included serum vitamin D metabolites (required only for subjects receiving 1 α -OH-D₂), and serum/plasma TGF β 1 and 2 (plasma); serum free/total PSA; plasma IGF-1, IGFBP-3, plasma bFGF and VEGF (normalization to platelet count was done at each serum/plasma biomarker time point). VEGF, basic FGF, plasma TGF β 1 and 2, free/total PSA; IGF-1, IGFBP-3 levels were measured by a commercially available sandwich immunoassay (Quantikine human, R&D Systems, Minneapolis, MN, Tandem-MP Hybritech, Beckman Coulter, Fullerton, CA). Vitamin D metabolites were evaluated in a clinical laboratory by standard methods. (Bone Care International, Madison, WI).

Tissue Histology and Intermediate Endpoint Biomarkers

Diagnostic biopsy tissue (pre-treatment) and prostatectomy tissue (post-treatment) were examined for changes in IEB. Cancerous, PIN, and normal prostate tissue areas were examined in two cohorts of patients, control (untreated) and 1 α -OH-D₂ treated.

In addition, samples from diagnostic biopsies and prostatectomy tissue were evaluated for proliferative index—MIB-1 immunohistochemistry; apoptotic index (TUNEL staining); microvessel density and factor VIII staining; differentiation markers—immunochemistry of androgen receptor (AR) and PSA; vitamin D receptor assay by ELISA and immunohistochemistry; nuclear morphometry (computer assisted).

Statistical Considerations and Data Analysis

In this study nearly all the intermediate endpoint biomarkers in the study were viewed as continuous random variables, and statistical power was computed based on a two-sample *t*-test for group differences in mean changes from baseline, at a two-sided overall significance level 0.10. Because of a long panel of nearly 15 IEBs, each test was performed at a significance level 0.10/15 (Bonferroni adjustment) to account for multiplicity. Power calculation was based on the standardized variables, where the effect size was defined by the difference between the means for the two groups divided by the standard deviation of the variable. A sample size of 30 evaluable subjects per group provided 85% power for an effect size of 1 standard deviation. An evaluable subject was one who had a prostatectomy performed and received at least one dose of drug. Unevaluable patients in the treatment arm were replaced.

Patients were assigned to the treatment group or observation only group using a simple randomization. Randomization was performed centrally by the University of Wisconsin site coordinator, and eligible patients randomized to 1 α -OH-D₂ must have been at least 75% compliant to be included in the analysis. Separate analyses based on the intent-to-treat principle were also performed and compared. Median and mean changes in all IEBs were reported by group, and group differences in the means of the IEBs at the time of prostatectomy were assessed using two-sample *t*-statistics. In instances where assays associated high values with higher measurement error variance, log-transformations were required. Serum/plasma biomarkers were collected weekly, providing the opportunity to see if they stabilize over time in the treatment group. Nuclear morphometric features were assessed, with summary values describing radius, perimeter, area, compactness, smoothness, concavity and symmetry. The correlation of these features with other IEBs was examined, using multiple regression to see which features are most highly associated.

In general, statistical tests were performed on differences between measurements, as well as between change from baseline values. To compare continuous and ordinal biomarkers between study arms, Wilcoxon rank-sum test was used. To test for significant change in levels within one group (such as with serum vitamin D metabolite) Wilcoxon signed-rank test was performed. Data was analyzed using SAS version 9.1. (SAS software, Version 9.1 of the SAS System for Linux. Copyright (c) 2002–2003 SAS Institute Inc., Cary, NC, USA).

RESULTS

Accrual and Patient Characteristics

The study was activated for patient accrual in July 2001, but patient accrual was terminated early due to 1 α -OH-D₂ supply issues with the last patient randomized in May 2005. Ultimately 31 patients were enrolled (approximately 8 subjects/year) and randomized to

receive either 1α -OH-D₂ 10 µg daily (n = 16) versus observation alone (n = 15) for 4 weeks (28 days) prior to RRP (Fig. 1).

