

Phase I Trial of 1α -Hydroxyvitamin D_2 in Patients with Hormone Refractory Prostate Cancer¹

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ABSTRACT

This Phase I study of 1α -hydroxyvitamin D_2 , an *p.o.* administered vitamin D analogue, in patients with advanced hormone-refractory prostate cancer was designed to assess the toxicity, pharmacokinetic and biological markers of drug activity, and lastly tumor response data to recommend a dose for Phase II studies.

1α -Hydroxyvitamin D_2 was administered daily at doses ranging from 5 to 15 μ g/day. Patients were monitored for toxicity and tumor response, and blood and urine samples were collected for pharmacokinetics ($1\alpha,25$ -dihydroxyvitamin D_2 levels) and other parameters of biological activity (bone markers, parathyroid hormone, urine calcium, and serum phosphorus levels).

Twenty-five patients were enrolled. Main toxicities were hypercalcemia with associated renal insufficiency. No other significant toxicity was seen. Pharmacokinetics showed an increase in the active metabolite $1\alpha,25$ -dihydroxyvitamin D_2 that reached a plateau by week 4 despite continuous drug dosing. Elevation in daily urinary calcium excretion and serum phosphorus levels was seen, whereas a decrease in serum parathyroid hormone was evident. Two patients showed evidence of a partial response, whereas 5 others achieved disease stabilization for ≥ 6 months.

1α -Hydroxyvitamin D_2 was well tolerated with main toxicities being hypercalcemia and renal insufficiency. All of the toxicity was reversible with drug discontinuation. Evidence for drug activity was seen in surrogate markers, and pharmacokinetic analysis showed substantial increases in

vitamin D metabolite levels among the various cohorts. Whereas the defined maximum tolerated dose was not reached, the recommended Phase II dose was 12.5 μ g/day given continuously.

INTRODUCTION

Prostate cancer is the most common malignancy among males in the United States and will be responsible for 189,000 new cases and 30,200 deaths for the year 2002 alone (1). Whereas the standard approach in patients with advanced disease is to initiate androgen ablation therapy, nearly all of these patients will unfortunately develop androgen-independent disease after a median of 18–24 months of hormonal therapy. Second- or third-line hormonal manipulations are minimally effective. Despite palliative activity, chemotherapeutic regimens have yet to show a clear improvement in terms of overall survival (2–4), and other therapeutic options remain limited. The median survival in patients with advanced androgen-independent disease remains relatively short (median 12 months). Thus, the search for newer agents in the treatment of HRPC³ is needed. Whereas different chemotherapy regimens are always being investigated, novel agents targeting angiogenesis, signal transduction, and tumor differentiation may be more promising.

In 1990, it was observed by Schwarz and Hulka (5) that major risk factors for developing prostate cancer included age, black race, and residence in a northern latitude. They questioned whether vitamin D deficiency was the unifying factor, because all three of the risks were associated with decreased levels of the vitamin. It was hypothesized that vitamin D maintains the differentiated phenotype of prostate cells, and in the presence of low-levels of vitamin D, subclinical prostate cancer may progress to clinical disease. This hypothesis was supported by a study suggesting an increase in prostate cancer mortality in patients with a decreased exposure to UV radiation, as determined by their geographic location of residence (6). This observation was additionally supported by a retrospective analysis measuring vitamin D levels from stored blood of 181 men who later developed prostate cancer. Controls were taken from same-day blood samples in men who did not develop prostate cancer. A significantly lower mean serum $1,25$ -dihydroxyvitamin D_3 (calcitriol) was found in patients who later developed prostate cancer compared with controls ($P = 0.002$). In men > 57 years of age, the calcitriol level was predictive of risk in palpable and anaplastic tumors but not incidental or well-differentiated tu-

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³ The abbreviations used are: HRPC, hormone-refractory prostate cancer; VDR, vitamin D receptor; PSA, prostate-specific antigen; BCI, Bone Care International; MTD, maximum tolerated dose; 1α -OH- D_2 , doxercalciferol; DLT, dose-limiting toxicity; PTH, parathyroid hormone; CT, computed tomography; SD, stable disease; PR, partial remission.

mors (7). A similar study in Finland looking at stored sera for 25-hydroxyvitamin D showed similar results (8). Thus, vitamin D may be protective with regards to prostate cancer.

Vitamin D plays a vital role in calcium homeostasis, and VDRs are found in the bones, intestines, and kidneys. Surprisingly, VDRs have also been found in many other cell types where this seco-hormone likely plays a key part in the growth and differentiation of these tissues. VDRs in human prostatic tissue were first demonstrated in 1988 (9). In 1992, Miller *et al.* (10) demonstrated biologically active VDR in the human prostate cancer cell line LNCaP, and subsequently, VDR were found by Skowronski *et al.* (11) not only in LNCaP, but also in DU145 and PC3 prostate cancer cell lines. *In vitro* cultures of these prostate cancer lines with physiological levels of calcitriol inhibited proliferation of LNCaP and PC3 but not DU145. Calcitriol also caused a dose-dependent stimulation of PSA production in LNCaP cells suggesting that vitamin D was both antiproliferative and differentiating in prostate cancer cells (12).

