Open Access

INVITED REVIEW

Prostate Cancer

Vitamin D in prostate cancer

Donald L Trump, Jeanny B Aragon-Ching

Signaling through the vitamin D receptor has been shown to be biologically active and important in a number of preclinical studies in prostate and other cancers. Epidemiologic data also indicate that vitamin D signaling may be important in the cause and prognosis of prostate and other cancers. These data indicate that perturbation of vitamin D signaling may be a target for the prevention and treatment of prostate cancer. Large studies of vitamin D supplementation will be required to determine whether these observations can be translated into prevention strategies. This paper reviews the available data in the use of vitamin D compounds in the treatment of prostate cancer. Clinical data are limited which support the use of vitamin D compounds in the management of men with prostate cancer. However, clinical trials guided by existing preclinical data are limited.

INTRODUCTION

A potential role for vitamin D compounds in the causation and treatment of cancer has been considered since the early 1970s. Treatment with vitamin D compounds was shown to inhibit the development of cancer in a carcinogen-induced model in the hamster; vitamin D receptor (VDR) was detected in human cancer cells and growth arrest *in vitro*.[1-3] This paper seeks to provide an overview of studies of vitamin D in prostate cancer. However, given the ubiquitous expression of VDR as well as the vitamin D synthesizing and degrading enzymes (*e.g.*, CYP 24a1, CYP27b1) in almost all human tissues, and cancers that arise from those tissues, there is every reason to believe that vitamin D signaling may play a role in the genesis, outcome, and treatment of other types of cancer.

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF VITAMIN D

Vitamin D compounds are important components of the vitamin D hormone system. Vitamin D is synthesized in the body through a complex series of steps beginning in the skin under the influence of ultraviolet light, when a cholesterol precursor molecule (7-dehydrocholesterol) is changed into the vitamin D hormone precursor, cholecalciferol (vitamin D_3). Hence, strictly speaking, vitamin D is not a vitamin. Vitamin D_3 is subsequently hydroxylated in the liver (yielding $25(OH)D_3$) and then in the kidney to yield the most active hormone form of these compounds, 1,25-dihydroxycholecalciferol or calcitriol. "Inactivation" of calcitriol and other vitamin D compounds is accomplished primarily by 24-hydroxylation yielding 1,24,25(OH) cholecalciferol; this step also occurs predominantly in the kidney. These hydroxylations are mediated primarily by cytochrome P450 (CYP) enzymes CYPR1, CYP27B1, and CYP24A1, respectively. As is the case for estrogenic and androgenic hormones, while major organs of metabolism and excretion are liver and kidneys, tissue level and even tumor cell and microenvironment cells may also metabolize these hormones. Vitamin D hormones are transported in the circulation bound to vitamin D-binding protein (DBP or Gc-MAF), a multifunctional protein in the albumin family. Alternatively spliced transcript variants encoding different isoforms have been found for the gene encoding this protein, and the prevalence of expression of these isoforms varies among different racial and ethnic groups. The physiologic importance of these differences is unclear. $25(OH)D_3$ is the compound measured in the blood to assess "vitamin D sufficiency" status. While the liver and kidneys are the predominant sites of D_3 metabolism, it is important to realize that these CYP enzymes are expressed in most tissues studied; extrahepatic and extrarenal production and catabolism of vitamin D hormones has been shown to occur, may be relevant in disease, and is a potential therapeutic target. There is also the potential for genetic variants of these metabolizing enzymes and the Gc transport protein to result in complex combinations of moieties that may impact tissue-specific vitamin D hormone signaling.[4]

After $1,25D_3$ enters cells, primarily by passive diffusion, it binds to the VDR which then heterodimerizes with the retinoid X receptor (RXR) and its cognate ligand, 9-cis-retinoic acid. This complex binds to promoter regions of vitamin D-responsive genes to modulate gene expression. More than 2000 genes are modulated by $1,25D_3$. The complexities of variants in D_3 metabolism, protein binding, partner heterodimerization, and the multitude of genes modulated by the vitamin D hormone systems suggest that dissection of the role of this system in cancer or other diseases will require very careful and detailed study.

EPIDEMIOLOGIC STUDIES OF VITAMIN D IN PROSTATE CANCER

One of the reasons to examine the role of vitamin D in prostate cancer is the large number of epidemiologic studies linking vitamin D and prostate cancer risk and outcome. Similar to the case in many other tumors (*e.g.*, colorectal, breast, lung, and non-Hodgkin's lymphoma), studies have reported a higher risk of prostate cancer or lethal prostate

Inova Schar Cancer Institute, Inova Health System, Fairfax, VA 22037, USA.
Correspondence: Dr. DL Trump (donald.trump@inova.org)

cancer in men living in Northern latitudes, and higher overall prostate cancer risk and/or poor prognosis among men whose estimated vitamin D intake is low or in whom $25(OH)D_3$ has been measured. One of the most persuasive cases for an association between vitamin D and prostate cancer is based on studies of African-American men. In these men, $25(OH)D_3$ levels are often low (primarily due to the effect of skin pigmentation reducing intracutaneous synthesis of vitamin D), and prostate cancer risk and mortality are clearly higher than that of Caucasian men. While this relationship has been described many times, the mechanisms for this association are unclear. Clearly, a factor contributing to unfavorable outcome among African-American men is disparities in access to medical care. If it was true that reduced serum levels of $25(OH)D_3$ contributed to the risk of prostate and other cancers, one would imagine that isoforms of vitamin D-metabolizing genes and perhaps even vitamin D-binding protein might be associated with different cancer risks or outcomes. While there are clear associations between polymorphisms in vitamin D pathway genes and vitamin D serum levels, an association between such polymorphisms and prostate cancer risk or prognosis remains elusive. The VITAL study (vitamin D and OmegA-3 Trial, a randomized trial in 20 000 individuals who are 55 years of age or greater and who receive 2000 IU vitamin D_3 or omega-3 fatty acid or both or placebo) provides important information regarding the role of vitamin D supplementation and the risk of cancer and cardiovascular disease.[5] This is a very important trial for two critical reasons: (1) the accrual objective is sufficient to make it likely that the effects of supplementation will be able to be determined with substantial statistical power; (2) it is the one of only two "large" trials in which individuals receive a dose of vitamin D likely to raise the $25(OH)D_3$ level in most patients. The outcomes of this trial will do much to clarify the role of vitamin D supplementation on health outcomes.

ANTICANCER EFFECTS OF VITAMIN D COMPOUNDS

While the biochemical changes associated with anticancer effects of vitamin D treatment of cells have been extensively studied in prostate cancer and other cancer cell lines, the detailed mechanisms underlying inhibition of the survival and proliferation of cancer cells remain poorly understood. Calcitriol (1,25-dihydroxycholecalciferol) is the compound most carefully studied *in vitro* and *in vivo*. Calcitriol inhibits tumor growth in association with the following biochemical effects:

- Cell cycle arrest with increased numbers of cells in G0/G1 and modulation of cyclin-dependent kinase (CDK) inhibitors, such as p21 and p27;[6–9]
- Induction of apoptosis with poly (ADP-ribose) polymerase (PARP) cleavage, annexin binding, and increased bax/bcl-2 ratio;[8–13]
- Suppression of the "pro-proliferative" signaling molecules such as phosphorylated mitogen-activated protein kinase (P-MAPK) (extracellular signal-regulated kinase [ERK] 1/2), phosphorylated-AKT (P-AKT), AKT, and MAPK/ERK kinase (MEKK)-1;[10]
- Induction of caspase-dependent MAPK/ERK kinase (MEK)-cleavage;[14,15]
- Induction of the p53 homolog p73;[15]
- Inhibition of angiogenesis;[16,17]
- Inhibition of motility and invasion;[18,19]
- Induction of differentiation;[5,20,21]
- Modulation of the expression of tumor-associated growth factors.[22–24]

Each of these mechanisms of tumor inhibition has been described to occur in prostate and other cancer models *in vitro* and *in vivo*. In addition, considerable evidence supports the role of vitamin D signaling

in immune function and inflammation. Immune dysregulation and inflammation are increasingly recognized as viable targets in cancer therapy and prevention. While the precise role of vitamin D in regulating immune function is still being defined, there are many studies demonstrating the impact of vitamin D signaling on monocyte/macrophage differentiation, T cell function, and cytokine production.[25–27] Cyclooxygenase-2 (COX-2), the enzyme that catalyzes prostaglandin (PG) synthesis, has been extensively investigated as a target in cancer therapy and prevention.[28] COX-2 is overexpressed in putative cancer precursor inflammatory lesions of the prostate, established prostate carcinoma, and tumor-infiltrating macrophages and other cells in the microenvironment of prostate.[29–31] Calcitriol regulates the expression of several genes in the PG pathway in prostate; *in vitro* and *in vivo* studies demonstrate that calcitriol + nonsteroidal anti-inflammatory agents which inhibit COX-2 potentiate the growth inhibitory effects of calcitriol.[32–34] $1,25(OH)_2D$ analogs may suppress inflammation as well as COX-2 expression and activity either directly or indirectly.[35,36]

