

The Role of Vitamin D in Cancer Prevention and Treatment

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- Vitamin D • Gene regulation • Antiproliferation
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Calcitriol (1,25-dihydroxyvitamin D₃), the biologically most active form of vitamin D, maintains calcium homeostasis through its actions in intestine, bone, kidneys, and the parathyroid glands.¹ The hormone exerts its effects through the vitamin D receptor (VDR), a member of the nuclear receptor superfamily.¹ VDR is present not only in cells and tissues involved in calcium regulation but also a wide variety of other cells including malignant cells. In recent years it has been recognized that calcitriol exerts antiproliferative and prodifferentiating effects in many malignant cells, and retards the development and growth of tumors in animal models raising the possibility of its use as an anticancer agent.²

EPIDEMIOLOGY

Epidemiologic studies have noted lower incidence and mortality rates from several cancers in regions with greater solar ultraviolet (UV)-B exposure.^{3–5} The potential benefit from sunlight is attributed to vitamin D, because UV light is essential for the cutaneous synthesis of vitamin D.¹ The sunlight hypothesis (assuming that sunlight is a surrogate for vitamin D levels in circulation) has been proposed to determine the risk for several cancers^{6,7} including colorectal cancer (CRC)³ prostate cancer (PCa),^{4,5} and breast cancer (BCa).⁸ An inverse association between cancer risk and

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circulating levels of 25-hydroxyvitamin D (25(OH)D, the circulating precursor to calcitriol), which reflect sun exposure and dietary vitamin D intake, has also been reported.⁷ The evidence is strongest for CRC; circulating 25(OH)D levels and vitamin D intake are inversely associated with colorectal adenoma incidence and recurrence.^{9,10} In addition, higher prediagnosis plasma 25(OH)D levels were associated with a significant improvement in overall survival in CRC patients.¹¹ A recent reanalysis of data from the Women's Health Initiative (WHI) randomized trial concluded that concurrent estrogen therapy modified the effect of calcium and vitamin D supplementation on CRC risk and in the women assigned to placebo arms of the estrogen trials, the supplementation was beneficial.¹² The evidence is somewhat weaker for PCa, with some studies suggesting an inverse correlation between serum 25(OH)D levels and PCa risk^{13,14} although others do not support such a correlation.^{15,16} In general, a serum 25(OH)D level exceeding 20 ng/mL was associated with a 30% to 50% reduction in the risk of developing CRC and PCa,^{17,18} and a level of approximately 52 ng/mL was associated with a reduction by 50% in the incidence of BCa.⁸ Higher dietary intake of vitamin D has been associated with a lower incidence of pancreatic cancer.¹⁹

MECHANISMS OF THE ANTICANCER EFFECTS OF CALCITRIOL

In addition to the epidemiologic evidence described earlier, data from *in vitro* studies in cultured malignant cells reveal that calcitriol exerts antiproliferative and prodifferentiating effects; *in vivo* studies in animal models of cancer demonstrate that calcitriol retards tumor growth.^{2,20-31} Several important mechanisms have been implicated in the anticancer effects of calcitriol. The molecular mediators of these calcitriol actions are currently being intensively investigated and characterized.²

Growth Arrest and Differentiation

Calcitriol inhibits the proliferation of many malignant cells by inducing cell cycle arrest and the accumulation of cells in the G₀/G₁ phase of the cell cycle.^{20,31,32} In PCa cells calcitriol causes G₁/G₀ arrest^{26,30,33} in a p53-dependent manner³⁰ by increasing the expression of the cyclin-dependent kinase inhibitors p21^{Waf/Cip1} and p27^{Kip1},³²⁻³⁴ decreasing cyclin-dependent kinase 2 (CDK2) activity,³³ and causing the hypophosphorylation of the retinoblastoma protein (pRb).³⁵ Calcitriol also enhances the expression of p73, a p53 homolog, which has been shown to be associated with apoptosis induction in several human and murine tumor systems. Suppression of p73 abrogates calcitriol-induced apoptosis and reduces the ability of calcitriol to augment the cytotoxic effects of agents such as gemcitabine and cisplatin in a squamous cell carcinoma (SCC) model.³⁶ Calcitriol also increases the expression of CDK inhibitors in other cancer cells.^{2,20,31} It has been shown that calcitriol controls cell growth in part by modulating the expression and activity of key growth factors in cancer cells.^{2,20,26,27,30,31} For example, in PCa cells calcitriol up-regulates the expression of insulinlike growth factor binding protein-3 (IGFBP-3),^{37,38} which functions to inhibit cell proliferation in part by increasing the expression of p21^{Waf/Cip1}.³⁷

