

Vitamin D and Intervention Trials in Prostate Cancer: From Theory to Therapy

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Studies of vitamin D and prostate cancer have advanced rapidly from the hypothesis that vitamin D deficiency increases the risk of prostate cancer to intervention trials of vitamin D administration in clinical cancer. The hormonal form of vitamin D, 1,25(OH)₂D, exerts prodifferentiating, antiproliferative, anti-invasive, and antimetastatic effects on prostate cells. Moreover, normal prostate cells synthesize 1,25(OH)₂D from serum levels of the prohormone, 25-hydroxyvitamin D. The autocrine synthesis of 1,25(OH)₂D by prostatic cells provides a biochemical mechanism whereby vitamin D may prevent prostate cancer. Many prostate cancer cells have lost the ability to synthesize 1,25(OH)₂D but still possess 1,25(OH)₂D receptors. This suggests that whereas vitamin D (e.g., cholecalciferol) might prevent prostate cancer, existing prostate tumors likely would require treatment with 1,25(OH)₂D and/or its analogs. The major obstacle to the use of 1,25(OH)₂D in patients therapeutically is the risk of hypercalcemia. Several maneuvers to reduce this risk, including pulse dosing and the use of less calcemic 1,25(OH)₂D analogs, have been explored in Phase I-III clinical trials. Once merely a promise, vitamin D-based therapies for prostate cancer may soon be medical practice.

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“Everybody talks about the weather” Mark Twain famously remarked, “but nobody does anything about it.” Like meteorology, epidemiology is primarily an observational science; interventions are rare. The topic of vitamin D in prostate cancer is an exception: the first vitamin D-based intervention in prostate cancer—and there are now more than 20—appeared just 5 years after vitamin D deficiency was hypothesized as a risk factor. The subject of vitamin D and prostate cancer epidemiology currently is undergoing intensive investigation (1–3) and is reviewed elsewhere (4, 5). This research highlights developments in vitamin D-based interventions in prostate cancer. I first summarize key clinical aspects of prostate cancer and of vitamin D synthesis.

PROSTATE CANCER, CLINICAL CONSIDERATIONS

Prostate cancer is the most common (nonskin) cancer among men in the Western world. Age-adjusted mortality rates worldwide vary more than 20-fold and are highest among African American and northern European men (6). Unlike mortality rates, the prevalence of subclinical

prostate cancer (also known as “latent” or “autopsy” prostate cancer) is ubiquitous among older men regardless of race or geographic location (7), which suggests that clinical prostate cancer is the result of factors that govern the growth of subclinical prostate tumors.

Prostate cancers that are confined to the prostate gland are potentially curable (via surgical removal of the prostate gland, prostatectomy, or via radiation therapy). Extraprostatic cancers can be treated with ionizing radiation and hormonal therapy. Within 10 years of radical prostatectomy or radiation therapy, 20–40% of men will experience detectable prostate-specific antigen (PSA), indicating a biochemical recurrence of their cancer (8). The optimal treatment—if any—for men with biochemically recurrent prostate cancer is unclear, because only one-third of these men will progress to clinical disease within 8 years (9).

Most prostate cells depend upon androgen for growth, and androgen deprivation (via medical or surgical castration) results in their death. Androgen independence—the growth of prostate cancer despite androgen withdrawal—is a turning point in the natural history of prostate cancer. Androgen deprivation therapy is effective as palliation for most men, but the duration of response for men with metastatic disease typically is only 14 to 20 months, after which most cancers become androgen-independent (10). Docetaxel-based therapies provide a modest survival benefit for men with androgen-independent metastatic prostate cancer, the median survival for which is 19 months (11).