$1,25(OH)_2D$ may alter androgen metabolism in prostate cancer cells and provide another antitumor mechanism. CYP3A4, CYP3A5, CYP3A43, AKR1C1-3, UGT2B15/17, HSD17B2, and SULT2B1b are enzymes important in cholesterol and steroid hormone metabolism; activity of these enzymes may reduce intracellular testosterone, dehydroepiandrosterone (DHEA), and androstanediol concentrations. Vitamin D compounds activate these enzymes in prostate cell lines and ultimately can reduce the availability of these pro-survival androgenic steroids. There is no direct evidence that vitamin D compounds modulate "intracrine" androgen metabolism in patients, but preclinical studies are consistent with the hypothesis that this is an additional mechanism whereby $1,25(OH)_2D$ compounds may suppress prostate tumor growth.[37–39]

ANALOGS OF $1,25(OH)_2D$

Considerable work has been done seeking to delineate analogs of $1,25(OH)_2D$ that may have greater antitumor activity and/or less potential to induce hypercalcemia, the only known toxic effect of vitamin D compounds. The analogs EB 1089, MC903, 22-oxacalcitriol, BGP-13(a 24-chloro calcipotriene-based D_3 analog), R024-2637, 19-nor-14-epi-23-yne-$1,25(OH)_2D_3$ (TX 522, inecalcitol), and 19-nor-14,20-bisepi-23-yne-$1,25(OH)_2D_3$ (TX 527) are reported to be less likely to cause hypercalcemia than the parent compound calcitriol. Each of these analogs appears to have activity in preclinical prostate cancer models.[40–47]

Inecalcitol (TX 522) has been tested clinically, a safe dose has been defined (4000 mcg daily [QD]), and a Phase II trial in combination with docetaxel suggests that this combination is superior to docetaxel alone.[48,49] A definitive trial has not been done, however. While appealing conceptually, $1,25(OH)_2D_3$ analogs have not been evaluated in a way as to prove that for equitoxic doses of an analog and parent compound, the analog has antitumor activity superior to $1,25(OH)_2D_3$ or that the potential for a given analog to cause hypercalcemia is less than $1,25(OH)_2D_3$, when given at "equi-effective" antitumor doses. Much of the apparent reduction in the potential to cause hypercalcemia for many analogs can be explained by differences in protein binding and catabolism of analog compared to the parent compound. For example, "resistance" to CYP24A1 breakdown will extend the half-life of an analog intracellularly. Resistance to CYP24A1-mediated catabolism would mean that a given concentration of an analog would be "more potent" since intracellular removal would be delayed. Such compounds would

likely cause more hypercalcemia at a molecularly equivalent dose of $1,25(OH)_2D_3$. Similarly, if an analog is more tightly protein bound, it will take a larger dose of said analog to cause hypercalcemia in an intact animal, since the active moiety of a drug is that portion which is "free" and physiologically active in tissues. Demonstrating that the dose of an analog which causes hypercalcemia is larger than the dose of calcitriol that causes hypercalcemia does not establish that an analog is intrinsically "less hypercalcemic." Ma and colleagues have demonstrated that inecalcitol and calcitriol have different maximum tolerable doses in mice and that antitumor effects of inecalcitol were seen at lower concentrations of this agent than calcitriol. However, in a xenograft model of squamous cell carcinoma, doses of these two compounds that caused similar degrees of hypercalcemia also had similar antitumor effects.[50] No vitamin D analog has been developed which clearly dissociates the hypercalcemic effects of the agent from the anticancer or other biological effects.

RESISTANCE TO THE ANTITUMOR EFFECTS OF VITAMIN D ANALOGS

As will be discussed below, the clinical activity of $1,25(OH)_2D_3$ and analogs has been much harder to demonstrate than might be expected given the extent of the preclinical data indicating substantial anticancer effects. One of the factors contributing to this could be the existence of substantial "resistance" mechanisms which may confound the clinical trials. Resistance to the antiproliferative effects of vitamin D analogs has been demonstrated in a number of preclinical models – *in vitro* and *in vivo*. The two best-characterized mechanisms of resistance to vitamin D compounds are loss or diminished function of VDR and enhanced CYP24A1-mediated catabolism. The absence or diminished expression of the VDR is clearly associated with diminished responsiveness to vitamin D analogs *in vivo* and *in vitro*.[51,52] Polymorphisms in VDR structure and varying levels of cofactors important in vitamin D signaling could change the sensitivity of tumor cells to $1,25(OH)_2D_3$. In a related fashion, treatment with a proteasome inhibitor, which impedes degradation of intracellular proteins, enhances the intracellular content of VDR and potentiates the antitumor effects of calcitriol *in vitro* in a bone tumor cell line.[53]

Changes in CYP24A1 activity and subsequent modulation of the antitumor effect of $1,25(OH)_2D_3$ and analogs has been demonstrated clearly *in vitro*, *in vivo* and potentially in the clinic.[54–58] Several different classes of CYP24A1 inhibitors have been developed and preclinical activity demonstrated; few studies have been done seeking to combine such inhibitors and vitamin D compounds as therapy for cancer.[59–63]

Ajibade and colleagues presented an interesting study, which from the standpoint of tumor biology is entirely plausible and not unexpected, but provides a cautionary note in the study of broadly active biologic agents in cancer therapy. In a transgenic murine model of prostate cancer (TRAMP), these investigators found that calcitriol and the calcitriol analog, QW, when administered weekly to 4-week-old mice inhibited the growth of prostate cancer (as indicated by reduced urogenital tract [$P=0.0022$ for calcitriol, $P=0.0009$ for QW] and prostate weights [$P=0.0178$ for calcitriol, $P=0.0086$ for QW]). Neither vitamin D compound had any effect on castration-resistant TRAMP prostate cancer. In a second experiment, TRAMP mice were treated for 20–25 weeks with calcitriol and the number of distant organ metastases was enhanced ($P = 0.0003$). These data suggest that the effects of $1,25(OH)_2D_3$ may differ in this model between castration-responsive and castration-resistant populations.[64]

COMBINATION THERAPIES WITH VITAMIN D COMPOUNDS

While $1,25(OH)_2D_3$ compounds have shown encouraging activity in preclinical models, single agents usually have limited effect in clinical cancer therapy. $1,25(OH)_2D_3$ compound-based combination therapies are being explored and enhance antitumor efficacy.

Glucocorticoids

Among the first compounds whose interaction with vitamin D was examined were glucocorticoids. Glucocorticoids have direct anticancer effects in their own right and are effective in reducing vitamin D-induced hypercalcemia. Glucocorticoids enhance VDR expression in many cell types. The extent to which this occurs varies with tissue type, tumor types, and species. Preclinical studies demonstrate synergistic antitumor effects of calcitriol and glucocorticoids in human prostate cancer xenografts, and the ability of dexamethasone to counteract calcitriol-induced hypercalcemia led to many clinical trials incorporating dexamethasone with calcitriol.[8,65]

Inhibitors of CYP24A1

Nonspecific (*e.g.*, ketoconazole) P450 inhibitors reduce the activity of CYP24A1 and accentuate the antitumor activity of liarazole and calcitriol. Specific inhibitors such as secosteroid derivatives of $1,25(OH)_2D$, as well as natural products such as soy or its component isoflavones such as genistein and daidzein and also progesterone all inhibit CYP24A1 (directly or indirectly) and potentiate the antitumor effects of vitamin D compounds.[51,52,55,66–72] The combination of calcitriol and genistein (which competitively inhibits CYP24a1 activity) synergistically inhibits tumor growth in human prostate models.[57,68] Muindi and colleagues clearly demonstrated in a prostate cancer model (PC-3) that ketoconazole potentiates the antitumor effect of high dose, intermittently administered calcitriol.[58] Clinical evaluation of this regimen is ongoing.