In many neoplastic cells, calcitriol also induces differentiation resulting in the generation of cells that acquire a more mature and less malignant phenotype. The mechanisms of the prodifferentiation effects of calcitriol in various cancer cells are specific to the cell type and cell context and include, for example, the regulation of signaling pathways involving β -catenin, Jun-N-terminal kinase (JNK), phosphatidylinositol 3-kinase, nuclear factor κ B (NF κ B) as well as the regulation of the activity of several transcription factors such as the activator protein-1 (AP-1) complex and CCAAT/enhancer-binding protein (C/EBP).^{2,39}

Apoptosis

Calcitriol induces apoptosis in several cancer cells, although this effect is not uniformly seen in all malignant cells. In PCa and BCa cells, calcitriol activates the intrinsic pathway of apoptosis causing the disruption of mitochondrial function, cytochrome release and production of reactive oxygen species.^{31,40–42} These effects are related to the repression of the expression of antiapoptotic proteins such as Bcl₂^{31,40} and enhancement of the expression of proapoptotic proteins such as Bax and Bad.⁴² In some cells calcitriol also directly activates caspases to induce apoptosis.^{42,43} In addition, calcitriol analogues have been shown to enhance cancer cell death in response to radiation and chemotherapeutic drugs.^{44,45}

Inhibition of Invasion and Metastasis

Calcitriol reduces the invasive and metastatic potential of many malignant cells as demonstrated in murine models of prostate and lung cancer.^{46,47} The mechanisms underlying this effect include the inhibition of angiogenesis (as discussed later) and the regulation of the expression of key molecules involved in invasion and metastasis such as the components of the plasminogen activator (PA) system and matrix metalloproteinases (MMPs),⁴⁸ decreasing the expression of tenascin-C, an extracellular matrix protein that promotes growth, invasion, and angiogenesis,⁴⁹ down-regulation of the expression of $\alpha 6$ and $\beta 4$ integrins,⁵⁰ and increase in the expression of E-cadherin, a tumor suppressor gene whose expression is inversely correlated to metastatic potential.³² Calcitriol-mediated suppression of MMP-9 activity and increase in tissue inhibitor of metalloproteinase-1 (TIMP-1) also decrease the invasive potential of PCa cells.⁵¹

Antiinflammatory Effects

Chronic inflammation, triggered by a variety of stimuli such as injury, infection, carcinogens, autoimmune disease, the development of tumors, hormonal factors, and so forth, has been recognized as a risk factor for cancer development.^{52–54} Cancer-related inflammation is characterized by the presence of inflammatory cells at the tumor sites and over-expression of inflammatory mediators such as cytokines, chemokines, prostaglandins (PGs) and reactive oxygen and nitrogen species in tumor tissue.^{52–56} Many of these proinflammatory mediators activate angiogenic switches usually under the control of vascular endothelial growth factor (VEGF) and thereby promote tumor progression, metastasis, and invasion.^{57,58} Epidemiologic studies show a decrease in the risk of developing several cancers associated with the intake of antioxidants and nonsteroidal antiinflammatory drugs (NSAIDs).^{58–60} Recent research suggests that calcitriol exhibits antiinflammatory actions that may contribute to its beneficial effects in several cancers in addition to the other antiproliferative actions discussed earlier. We used cDNA microarrays to uncover the molecular pathways that mediate the anticancer effects of calcitriol in PCa cells.^{61,62} The results show that calcitriol regulation of gene expression leads to the inhibition of the synthesis and biologic actions of prostaglandins (PGs), stress-activated kinase signaling, and production of proinflammatory cytokines. Calcitriol also suppresses the activation and signaling of NF κ B, a transcription factor that regulates the expression of genes involved in inflammatory and immune responses and cellular proliferation⁶³ and believed to play a key role in the process leading from inflammation to carcinogenesis.⁶⁴

Regulation of prostaglandin metabolism and signaling

In multiple PCa cell lines as well as primary prostatic epithelial cells established from prostatectomy samples, calcitriol decreases the mRNA and protein levels of

cyclooxygenase-2 (COX-2), the enzyme responsible for PG synthesis, and increases the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the enzyme that initiates PG catabolism.⁶⁵ As a result calcitriol decreases the levels of biologically active PGs in these cells. In addition, calcitriol decreases the expression of EP and FP PG receptors and thereby attenuates PG-mediated functional responses including stimulation of cell growth.⁶⁵ Thus, reduction in the levels of biologically active PGs and inhibition of PG signaling through their receptors by calcitriol results in suppression of the proliferative and angiogenic stimuli provided by PGs. Combinations of calcitriol with NSAIDs cause a synergistic enhancement of the inhibition of PCa cell proliferation, suggesting that this drug combination might have clinical usefulness in PCa therapy.^{65,66}