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Selected Abbreviations and Acronyms

PSA = prostate-specific antigen
 VDR = vitamin D receptor
 PTH = parathyroid hormone

PROSTATE CANCER AND THE VITAMIN D HYPOTHESIS

In 1990, we noted that the major risk factors for prostate cancer: older age, black race, and residence at northern latitudes all are associated with a decreased synthesis of vitamin D. We proposed that vitamin D maintained the normal phenotype of prostatic cells and that vitamin D deficiency promoted the development of clinical prostate cancer from its preclinical precursors (12). This idea was supported by cartographic analyses published in 1992, in which we showed that U.S. county-wide mortality rates for prostate cancer among white men were correlated inversely with the availability of ultraviolet radiation, the major source of Vitamin D (13, 14). The same year, Miller and colleagues (15) demonstrated that prostate cancer cells possessed high-affinity receptors for the hormonal form of vitamin D, 1,25(OH)₂D (vitamin D receptors, VDR). Pleiotropic anticancer effects of 1,25(OH)₂D on normal and cancerous prostate cells later were described by numerous laboratories. The mechanisms for these effects in the prostate are not completely characterized but include marked inhibition of: cell proliferation (e.g., via cell cycle arrest) (16); invasion (e.g., inhibition of matrix metalloproteinases) (17); migration (18); metastasis (19, 20); and angiogenesis (21) (Fig. 1).

SYNTHESIS OF VITAMIN D METABOLITES

The synthesis of 1,25(OH)₂D (calcitriol) begins with the production of vitamin D₃ (cholecalciferol) after 7-dehydrocholesterol in the skin is exposed to UV-B radiation or after vitamin D is ingested from the diet. Approximately 90% of vitamin D is derived from sunlight (22). Vitamin D is hydroxylated first in the liver at the 25th carbon, forming the prohormone, 25-hydroxyvitamin D (25-OHD), and again at the 1- α position, forming 1,25(OH)₂D, the active, hormonal form of vitamin D (23). Classically, the hydroxylation of 25-OHD at the 1- α position was presumed to occur exclusively in the kidney and the function of 1,25(OH)₂D was thought to be in the control of serum calcium and phosphorus (24). However, we now know that local synthesis of 1,25(OH)₂D occurs in an autocrine or paracrine fashion in prostate (and other) cells, where 1,25(OH)₂D controls key processes involving cell differentiation and proliferation (25, 26) (Fig. 2).

The clue to the discovery of the autocrine synthesis of 1,25(OH)₂D by prostate cells came not from biochemistry,

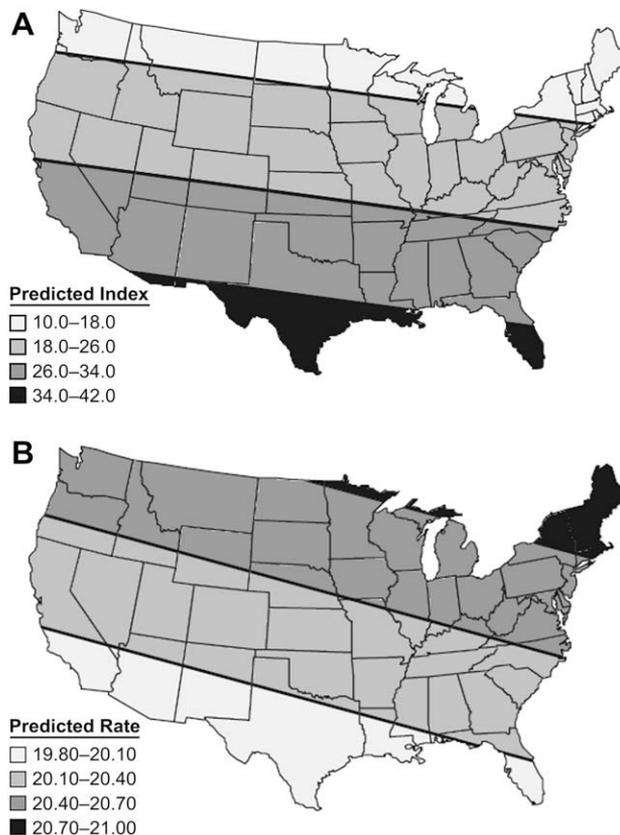


FIGURE 1. (A) Linear trend surface map of ultraviolet radiation in 3073 counties of the contiguous United States. (Redrawn from Cancer 70:2865, 1992.) (B) Trend surface analysis of age-adjusted prostate cancer mortality by county among White men, 1970–1979, in the contiguous United States. (From Cancer 1992;70:2864. Reprinted with permission from Wiley-Liss, Inc.)