The baseline patient characteristics are summarized in Table I.

Study Compliance

Compliance with study drug was excellent with 15 of the 16 patients on 1α -OH-D₂ taking 95% of study drug and 11 of these 15 taking 99% of study drug.

Serum concentrations of 1α -OH-D₂, endogenous vitamin D metabolite (1,25(OH)₂D₃) levels and plasma intact PTH levels were measured at baseline, days 8, 15, 21, and post-treatment.

Figure 2a summarizes the absolute change from baseline in serum vitamin D metabolite levels (metabolites of both the analog and endogenous vitamin D collectively measured as total 1,25 dihydroxy vitamin D) by visit for the treatment arm only. Absolute mean levels were 54.0, 73.6, 53.3, 62.6, and 64.5 ng/ml at baseline and days 8, 15, 21, and 28, respectively. A non-significant increase in vitamin D metabolite on days 8 and 28 ($P = 0.219$ and $P = 0.148$) was seen. Of note there was no significant difference ($P = 0.790$) in endogenous serum vitamin D levels between the two arms at baseline (62.1 vs. 54.0 ng/ml, observation vs. treatment arms, respectively).

Figure 2b summarizes the absolute change from baseline in plasma intact PTH by visit. Absolute mean PTH levels at baseline and days 8, 15, 22, and 28 were 50.7, 51.5, 55.1, 68.6, and 60.9 ng/ml versus 53.2, 26.8, 22.9, 23.2, and 23.3 ng/ml for the observation and treatment arms, respectively. At baseline, there was no significant difference in PTH across the arms ($P = 0.648$). On days 8, 15, 22 and 28, there were significant differences ($P < 0.05$) in PTH in absolute changes from baseline across the arms ($P = 0.030, 0.010, 0.003$, and < 0.001 , respectively). Serum testosterone concentrations for the observation and treatment arms were respectively 334.6 and 337.3 ($P = 0.442$) at baseline. A non-significant increase in testosterone levels was observed in the treatment arm off study (337.2 vs. 389.5, $P = 0.350$).

Toxicity and Adverse Events

Adverse events by worst grade is given in Table II.

The vitamin D analog 1α -OH-D₂ was well tolerated with only one grade 3 adverse event (6.2%) reported for the treatment arm and none reported for the control arm ($P = 0.325$). The control arm had eight (53.3%) patients with an adverse event while on study as compared to six (37.5%) in the treatment arm. In terms of treatment arm subjects with adverse events and attribution, one subject had grade 1 elevated serum creatinine and calcium deemed probably related to the study treatment, two subjects had grade 1 events (diarrhea, flatulence, hypercalcemia) considered possibly or unlikely related to the study treatment and three treatment arm patients had events considered unlikely or not related to study treatment.

Tissue Histology and Intermediate Endpoint Biomarkers

Assessment of histologic parameters of neoplasia (PIN, Gleason grade of adenocarcinoma) revealed no significant differences between the groups at baseline ($P = 0.148$ for PIN, $P = 0.365$ for Gleason grade) or end of study prostatectomy samples ($P = 0.089$ for PIN, $P = 0.647$ for Gleason grade). No differences between the groups were noted in other tissue parameters including MIB-1 expression, TUNEL staining, microvessel density (MVD) or

factor VIII expression. Additionally, expression of AR and PSA in adenocarcinoma did not significantly differ between groups.

Nuclear morphometry measurements with summary values describing radius, perimeter, area, compactness, smoothness, concavity and symmetry revealed no observed significant differences between groups in this study.

Attempts to measure prostatic vitamin D receptor (VDR) activity by VDR ELISA in fresh frozen tissue samples from prostatectomy were unsuccessful with activity levels below the limits of the assay.

Serum and Plasma Intermediate Endpoint Biomarkers

Potential surrogate tumor markers including serum total and free/total PSA, and IGF/IGFBP-3, as well as bFGF, VEGF, and TGF β 1/2 as markers of systemic response with normalization by platelet count are summarized in Table III.