Induction of mitogen-activated protein kinase phosphatase-5 (MKP5) and inhibition of stress-activated kinase signaling

cDNA microarray analysis in normal human prostate epithelial cells⁶² revealed a novel calcitriol-responsive gene, MKP5, also known as DUSP10, a member of the dual specificity MKP family of enzymes that dephosphorylate, and thereby inactivate, mitogen-activated protein kinases (MAPKs). MKP5 specifically dephosphorylates p38 MAPK and the stress-activated protein kinase JNK, leading to their inactivation. Calcitriol increases MKP5 transcription by the activation of VDR and its binding to a vitamin D response element (VDRE) in the MKP5 promoter.⁶⁷ The calcitriol-mediated increase in MKP5 causes the dephosphorylation and inactivation of the p38 stress-induced kinase, resulting in a decrease in the production of proinflammatory cytokines that sustain and amplify the inflammatory response, such as interleukin-6 (IL-6). IL-6 stimulates PCa growth and progression⁶⁸ and IL-6 synthesized in periprostatic adipose tissue has recently been shown to modulate PCa aggressiveness.⁶⁹ Calcitriol up-regulation of MKP5 is seen in primary cells derived from normal prostate epithelium and primary localized adenocarcinoma but not in the established cell lines derived from metastatic PCa,⁶⁷ suggesting that the loss of MKP5 might occur during PCa progression, as a result of a selective pressure to eliminate the tumor suppressor activity of MKP5 and/or calcitriol.

Inhibition of NF κ B activation and signaling

NF κ B comprises a family of inducible transcription factors ubiquitously present in cells that are important regulators of innate immune responses and inflammation.⁷⁰ In the basal state, most NF κ B dimers are bound to specific inhibitory proteins called I κ B and proinflammatory signals activate NF κ B mainly through I κ B kinase (IKK)-dependent phosphorylation and degradation of the inhibitory I κ B proteins.⁶⁴ Free NF κ B then translocates to the nucleus and activates the transcription of proinflammatory cytokines, chemokines, and antiapoptotic factors.⁷¹ In contrast to normal cells, many malignant cells have increased levels of active NF κ B.^{72,73} Calcitriol directly modulates basal and cytokine-induced NF κ B activity in many cells including human lymphocytes,⁷⁴ fibroblasts,⁷⁵ and peripheral blood monocytes.⁷⁶ A reduction in the levels of the NF κ B inhibitory protein I κ B has been reported in mice lacking the VDR.⁷⁷ Addition of a VDR antagonist to colon cancer cells up-regulates NF κ B activity by decreasing the levels of I κ B, suggesting that VDR ligands suppress NF κ B activation.⁷⁸ Calcitriol decreases the production of the angiogenic and proinflammatory cytokine IL-8 in immortalized normal human prostate epithelial cell lines and established PCa cell lines by inhibiting the nuclear translocation of the NF κ B subunit p65 and subsequent transcriptional stimulation of the NF κ B downstream target IL-8.⁷⁹ Thus calcitriol could delay the progression of PCa by suppressing the expression of

angiogenic and proinflammatory factors such as VEGF and IL-8. In addition, calcitriol indirectly inhibits NF κ B signaling by up-regulating the expression of IGFBP-3, which has been shown to interfere with NF κ B signaling in PCa cells.⁸⁰ NF κ B also provides an adaptive response to PCa cells against cytotoxicity induced by redox active therapeutic agents and is implicated in radiation resistance of cancers.^{81,82} Calcitriol significantly enhances the sensitivity of PCa cells to ionizing radiation by selectively suppressing radiation-mediated RelB activation.⁸³ Thus calcitriol may serve as an effective agent for sensitizing PCa cells to radiation therapy via suppression of the NF κ B pathway. There is also considerable evidence that calcitriol potentiates the anti-tumor activity of a wide variety of cytotoxic chemotherapy agents (described later). As noted earlier, the induction of p73 by calcitriol seems to contribute to the synergistic activity of calcitriol and platinum analogues and some antimetabolites.³⁶

The Role of Antiinflammatory Effects of Calcitriol in Cancer Prevention and Treatment