Because of the preclinical data suggesting that vitamin D inhibits the growth and development of certain tumors, clinical trials using oral calcitriol in prostate (13) and hematologic malignancies (14, 15) were conducted. As expected, the DLT observed was hypercalcemia. No antitumor activity was seen, but this was attributed to difficulties in administering an adequate dose of calcitriol secondary to the development of hypercalcemia. Subsequently, a small pilot study in patients with biochemical failure after radical prostatectomy or radiation therapy showed prolongation of the PSA doubling time using calcitriol (16). Because the therapeutic benefit of calcitriol was difficult to exploit because of hypercalcemic side effects, less hypercalcemic vitamin D analogs have been developed for clinical testing. Biological effects have been observed with these analogs in colon (17, 18), bone (19), and breast cancer cell lines (20). Many of these have also shown similar or increased activity compared with calcitriol in prohibiting prostate cancer growth *in vitro* with decreased hypercalcemia. These studies confirm that the inhibitory growth properties of vitamin D can be enhanced with analogs, as long as the binding affinity for the VDR is retained (21–23). One such analogue of interest is 1α -OH- D_2 .

1α -OH- D_2 is a vitamin D analogue developed by BCI (Madison, WI). This analogue is an inactive precursor of two naturally occurring hormones, $1\alpha,25$ -dihydroxyvitamin D_2 and $1\alpha,24$ -dihydroxyvitamin D_2 , which are produced *in vivo* by hepatic target cell hydroxylation at carbons 25 and 24 respectively. Preclinical studies have shown decreased calcemic effects relative to calcitriol with equal or greater growth inhibitory effects (24, 25). The reduced calcemic effects of 1α -OH- D_2 have been attributed to: (a) the lack of first pass stimulation of the intestinal calcium transport; (b) the controlled and sustained hepatic release of its two active forms into the circulation; and (c) the markedly reduced calcemic activity of $1\alpha,24$ -dihydroxyvitamin D_2 .

Clinical testing of 1α -OH- D_2 has been conducted in healthy males, women with postmenopausal osteoporosis, and in patients with end-stage renal disease. Single doses ranging from 2 to 8 μ g resulted in no adverse events in the healthy volunteers (26). Similarly, 15 postmenopausal osteopenic women were given increasing oral doses of 1α -OH- D_2 , at 0.5, 1,

2, 4, and 5 μ g/day. In 5 of these subjects, the dose was additionally increased to 8 or 10 μ g/day. No subjects had hypercalciuria at doses <5.0 μ g/day; 5 subjects had hypercalciuria at or above 5 μ g/day (3 at 5 μ g/day, 1 at 8 μ g/day, and 1 at 10 μ g/day). Mean serum calcium increased slightly on the 4 μ g dose ($P < 0.05$) but remained well within the normal range. Mean creatinine clearance and blood urea nitrogen, used as measures of renal function, showed no significant changes (27). In 1999, an oral formulation of 1α -OH- D_2 (Hectoral) was approved for use in patients with hyperparathyroidism undergoing chronic hemodialysis (28, 29).

Given the preclinical data as well as early clinical work, this dose escalation trial using 1α -OH- D_2 was conducted to determine the MTD in patients with advanced HRPC, starting at an established safe dose of 5 μ g/day.

PATIENTS AND METHODS

Eligibility

Patients with histologically confirmed evidence for adenocarcinoma of the prostate with advanced hormone-refractory disease were eligible for this study. All of the patients needed to have evaluable disease, which included either bidimensionally measurable disease or bone scan alone abnormalities associated with a serum PSA >10 μ g/ml. Furthermore, all of the patients had to show progressive prostate cancer with documentation of a rising PSA taken at least 2 weeks apart and being at least 50% over the baseline values obtained during successful hormonal interventions. PSA abnormalities alone were not considered evaluable, and those patients were excluded from this trial. Eligibility criteria also included: age >40 years, an Eastern Cooperative Oncology Group performance states ≤ 2 , and life expectancy >3 month. Other requirements included: adequate bone marrow function with absolute neutrophil count $\geq 1,200/\mu$ l, hemoglobin ≥ 8 g/dl, and platelets $\geq 100,000/\mu$ l; stable renal function with creatinine ≤ 1.8 mg/dl; and adequate hepatic reserve with normal bilirubin and aspartate aminotransferase ≤ 2.5 times the upper limit of normal. A corrected serum calcium ≤ 10.2 mg/dl was needed at study entry. Patients were excluded if they had received more than two prior cytotoxic chemotherapy regimens (or chemotherapy within 4 weeks of beginning study), received radiotherapy within 4 weeks (8 weeks for strontium) of start date, had received suramin in past, or have any history of brain metastasis. Any history of idiopathic urinary calcium stone disease, chronic hypercalcemia, gastrointestinal malabsorptive conditions, or current use of digitalis was prohibited. Patients who did not have an orchiectomy were continued on their luteinizing hormone-releasing hormone during the study although the antiandrogen was discontinued at least 4 weeks before start date. Uncontrolled infections or other serious intercurrent medical illnesses were not allowed. All of the patients gave written informed consent in compliance with state, federal, and institutional guidelines.