No significant differences in absolute values were observed between the groups when normalized by platelet count. The absolute change in these parameters from baseline was also determined, and the absolute change from baseline for TGF- β 2 was found to be significantly lower in the treatment arm on day 21 ($P = 0.016$) and on day 28 ($P = 0.001$), although it should be noted that there was a baseline imbalance for TGF- β 2; the baseline value of TGF- β 2 was significantly higher in the treatment arm ($P = 0.015$). This comparison with absolute values as well is shown in Figure 2c. There was no significant difference on day 15 ($P = 0.443$). With the exception of a decrease in total PSA at one time point (day 21, $P = 0.024$), no significant differences between arms for absolute change from baseline were observed for serum total PSA at remaining timepoints or for free/total PSA ratio, IGF-1, IGFBP-3, VEGF, bFGF, or TGF- β 1.

No significant differences between the groups were observed for safety serum labs, although a trend toward lower serum potassium in the treatment arm was observed at the off-study visit (a change from baseline of -0.10 mEq/L for treatment vs. 0.22 mEq/L for observation, $P = 0.051$), and significantly higher urinary calcium on day 15 (9.06 mg/dl for observation arm vs. 9.57 mg/dl in treatment arm, $P = 0.048$) and a difference in phosphate (2.64 mg/dl in observation arm vs. 3.67 mg/dl in treatment arm, $P = 0.004$) excretion was observed on day 15; the changes from baseline for urinary calcium and phosphate were not significantly different from each other.

DISCUSSION

The observed associations between Vitamin D parameters and prostate cancer risk and in vitro and in vivo evidence of beneficial biological activity in prostate cancer have led to considerable interest in Vitamin D and analogs. Yet in this open label, randomized phase II study of the vitamin D analog 1α -OH-D₂, in men undergoing prostatectomy for early stage prostatic neoplasia, no obvious beneficial effects were observed. Several possible explanations for this lack of observed effect exist. Leading considerations include being underpowered, due to low accrual, thus not being able to detect anything other than a large biological effect; the exposure time of 28 days may be insufficient in duration; or this analog at this dose has minimal biological effect. The lack of any trends in our data other than decreased serum TGF β imply only a small effect, if any exists with this dose and duration for 1α -OH-D₂, but clearly our study ended up being underpowered, so the lack of observed effect needs to be assessed relative to that. Similar to our prior clinical studies, this dose of 1α -OH-D₂ does result in a measurable biological effect on calcium homeostasis as evidenced by the decrease in serum PTH and increase in urinary calcium excretion.

However it may not be sufficient to produce other end-organ effects, for example, prostate tissue effects.

Reasons for slow accrual include patients' reluctance to undergo additional preoperative visits and blood sampling during the interval between study initiation and surgery. For many patients, it was simply a matter of transportation to the hospital. Our current pre-prostatectomy studies, which accrue better, have limited the number of required study visits before prostatectomy. Our current studies are also double-blinded as compared to the open label approach of this study.

Despite low accrual to the study, we were able to make several important observations. For instance, 1α -OH- D_2 vitamin D analog is well tolerated and can be safely administered to patients with prostate cancer with good compliance and no evidence of hypercalcemia over 28 days, similar to our prior clinical experience with this analog in subjects with more advanced prostate cancer (3/35 patients randomized to 1α -OH- D_2 had grade 2 hypercalcemia) [27]. Serum levels of vitamin D metabolites only slightly increased over a 28 day interval. Nevertheless, evidence of vitamin D biologic activity was observed with significant reductions in serum PTH levels. This decrease in serum PTH might be predicted as a compensatory response to vitamin D supplementation and has been previously reported [28].