As discussed earlier current perspectives in cancer biology suggest that inflammation plays a role in the development of cancer.^{52–54} De Marzo and colleagues⁸⁴ have proposed that precursor lesions called proliferative inflammatory atrophy (PIA) in the prostate, which are associated with acute or chronic inflammation, are the precursors of prostate intraepithelial neoplasia (PIN) and PCa. The epithelial cells in PIA lesions exhibit many molecular signs of stress including increased expression of COX-2.^{85,86} Similarly, inflammatory bowel disease is associated with the development of CRC.^{87–89} Based on the evidence demonstrating antiinflammatory effects of calcitriol, we postulate that calcitriol may play a role in delaying or preventing cancer development and/or progression. PCa generally progresses very slowly, likely over decades, before symptoms become obvious and the diagnosis is made.⁹⁰ The observed latency in PCa provides a long window of opportunity for intervention by chemopreventive agents. Dietary supplementation of COX-2 selective NSAIDs such as celecoxib has been shown to suppress prostate carcinogenesis in the Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model of PCa.⁹¹ The inhibitory effects of calcitriol on COX-2 expression and the PG pathway, production of proinflammatory cytokines, NF κ B signaling, and tumor angiogenesis suggest that calcitriol has the potential to be useful as a chemopreventive agent in malignancies such as PCa. Foster and colleagues have shown that administration of high-dose calcitriol (20 μ g/kg), intermittently 3 days per week for up to 14 to 30 weeks, suppresses prostate tumor development in TRAMP mice.^{92,93} The efficacy of calcitriol as a chemopreventive agent has also been examined in Nkx3.1;Pten mutant mice, which recapitulate stages of prostate carcinogenesis from PIN lesions to adenocarcinoma.⁹⁴ The data reveal that calcitriol significantly reduces the progression of PIN from a lower to a higher grade. Calcitriol is more effective when administered before, rather than after, the initial occurrence of PIN. These animal studies as well as in vitro observations suggest that clinical trials in PCa patients with PIN or early disease evaluating calcitriol and its analogues as agents that prevent and/or delay progression, are warranted.

Inhibition of Angiogenesis

Angiogenesis is the process of formation of new blood vessels from existing vasculature and is a crucial step in the continued growth, progression, and metastasis of tumors.⁹⁵ VEGF is the most potent stimulator of angiogenesis. PGs are also important proangiogenic factors. The initiation of angiogenesis is controlled by local hypoxia, which induces the synthesis of proangiogenic factors that activate signaling pathways leading to the structural reorganization of endothelial cells favoring new capillary formation.⁹⁶ Stimulation of angiogenesis in response to hypoxia is mediated by

hypoxia-inducible factor 1 (HIF-1), which directly increases the expression of several proangiogenic factors including VEGF.^{97,98} Early studies indicate that calcitriol is a potent inhibitor of tumor cell-induced angiogenesis in experimental models.⁹⁹ Calcitriol inhibits VEGF-induced endothelial cell tube formation *in vitro* and decreases tumor vascularization *in vivo* in mice bearing xenografts of BCa cells over-expressing VEGF.¹⁰⁰ Calcitriol and its analogues also directly inhibit the proliferation of endothelial cells^{101–103} leading to the inhibition of angiogenesis.

At the molecular level, calcitriol may exert its antiangiogenic effects through a direct antiproliferative action on endothelial cells in the tumor microenvironment and/or by regulating the expression of key factors that control angiogenesis. Calcitriol reduces VEGF expression in PCa cells through transcriptional repression of HIF-1.¹⁰¹ Calcitriol also suppresses the expression of the proangiogenic factor IL-8 in an NF κ B-dependent manner.⁷⁹ Chung and colleagues¹⁰³ established TRAMP-2 tumors in wild-type mice and VDR knockout mice and found enlarged vessels and increased vessel volume in TRAMP tumors in the VDR knockout mice, suggesting an inhibitory role for VDR and calcitriol in tumor angiogenesis. Their study further showed increased expression of proangiogenic factors such as HIF-1 α , VEGF, angiopoietin-1 and platelet-derived growth factor (PDGF) in the tumors in the VDR knockout mice.¹⁰³ Another important mechanism by which COX-2 promotes tumor progression is through the stimulation of angiogenesis. The proangiogenic effect of COX-2-generated PGE₂ might be a result of its action to increase HIF-1 α protein synthesis in cancer cells.¹⁰⁴ Suppression of COX-2 expression by calcitriol therefore provides an important indirect mechanism by which calcitriol inhibits angiogenesis, in addition to its direct suppressive effects on proangiogenic factors such as HIF-1 and VEGF.