Drug Formulation

1α -OH- D_2 was provided by BCI in soft, gelatinized capsules in units of 2.5 μ g/capsule. Inactive ingredients in order of decreasing weight included: fractionated coconut oil, gelatin,

glycerin, titanium dioxide, D&C yellow no. 10, ethanol, and butylated hydroxyanisole.

Dosage and Drug Administration

On the basis of prior clinical experience in postmenopausal women with osteoporosis (27), 1 α -OH-D₂ at 5 μ g/day p.o. was chosen as a safe starting dose. Capsules were administered on an empty stomach before the first meal of each day. Dose escalation for each new cohort then increased by one capsule or 2.5 μ g/day increments. No attempts were made to ameliorate the calcemic effects of the drug with dietary manipulations or other medications.

Study Design

This study was planned using a standard design with patients enrolled in cohorts of three. Subsequent dose escalation was not begun until at least 3 patients were treated for a minimum of 4 weeks without complication. On the basis of the number of patients that experience a DLT, additional patients would be added at the same or previous level. Once ≥ 2 of 6 patients experience a DLT at a given level, the MTD would be considered exceeded, and the previous dose level was considered the MTD. Additional patients would be added at the MTD to additionally define toxicity at this dose.

Toxicity was graded using the National Cancer Institute common toxicity criteria. A DLT was defined as any toxicity grade ≥ 3 , occurring at any time while on study, which was probably or definitely related to 1 α -OH-D₂ therapy. For this study, asymptomatic hypercalciuria was not considered a DLT. A grade 3 or higher hematologic toxicity manifesting as low hemoglobin alone was also not considered a DLT, and transfusions were allowed without the need for dose modification. Otherwise, any grade 3 or higher toxicity required holding drug until resolution of toxicity (grade ≤ 2), then resuming at the next lowest dose. Any patient experiencing a DLT at the lowest dose (2.5 μ g/day) was removed from the study. Only patients completing ≥ 1 course (4 weeks) of therapy were evaluable for toxicity (unless DLT achieved).

Pretreatment Evaluation and Follow-Up Assessments

Standard Tests. All of the patients were evaluated before enrollment with a full history, complete physical examination, and estimation of performance status. In addition, a complete blood count, liver function tests, fasting chemistry profile (including blood urea nitrogen and creatinine, electrolytes with calcium and phosphorus, and albumin), and PSA were obtained. Patients on study had serum calcium and phosphorus levels repeated weekly for the first month of treatment. Before each new course (28-day cycle), all of the patients had their complete blood count, liver function tests, fasting chemistry profile, electrolytes, calcium, phosphorus, and albumin repeated. PSA levels were repeated at the beginning of each new cycle as well.

Study-specific Metabolic Tests. PTH levels were obtained at enrollment in all of the eligible patients. Fasting spot urine samples (from second void in the morning) for calcium, phosphorus, and creatinine, as well as 24-hour urine collections for calcium, phosphorus, and creatinine were also collected at enrollment. Weekly spot urine calcium, phosphorus, and creat-

inine were obtained for the first 4 weeks of treatment. Thereafter, PTH and 24-hour urine samples were collected every 4 weeks (before each new cycle of treatment).

Metabolite Concentration Determination. The vitamin D metabolite (1,25-dihydroxyvitamin D₂) was collected at enrollment and every 4 weeks thereafter. This level was obtained predose (trough level) on day 1 of each new cycle. The vitamin D metabolite samples were collected and frozen at -70°C until time of analysis. These samples were then analyzed by BCI using a specific high-performance liquid chromatography radioimmunoassay method (27).

Bone Markers. Serum bone markers (*i.e.*, osteocalcin and bone-specific alkaline phosphatase) and 24-hour urine collections for urinary bone markers (*i.e.*, pyridinoline and deoxypyridinoline; Ref. 27) were collected at enrollment and repeated before each new cycle of treatment.