Even with evidence of a metabolic response with a reduction in PTH levels, TGF- β 2 was the only intermediate endpoint biomarker significantly altered by vitamin D supplementation. Interestingly, both benign and malignant prostate cells have been shown to produce latent TGF- β 2 which in turn is activated by PSA [29]. Furthermore, other research has shown that TGF- β 2 plays a role in NF κ B induction and resistance to apoptosis in prostate cancer [30]. Whether reduced TGF- β 2 levels in our study is an early indicator of response to vitamin D or may provide benefit in terms of preventing prostate cancer progression remains unclear.

Serum PSA was not significantly altered, suggesting that either there were insufficient tissue levels of vitamin D in the prostate, inadequate time of exposure to produce an effect, or simply a lack of activity of vitamin D in the human prostate, contrary to preclinical evidence [9–17]. Similarly, IGF/IGFBP3 has been reported as a surrogate tumor marker of response in prostate cancer [31] whereby normalized levels of IGF/IGFBP3 were unaltered in our study. No other serum biomarkers were significantly altered by vitamin D in our study. Activity levels of prostatic vitamin D receptor (VDR) activity were undetectable by VDR ELISA, and while reasons for this are unclear, this may have been due to unavailability of the antigen due to nuclear localization of this receptor. Alternatively, all available receptor sites may have been occupied such that detection of VDR activity was not possible.

Essentially no significant effect of vitamin D was observed at the tissue level. Specifically, no difference in tissue histology was observed in the two groups either pre-study or at surgery. Furthermore, no difference was observed in tissue markers of biologic activity, including MIB-1 as a marker of proliferation, apoptotic index, microvessel density or corresponding expression of factor VIII. Markers of differentiation including expression of PSA and the androgen receptor were also unaltered. Finally, nuclear morphometry, measurements of nuclear size and shape, has also been reported to be of prognostic significance in prostate cancer [32]. In a study of Gleason score, stage, and 10 nuclear morphometric factors (including mean nuclear area, nuclear perimeter, shortest and longest nuclear axis, and form factor), nuclear area had independent prognostic significance in low stage (T1) tumors [32]. While this is a promising surrogate marker of response in prostate cancer, no difference was observed between groups in this study.

Aside from the issue of low accrual, another reason for lack of observed effect in tissue is that tissue levels of vitamin D may have been inadequate. We were unable to determine tissue levels of vitamin D in this study, which would have required frozen tissue. One other potential problem which may have been addressed by frozen tissue harvesting is that with evaluation of molecular endpoints in biological specimens, molecular changes may have occurred in the interval between prostatectomy and paraffinization of the tissue specimens, and frozen specimen acquisition may be more advantageous in future studies.

CONCLUSION

The vitamin D analog 1 α -OH-D₂ is well tolerated in patients with prostate cancer. However, biologic activity of this agent was minimal in both serum and tissue assays of biomarker response in patients with organ-confined prostate cancer, which is evident even given limitations imposed by low accrual to this study. While further investigation of vitamin D may be warranted based on preclinical studies, results of the present trial do not appear to justify further evaluation of 1 α -OH-D₂ in larger clinical prostate cancer prevention studies.

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Fig. 1.
Studyschema.

Fig. 2.

a: Serum vitamin D metabolite (ng/ml) by visit for the treatment arm. A slight increase in vitamin D metabolite on days 8 and 28 was observed which is non-significant ($P=0.219$ and $P=0.148$ for Wilcoxon signed-rank test). **b:** Absolute change for plasma intact PTH by visit. Absolute changes from baseline show significant differences across the arms for each visit with $P=0.030$ for day 8, $P=0.010$ for day 15, $P=0.003$ for day 21, and $P=0.001$ for day 28. **c:** Absolute change from baseline for TGF-β2 (with platelet normalization), found to exhibit a lower trend for the treatment arm for day 21 ($P=0.068$) and was significantly lower for day 28 ($P=0.007$, $P=0.012$ [LOCF]) as shown. No significant difference between arms was observed for day 15 ($P=0.318$).