Nonsteroidal anti-inflammatory drugs

As noted above, inhibition of prostaglandin synthesis may potentiate the activity of vitamin D compounds. A Phase II trial evaluating the combination of the nonselective nonsteroidal anti-inflammatory drug (NSAID) naproxen and high-dose calcitriol in patients with early recurrent prostate cancer demonstrated some benefits in terms of reduction in prostate-specific antigen (PSA) doubling time.[73]

Retinoids

Given the interaction between $1,25(OH)_2D_3$ – VDR and retinoid X receptor (RXR)-9-cisretinoic acid, it is plausible to hypothesize that ligands for the retinoid receptors, retinoic acid receptors (RARs), and RXRs would modify $1,25(OH)_2D_3$ action. Several studies report potentiation of calcitriol antitumor effects by retinoid compounds.[74–79]

Cytotoxic agents

Studies, *in vitro* and *in vivo*, show that vitamin D compounds potentiate the cytotoxicity of many anticancer agents; substantial potentiation and often synergy has been reported with the combination of calcitriol or other $1,25(OH)D_3$ analogs and platinum compounds (cisplatin [Platinol®] and carboplatin [Paraplatin®]), anthracyclines (doxorubicin [Adriamycin®] and mitoxantrone [Novantrone®]), topoisomerase inhibitors (irinotecan [Camptosar®] and etoposide [Etoposide®]), antimetabolites (cytosine arabinoside [Cytarabine®], gemcitabine [Gemzar®], and 5-fluorouracil [Adrucil®]), and taxanes (docetaxel [Docetaxel®] and paclitaxel [Taxol®]). This interaction is associated with increased expression of p21 and perturbation of cell cycle kinetics, enhanced induction of apoptosis, and increased expression of p73.[80–88] These effects are most pronounced when vitamin

D compounds are administered before or simultaneously with the cytotoxic agent (**Table 1**).

Ionizing radiation and photodynamic therapy

Vitamin D compounds potentiate the antitumor effects of ionizing radiation as well as photodynamic therapy. Little clinical work has been done to explore this potentially useful combination.[89,90]

CLINICAL TRIALS OF VITAMIN D COMPOUNDS IN PROSTATE CANCER THERAPY

Preclinical studies of $1,25(OH)_2D_3$ and analogs clearly demonstrate principles on which the conduct of clinical trials of these compounds in cancer therapy should be based. As we will see, such principles have often been ignored:

- The anticancer activity of $1,25(OH)_2D_3$ is clearly dose dependent. Based on this concept, and in keeping with the general tenets of cancer drug development, clinical studies should be conducted utilizing the maximum safe dose of vitamin D compound
- Maximum exposure to $1,25(OH)_2D_3$ compounds in animal studies is achieved if these agents are administered on an intermittent schedule. For example, clinical trials demonstrate that the maximum oral dose of calcitriol is 1.5–2.0 mcg QD (~14 mcg per 2 weeks).[91] In contrast, intravenous calcitriol at a dose of 74 mcg weekly is safe and well tolerated (144 mcg per 2 weeks).[92] 125 mcg per week is safe if combined with dexamethasone (250 per 2 weeks).[85]

Muindi and colleagues demonstrated that the systemic exposure to calcitriol achieved in murine models in which calcitriol is effective in suppressing cancer growth can be achieved in humans at high, but safe and tolerable oral and intravenous doses of calcitriol.[88]

Single-agent Vitamin D compounds

Calcitriol, 1α-hydroxyvitamin D_2 (doxercalciferol), and 19-nor-1alpha-25-dihydroxyvitamin D_2 (paricalcitol) have been evaluated as single agents in patients with castration-resistant prostate cancer (CRPC) and castration-sensitive disease (**Table 2**). While a decrease in the rate of PSA rise in the castration-sensitive setting and some evidence of activity in CRPC (19% PSA response rate),[73,91–98] none of these studies provides convincing evidence of clinically important single-agent activity of calcitriol. Confounding the analysis of single-agent activity of calcitriol has been the fact that it has been combined with glucocorticoids in most studies. This was done to facilitate maximum, safe calcitriol dosing, as glucocorticoids have been shown to block the hypercalcemic effect of calcitriol. However, since glucocorticoids have anticancer activity in men with prostate cancer, determining the calcitriol response rate in single-arm studies using calcitriol + dexamethasone is problematic. In large trials in which single-agent glucocorticoids have served as the control arm for testing new agents, PSA response rates have been reported in the range of 3%–10%.[99–102] In view of these data, one might conclude that a PSA response rate of 19% following calcitriol po + dexamethasone is "interesting." However, in multiple small studies of glucocorticoids alone, rates of 25%–45% are reported.[83,103–106] Factors contributing to this wide variation in PSA response with glucocorticoids include the small number of patients entered on many trials, the variation in prior treatment and extent of disease, even though all were castration resistant. There are intriguing data that response rates following different glucocorticoids may differ. In summary, however, the data indicate a response rate to calcitriol + dexamethasone that is difficult to distinguish from the rate one might expect with dexamethasone alone.

Gross et al.[95] studied androgen deprivation therapy (ADT)-naïve patients and noted a decrease in the "rate of rise" of PSA. While intriguing, this is a measure of uncertain significance in such patients. As was noted in the study of Ajibade et al.,[64] calcitriol and a related compound had different effects in castration-naive and castration-resistant prostate cancer; the same could be true in men with prostate cancer. Most studies of calcitriol alone have been conducted in men with castration-resistant disease.

1 alpha (OH) vitamin D_2 (doxercalciferol) has been studied as a single agent in CRPC (12.5 mcg orally QD). Among 26 patients, there was no evidence of antitumor activity and no substantial decreases in PSA were noted.[100] Paricalcitol was evaluated on a 3 × per week schedule. This schedule and dose of paricalcitol proved to be very safe and well tolerated, but PSA responses were not seen; parathyroid hormone (PTH) levels were suppressed, indicating biologic activity of this dose and schedule.[95]

While limited data exist that single-agent calcitriol or its analogs have important clinical activity, these trials clearly demonstrate that calcitriol can be administered at a very high dose on an intermittent schedule and that the safe dose of calcitriol administered can be increased if concomitant glucocorticoids are employed.

However, there is not a suitable formulation of calcitriol for high-dose oral administration. Two groups have shown that the

Table 1: Cytotoxic agents with which vitamin D compounds have demonstrated synergy *in vivo*

Vitamin D compounds	Cytotoxic agents
Platinum compounds	Cisplatin (Platinol®)
	Carboplatin (Paraplatin®)
Anthracyclines	Doxorubicin (Adriamycin®)
	Mitoxantrone (Novantrone®)
Topoisomerase inhibitors	Cytosine arabinoside (Cytarabine®)
	Gemcitabine (Gemzar®)
	5-fluorouracil (Adrucil®)
Antimetabolites	Cytosine arabinoside (Cytarabine®)
	Gemcitabine (Gemzar®)
	5-fluorouracil (Adrucil®)
Taxanes	Docetaxel (Docetaxel®)
	Paclitaxel (Taxol®)

Table 2: Clinical trials of calcitriol "only" in prostate cancer

	Calcitriol dose	Concomitant medications	Response	Reference
ADT-naive	45 mcg po weekly	Naproxen	14/21 prolonged PSADT	Srinivas and Feldman[73]
	0.5–2.5 mcg po daily	None	6/7 prolonged PSADT	Gross et al.[95]
CRPC	74 mcg iv weekly	Dexamethasone 4 mg QD × 2, weekly	0/18	Chadha et al.[92]
	8–12 mcg QD × 3, po weekly	Dexamethasone 4 mg QD × 4, weekly	8/37 (19%)	Trump et al.[93]
	0.5–1.5 mcg po QD	None	0/14	Osborn et al.[91]
	4–30 mcg po QD	Zoledronic acid	"Minimal"	Morris et al.[94]

ADT: androgen deprivation therapy; CRPC: castration-resistant prostate cancer; mcg: micrograms; QD: daily; PSADT: prostate-specific antigen doubling time; po: per os; iv: intravenous

standardly available oral formulation (Rocaltrol®) is inappropriate for high-dose administration. Doses of Rocaltrol® as high as 40 mcg QD × 3 have been investigated; this schedule is both inconvenient (requiring 80 tablets daily) and more importantly pharmaceutically flawed. At doses greater than 15–20 mcg QD, the expected linear relationship between dose administered and plasma levels of $1,25(OH)_2D$ achieved is lost, apparently due to impaired absorption. Use of the liquid formulation of calcitriol does not overcome this problem.[83,107]

The now defunct pharmaceutical company Novocea developed a new formulation of calcitriol referred to as DN-101. This formulation was carefully studied and provided a linear relationship between dose and systemic exposure over a wide dose range.[108]

Combinations of vitamin D compounds and cytotoxic agents in prostate cancer

Many classes of agents are synergistic or additive in preclinical studies providing strong rationale for combinations of calcitriol and cytotoxic agents (**Table 3**). Examination of the sequence of trials with vitamin D compounds and cytotoxic agents illustrates the challenges of drug development.[87,109,110,112] There was limited pharmaceutical company interest in such studies; hence, resources for the conduct of such trials were limited. This led to the conduct of many small, Phase 2 trials often with less than maximal (and hence likely suboptimal) doses of calcitriol.