Radiographic Assessments. CT, magnetic resonance imaging, and/or bone scan was required within 2 weeks of treatment initiation. Repeat imaging was performed routinely every 12 weeks (3 cycles) or sooner if clinically indicated.

Response Criteria

Only patients completing at least 8 weeks of therapy were evaluated for objective disease responses. Responses were classified as follows: a complete response included the disappearance of all known disease by two separate observations not < 4 weeks apart, during which no new lesions develop. For patients with bone only metastasis, a complete response means recalcification of lytic lesions or biopsy proven absence of tumor cells. Normalization of the bone scan was not necessary. A PR was defined as a $> 50\%$ decrease in the sum of the products of the perpendicular tumor diameters of all of the measurable lesions for ≥ 4 weeks. No simultaneous increase in the size of any lesion or the appearance of any new lesion may occur. For bone scan abnormalities, stability or improvements in images must be seen. SD is response less than a PR or progression for a minimum of 8 weeks. Progression was defined as an unequivocal increase of at least 25% in the size of any measurable lesion (as defined above) or the appearance of any new lesion. Because of preclinical data observing increased PSA expression despite conditions of vitamin D-induced growth inhibition (12), PSA changes were recorded but not used in the primary response criteria.

RESULTS

General. Twenty-five patients, whose pertinent characteristics are shown in Table 1, were entered into this Phase I study, all but 4 (21 of 25) of which were evaluable for toxicity. These four unevaluable patients (1 patient from cohort 2 and 3 from cohort 5) all developed pain (2 patients at week 2 and 2 at week 3) requiring radiation therapy. In all, 110 total courses of 1 α -OH-D₂ were administered in doses ranging from 5 μ g/day up to 15 μ g/day. This is depicted in Table 2, which shows each cohort and dose of 1 α -OH-D₂ received, the number of patients per cohort, the number of patients completing ≥ 8 weeks of therapy and, therefore, evaluable for response, and lastly the number of full courses given per cohort.

Toxicity. 1 α -OH-D₂ was well tolerated with no unexpected toxicities. As predicted, the main toxicity observed was

Table 1 Patient characteristics

Patients entered	25
Patients evaluable for toxicity ^a	21
Median age (range), in years	72 (54–83)
Performance status (Eastern Cooperative Oncology Group)	
0	14
1	10
2	1
Prior treatment for metastatic disease	
Hormonal	25
Radiation	10
Chemotherapy	1
Disease sites	
Bone only	14
Soft-tissue only	2
Bone and soft-tissue	9

^a Only patients completing one full course of therapy (4 weeks) were considered evaluable for toxicity (unless DLT observed).

Table 2 Dose escalation scheme

Cohort	Dose (μg/day)	Patients, no.	Patients on ≥8 weeks ^a	Courses, no.
1	5.0	3	3	17
2	7.5	4	2	19
3	10.0	3	3	10
4	12.5	3	3	29
5	15.0	12	5	35

^a Only patients completing ≥8 weeks of therapy were considered evaluable for response.

Table 3 Hypercalcemia toxicity data

Cohort (dose)	Number of evaluable ^a patients	Grade of toxicity			
		1	2	3	4
1 (5.0 μg/day)	3	0	0	0	0
2 (7.5 μg/day)	3	1	0	0	0
3 (10.0 μg/day)	3	0	0	0	0
4 (12.5 μg/day)	3	0	1	0	0
5 (15.0 μg/day)	9	2	1 ^b	1 ^c	0

^a Evaluable course for toxicity was ≥4 weeks of therapy.

^b Patient developed associated reversible grade 2 increase in serum creatinine.

^c Patient developed associated reversible grade 3 increase in serum creatinine.

hypercalcemia, with its associated effects like constipation, dehydration, and renal insufficiency. This is shown in Table 3, which compares each cohort with the degree of hypercalcemia observed. The 1 patient in cohort 2 (7.5 μg/day) that developed grade 1 hypercalcemia showed no additional increase in serum calcium levels and remained on study. The single patient in cohort 4 (12.5 μg/day) that experienced grade 2 hypercalcemia had a spontaneous improvement in calcium level to a grade 1. Neither of these patients required any dose modification during the study. At the 15 μg/day dose, 2 patients developed grade 1 hypercalcemia without associated complication. Another patient experienced a grade 3 hypercalcemia (calcium 12.8 mg/dl) by week 3 of treatment, which was complicated by dehydration and a grade 3 creatinine increase (serum creatinine 1.2 mg/dl to 3.4