but from descriptive epidemiology. The north–south gradient in prostate cancer mortality and greater rates among black men suggested a deficiency in 25-OHD, whose serum levels are known to be lower at higher latitudes and among persons with dark pigmentation. However, the active vitamin D hormone is 1,25(OH)₂D, not 25-OHD. Serum levels of 1,25(OH)₂D are tightly regulated, are not lower in black than white men, and (in normal individuals) are not correlated with serum levels of 25-OHD (27).

Thus, it was unclear how the north–south gradient in prostate cancer mortality and the higher risk among black men could be causally related to vitamin D deficiency. We reasoned that this paradox would be resolved if prostate cells themselves synthesized 1,25(OH)₂D from circulating levels of 25-OHD (28). In 1998, we demonstrated that normal human prostate cells possess functional 25-hydroxyvitamin D₃-1 α -hydroxylase (1 α -Oase) and convert 25-OHD into 1,25(OH)₂D (28). Moreover, 25-OHD, which was previously thought to be inert, could inhibit the proliferation of prostate cells that possessed 1 α -Oase (29). The

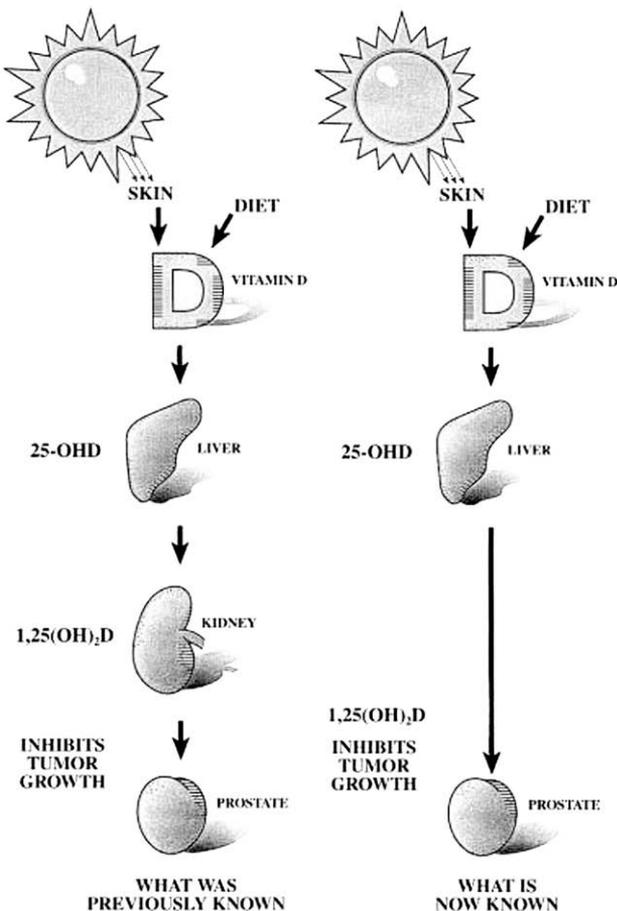


FIGURE 2. Current understanding of the endocrine and autocrine role of 1,25(OH)₂D on prostate cells. In addition to the kidney (endocrine system), the prostate synthesizes its own 1,25(OH)₂D (autocrine system) that controls local growth and proliferation (from Schwartz, *Semin Dialysis* 2005;18:276-289).

implications of the discovery of the autocrine synthesis of 1,25(OH)₂D in normal prostate cells are potentially profound; this property provides a biochemical mechanism by which exposure to vitamin D (cholecalciferol, vitamin D₃ or ergocalciferol, vitamin D₂) might prevent prostate cancer (30).