Novocea, to its credit, initiated randomized Phase III trials as soon as a safe and pharmacologically dependable dose of DN-101 was determined. However, the dose of DN-101 chosen was based on convenience, not scientific data. In light of safety and response rate seen in a single-institution trial of calcitriol (0.5 mcg kg^{-1} day 1) + docetaxel (day 1),[109] Novocea conducted a randomized Phase III study (ASCENT I) to determine the PSA response rate (defined as a >50% decline in PSA for >1 month) following standard therapy for CRPC (docetaxel 36 mg sqm^{-1} weekly intravenously × 4 weeks every 6 weeks) compared to the same dose and schedule of docetaxel + calcitriol (DN-101), 45 mcg weekly.[113] The dose of DN-101 was based on the safety and response rate in the earlier calcitriol trial and the fact that transient, asymptomatic, and readily reversible hypercalcemia (Grade 2) occurred in 2 of 6 patients treated with 60 mcg of DN-101.[108] Two hundred and fifty patients were randomized. PSA response rates were 63% (DN-101) and 52% (placebo) ($P = 0.07$). While the primary goal (superior PSA response for DN-101 + docetaxel) of this trial was not achieved, there was a numeric difference in the PSA response rate favoring the DN-101 arm. While survival was not a primary endpoint of this trial, patients receiving DN-101 did fare better. The median survival for the placebo arm was around 16.4 months and for the DN-101 arm around 24.5 months. Hazard rate for death in the DN-101 group was 0.67 ($P = 0.04$). All toxicities appeared equal between the two arms and no limiting hypercalcemia was noted. These results were encouraging, especially in view of the fact that this was a relatively large, randomized trial. However, drug approval was not possible based on this trial. A larger trial was

initiated (ASCENT II) in order to evaluate the apparent survival advantage of DN-101 + docetaxel. When ASCENT II was designed, the weekly docetaxel regimen employed in ASCENT I had been shown to be inferior in terms of patient survival compared to an every 3-week regimen (75 mg sqm^{-1} every 3 weeks).[114]

Despite this change in the standard of care, the ASCENT II trial designers decided to compare the experimental treatment arm of ASCENT I (docetaxel, 36 mg sqm^{-1} + DN-101, 45 mcg weekly × 4, every 6 weeks) to docetaxel 75 mg sqm^{-1} every 3 weeks + placebo.[115] One thousand patients were to be accrued to this study. ASCENT II was halted when 953 had been accrued because at an interim analysis, survival was statistically inferior in the DN-101 arm. The median overall survival was 17.8 months (95% CI: 16.0–19.5) in the calcitriol arm and 20.2 months (95% CI: 18.8–23.0) in the docetaxel arm (log-rank $P = 0.002$). Unfortunately, this trial result has been interpreted to show that calcitriol does not potentiate the antitumor efficacy of docetaxel in CRPC. This trial demonstrates that a calcitriol dose less than that which can be safely administered does not overcome the inferiority of weekly versus q 3 weeks of docetaxel. This trial was seriously flawed in two critical ways:

- The design of ASCENT II violated a basic principle of clinical trial development. The appropriate standard for trial design is standard therapy ± investigational regimen. ASCENT II compared a superior regimen + placebo to an inferior regimen + investigational agent. The appropriate conclusion from ASCENT II is that calcitriol at the dose studied did not overcome the intrinsic inferiority of the weekly docetaxel regimen

- There are no data that indicate that the dose of calcitriol administered in ASCENT II was biologically or therapeutically ideal. The dose studied was less than 50% of the maximum tolerated dose of calcitriol that can be given on an intermittent schedule. The dose of calcitriol chosen was based on a study of 37 patients by Beer and colleagues which demonstrated an 81% response rate in CRPC,[109] and the Phase 1 study of DN-101 which determined that the appropriate dose of this calcitriol formulation was 45 mcg weekly – based on the occurrence of Grade 2 hypercalcemia (11.6–12.5 mg dl^{-1}) in 2 of 6 patients treated with 60 mcg of calcitriol as DN-101.[108] Many would view this as an overly conservative definition of tolerable dose for use in men with advanced cancer. Ramnath et al.[116] safely administered 80 mcg sqm^{-1} (~120 mcg total dose) of calcitriol intravenously with docetaxel and cisplatin and no patient had hypercalcemia. Myelosuppression was the limiting toxicity in that trial, very likely related solely to the cytotoxic agents. Muindi et al.[85] and Fakih et al.[86] studied intravenous calcitriol weekly with gefitinib (Iressa®), an oral epidermal growth factor tyrosine kinase inhibitor. 74 mcg weekly alone and 125 mcg weekly with dexamethasone were the defined "Phase II" doses. The ASCENT I and II trials were done with a dose of calcitriol that was one-quarter to one-half the calcitriol dose that would have

Table 3: Selected results of Phase II trials of vitamin D compounds + interacting agents in men with prostate cancer

Vitamin D compound	Combination agent	Patients (n)	Response rate (%)	Reference
Calcitriol	Docetaxel (Taxotere®)	37	81	Beer et al.[109]
Calcitriol	Mitoxantrone (Novantrone®)	19	26	Chan et al.[87]
Calcitriol	Carboplatin (Paraplatin®)	17	38	Beer et al.[108]
			6	Flaig et al.[110]
Inecalcitol	Docetaxel (Taxotere®)	54	76	Medioni et al.[49]
Calcitriol	Ketoconazole	33	58	Trump et al.[111]

been safe. Considerable evidence in preclinical studies indicates that the anticancer effect of calcitriol is dose/exposure related.

In another double-blind, randomized study, Attia and colleagues compared docetaxel plus 1-alpha hydroxyvitamin D_2 (doxercalciferol) to docetaxel plus placebo in patients with CRPC receiving weekly docetaxel. Doxercalciferol was administered orally, 10 mcg daily. Seventy patients were randomized to doxercalciferol or placebo. While this dose and schedule of doxercalciferol was safe and well tolerated, no benefit of the vitamin D compound was seen. PSA response rate was 39.4% for the placebo arm and 46.7% for the doxercalciferol arm; survival was 17.8 and 16.4 months in the vitamin D and placebo arms, respectively.[117]