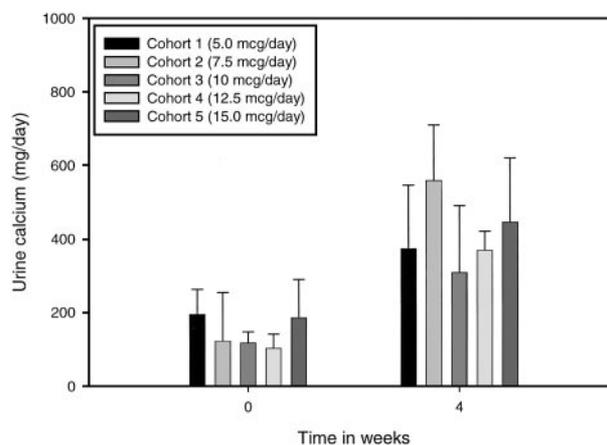


Fig. 1 Measurement of 24-h urine calcium levels at baseline and after 4 weeks of 1α-OH-D₂. For comparison, the normal urine calcium level in men is <300 mg/day. The observed hypercalciuria was seen in all of the patients at every dose level. This change remained stable while patients received drug; bars, ±SD.

mg/dl). He met criteria for a DLT, and the drug was discontinued with resolution of symptoms. Lastly, another patient developed a grade 2 hypercalcemia (calcium 11.8 mg/dl) at week 3 of treatment that was associated with a serum creatinine increase from 1.1 mg/dl to 1.8 mg/dl. Neither patient with grade ≥2 hypercalcemia was rechallenged with the study drug, and both of these patients normalized their laboratory abnormalities within 1 week after drug cessation. Mild transient hyperphosphatemia was seen in 8 patients, but this required no dose adjustments or other therapy. Hypercalciuria was seen in nearly all of the patients (Fig. 1) at the 4-week evaluation by quantitative 24-h urine calcium determination, and this elevation persisted while the patient was on therapy. There was no associated observed acute toxicity with the hypercalciuria. Lastly, no significant hematological, gastrointestinal, hepatic, or neurological toxicity was noted.

Given the observation of grade ≥2 hypercalcemia with associated grade ≥2 renal insufficiency in 2 of 9 patients at the 15 μg/day dose, safety concerns were raised given the need for long-term drug administration. Although the MTD was not reached, additional dose-escalation was not conducted, and the previous dose was recommended to be the Phase II starting dose.

Antitumor Activity. Two patients, 1 in cohort 1 and the other in cohort 2, were observed to have an objective response during this study. The first patient (cohort 1, 5 μg/day dose) had soft-tissue disease at the time of enrollment with an enlarged and lobular prostate gland, and new patchy pelvic lymphadenopathy by CT scan. By week 12 of treatment, a partial response was seen with resolution of the pelvic adenopathy, despite the prostate remaining enlarged. At week 24, the CT scans remained stable with continued absence of adenopathy. This patient eventually did progress with evidence of new pulmonary metastasis, as well as recurrent pelvic and new retroperitoneal lymphadenopathy by week 36 of the study. The second patient (cohort 2, 7.5 μg/day dose) had bony metastasis and soft-tissue disease with a radiographically measurable right obturator and inguinal



Fig. 2 A, pretreatment CT scan of pelvic lymphadenopathy (identified by white arrow) in 1 patient with documented response to 1 α -OH-D₂. B, week 36 CT scan of the same patient showing regression of pelvic lymphadenopathy (identified by white arrow) meeting definition for a partial response.

lymph node. These lesions remained stable at week 12, but by week 24, some evidence for a response was observed with slight decrease in the size of these lymph nodes. By week 36, a soft-tissue response consistent with a PR was noted (Fig. 2). Curiously, a concurrent bone scan at week 36 showed 3 new lesions suspicious for metastatic disease despite the clear improvement in disease by CT scan, a dropping PSA, and a normal alkaline phosphatase. Per patient and the treating physician's decision, the patient remained on study until week 47 when he then developed progressive disease (increased lymphadenopathy). No other objective responses were observed. Five other patients had SD for ≥ 6 months (1 patient from cohort 2, 2 from cohort 4, and 2 from cohort 5) with 2 of these patients having SD for ≥ 12 months. All of the others patients progressed or were unevaluable for response because of early drug discontinuation.

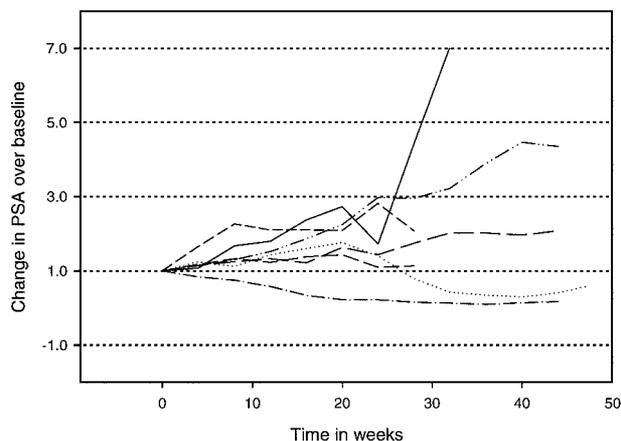


Fig. 3 Change in PSA in all of the patients with SD (> 6 months) or a partial response. Shown as a ratio of current over baseline PSA plotted against time in weeks.