PROSTATE CANCER CELLS EXPRESS VDR BUT LOSE EXPRESSION OF 1 OHASE

The biological activity of 1,25(OH)₂D in tissues requires the presence of VDR, a ligand-dependent transcription factor that is a member of the steroid nuclear receptor super family (31,32). Krill and colleagues (33) examined the expression patterns of VDR by immunohistochemistry in 27 clinical samples of normal human prostates that were free from adenocarcinoma or suspected carcinoma. They showed that VDR are widely expressed in human prostate cells and

were expressed more predominantly in the peripheral zone of the prostate (the site of origin of most prostate cancers) than in the central zone. Numerous studies indicate that VDR also are widely expressed on human prostate cancer cells and cell lines (34-37).

Although normal prostate cancer cells express high levels of 1- α Ohase, 1- α Ohase expression is greatly diminished in prostate cancer cells. Hsu and colleagues (38) compared 1- α -hydroxylase activity in samples from normal prostate epithelial cells, cancer-derived prostate epithelial cells, prostate cancer cells lines, and samples of benign prostatic hyperplasia. 1- α OHase expression was significantly reduced in the benign prostatic hyperplasia cells and was reduced further in the cancer-derived cells and cell lines. Decreased expression of 1- α -OHase was correlated with a decrease in growth inhibition in response to 25-OHD₃. Similar data showing a loss of 1- α -OHase expression in prostate cancers vs. benign and noncancerous prostates were shown by Whitlatch et al. (39). These authors demonstrated further that transfection of the cDNA for 1- α OHase into prostate cancer cells that did not express 1- α OHase (LNCaP cells) and were not growth inhibited by 25-hydroxyvitamin D₃, conferred growth inhibition by 25-OHD₃ in these cells. Together, these findings have important therapeutic implications: Because prostate cancers have lost 1- α OHase expression, they are unlikely to respond to treatment with vitamin D or 25-OHD. However, because they retain VDR, prostate cancer cells could be treated with 1,25(OH)₂D and its analogs (40).

CLINICAL TRIALS OF VITAMIN D METABOLITES IN PROSTATE CANCER

The birth of vitamin D-based therapies in cancer can be dated from 1981 when Abe et al. (41) reported that myeloid leukemic cells differentiated into macrophages by nanomolar concentrations of 1,25(OH)₂D₃. Two years later, 1,25(OH)₂D₃ and a prodrug, 1(OH)D₃, given to mice inoculated with myeloid leukemia cells were shown to prolong the survival of the mice (42). This led to the first human vitamin D-based clinical trial in cancer 1987, when Tobler and Koeffler gave 1,25(OH)₂D₃ at 2 μ g/day, orally, to 18 patients with myelodysplastic syndrome (43). There were no clinical improvements and half of the patients developed hypercalcemia. These authors identified the central problem in vitamin D-based therapeutic trials in cancer, i.e., how to get a therapeutic response without inducing hypercalcemia. Although the problem is complex and involves pharmacokinetics, peak drug levels and other parameters (44), essentially it is this: 2 μ g/day 1,25(OH)₂D₃ in normal individuals produces serum 1,25(OH)₂D₃ levels of about 2.0×10^{-10} M. Yet most antiproliferative responses to 1,25(OH)₂D₃ in cancer cells in culture require nanomolar

(10^{-9} M) levels of $1,25(\text{OH})_2\text{D}_3$ or higher. “In the future,” Tobler and Koeffler concluded prophetically, “vitamin D analogs that induce [hematopoietic] cell differentiation without inducing hypercalcemia might be medically useful compounds for selected patients ... Likewise, combinations of inducers of differentiation may be more effective than any one agent” (p. 131).

Research in the early 1990s showed that, like leukemic cells, prostate cancer cells exhibited increased differentiation and an inhibition of proliferation in response to $1,25(\text{OH})_2\text{D}_3$ in vitro and in vivo (e.g., 45–47). These studies led to the first vitamin D-based trial in prostate cancer in 1995 (48). Osborn et al. treated 13 men with advanced, androgen-independent prostate cancer with oral $1,25(\text{OH})_2\text{D}_3$ with doses as high as 1.5 $\mu\text{g}/\text{day}$ (49). No therapeutic benefit was shown, as defined by the standard criterion, a 50% sustained drop in PSA. As observed by Tobler and Koeffler, hypercalcemia limited the ability to give $1,25(\text{OH})_2\text{D}_3$ at greater doses.