VITAMIN D ANALOGS ADMINISTERED TO PATIENTS PRIOR TO PROSTATE REMOVAL: EXPLORATION OF BIOLOGIC EFFECTS

Several studies have been conducted to explore the biologic effect of the administration of vitamin D compounds in the preoperative period before prostatectomy to evaluate potential biologic changes. This study design has considerable merit in allowing exploration of clinically relevant dose and biologic response relationships in human tissues. Beer and colleagues gave 39 patients either calcitriol (0.5 mcg kg^{-1} weekly × 4) or placebo and after 4 weeks patients underwent scheduled prostatectomy for prostate cancer. While in the calcitriol group, the percentage of cells expressing VDR was lower (75.3%) compared to the patients receiving placebo (98.6%), there was no change in the percentage of cells expressing TGF beta RII, phosphatase and tensin homolog (PTEN), or proliferating cell nuclear antigen (PCNA). Bcl-2 and c-Myc expression could not be evaluated because of low levels of expression.[118] Gee and colleagues studied doxercalciferol in similar patients randomized to doxercalciferol (10 mcg QD × 28 days) versus placebo for 28 days prior to radical prostatectomy.[119] Serum markers examined included vitamin D metabolites, TGF-β 1/2, free/total PSA, insulin-like growth factor (IGF)-1, IGF-binding protein (IGFBP)-3, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF); tissue markers studied included histology, MIB-1 and TUNEL staining, microvessel density and factor VIII, staining, androgen receptor and PSA, VDR expression and nuclear morphometry. TGF-β2 was the only biomarker significantly altered by doxercalciferol supplementation (~2–4 fold).[87] The meaning of this change has not been further studied. Wagner and colleagues evaluated the biologic effect of vitamin D_3 (cholecalciferol) administration prior to prostatectomy on intraprostatic concentrations of vitamin D metabolites as well as on markers of potential biologic importance.[120] Sixty-three patients completed the administration of either 400 IU, 10 000 IU, or 40 000 IU daily by mouth for up to 4 weeks prior to prostatectomy. In prostate tissue, vitamin D metabolite levels and Ki67 labeling were assessed and serum PTH and PSA were measured. This study demonstrated the safety of these dosing regimens and showed clearly that tissue and serum levels of vitamin D metabolites, including calcitriol, increased in a dose-dependent manner ($P < 0.03$). Vitamin D compound concentrations were highest in the 40 000 IU day^{-1} group ($P < 0.03$). Tissue vitamin D metabolite levels were positively correlated with vitamin D serum levels ($P < 0.0001$). While Ki67 labeling indices were not different among the dosing groups, there was evidence that intraprostatic calcitriol level was inversely associated with the proliferation marker, Ki67 intensity and percent Ki67 "positive" nuclei in prostate cancer and benign tissue ($P < 0.05$). The two highest dose supplementation groups were combined in an analysis of the impact of dosing on PTH and PSA serum levels which indicated that high-dose D_3 administration suppressed PTH and PSA levels ($P < 0.02$).[120] This group conducted further exploratory analyses on the tissues available from this clinical trial. VDR was detected in both epithelial and stromal tissues, and vitamin D hydroxylases were present only in prostate stromal cells. VDR expression was suppressed in those tissues with the highest $1,25(OH)_2D_3$ content. In specimens with the highest $1,25(OH)_2D_3$ content, epithelial cell interleukin (IL)-6 was the highest and stromal cell COX-2 was the lowest. This group has also described *in vitro* studies in prostate stroma and found that the expression of miR-126-3p, miR-154-5p, and miR-21-5p was positively correlated with $1,25D_3$ prostate tissue content. These miRs are associated with pro-inflammatory and proliferative pathways. These investigators also reported that high epithelial/stromal ratio of the miR processing ribonuclease, DICER1, was predictive of biochemical recurrence of prostate cancer in a study of 170 matched prostatectomy specimens (OR = 3.1, $P = 0.03$). These data support the role of vitamin D compounds in influencing the biology of prostate tissues including prostate cancer.[111,121-123]

SUMMARY

There are considerable data indicating the importance of vitamin D signaling in prostate cancer. Vitamin D signaling is a plausible target for the treatment of established cancers – either as vitamin D agents alone or such agents combined with other antineoplastic agents. Careful studies of vitamin D supplementation will be required to determine whether these biologic observations can be translated into prevention strategies. Unfortunately, there is limited information regarding the role of vitamin D compounds in the treatment of prostate cancer. Two approaches could be employed to refine the clinical studies of vitamin D in prostate cancer: (1) studies which establish dependable biomarkers of vitamin D response would allow selection of patients with a greater likelihood of response and (2) well-designed clinical trials of biologically appropriate doses of vitamin D compounds are needed. Existing data strongly support the continued development of these approaches.

COMPETING INTERESTS

Both authors declare no competing interests.

REFERENCES

1 Rubin D, Levij IS. Suppression by vitamins D2 and D3 of hamster cheek pouch carcinoma induced with 9,10-dimethyl-1,2-benzanthracene with a discussion of the role of intracellular calcium in the development of tumors. *Pathol Microbiol* (*Basel*) 1973; 39: 446–60.

2 Murphy LC, Wild J, Posen S, Stone G. 25-Hydroxycholecalciferol receptors in human breast cancer. *Br J Cancer* 1979; 39: 531–5.

3 Colston K, Colston MJ, Feldman D. 1,25-dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. *Endocrinology* 1981; 108: 1083–6.

4 Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, *et al.* Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013; 369: 1991–2000.

5 Olsson I, Gullberg U, Ivhed I, Nilsson K. Induction of differentiation of the human histiocytic lymphoma cell line U-937 by 1 alpha,25-dihydroxycholecalciferol. *Cancer Res* 1983; 43: 5862–7.

6 Wang QM, Jones JB, Studzinski GP. Cyclin-dependent kinase inhibitor p27 as a mediator of the G1-S phase block induced by 1,25-dihydroxyvitamin D3 in HL60 cells. *Cancer Res* 1996; 56: 264–7.

7 Simboli-Campbell M, Narvaez CJ, van Weelden K, Tenniswood M, Welsh J. Comparative effects of 1,25(OH)2D3 and EB1089 on cell cycle kinetics

and apoptosis in MCF-7 breast cancer cells. *Breast Cancer Res Treat* 1997; 42: 31–41.

8   Verlinden L, Verstuyf A, Convents R, Marcelis S, Van Camp M, *et al*. Action of 1,25(OH)2D3 on the cell cycle genes, cyclin D1, p21 and p27 in MCF-7 cells. *Mol Cell Endocrinol* 1998; 142: 57–65.

9   Bernardi RJ, Trump DL, Yu WD, McGuire TF, Hershberger PA, *et al*. Combination of 1alpha,25-dihydroxyvitamin D(3) with dexamethasone enhances cell cycle arrest and apoptosis: role of nuclear receptor cross-talk and Erk/Akt signaling. *Clin Cancer Res* 2001; 7: 4164–73.

10   Welsh J. Induction of apoptosis in breast cancer cells in response to vitamin D and antiestrogens. *Biochem Cell Biol* 1994; 72: 537–45.

11   James SY, Mackay AG, Colston KW. Effects of 1,25 dihydroxyvitamin D3 and its analogues on induction of apoptosis in breast cancer cells. *J Steroid Biochem Mol Biol* 1996; 58: 395–401.

12   Pintado CO, Carracedo J, Rodriguez M, Perez-Calderon R, Ramirez R. 1 alpha, 25-dihydroxyvitamin D3 (calcitriol) induces apoptosis in stimulated T cells through an IL-2 dependent mechanism. *Cytokine* 1996; 8: 342–5.

13   Narvaez CJ, Welsh J. Differential effects of 1,25-dihydroxyvitamin D3 and tetradecanoylphorbol acetate on cell cycle and apoptosis of MCF-7 cells and a vitamin D3 resistant variant. *Endocrinology* 1997; 138: 4690–8.

14   Colston KW, Hansen CM. Mechanisms implicated in the growth regulatory effects of vitamin D in breast cancer. *Endocr Relat Cancer* 2002; 9: 45–59.

15   Johnson CS, Muindi JR, Hershberger PA, Trump DL. The antitumor efficacy of calcitriol: preclinical studies. *Anticancer Res* 2006; 26: 2543–9.

16   Miyata Y, Ohba K, Matsuo T, Watanabe S, Hayashi T, *et al*. Tumor-associated stromal cells expressing E-prostanoid 2 or 3 receptors in prostate cancer: correlation with tumor aggressiveness and outcome by angiogenesis and lymphangiogenesis. *Urology* 2013; 81: 136–42.

17   Chung I, Han G, Seshadri M, Gillard BM, Yu WD, *et al*. Role of vitamin D receptor in the antiproliferative effects of calcitriol in tumor-derived endothelial cells and tumor angiogenesis *in vivo*. *Cancer Res* 2009; 69: 967–75.

18   Hou YF, Gao SH, Wang P, Zhang HM, Liu LZ, *et al*. 1α,25(OH)$_2$D$_3$ suppresses the migration of ovarian cancer SKOV-3 cells through the inhibition of epithelial-mesenchymal transition. *Int J Mol Sci* 2016; 17. pii: E1285.

19   Chen S, Zhu J, Zuo S, Ma J, Zhang J, *et al*. 1,25(OH)2D3 attenuates TGFbeta1/beta2-induced increased migration and invasion via inhibiting epithelial-mesenchymal transition in colon cancer cells. *Biochem Biophys Res Commun* 2015; 468: 130–5.

20   Koeffler HP, Hirji K, Itri L. 1,25-Dihydroxyvitamin D3: *in vivo* and *in vitro* effects on human preleukemic and leukemic cells. *Cancer Treat Rep* 1985; 69: 1399–407.

21   Abe J, Moriya Y, Saito M, Sugawara Y, Suda T, *et al*. Modulation of cell growth, differentiation, and production of interleukin-3 by 1 alpha,25-dihydroxyvitamin D3 in the murine myelomonocytic leukemia cell line WEHI-3. *Cancer Res* 1986; 46: 6316–21.

22   Irani M, Seifer DB, Grazi RV, Julka N, Bhatt D, *et al*. Vitamin D supplementation decreases TGF-beta1 bioavailability in PCOS: a Randomized Placebo-Controlled Trial. *J Clin Endocrinol Metab* 2015; 100: 4307–14.