PSA Response. The PSA values tended to initially increase in nearly all of the patients as drug was being administered. This upward trend was also seen in the 2 patients experiencing a partial response. The first patient (5.0 $\mu\text{g/day}$ dosage) had continuously rising PSA during the first 20 weeks of treatment despite radiological disease regression. At that point, the PSA appeared to drop as corresponding CT scans showed near complete resolution of the soft-tissue disease. Interestingly, between weeks 24 and 32, the PSA again rose, but this time the rate of rise was much steeper than previous. This coincided with radiographic disease progression. The second patient (7.5 $\mu\text{g/day}$ dosage) also showed a rising PSA during the first 20 weeks of treatment despite stable to improving disease. At that point, his PSA dropped from a peak of 65 ng/dl down to a value of 12.3 ng/dl with CT scans showing regression of soft-tissue disease. The patient subsequently progressed, but his PSA actually decreased additionally to a nadir of 10.3 ng/dl before increasing to only 14.5 ng/dl at study termination. This is in comparison to his pretrial PSA of 35.9 ng/dl. The 5 other patients with SD for ≥ 6 months also showed rising PSA while on study (Fig. 3). No correlation between PSA and disease response was observed.

Pharmacologic Studies and Physiological Markers.

The active metabolite 1 α ,25-dihydroxyvitamin D₂ was determined at the time of enrollment, then every 4 weeks while the patient was on protocol. Results showed low levels of 1 α -OH-D₂ at enrollment with a range between < 5.0 pg/ml and 15.7 pg/ml (median < 5.0 pg/ml) in our patients. These levels did increase with drug administration and appeared to plateau by weeks 4–8 with mean trough values between 50 and 60 pg/ml (Fig. 4). Wide interpatient, as well as inpatient, variability was observed, and the elevated concentrations persisted while the patient was on therapy with drug.

Serum PTH levels were observed to decrease in nearly every patient during treatment with 1 α -OH-D₂ (mean PTH level: baseline, 44.6 pg/ml; week 4 value, 22.9 pg/ml). Likewise, serum phosphorus was seen to increase. This increase was not clinically significant, and only 1 patient developed a serum

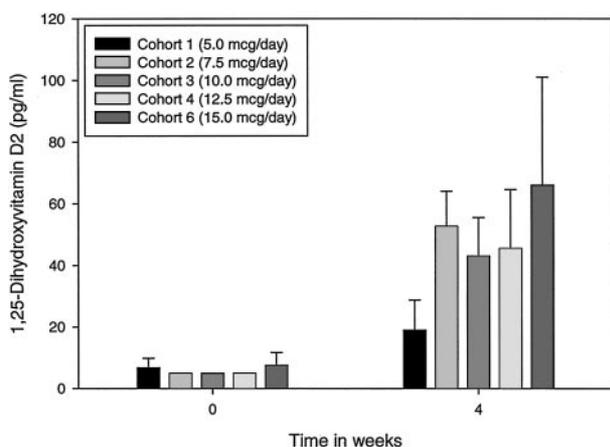


Fig. 4 Serum 1,25-dihydroxyvitamin D₂ levels at baseline and after 4 weeks of 1 α -OH-D₂ (stratified by cohort). A dose-dependent increase in the 1,25-dihydroxyvitamin D₂ level was observed at the lowest two dose-levels, but this dose-dependency was lost after the 7.5 μ g/day level. 1,25-Dihydroxyvitamin D₂ levels remained stable while patients remained on drug.

phosphorus >5.0 mg/dl. This patient (15 μ g/day dose) had a peak phosphorus of 6.8 mg/dl at week 8, which normalized spontaneously without dose modification.

The mean baseline value of osteocalcin was 31.7 ng/ml (range, 15.3–47.5 ng/ml). By week 4, the mean value was 41.9 ng/ml (range, 20.6–64.1 ng/ml). Whereas a small average increase in osteocalcin levels were seen, no obvious trends were observed between the dose levels and various time points. Similarly, the mean baseline value for bone alkaline phosphatase was 44.4 units/liter (range, 21.2–118.1 units/liter) with week 4 values showing a mean 47.9 units/liter (range, 18.7–128.0 units/liter). The values of the N- and C-telopeptide concentrations showed no significant differences among the cohorts when baseline levels were compared with week 4 or higher time points. Overall, large intra- and interpatient variability was seen in all of these values making trends difficult to distinguish in this study.