Similar results were reported for the prodrug, doxercalciferol ($1\alpha\text{OH}_2\text{D}_2$). Liu et al. (50) treated 16 men with androgen-insensitive prostate cancer for 12 weeks at doses ranging from 5 to 15 $\mu\text{g}/\text{day}$. Two patients showed significant decreases in PSA and 9 showed PSA stabilization. Numerous Phase I and Phase II trials of $1,25(\text{OH})_2\text{D}_3$ alone or in combination with other therapies showed the feasibility of administering $1,25(\text{OH})_2\text{D}_3$ intermittently at high dose, a maneuver that lowers the calcemic effects of calcitriol (51). Additionally, Trump et al. (52) demonstrated the feasibility of administering $1,25(\text{OH})_2\text{D}_3$ with drugs such as dexamethasone. Dexamethasone itself has biologic activity against prostate cancer and also reduces the calcemic effects of $1,25(\text{OH})_2\text{D}_3$.

A series of Phase II trials led by Beer and colleagues (53) ought to exploit maneuvers that permit higher doses of $1,25(\text{OH})_2\text{D}_3$ without inducing hypercalcemia, which led to the development of a proprietary formulation of high-dose calcitriol, DN-101 (Novacea, Inc., South San Francisco, CA) Pharmacokinetic evaluation of this compound showed that oral dosing with DN-101 could achieve $1,25(\text{OH})_2\text{D}_3$ exposures 5- to 8-fold greater than that attainable with commercial oral formulations of $1,25(\text{OH})_2\text{D}_3$. In 2007, Beer et al. (54) reported the results of a trial of DN-101 in conjunction with docetaxel (the ASCENT trial). Two hundred fifty men with androgen-independent prostate cancer were randomly assigned to either weekly docetaxel (36 mg/m^2 intravenously) combined with 45 μg of calcitriol (as DN-101) or weekly docetaxel combined with placebo, taken orally. The primary endpoint was a PSA response within 6 months of enrollment. PSA responses were not significantly different in the DN-101 or placebo arms (63% vs. 52%, respectively, $p = 0.07$). However, in a multivariate analysis,

the median survival (not a primary endpoint in the trial) was estimated at 24.5 months in the DN-101 arm versus 16.4 months for placebo. This large survival advantage led to a Phase III trial (ASCENT-2), which had a planned enrollment of 1200 men and in which survival was a primary endpoint. Unfortunately, after more than 900 men were enrolled, the Phase III trial was terminated abruptly due to excess deaths in the DN-101 arm (55).

The cause of the excess deaths in the treatment arm of ASCENT-2 is not yet known and will be critical to understand. It also will be important to understand the mechanism(s) underlying the survival advantage for $1,25(\text{OH})_2\text{D}$ in the original ASCENT trial, as it is possible that this effect could be replicated with lower levels of calcitriol or with less calcemic calcitriol analogs. Interestingly, although DN-101 has been shown to produce peak levels of $1,25(\text{OH})_2\text{D}$ of approximately 2 nM (56); these doses are only marginally effective at inhibiting prostate cancer cell proliferation in culture (57). This suggests that the survival advantage may reflect the effects of $1,25(\text{OH})_2\text{D}$ on the metastatic process rather than on prostate cancer cells directly.

In this regard, we recently reported a phase I/II trial of the $1,25(\text{OH})_2\text{D}$ analog, paricalcitol (19-nor- 1α -25-dihydroxyvitamin D_2) in advanced androgen-insensitive prostate cancer (58). None of the 18 enrolled men had an objective response to treatment as measured by a sustained decline in PSA. However, we observed that serum levels of parathyroid hormone (PTH) were significantly and negatively associated with prostate cancer survival; the higher the serum PTH, the shorter the survival. This effect was independent of disease severity, as measured by serum PSA. Recent data indicate that PTH accelerates the growth of prostate cancers in bone (59). Increased serum levels of PTH are known to be associated with an increased risk of bone fractures and other skeletal events that are significant predictors of mortality in prostate cancer (60). Thus, these findings raise the possibility that suppression of serum PTH—a “classical effect” of $1,25(\text{OH})_2\text{D}$ —may be a novel therapeutic approach to retard the growth of bony metastases in advanced prostate cancer (61, 62) (Fig. 3).