ANTICANCER EFFECTS OF CALCITRIOL IN ANIMAL MODELS

Considerable data indicate antitumor effects of vitamin D compounds in *in vivo* models and calcitriol and calcitriol analogues also potentiate the antitumor actions of many more traditional anticancer agents. In model systems of murine SCC¹⁰⁵ and human carcinomas arising in the prostate,⁴⁶ lung,⁴⁷ ovary,¹⁰⁶ breast,^{107,108} bladder,¹⁰⁹ pancreas,¹¹⁰ as well as neuroblastoma,¹¹¹ calcitriol or calcitriol analogues have substantial anticancer effects. Significant inhibition of metastasis is observed in murine models of prostate and lung cancer treated with calcitriol; these effects may be based, at least in part, on antiangiogenic effects.^{46,47} In tumor-derived endothelial cells (TDECs), calcitriol induces apoptosis and cell cycle arrest; however, these effects are not seen in endothelial cells isolated from normal tissues or from Matrigel.^{103,112} Recently, Chung and colleagues¹¹³ reported that tumor-derived endothelial cells may be more sensitive to calcitriol as a result of novel epigenetic silencing of *CYP24A1*. Because *Cyp24* (24-hydroxylase) initiates the degradation of calcitriol,¹ inhibition of this enzyme has been shown to enhance calcitriol actions and tumors expressing high levels of the enzyme are resistant to calcitriol action.^{114–116} Direct effects of calcitriol on endothelial cells may play a primary role in the calcitriol-mediated antitumor activity that is observed in animal tumor models. There are no *in vitro* data to suggest that particular histotypes of cancer are more, or less, responsive to calcitriol-mediated antitumor effects except in the case of cells that have lost their VDR³¹ or over-express *CYP24*.^{114–116}

In Vivo Animal Studies of Calcitriol in Combination Regimens

In vivo analyses in mouse tumor models indicate that calcitriol acts synergistically with a wide range of chemotherapeutic agents. Calcitriol potentiates the anticancer activity of platinum analogues,^{117,118} taxanes,¹¹⁹ and DNA-intercalating agents.³⁶ Optimal

potentiation is seen when calcitriol is administered before or simultaneously with chemotherapy treatment; administration of calcitriol after the cytotoxic agent does not provide potentiation.¹¹⁹ In SCC and PC-3 (PCa) xenografts in immunodeficient mice, pretreatment with calcitriol or calcitriol analogues followed by paclitaxel results in enhanced antitumor effects.^{119,120} In vivo studies also indicate that the antitumor effects of calcitriol can be potentiated by agents that inhibit calcitriol metabolism. Azole antagonists of the primary catabolic enzyme (CYP24A1) responsible for vitamin D breakdown enhance the antitumor effects of calcitriol in vitro and in vivo.^{114,116,121} Ketoconazole is the most readily available of such agents and this drug has significant utility in the treatment of men with prostate cancer in whom disease progression has occurred despite androgen deprivation (so-called androgen-independent or castration-resistant PCa). The activity of ketoconazole in tumor cells (prostate and nonprostate) that are apparently unresponsive to androgens, supports the hypothesis that there are extra-androgenic mechanisms underlying ketoconazole activity.^{116,122} There are more specific inhibitors of CYP24 than azoles and secosteroid vitamin D analogues. These agents have antitumor activity in in vitro and in vivo models and potentiate the antitumor activity of calcitriol.^{123,124}

CLINICAL STUDIES

Single-Agent Calcitriol Trials: Phase I Studies and Toxicity

Most anticancer clinical trials of vitamin D analogues have been conducted with calcitriol because it is readily available as an injectable (Calcijex, Abbott Pharmaceuticals, Abbott Park, IL, USA) or oral (Rocaltrol, Hoffman-Roche Laboratories Inc, Nutley, NJ, USA) formulation. As described earlier, preclinical studies indicate that calcitriol has substantial antitumor activity when used in high doses. Most discussions of the role of vitamin D in cancer therapy express the concern that high-dose calcitriol is too toxic to be administered to patients with cancer. There are now many clinical studies that clearly establish that calcitriol can be safely administered in very high doses if an intermittent treatment schedule is used. Administration of oral calcitriol on a daily schedule (1.5–2.5 µg/d, weekly dose intensity ~ 10.5–17.5 µg/wk) is associated with a 20% to 30% frequency of hypercalcemia in men with PCa and in postmenopausal women.^{125–128} However, in in vivo settings demonstrating calcitriol efficacy, high-dose intermittent administration schedules are used (see later).

