Vitamin D in prostate cancer

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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.

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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. 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 D3). Hence, strictly speaking, vitamin D is not a vitamin. Vitamin D3 is subsequently hydroxylated in the liver (yielding 25(OH)D3) 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)3 cholecalciferol; this step also occurs predominantly in the kidney. These hydroxylations are mediated primarily by cytochrome P450 (CYP) enzymes CYP24A1, 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)D3 is the compound measured in the blood to assess “vitamin D sufficiency” status. While the liver and kidneys are the predominant sites of D3 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.

After 1,25D3 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 1,25D3. The complexities of variants in D3 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
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$_3$), 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. 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.
- **Induction of apoptosis with poly (ADP-ribose) polymerase (PARP) cleavage, annexin binding, and increased bax/bcl-2 ratio**.
- **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**.
- **Induction of caspase-dependent MAPK/ERK kinase (MEK)-cleavage**.
- **Induction of the p53 homolog p73**.
- **Inhibition of angiogenesis**.
- **Inhibition of motility and invasion**.
- **Induction of differentiation**.
- **Modulation of the expression of tumor-associated growth factors**.

Each of these mechanisms of tumor growth 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. Cyclooxygenase-2 (COX-2), the enzyme that catalyzes prostaglandin (PG) synthesis, has been extensively investigated as a target in cancer therapy and prevention. 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. 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. 1,25(OH)$_2$D$_3$ analogs may suppress inflammation as well as COX-2 expression and activity either directly or indirectly.

1,25(OH)$_2$D$_3$ 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 androstenediol 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)$_2$D$_3$ compounds may suppress prostate tumor growth.

**ANALOGS OF 1,25(OH)$_2$D**

Considerable work has been done seeking to delineate analogs of 1,25(OH)$_2$D$_3$ 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 calciropirole-based D$_3$ analog), R024-2637, 19-nor-14-epi-23-yne-1,25(OH)$_2$D$_3$ (TX 522, inecalcitol), and 19-nor-14,20-bisepi-23-yne-1,25(OH)$_2$D$_3$ (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.

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. A definitive trial has not been done, however. While appealing conceptually, 1,25(OH)$_2$D$_3$ 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)$_2$D$_3$ or that the potential for a given analog to cause hypercalcemia is less than 1,25(OH)$_2$D$_3$ 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)\textsubscript{2}D\textsubscript{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 hypercalemic.” 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.\textsuperscript{30} 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)\textsubscript{2}D\textsubscript{3} and analogs has been much harder to demonstrate than might be expected given the extent of the preclinical data indicating substantial anticaner 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 vitro and in vivo.\textsuperscript{31,32} 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)\textsubscript{2}D\textsubscript{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.\textsuperscript{15}

Changes in CYP24A1 activity and subsequent modulation of the antitumor effect of 1,25(OH)\textsubscript{2}D\textsubscript{3} and analogs has been demonstrated clearly in vitro, in vivo and potentially in the clinic.\textsuperscript{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.\textsuperscript{36–43}

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).\textsuperscript{74} 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)\textsubscript{2}D\textsubscript{3} may differ in this model between castration-responsive and castration-resistant populations.\textsuperscript{64}

**COMBINATION THERAPIES WITH VITAMIN D COMPOUNDS**

While 1,25(OH)\textsubscript{2}D\textsubscript{3} compounds have shown encouraging activity in preclinical models, single agents usually have limited effect in clinical cancer therapy. 1,25(OH)\textsubscript{2}D\textsubscript{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.\textsuperscript{54–55}

**Inhibitors of CYP24A1**

Nonspecific (e.g., ketoconazole) P450 inhibitors reduce the activity of CYP24A1 and accentuate the antitumor activity of liarazol and calcitriol. Specific inhibitors such as secosteroid derivatives of 1,25(OH)\textsubscript{2}D\textsubscript{3}, as well as natural products such as soy or its component isoflavones such as genistene and daidzein, and progesterone also inhibit CYP24A1 (directly or indirectly) and potentiate the antitumor effects of vitamin D compounds.\textsuperscript{35,52,66–72} The combination of calcitriol and genistein (which competitively inhibits CYP24a1 activity) synergistically inhibits tumor growth in human prostate models.\textsuperscript{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.\textsuperscript{30} 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.\textsuperscript{73}