INTERVENTIONS IN RECURRENT PROSTATE CANCER AND BEYOND

Just as it is easier to extinguish a smoldering cigarette than a flaming house, vitamin D therapies might be more effective in recurrent prostate cancer, when there are only a few cancerous cells, than in advanced cancer, when metastases are widespread and tumors may be less differentiated. This was the rationale for a small study by Gross et al. (63), who treated 7 men with recurrent prostate cancer with 2 $\mu\text{g}/\text{day}$ oral $1,25(\text{OH})_2\text{D}_3$. Treatment caused

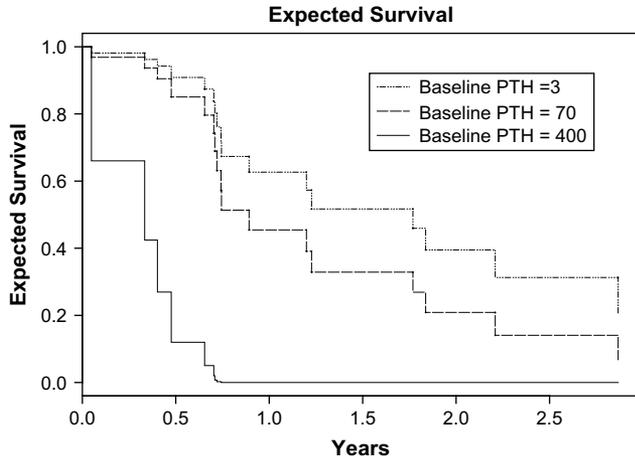


FIGURE 3. Kaplan-Meier plot of survival for 17 patients as a function of initial PTH. The estimated median survivals for baseline PTH of 3, 70, and 400 (the minimum, median, and maximum baseline values in our trial) are 21.2, 10.7, and 4.0 months, respectively. From Schwartz et al., *Clin Cancer Res.* 2005;11: 8680-8685).

a decrease in the rate of rise of PSA in 6 of 7 men, and caused an absolute decrease in several men. However, hypercalcemia occurred in most men and was dose-limiting.

A follow-up to the study of Gross et al. (63) was made by Woo et al. (64), who studied 15 men with recurrent disease using vitamin D₃. They reasoned that because 1,25(OH)₂D should be synthesized intraprostatically by normal prostate cells, vitamin D₃ might have similar therapeutic effects without the calcemic effects of 1,25(OH)₂D. (Although prostate cancer cells have less 1- α OHase than normal prostate cells, 1,25(OH)₂D synthesized by normal prostatic cells might influence cancerous cells in a paracrine manner.) Fifteen men with recurrent disease were given 2000 IU (50 μ g) vitamin D₃ and were monitored prospectively every 2 to 3 months. PSA levels in 9 of 15 men decreased or remained unchanged for as long as 21 months. The authors reported that vitamin D treatment was associated with a significant prolongation of the PSA-doubling time (64).

In addition to its possible therapeutic role in advanced prostate cancer, vitamin D metabolites may be effective in disease palliation (i.e., tertiary prevention). Many men with advanced prostate cancer experience significant disease-related pain, especially bone pain. In a small, single-arm study, Beer and colleagues (65) reported significant analgesic activity for the combination of calcitriol and docetaxel in men with metastatic androgen-independent prostate cancer. Interestingly, some patients with bone pain may respond to vitamin D₃. Van Veldhuizen et al. (66) treated 16 men with metastatic androgen-insensitive prostate cancer with 2,000 IU vitamin D₃ for 12 weeks to study improvement in bone pain. At enrollment, 8 (50%) men

had vitamin D levels below currently recognized cutpoints for vitamin D deficiency (20 ng/mL). Four men (25%) experienced improvement in pain scores and one showed a decrease in PSA from 99.2 to 55.3 ng/mL. This result suggests that cholecalciferol may have therapeutic effects in some men with advanced disease.