Calcitriol administered by mouth daily for 3 days every week (28 µg daily for 3 days) + dexamethasone (4 mg daily for 4 days) weekly is very safe and well tolerated in men with advanced PCa.¹²⁰ We have conducted studies of escalating doses of calcitriol (QDX3 weekly) + paclitaxel (80 mg/kg weekly for 4 weeks), as well as calcitriol (QDX3 monthly) + carboplatin (320 mg/sqm, monthly). In these 2 studies, doses of calcitriol of 38 µg every day for 3 days weekly and 28 µg every day for 3 days monthly were safely administered together with paclitaxel and carboplatin, respectively.¹²⁹ Pharmacokinetic studies in these trials demonstrated that calcitriol as Rocaltrol was unsuitable for high-dose administration because of inconvenience (38 µg requires the administration of 76 caplets) and unsuitable pharmacokinetics.¹³⁰ Administration of high doses of this formulation does not lead to a proportional increase in serum levels or systemic exposure. Similar findings were noted by Beer and colleagues,¹³¹ who studied a once weekly oral regimen. Novacea Pharmaceuticals undertook the development of a more suitable formulation. Their drug, DN-101, did have a linear relationship between dose and exposure up to doses of 165 µg.^{132,133} Fakhri and colleagues¹³⁴ studied intravenous calcitriol (Calcijex, Roche Pharmaceutical Corporation) weekly + gefitinib and reported that very high doses of calcitriol can be

administered safely. The dose-limiting toxicity of weekly intravenous calcitriol + gefitinib was grade 3 hypercalcemia at a dose of 98 $\mu\text{g}/\text{wk}$. The phase II dose of this regimen is 77 μg weekly alone and 98 $\mu\text{g}/\text{kg}$ weekly when calcitriol is combined with high-dose dexamethasone.^{134,135} The systemic exposure of calcitriol following 98 μg is approximately 30 ng/h/24 h which is in the range of exposure we have reported in murine models in which calcitriol has clear-cut antitumor activity.¹³⁶

Beer and colleagues¹³¹ have studied high-dose oral calcitriol (as Rocaltrol) and concluded that 0.5 $\mu\text{g}/\text{kg}$, weekly is very safe. Studies with DN-101 demonstrated that 45 μg weekly was safe and well tolerated and that 165 μg given on week 1, followed by 45 μg weekly produced no toxicity.¹³³ A linear relationship between dose of DN-101 administered and area under the curve (AUC) was maintained up to 165 μg . Studies using an intermittent schedule of administration (weekly or every day for 3 days weekly) have encountered dose-limiting hypercalcemia only at doses ~ 100 μg following intravenous administration; transient increase in serum calcium (11–13 mg/dL) does occur 1 to 3 days after completion of a single or daily for 3 days schedule. However, only at doses achieving AUC more than ~ 30 ng/h/ml has dose-limiting hypercalcemia been encountered. Hypercalciuria is universal following administration of high-dose calcitriol. Dietary calcium restriction is very difficult for patients to maintain and there is little evidence that it reduces hypercalciuria. There has been no deterioration of renal function in patients receiving high-dose intermittent calcitriol for more than 12 months. Radiographic monitoring for urinary tract stones (ultrasound or computed tomography) in our studies suggests that newly discovered urinary tract stones may occur in 1% to 3% of patients.^{129,130}

Trials of single-agent calcitriol and other vitamin D analogues in PCa have resulted in a few partial responses and prostate-specific antigen (PSA) responses have been seen. However, important clinical antitumor effects are quite infrequent. Very few studies of calcitriol or any other analogue have been conducted using doses approaching the maximum tolerated dose (MTD). In view of the many unresolved questions regarding the MTD, optimal biologic dose, optimal schedule, and pharmaceutical concerns about the available vitamin D formulations, it is not surprising that thus far limited antitumor activity has been seen in phase I and II trials.

Other Calcitriol Analogues

There is only limited information regarding the use of other vitamin D analogues as cancer therapy. EB1089 (seocalcitol),^{137–139} 1- α -vitamin D₂,^{140–142} inecalcitol (19-nor-, 14-epi-, 23-yne, 1,25-dihydroxyvitamin D₃),¹⁴³ and paricalcitol (19-nor-, 1- α , 25-dihydroxyvitamin D₂, Zemplar)¹⁴⁴ have each been studied, but always at relatively low doses and on schedules that would be predicted to make high-dose therapy impossible. No consistent and convincing evidence of antitumor activity has been seen in any of these studies.