**Retinoids**

Given the interaction between 1,25(OH)\textsubscript{2}D\textsubscript{3} and VDR, retinoids and 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)\textsubscript{2}D\textsubscript{3} effects in their own right and are effective in reducing vitamin D-induced hypercalcemia. The combination of calcitriol and retinoids (which competitively inhibits CYP24a1 activity) synergistically inhibits tumor growth in human prostate model.\textsuperscript{57,68} 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.\textsuperscript{54–55}

**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)\textsubscript{2}D\textsubscript{3} analogs and platinum compounds (cisplatin [Platinol\textsuperscript{®}] and carboplatin [Paraplatin\textsuperscript{®}]), anthracyclines (doxorubicin [Adriamycin\textsuperscript{®}] and mitoxantrone [Novantrone\textsuperscript{®}]), topoisomerase inhibitors (irinotecan [Camptosar\textsuperscript{®}] and etoposide [Etoposide\textsuperscript{®}]), antimetabolites (cytosine arabinoside [Cytarabine\textsuperscript{®}], gemcitabine [Gemzar\textsuperscript{®}], and 5-fluorouracil [Adrucil\textsuperscript{®}]), and taxanes (docetaxel [Docetaxel\textsuperscript{®}] and paclitaxel [Taxol\textsuperscript{®}]). This interaction is associated with increased expression of p21 and perturbation of cell cycle kinetics, enhanced induction of apoptosis, and increased expression of p73.\textsuperscript{80–89} 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.

CLINICAL TRIALS OF VITAMIN D COMPOUNDS IN PROSTATE CANCER THERAPY
Preclinical studies of 1,25(OH)₂D₃ 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)₂D₃ 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)₂D₃ 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). In contrast, intravenous calcitriol at a dose of 74 mcg weekly is safe and well tolerated (144 mcg per 2 weeks). 125 mcg per week is safe if combined with dexamethasone (250 per 2 weeks).

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.

Single-agent Vitamin D compounds
Calcitriol, 1α-hydroxyvitamin D₃ (doxercalciferol), and 19-nor-1α,25-dihydroxyvitamin D₂ (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), 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%. 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. 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. 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., calcitriol and a related compound had different effects in castration-naïve 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 D₃ (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. 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.

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

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**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 Feldman73 |
|                 | 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 x 2, weekly | 0/18 | Chada et al.92 |
|                 | 8–12 mcg QD x 3, po weekly | Dexamethasone 4 mg QD x 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
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), the dose was increased to DN-101, 45 mcg weekly.\(^108\) 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. Muiindi et al,\(^85\) and Fakhil et al,\(^86\) studied intravenous calcitriol weekly with gefitinib (Iressa\(^\text{®}\)), 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

| 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\(^{109}\) |
| Calcitriol          | Carboplatin (Paraplatin®) | 17          | 38                | Beer et al\(^{109}\) |
| Inecalcitol         | Docetaxel (Taxotere®) | 54          | 76                | Medioni et al\(^{110}\) |
| 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$_3$ (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.\textsuperscript{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 $\times$ 4) or placebo and after 4 weeks patients underwent scheduled prostatectomy for prostate cancer. While in the calcitriol group, 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.\textsuperscript{118} Gee and colleagues studied doxercalciferol in similar patients randomized to doxercalciferol (10 mcg QD $\times$ 28 days) versus placebo for 28 days prior to radical prostatectomy.\textsuperscript{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, MI-B-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).\textsuperscript{47} 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.\textsuperscript{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 ($P < 0.02$).\textsuperscript{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)$_2$D$_3$ content. In specimens with the highest 1,25(OH)$_2$D$_3$ 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.\textsuperscript{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.

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