DISCUSSION

The natural history of prostate cancer affords multiple opportunities for intervention with vitamin D metabolites (Fig. 4). The stages of prostate cancer can be visualized as an iceberg, where the “water line” separates clinical disease from (submerged) subclinical disease. At the tip of the iceberg are men with androgen-insensitive and men with recurrent disease; at the base, men who are apparently prostate cancer free but who are at risk for prostate cancer. At each stage of the natural history of prostate cancer, there is some form of vitamin D-based therapy that could be a logical choice for investigational therapy.

To date, the authors of most interventions have studied men with advanced androgen-independent disease. This population is a challenging one, as these are men for whom virtually all other interventions have failed. Early trials in this population with calcitriol alone were unable to inhibit cancer without inducing hypercalcemia. In the AIPC Study of Calcitriol Enhancing Taxotere (ASCENT) trial, a high-dose formulation of calcitriol appeared to significantly lengthen survival in comparison to standard therapy, yet a Phase III trial (ASCENT-2) of the same formulation proved excessively toxic. The use of 1,25(OH)₂D at a lower dose, or the use of calcitriol analogs, are logical next steps. In this regard it is noteworthy that a recent Phase II trial of calcitriol in advanced disease at 32 μ g per week (vs. 45 μ g used

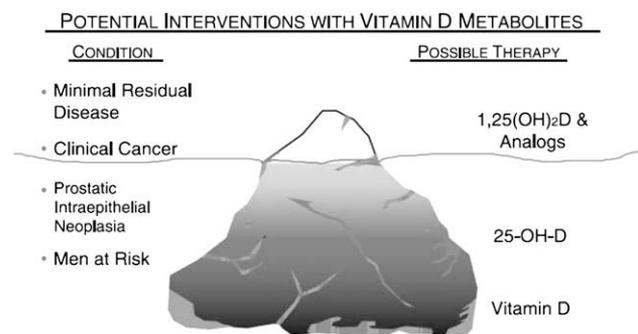


FIGURE 4. The natural history of prostate cancer and possible treatments with vitamin D. At each stage of prostate cancer there is a corresponding treatment with some form of vitamin D, ranging from the hormonal precursor (vitamin D₃) in men at risk for prostate cancer to active vitamin D, 1,25(OH)₂D₃, and its analogs for men with advanced disease.

in ASCENT-2) was associated with a PSA response in 8 of 26 patients (31%) (67).

Approximately 50,000 new cases of recurrent disease occur annually in the United States (68). There is presently no standard care for men with recurrent disease. These men comprise an attractive group for vitamin D-based interventions because their disease burden is relatively small and their cancers may be better differentiated than in advanced disease. Men with slow-growing cancers who defer active treatment (i.e., men undergoing expectant management or “watchful waiting”) also may be candidates for low-morbidity interventional therapies.

Finally, it is possible that high dose vitamin D (i.e., vitamin D₃) alone or in combination with other agents may be effective in primary prevention. Just as combination therapies may extend the reach of calcitriol in advanced disease, combination therapies could extend the reach of vitamin D metabolites in prostate cancer prevention. For example, soy derivatives are known to reduce the catabolism of 1,25-dihydroxyvitamin D and thereby potentiate the antiproliferative effects of 1,25(OH)₂D on prostate cancer cells (69). This suggests a form of combination therapy that could be useful at several levels, e.g., primary prevention and recurrent disease.

In conclusion, the subject of vitamin D in prostate cancer has evolved rapidly from theory to proof-of-principle therapy. The next steps in this clinical evolution—those of defining and testing vitamin D-based interventions that are safe and potentially effective for each stage in the natural history of prostate cancer—should challenge—and reward—the combined talents of basic scientists, clinicians, and public health investigators.

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