Combination studies: phase I studies and toxicity

Several phase I clinical trials of calcitriol in combination with cytotoxic agents have been completed. Interpretation of the results of phase I and II clinical trials are hampered by the same challenges that limit our knowledge with regard to the interpretation of studies of calcitriol used as a single agent: lack of clear delineation of an optimal biologic dose and limited data on the MTD of calcitriol. Beer and colleagues¹⁴⁵ studied the combination of calcitriol + docetaxel with the intent of applying these studies to the treatment of men with advanced PCa progressing despite castration. These investigators used the commercially available oral

formulation of calcitriol (Rocaltrol). They conducted a phase II trial of weekly docetaxel (36 mg/m², weekly for 6 weeks) on day 2 + their phase II dose of calcitriol (0.5 µg/kg orally weekly) on day 1. No unusual toxicity was seen in this trial and PSA response (>50% reduction on 2 successive measurements maintained for >28 days) was seen in 30 of 37 patients (81%; 95% confidence interval [CI], 68%–94%). Pharmacokinetics of calcitriol and docetaxel were indistinguishable from those expected from single-agent therapy. These results were encouraging and provided the rationale for Novacea Company to develop a new formulation of calcitriol (DN-101) and to undertake 2 studies: first, a large randomized, double-blind trial of docetaxel ± DN-101 (ASCENT I = AIPC Study of Calcitriol Enhancing Taxotere)¹⁴⁶; the end point of this trial was PSA response. ASCENT I enrolled 250 patients and the PSA response rates were 63% (DN-101) and 52% (placebo), *P* = .07. Patients in the DN-101 group had a hazard ratio for death of 0.67 (*P* = .04) in a secondary multivariate analysis that included baseline hemoglobin and performance status.¹⁴⁶ Median survival was not reached for the DN-101 arm and was estimated to be 24.5 months, compared with 16.4 months for placebo. Clinically important adverse events occurred in 58% of DN-101 patients and in 70% of placebo-treated patients (*P* = .07). Neither significant hypercalcemia nor renal dysfunction was seen. The addition of weekly DN-101 did not increase the toxicity of weekly docetaxel and might even have decreased it.¹⁴⁶ These preliminary results showing increased survival in the DN-101 arm were very encouraging and led to ASCENT II, a 900-patient randomized, double-blinded, placebo-controlled phase III trial, in which survival was the end point. The goal of ASCENT II was to define any survival difference associated with calcitriol treatment in combination with docetaxel with the goal of achieving approval from the US Food and Drug Administration (FDA) of this combination. Unfortunately, in designing ASCENT II, 2 issues were unaddressed that ultimately proved to be problematic in the interpretation of ASCENT II:

1. ASCENT II was designed as a randomized study comparing docetaxel (every 3 weeks, 75 mg/m², the FDA-approved regimen) + prednisone (daily, 10 mg) + placebo versus docetaxel (weekly, 36 mg/m², a regimen that at the time ASCENT II was initiated had been shown to be inferior to the weekly every 3 weeks docetaxel regimen) + prednisone (daily, 10 mg) + calcitriol (DN-101, 0.5 µg/kg 1 day before docetaxel). This asymmetric design violates 1 of the primary tenets of randomized trial design; that is, to eliminate all variables between standard and experimental arms, except 1.
2. There are no data that define either the optimal or maximal dose of oral calcitriol. The 0.5 µg/kg weekly oral dose was a dose of convenience. A dose of approximately 77 µg (>1 µg/kg in a 70-kg patient) of calcitriol intravenously is required to achieve the AUC that is associated with antitumor effects in mice.

With these concerns in mind, perhaps it is not surprising that ASCENT II was halted in November 2007 when the data safety monitoring committee noted that the death rate in the investigational arm (weekly docetaxel + calcitriol + prednisone) was greater than in the standard therapy arm (every 3 weeks docetaxel + placebo + prednisone). Subsequent analysis of this trial to June 2008 indicated that all deaths in this study were caused by progressive prostate cancer and there was no excess of toxicity related to administration of calcitriol (John Curd, MD, personal communication, 2008). The result of ASCENT II is a discouraging finding in the quest to define a role for high-dose calcitriol in cancer therapy. However, there are several unaddressed questions in the development of calcitriol as a cancer therapy. The negative findings

in ASCENT II may be related to inappropriate trial design and drug dose rather than failure of the overall concept.