DISCUSSION

In preclinical studies, vitamin D (calcitriol) has shown both antiproliferative and differentiating effects on prostate cancer cells. This was evident by inhibition of tumor growth *in vitro* with a concurrent stimulation of PSA production in certain prostate cancer cell lines (12). Unfortunately, calcitriol did not result in significant clinical responses, as the hypercalcemic toxicity prohibited adequate dose-intensive therapy. Although pulse-dose calcitriol has now been shown to be safe with less clinically significant hypercalcemia (30), its activity against prostate cancer is less clear given that the preclinical work was all conducted using a chronic daily dosing. On the basis of extensive work by multiple researchers, it is strongly implied that chronic administration of a vitamin D ligand is needed to optimally maintain the antiproliferative and differentiating effects of vitamin D seen *in vitro*; thus, pursuing chronic daily vitamin D therapies remains of much interest. Vitamin D ana-

logs have since been shown to have less hypercalcemic toxicity, despite similar or improved *in vitro* antitumor effects. One such analogue is 1 α -OH-D₂, which has already been studied for use in women with postmenopausal osteoporosis, as well as patients with renal osteodystrophy (31). Here we conduct a Phase I, dose-escalating study of 1 α -OH-D₂ in patients with advanced HRPC to determine the MTD and antitumor activity.

In this Phase I study, separate approaches to assess the activity of 1 α -OH-D₂ were used because of the noncytotoxic nature of the drug and potential need for long-term administration. The first was a traditional assessment of toxicity using established criteria from cytotoxic drug development. The second was pharmacokinetic monitoring with measurement of an active metabolite of 1 α -OH-D₂ to correlate drug dose to plasma levels. Lastly, the biological activity was determined by measuring the specific markers (*i.e.*, markers of bone turnover, effects on PTH, and serum phosphorus) used in early clinical and preclinical studies.

There were two main toxicities seen associated with 1 α -OH-D₂ administration. The first was hypercalcemia, which appeared soon after drug initiation, and the other was renal insufficiency likely because of complication from the former. Only 1 patient experienced a grade 1 hypercalcemia at a 1 α -OH-D₂ dose of \leq 10.0 μ g/day. However, at the 15.0 μ g/day dose, reversible grade 2 and 3 hypercalcemia, and renal insufficiency were observed by week 3 of treatment, and these 2 patients were promptly withdrawn from the study because of toxicity. Whereas only one DLT was seen at 15 μ g/day, the observation of grade \geq 2 hypercalcemia with associated grade \geq 2 renal insufficiency in 2 of 9 patients raised concerns about the safety of long-term drug administration at this dose level. Therefore, 12.5 μ g/day was recommended as the Phase II dose when administered on a chronic daily basis. Otherwise, 1 α -OH-D₂ was well tolerated with no signs of irreversible toxicity.

We did observe hypercalciuria at all of the dose levels, but no case of clinical nephrolithiasis was seen during this trial. The finding of a hypercalciuric state may have long-term consequences, which we acknowledge, given that the therapy would potentially be administered chronically. The exact risk of developing nephrolithiasis from chronic hypercalciuria is unknown, as other factors like urinary pH and citrate levels are important as well. What can be said is that chronic hypercalciuria is not uncommon and that long-term studies in children with idiopathic hypercalciuria have shown that many patients remain asymptomatic despite persistently elevated urinary calcium levels (32). In any event, in patients with 1 α -OH-D₂-induced calciuria, early clinical studies showed normalization of urinary calcium excretion in \sim 1 week after discontinuation of drug (27).

1 α -OH-D₂ is metabolized to 1 α ,25-dihydroxyvitamin D₂ and 1 α ,24-dihydroxyvitamin D₂. To assess plasma drug levels from 1 α -OH-D₂ administration, only the more calcium-inducing metabolite 1 α ,25-dihydroxyvitamin D₂ was measured. From the early clinical trials in postmenopausal women with osteoporosis, there was a dose-dependent increase in 1 α ,25-dihydroxyvitamin D₂ levels from a baseline of 4 pg/ml to a mean of 23 pg/ml at the 5 μ g/day dose of 1 α -OH-D₂. At a dose of 8.0 μ g/day, the mean 1 α ,25-dihydroxyvitamin D₂ level was 41 pg/ml (27). This is similar to our data in which 1 α ,25-dihydroxyvitamin D₂ rose