23   Chen PT, Hsieh CC, Wu CT, Yen TC, Lin PY, *et al*. 1alpha,25-Dihydroxyvitamin D3 inhibits esophageal squamous cell carcinoma progression by reducing IL6 signaling. *Mol Cancer Ther* 2015; 14: 1365–75.

24   Mohapatra S, Saxena A, Gandhi G, Koner BC, Singh T, *et al*. Does vitamin D mediate inhibition of epithelial ovarian cancer by modulating cytokines? *Clin Transl Oncol* 2015; 17: 590–5.

25   Gonzalez-Cao M, Karachaliou N, Viteri S, Morales-Espinosa D, Teixido C, *et al*. Targeting PD-1/PD-L1 in lung cancer: current perspectives. *Lung Cancer* (*Auckl*) 2015; 6: 55–70.

26   Tran E, Robbins PF, Rosenberg SA. 'Final common pathway' of human cancer immunotherapy: targeting random somatic mutations. *Nat Immunol* 2017; 18: 255–62.

27   Byun DJ, Wolchok JD, Rosenberg LM, Girotra M. Cancer immunotherapy-immune checkpoint blockade and associated endocrinopathies. *Nat Rev Endocrinol* 2017; 13: 195–207.

28   Wang W, Bergh A, Damber JE. Cyclooxygenase-2 expression correlates with local chronic inflammation and tumor neovascularization in human prostate cancer. *Clin Cancer Res* 2005; 11: 3250–6.

29   Zha S, Gage WR, Sauvageot J, Saria EA, Putzi MJ, *et al*. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res* 2001; 61: 8617–23.

30   Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *Prostate* 2000; 42: 73–8.

31   Yoshimura R, Sano H, Masuda C, Kawamura M, Tsubouchi Y, *et al*. Expression of cyclooxygenase-2 in prostate carcinoma. *Cancer* 2000; 89: 589–96.

32   Krishnan AV, Feldman D. Molecular pathways mediating the anti-inflammatory effects of calcitriol: implications for prostate cancer chemoprevention and treatment. *Endocr Relat Cancer* 2010; 17: R19–38.

33   Krishnan AV, Srinivas S, Feldman D. Inhibition of prostaglandin synthesis and actions contributes to the beneficial effects of calcitriol in prostate cancer.

34   *Dermatoendocrinol* 2009; 1: 7–11.

34   Moreno J, Krishnan AV, Swami S, Nonn L, Peehl DM, *et al*. Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. *Cancer Res* 2005; 65: 7917–25.

35   Nonn L, Peng L, Feldman D, Peehl DM. Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. *Cancer Res* 2006; 66: 4516–24.

36   Aparna R, Subhashini J, Roy KR, Reddy GS, Robinson M, *et al*. Selective inhibition of cyclooxygenase-2 (COX-2) by 1alpha,25-dihydroxy-16-ene-23-yne-vitamin D3, a less calcemic vitamin D analog. *J Cell Biochem* 2008; 104: 1832–42.

37   Doherty D, Dvorkin SA, Rodriguez EP, Thompson PD. Vitamin D receptor agonist EB1089 is a potent regulator of prostatic "intracrine" metabolism. *Prostate* 2014; 74: 273–85.

38   Seo YK, Mirkheshti N, Song CS, Kim S, Dodds S, *et al*. SULT2B1b sulfotransferase: induction by vitamin D receptor and reduced expression in prostate cancer. *Mol Endocrinol* 2013; 27: 925–39.

39   Maguire O, Pollock C, Martin P, Owen A, Smyth T, *et al*. Regulation of CYP3A4 and CYP3A5 expression and modulation of "intracrine" metabolism of androgens in prostate cells by liganded vitamin D receptor. *Mol Cell Endocrinol* 2012; 364: 54–64.

40   Skowronski RJ, Peehl DM, Feldman D. Actions of vitamin D3, analogs on human prostate cancer cell lines: comparison with 1,25-dihydroxyvitamin D3. *Endocrinology* 1995; 136: 20–6.

41   Campbell MJ, Reddy GS, Koeffler HP. Vitamin D3 analogs and their 24-oxo metabolites equally inhibit clonal proliferation of a variety of cancer cells but have differing molecular effects. *J Cell Biochem* 1997; 66: 413–25.

42   Berkovich L, Sintov AC, Ben-Shabat S. Inhibition of cancer growth and induction of apoptosis by BGP-13 and BGP-15, new calcipotriene-derived vitamin D3 analogs, *in-vitro* and *in-vivo* studies. *Invest New Drugs* 2013; 31: 247–55.

43   Okamoto R, Delansorne R, Wakimoto N, Doan NB, Akagi T, *et al*. Inecalcitol, an analog of 1alpha,25(OH)(2)D(3), induces growth arrest of androgen-dependent prostate cancer cells. *Int J Cancer* 2012; 130: 2464–73.

44   Verlinden L, Verstuyf A, Van Camp M, Marcelis S, Sabbe K, *et al*. Two novel 14-Epi-analogues of 1,25-dihydroxyvitamin D3 inhibit the growth of human breast cancer cells *in vitro* and *in vivo*. *Cancer Res* 2000; 60: 2673–9.

45   Van Belle TL, Vanherwegen AS, Feyaerts D, De Clercq P, Verstuyf A, *et al*. 1,25-Dihydroxyvitamin D3 and its analog TX527 promote a stable regulatory T cell phenotype in T cells from type 1 diabetes patients. *PLoS One* 2014; 9: e109194.

46   Ferreira GB, Overbergh L, Verstuyf A, Mathieu C. 1alpha,25-Dihydroxyvitamin D3 and its analogs as modulators of human dendritic cells: a comparison dose-titration study. *J Steroid Biochem Mol Biol* 2013; 136: 160–5.

47   Verlinden L, Leyssens C, Beullens I, Marcelis S, Mathieu C, *et al*. The vitamin D analog TX527 ameliorates disease symptoms in a chemically induced model of inflammatory bowel disease. *J Steroid Biochem Mol Biol* 2013; 136: 107–11.

48   Medioni J, Deplanque G, Ferrero JM, Maurina T, Rodier JM, *et al*. Phase I safety and pharmacodynamic of inecalcitol, a novel VDR agonist with docetaxel in metastatic castration-resistant prostate cancer patients. *Clin Cancer Res* 2014; 20: 4471–7.

49   Medioni J, Deplanque G, Ferrero J, Maurina T, Rodier JP, *et al*. Dose-finding and efficacy phase II study of inecalcitol, a new VDR agonist, in combination with docetaxel-prednisone regimen for castration-resistant prostate cancer (CRPC) patients (pts). *J Clin Oncol* 2011; 29 15 Suppl: 4605.

50   Ma Y, Yu WD, Hidalgo AA, Luo W, Delansorne R, *et al*. Inecalcitol, an analog of 1,25D3, displays enhanced antitumor activity through the induction of apoptosis in a squamous cell carcinoma model system. *Cell Cycle* 2013; 12: 743–52.

51   Farhan H, Wahala K, Cross HS. Genistein inhibits vitamin D hydroxylases CYP24 and CYP27B1 expression in prostate cells. *J Steroid Biochem Mol Biol* 2003; 84: 423–9.

52   Swami S, Krishnan AV, Moreno J, Bhattacharyya RB, Peehl DM, *et al*. Calcitriol and genistein actions to inhibit the prostaglandin pathway: potential combination therapy to treat prostate cancer. *J Nutr* 2007; 137: 205S–10S.

53   Kaiser MF, Heider U, Mieth M, Zang C, von Metzler I, *et al*. The proteasome inhibitor bortezomib stimulates osteoblastic differentiation of human osteoblast precursors via upregulation of vitamin D receptor signalling. *Eur J Haematol* 2013; 90: 263–72.

54   Hedlund TE, Moffatt KA, Miller GJ. Vitamin D receptor expression is required for growth modulation by 1 alpha,25-dihydroxyvitamin D3 in the human prostatic carcinoma cell line ALVA-31. *J Steroid Biochem Mol Biol* 1996; 58: 277–88.

55   Zhang Q, Kanterewicz B, Buch S, Petkovich M, Parise R, *et al*. CYP24 inhibition preserves 1alpha,25-dihydroxyvitamin D(3) anti-proliferative signaling in lung cancer cells. *Mol Cell Endocrinol* 2012; 355: 153–61.

56   Peehl DM, Seto E, Hsu JY, Feldman D. Preclinical activity of ketoconazole in combination with calcitriol or the vitamin D analogue EB 1089 in prostate cancer cells. *J Urol* 2002; 168: 1583–8.

57   Swami S, Krishnan AV, Peehl DM, Feldman D. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: role of the direct inhibition of CYP24 enzyme activity. *Mol Cell Endocrinol* 2005; 241: 49–61.