TABLE I**Baseline Patient Characteristics ***

Characteristic	Placebo (n = 15)	Vitamin D (n = 16)
White (%)	14 (93)	16 (100)
Black (%)	1 (7)	0 (0)
Age (years)	57.9 ± 6.2	59.9 ± 5.8
Body mass (kg)	94.6 ± 12.4	88.0 ± 11.6
Body mass index (kg/m ²)	29.6 ± 3.3	28.6 ± 4.0
Systolic blood pressure	132.0 ± 13.5	127.3 ± 11.0
Diastolic blood pressure	79.0 ± 7.9	80.0 ± 6.7
Mean Gleason score	6.56 ± 0.88	6.20 ± 1.32
Serum PSA	6.8 ± 5.3	11.7 ± 12.4

* Presented as percentage of group or mean ± SD.

TABLE II

Adverse Events by Worst Grade: All Grade II and Grade III Adverse Events and Any Adverse Events Occurring in More than One Patient

Toxicity	Observation (n = 15)			Vitamin D (n = 16)		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Chills	0	0	0	0	0	1 (6.3%)
Cough	1 (6.7%)	0	0	1 (6.3%)	0	0
DIC	0	0	0	0	0	1 (6.3%)
Headache	1 (6.7%)	0	0	1 (6.3%)	0	0
Hypophosphatemia	0	1 (6.7%)	0	0	0	0
Low ANC	0	0	0	0	0	1 (6.3%)
Low WBC	0	0	0	0	0	1 (6.3%)
Low platelets	0	0	0	0	0	1 (6.3%)
Sweats	0	0	0	0	1 (6.3%)	0

TABLE III

Serum Intermediate Endpoint Biomarkers

	Baseline	Day 15	Day 21	Off Study	Off study (LOCF)	Standard deviation
Total PSA (ng/ml)						
Obs	6.8	10	10.3	10.5	9.2	3.94
Vit D	11.7	8.9	8.3	11	9.9	8.89
<i>P</i> -value *		0.397	0.024	0.077	0.156	
Free/total PSA ratio						
Obs	0.59	0.78	0.88	0.59	0.52	0.40
Vit D	0.47	0.47	0.45	0.37	0.34	0.43
<i>P</i> -value *		0.458	0.156	0.532	0.678	
VEGF (pg/10E06 platelets)						
Obs	0.441	0.213	0.392	0.236	0.219	0.66
Vit D	0.21	0.158	0.15	0.129	0.141	0.09
<i>P</i> -value *		0.609	0.953	0.289	0.458	
IGF-1 (μg/10E06 platelets)						
Obs	0.433	0.426	0.435	0.419	0.418	0.0823
Vit D	0.408	0.433	0.458	0.4	0.4	0.0535
<i>P</i> -value *		0.599	0.413	0.682	0.743	
IGFBP-3 (μg/10E06 platelets)						
Obs	0.008	0.007	0.008	0.007	0.008	0.0011
Vit D	0.012	0.008	0.008	0.008	0.011	0.0013
<i>P</i> -value *		0.518	0.829	0.484	0.536	
bFGF (pg/10E06 platelets)						
Obs	0.034	0.017	0.027	0.051	0.045	0.1203
Vit D	0.007	0.009	0.011	0.008	0.007	0.0058
<i>P</i> -value *		0.701	0.71	0.4	0.432	
TGF β1 (pg/10E06 platelets)						
Obs	0.036	0.045	0.048	0.039	0.039	0.0198
Vit D	0.043	0.026	0.023	0.034	0.037	0.0378
<i>P</i> -value *		0.25	0.386	0.26	0.471	
TGF β2 (pg/10E06 platelets)						
Obs	0.74	0.77	0.76	0.77	0.73	0.25
Vit D	1.23	0.75	0.7	0.71	0.83	0.36
<i>P</i> -value *		0.443	0.016	0.001	0.005	

* *P*-value comparing absolute change from baseline.