from a baseline of <5.0 pg/ml to 19 pg/ml at the 5.0 μ g/day dose. We did observe additional increases in serum 1 α ,25-dihydroxyvitamin D₂ levels in the higher cohorts (7.5–15.0 μ g/day doses) with mean values between 40.5 pg/ml and 68.7 pg/ml, but a linear relationship in these higher dose levels could not be established. An explanation for the lack of obvious linearity from 7.5 μ g/day to 15.0 μ g/day may be the large variability observed within and between the patients, as well as the small numbers of individuals involved. The pharmacokinetics confirm that drug is absorbed, but whether the lack of increase seen in the measured 1 α ,25-dihydroxyvitamin D₂ with the higher dose levels of administered 1 α -OH-D₂ is due to saturation of absorptive mechanisms is unclear. It is possible that at the higher dose levels, more 1 α ,24-dihydroxyvitamin D₂ was generated, instead of 1 α ,25-dihydroxyvitamin D₂ (which was measured). Given that 1 α ,24-dihydroxyvitamin D₂ also possesses potential antitumor properties, higher doses of 1 α -OH-D₂ may be beneficial despite what was observed with measurement of 1 α ,25-dihydroxyvitamin D₂ levels alone.

Serum PTH was noted to decrease in nearly all of the patients. This is because of the binding of vitamin D to its receptor resulting in a decrease in PTH transcription. Conversely, serum phosphorus tended to increase with 1 α -OH-D₂ administration, but this increase did not appear dose-dependent or achieve any clinical significance. This increase in phosphorus is because the net effect of vitamin D is to increase cellular phosphorus transport at the brush borders in the intestines. Because, the sodium-dependent phosphorus uptake process can be saturated (33), the lack of correlation between serum phosphorus at the higher doses of 1 α -OH-D₂ was of little surprise. The presence of both of these effects confirms physiological activity of the 1 α -OH-D₂ tested.

In the development of 1 α -OH-D₂, various markers of bone metabolism were measured such as serum osteocalcin and bone alkaline phosphatase. The data in postmenopausal women suggested that vitamin D stimulated osteoblastic activity in a dose-dependent manner, which was reflected by the linear rise in osteocalcin levels at the dose tested (up to 10.0 μ g/day; Ref. 27). However, we did not observe this trend in this study. Given the interpatient variability, little conclusion could be derived from our small patient population. One explanation is that in our patient population, the majority (23 of 25) had bone metastasis from their prostate cancer. As prostate cancer commonly results in blastic metastasis on plain radiographs, perhaps the presence or severity of bony disease could confound the measured serum osteocalcin levels and explain the variability seen within and among the various cohorts. The effects of the cancer on the osteoblasts could quite possibly be overshadowing any effects that one might see from the drug itself. This same rationale may be used to explain the lack of dose-effect seen in the measurement of bone-specific alkaline phosphatase.

In this study, two objective responses (partial responses) were seen with 5 other patients experiencing disease stabilization for \geq 6 months (7 patients with PR or SD out of 16 disease evaluable patients). As all of the patients had either rising PSA or new lesions on CT/bone scan before enrollment, presumably real antitumor activity is present. Whereas rising serum PSA levels have excellent correlation with disease progression in patients with prostate cancer, we found that for patients treated

with vitamin D analogs that the correlation between the change in PSA values and actual disease status (clinically and radiographically) was not so clear. Being that vitamin D is thought of as a differentiating agent, it can hypothetically induce PSA production regardless of any increase in tumor growth. This is demonstrated clearly by one of our patients with radiographically confirmed partial responses. This patient started with a baseline PSA of 132.6 ng/dl, which consistently rose to a value of 361.3 ng/dl (week 20) when his CT scan showed normalization of extraprostatic soft-tissue disease. His PSA subsequently began to decline (PSA 227.4 ng/dl at week 24) before peaking at 1020 ng/dl (week 36) when he radiographically progressed.

In summary, 1 α -OH-D₂ is a drug that is well tolerated with predictable toxicities. Because 1 α -OH-D₂ is a noncytotoxic agent with a presumed mechanism of action being induction of differentiation, minimal side-effects is desired given that chronic administration would be necessary. Given good drug tolerability, the differentiating action of this drug can have implications in the field of chemoprevention. For example, these novel agents are now being considered for use in the adjuvant setting in patients with prostate cancer with high likelihood of recurrence (*i.e.*, high Gleason tumors) after primary therapy. Also, the combination of these new analogs with current cytotoxic drugs may exploit potential synergism without adding toxicity. Lastly, studies combining bisphosphonates with these vitamin D analogs can be considered to improved dose intensity and possibly exploit synergistic activity in diseases like prostate cancer that are particularly prone toward bony metastasis.

In conclusion, we have conducted this Phase I trial of 1 α -OH-D₂ in patients with advanced HRPC. The drug was well tolerated with no unexpected toxicity. Whereas the defined MTD was not reached, surrogate markers showing drug activity of the 1 α -OH-D₂ was seen. Given the observed responses, this drug is currently being additionally pursued in patients with advanced HRPC at the recommended Phase II dose of 12.5 μ g/day given continuously.

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