58   Muindi JR, Yu WD, Ma Y, Engler KL, Kong RX, *et al*. CYP24A1 inhibition enhances the antitumor activity of calcitriol. *Endocrinology* 2010; 151: 4301–12.

59 Chiellini G, Rapposelli S, Zhu J, Massarelli I, Saraceno M, *et al*. Synthesis and biological activities of vitamin D-like inhibitors of CYP24 hydroxylase. *Steroids* 2012; 77: 212–23.

60 Komagata S, Nakajima M, Takagi S, Mohri T, Taniya T, *et al*. Human CYP24 catalyzing the inactivation of calcitriol is post-transcriptionally regulated by miR-125b. *Mol Pharmacol* 2009; 76: 702–9.

61 Lechner D, Manhardt T, Bajna E, Posner GH, Cross HS. A 24-phenylsulfone analog of vitamin D inhibits 1alpha,25-dihydroxyvitamin D(3) degradation in vitamin D metabolism-competent cells. *J Pharmacol Exp Ther* 2007; 320: 1119–26.

62 Yee SW, Simons C. Synthesis and CYP24 inhibitory activity of 2-substituted-3,4-dihydro-2H-naphthalen-1-one (tetralone) derivatives. *Bioorg Med Chem Lett* 2004; 14: 5651–4.

63 Schuster I, Egger H, Nussbaumer P, Kroemer RT. Inhibitors of vitamin D hydroxylases: structure-activity relationships. *J Cell Biochem* 2003; 88: 372–80.

64 Ajibade AA, Kirk JS, Karasik E, Gillard B, Moser MT, *et al*. Early growth inhibition is followed by increased metastatic disease with vitamin D (calcitriol) treatment in the TRAMP model of prostate cancer. *PLoS One* 2014; 9: e89555.

65 Trump DL, Hershberger PA, Bernardi RJ, Ahmed S, Muindi J, *et al*. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. *J Steroid Biochem Mol Biol* 2004; 89-90: 519–26.

66 Ly LH, Zhao XY, Holloway L, Feldman D. Liarozole acts synergistically with 1alpha,25-dihydroxyvitamin D3 to inhibit growth of DU 145 human prostate cancer cells by blocking 24-hydroxylase activity. *Endocrinology* 1999; 140: 2071–6.

67 Zhao J, Tan BK, Marcelis S, Verstuyf A, Bouillon R. Enhancement of antiproliferative activity of 1alpha,25-dihydroxyvitamin D3 (analogs) by cytochrome P450 enzyme inhibitors is compound- and cell-type specific. *J Steroid Biochem Mol Biol* 1996; 57: 197–202.

68 Rao A, Woodruff RD, Wade WN, Kute TE, Cramer SD. Genistein and vitamin D synergistically inhibit human prostatic epithelial cell growth. *J Nutr* 2002; 132: 3191–4.

69 Rodriguez GC, Turbov J, Rosales R, Yoo J, Hunn J, *et al*. Progestins inhibit calcitriol-induced CYP24A1 and synergistically inhibit ovarian cancer cell viability: an opportunity for chemoprevention. *Gynecol Oncol* 2016; 143: 159–67.

70 Lee LR, Teng PN, Nguyen H, Hood BL, Kavandi L, *et al*. Progesterone enhances calcitriol antitumor activity by upregulating vitamin D receptor expression and promoting apoptosis in endometrial cancer cells. *Cancer Prev Res* (*Phila*) 2013; 6: 731–43.

71 Lou YR, Tuohimaa P. Androgen enhances the antiproliferative activity of vitamin D3 by suppressing 24-hydroxylase expression in LNCaP cells. *J Steroid Biochem Mol Biol* 2006; 99: 44–9.

72 Yee SW, Campbell MJ, Simons C. Inhibition of Vitamin D3 metabolism enhances VDR signalling in androgen-independent prostate cancer cells. *J Steroid Biochem Mol Biol* 2006; 98: 228–35.

73 Srinivas S, Feldman D. A phase II trial of calcitriol and naproxen in recurrent prostate cancer. *Anticancer Res* 2009; 29: 3605–10.

74 Gocek E, Marchwicka A, Baurska H, Chrobak A, Marcinkowska E. Opposite regulation of vitamin D receptor by ATRA in AML cells susceptible and resistant to vitamin D-induced differentiation. *J Steroid Biochem Mol Biol* 2012; 132: 220–6.

75 Mernitz H, Smith DE, Wood RJ, Russell RM, Wang XD. Inhibition of lung carcinogenesis by 1alpha,25-dihydroxyvitamin D3 and 9-cis retinoic acid in the A/J mouse model: evidence of retinoid mitigation of vitamin D toxicity. *Int J Cancer* 2007; 120: 1402–9.

76 Mouratidis PX, Dalgleish AG, Colston KW. Investigation of the mechanisms by which EB1089 abrogates apoptosis induced by 9-cis retinoic acid in pancreatic cancer cells. *Pancreas* 2006; 32: 93–100.

77 Peehl DM, Feldman D. Interaction of nuclear receptor ligands with the vitamin D signaling pathway in prostate cancer. *J Steroid Biochem Mol Biol* 2004; 92: 307–15.

78 Ikeda N, Uemura H, Ishiguro H, Hori M, Hosaka M, *et al*. Combination treatment with 1alpha,25-dihydroxyvitamin D3 and 9-cis-retinoic acid directly inhibits human telomerase reverse transcriptase transcription in prostate cancer cells. *Mol Cancer Ther* 2003; 2: 739–46.

79 Elstner E, Campbell MJ, Munker R, Shintaku P, Binderup L, *et al*. Novel 20-epi-vitamin D3 analog combined with 9-cis-retinoic acid markedly inhibits colony growth of prostate cancer cells. *Prostate* 1999; 40: 141–9.

80 Kommagani R, Payal V, Kadakia MP. Differential regulation of vitamin D receptor (VDR) by the p53 family: p73-dependent induction of VDR upon DNA damage. *J Biol Chem* 2007; 282: 29847–54.

81 Smith DC, Johnson CS, Freeman CC, Muindi J, Wilson JW, *et al*. A phase I trial of calcitriol (1,25-dihydroxycholecalciferol) in patients with advanced malignancy. *Clin Cancer Res* 1999; 5: 1339–45.

82 Johnson CS, Hershberger PA, Bernardi RJ, McGuire TF, Trump DL. Vitamin D receptor: a potential target for intervention. *Urology* 2002; 60: 123–30. [Discussion 30-1].

83 Muindi JR, Peng Y, Potter DM, Hershberger PA, Tauch JS, *et al*. Pharmacokinetics of high-dose oral calcitriol: results from a phase 1 trial of calcitriol and paclitaxel. *Clin Pharmacol Ther* 2002; 72: 648–59.

84 Muindi JR, Potter DM, Peng Y, Johnson CS, Trump DL. Pharmacokinetics of liquid calcitriol formulation in advanced solid tumor patients: comparison with caplet formulation. *Cancer Chemother Pharmacol* 2005; 56: 492–6.

85 Muindi JR, Johnson CS, Trump DL, Christy R, Engler KL, *et al*. A phase I and pharmacokinetics study of intravenous calcitriol in combination with oral dexamethasone and gefitinib in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2009; 65: 33–40.

86 Fakih MG, Trump DL, Muindi JR, Black JD, Bernardi RJ, *et al*. A phase I pharmacokinetic and pharmacodynamic study of intravenous calcitriol in combination with oral gefitinib in patients with advanced solid tumors. *Clin Cancer Res* 2007; 13: 1216–23.

87 Chan JS, Beer TM, Quinn DI, Pinski JK, Garzotto M, *et al*. A phase II study of high-dose calcitriol combined with mitoxantrone and prednisone for androgen-independent prostate cancer. *BJU Int* 2008; 102: 1601–6.

88 Muindi JR, Modzelewski RA, Peng Y, Trump DL, Johnson CS. Pharmacokinetics of 1alpha,25-dihydroxyvitamin D3 in normal mice after systemic exposure to effective and safe antitumor doses. *Oncology* 2004; 66: 62–6.

89 Dunlap N, Schwartz GG, Eads D, Cramer SD, Sherk AB, *et al*. 1alpha,25-dihydroxyvitamin D(3) (calcitriol) and its analogue, 19-nor-1alpha,25(OH)(2)D(2), potentiate the effects of ionising radiation on human prostate cancer cells. *Br J Cancer* 2003; 89: 746–53.

90 Anand S, Rollakanti KR, Horst RL, Hasan T, Maytin EV. Combination of oral vitamin D3 with photodynamic therapy enhances tumor cell death in a murine model of cutaneous squamous cell carcinoma. *Photochem Photobiol* 2014; 90: 1126–35.

91 Osborn JL, Schwartz GG, Smith DC, Bahnson R, Day R, *et al*. Phase II trial of oral 1,25-dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer. *Urol Oncol* 1995; 1: 195–8.

92 Chadha MK, Tian L, Mashtare T, Payne V, Silliman C, *et al*. Phase 2 trial of weekly intravenous 1,25 dihydroxy cholecalciferol (calcitriol) in combination with dexamethasone for castration-resistant prostate cancer. *Cancer* 2010; 116: 2132–9.

93 Trump DL, Potter DM, Muindi J, Brufsky A, Johnson CS. Phase II trial of high-dose, intermittent calcitriol (1,25 dihydroxyvitamin D3) and dexamethasone in androgen independent prostate cancer. *Cancer* 2006; 106: 2136–42.

94 Morris MJ, Smaletz O, Solit D, Kelly WK, Slovin S, *et al*. High-dose calcitriol, zoledronate, and dexamethasone for the treatment of progressive prostate carcinoma. *Cancer* 2004; 100: 1868–75.

95 Gross C, Stamey T, Hancock S, Feldman D. Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D3 (calcitriol). *J Urol* 1998; 159: 2035–9. [Discussion 9-40].

96 Schwartz GG, Hall MC, Stindt D, Patton S, Lovato J, *et al*. Phase I/II study of 19-nor1alpha-25-dihydroxyvitamin D2 (paricalcitol) in advanced, androgen-insensitive prostate cancer. *Clin Cancer Res* 2005; 11: 8680–5.

97 Beer TM, Lemmon D, Lowe BA, Henner WD. High-dose weekly oral calcitriol in patients with a rising PSA after prostatectomy or radiation for prostate carcinoma. *Cancer* 2003; 97: 1217–24.

98 Liu G, Wilding G, Staab MJ, Horvath D, Miller K, *et al*. Phase II study of 1alpha hydroxyvitamin D (2) in the treatment of advanced androgen-independent prostate cancer. *Clin Cancer Res* 2003; 9: 4077–83.

99 de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, *et al*. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011; 364: 1995–2005.

100 Fizazi K, Jones R, Oudard S, Efstathiou E, Saad F, *et al*. Phase III, randomized, double-blind, multicenter trial comparing orteronel (TAK-700) plus prednisone with placebo plus prednisone in patients with metastatic castration-resistant prostate cancer that has progressed during or after docetaxel-based therapy: ELM-PC 5. *J Clin Oncol* 2015; 33: 723–31.

101 Smith M, De Bono J, Sternberg C, Le Moulec S, Oudard S, *et al*. Phase III study of cabozantinib in previously treated metastatic castration-resistant prostate cancer: COMET-1. *J Clin Oncol* 2016; 34: 3005–13.

102 Kantoff PW, Halabi S, Conaway M, Picus J, Kirshner J, *et al*. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. *J Clin Oncol* 1999; 17: 2506–13.

103 Venkitaraman R, Lorente D, Murthy V, Thomas K, Parker L, *et al*. A randomised phase 2 trial of dexamethasone versus prednisolone in castration-resistant prostate cancer. *Eur Urol* 2015; 67: 673–9.

104 Sartor O, Weinberger M, Moore A, Li A, Figg WD. Effect of prednisone on prostate-specific antigen in patients with hormone-refractory prostate cancer. *Urology* 1998; 52: 252–6.

105 Morioka M, Kobayashi T, Furukawa Y, Jo Y, Shinkai M, *et al*. Prostate-specific antigen levels and prognosis in patients with hormone-refractory prostate cancer treated with low-dose dexamethasone. *Urol Int* 2002; 68: 10–5.

106 Saika T, Kusaka N, Tsushima T, Yamato T, Ohashi T, *et al*. Treatment of androgen-independent prostate cancer with dexamethasone: a prospective study in stage D2 patients. *Int J Urol* 2001; 8: 290–4.

107 Beer TM, Munar M, Henner WD. A phase I trial of pulse calcitriol in patients with refractory malignancies: pulse dosing permits substantial dose escalation. *Cancer* 2001; 91: 2431–9.

108 Beer TM, Javle MM, Ryan CW, Garzotto M, Lam GN, *et al*. Phase I study of weekly DN-101, a new formulation of calcitriol, in patients with cancer. *Cancer Chemother Pharmacol* 2007; 59: 581–7.

109 Beer TM, Eilers KM, Garzotto M, Egorin MJ, Lowe BA, *et al*. Weekly high-dose calcitriol and docetaxel in metastatic androgen-independent prostate cancer. *J Clin Oncol* 2003; 21: 123–8.

110 Flaig TW, Barqawi A, Miller G, Kane M, Zeng C, *et al*. A phase II trial of dexamethasone, vitamin D, and carboplatin in patients with hormone-refractory prostate cancer. *Cancer* 2006; 107: 266–74.

111 Trump DL, Brady WE, Aragon-Ching JB, Pili R, Levine EG, *et al*. A phase I/II trial of ketoconazole + calcitriol [1,25(OH)2D3] in castration-resistant prostate cancer. *J Clin Oncol* 2016; 34: 5065.

112 Beer TM, Garzotto M, Katovic NM. High-dose calcitriol and carboplatin in metastatic androgen-independent prostate cancer. *Am J Clin Oncol* 2004; 27: 535–41.

113 Beer TM, Ryan CW, Venner PM, Petrylak DP, Chatta GS, *et al*. Double-blinded randomized study of high-dose calcitriol plus docetaxel compared with placebo plus docetaxel in androgen-independent prostate cancer: a report from the ASCENT Investigators. *J Clin Oncol* 2007; 25: 669–74.

114 Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, *et al*. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004; 351: 1502–12.

115 Scher HI, Jia X, Chi K, de Wit R, Berry WR, *et al*. Randomized, open-label phase III trial of docetaxel plus high-dose calcitriol versus docetaxel plus prednisone for patients with castration-resistant prostate cancer. *J Clin Oncol* 2011; 29: 2191–8.

116 Ramnath N, Daignault-Newton S, Dy GK, Muindi JR, Adjei A, *et al*. A phase I/II pharmacokinetic and pharmacogenomic study of calcitriol in combination with cisplatin and docetaxel in advanced non-small-cell lung cancer. *Cancer Chemother Pharmacol* 2013; 71: 1173–82.

117 Attia S, Eickhoff J, Wilding G, McNeel D, Blank J, *et al*. Randomized, double-blinded phase II evaluation of docetaxel with or without doxercalciferol in patients with metastatic, androgen-independent prostate cancer. *Clin Cancer Res* 2008; 14: 2437–43.

118 Beer TM, Myrthue A, Garzotto M, O'Hara MF, Chin R, *et al*. Randomized study of high-dose pulse calcitriol or placebo prior to radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 2225–32.

119 Gee J, Bailey H, Kim K, Kolesar J, Havighurst T, *et al*. Phase II open label, multi-center clinical trial of modulation of intermediate endpoint biomarkers by 1alpha-hydroxyvitamin D2 in patients with clinically localized prostate cancer and high grade pin. *Prostate* 2013; 73: 970–8.

120 Wagner D, Trudel D, Van der Kwast T, Nonn L, Giangreco AA, *et al*. Randomized clinical trial of vitamin D3 doses on prostatic vitamin D metabolite levels and ki67 labeling in prostate cancer patients. *J Clin Endocrinol Metab* 2013; 98: 1498–507.

121 Giangreco AA, Dambal S, Wagner D, Van der Kwast T, Vieth R, *et al*. Differential expression and regulation of vitamin D hydroxylases and inflammatory genes in prostate stroma and epithelium by 1,25-dihydroxyvitamin D in men with prostate cancer and an *in vitro* model. *J Steroid Biochem Mol Biol* 2015; 148: 156–65.

122 Giangreco AA, Vaishnav A, Wagner D, Finelli A, Fleshner N, *et al*. Tumor suppressor microRNAs, miR-100 and -125b, are regulated by 1,25-dihydroxyvitamin D in primary prostate cells and in patient tissue. *Cancer Prev Res (Phila)* 2013; 6: 483–94.

123 Dambal S, Giangreco AA, Acosta AM, Fairchild A, Richards Z, *et al*. microRNAs and DICER1 are regulated by 1,25-dihydroxyvitamin D in prostate stroma. *J Steroid Biochem Mol Biol* 2017; 167: 192–202.