There are considerable data indicating the synergistic potential of calcitriol and a variety of antitumor agents. Clinical trials of calcitriol and paclitaxel, docetaxel, carboplatin, and gefitinib have been conducted; no unusual toxicity was seen and anti-tumor responses were documented.^{134,147,148} However, the drug formulations used did not allow dose escalation to doses near the MTD, except in the trial with gefitinib.

Although there are preclinical data that would support the study of combinations of calcitriol and several other antitumor agents including antimetabolites (methotrexate, cytosine arabinoside, gemcitabine), anthracyclines and anthracenediones, and topoisomerase inhibitors, no clinical trials of such combinations have been conducted.

SUMMARY

Considerable data described in the first part of this review suggest that there is a role for vitamin D in cancer therapy and prevention. Although the preclinical data are persuasive and the epidemiologic data intriguing, no well-designed clinical trial of optimal administration of vitamin D as a cancer therapy has ever been conducted. Had there been the opportunity and insight to develop calcitriol as any other cancer drug, the following studies would have been completed:

1. Definition of the MTD
2. Definition of a phase II dose, as a single agent and in combination with cytotoxic agents
3. Studies to define a biologically optimal dose
4. Phase II (probably randomized phase II) studies of calcitriol alone and chemotherapy \pm calcitriol
5. Then, randomized phase III trials would be conducted and designed such that the only variable was the administration of calcitriol.

Prerequisites 1 to 5 have not been completed for calcitriol. Preclinical data provide considerable rationale for continued development of vitamin D analogue-based cancer therapies. However, design of future studies should be informed by good clinical trials design principles and the mistakes of the past not repeated. Such studies may finally provide compelling data to prove whether or not there is a role for vitamin D analogues in cancer therapy.

REFERENCES

1. Feldman D, Malloy PJ, Krishnan AV, et al. Vitamin D: biology, action and clinical implications. In: Marcus R, Feldman D, Nelson DA, et al, editors. 3rd edition, Osteoporosis, vol. 1. San Diego (CA): Academic Press; 2007. p. 317–82.
2. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007;7(9):684–700.
3. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 1980;9(3):227–31.
4. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. *Cancer* 1992;70(12): 2861–9.
5. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). *Anticancer Res* 1990;10(5A):1307–11.

6. Garland CF, Garland FC, Gorham ED, et al. The role of vitamin D in cancer prevention. *Am J Public Health* 2006;96(2):252–61.
7. Giovannucci E. Vitamin D status and cancer incidence and mortality. *Adv Exp Med Biol* 2008;624:31–42.
8. Garland CF, Gorham ED, Mohr SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol* 2007;103(3–5):708–11.
9. Wei MY, Garland CF, Gorham ED, et al. Vitamin D and prevention of colorectal adenoma: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2008;17(11):2958–69.
10. Yin L, Grandi N, Raum E, et al. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. *Aliment Pharmacol Ther* 2009;30(2):113–25.
11. Ng K, Meyerhardt JA, Wu K, et al. Circulating 25-hydroxyvitamin D levels and survival in patients with colorectal cancer. *J Clin Oncol* 2008;26(18):2984–91.
12. Ding EL, Mehta S, Fawzi WW, et al. Interaction of estrogen therapy with calcium and vitamin D supplementation on colorectal cancer risk: reanalysis of Women's Health Initiative randomized trial. *Int J Cancer* 2008;122(8):1690–4.
13. Corder EH, Friedman GD, Vogelman JH, et al. Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. *Cancer Epidemiol Biomarkers Prev* 1995;4(6):655–9.
14. Corder EH, Guess HA, Hulka BS, et al. Vitamin D and prostate cancer: a pre-diagnostic study with stored sera. *Cancer Epidemiol Biomarkers Prev* 1993;2(5):467–72.
15. Ahn J, Peters U, Albanes D, et al. Serum vitamin D concentration and prostate cancer risk: a nested case-control study. *J Natl Cancer Inst* 2008;100(11):796–804.
16. Platz EA, Leitzmann MF, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. *Cancer Causes Control* 2004;15(3):255–65.
17. Ahonen MH, Tenkanen L, Teppo L, et al. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* 2000;11(9):847–52.
18. Garland CF, Comstock GW, Garland FC, et al. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1989;2(8673):1176–8.
19. Skinner HG, Michaud DS, Giovannucci E, et al. Vitamin D intake and the risk for pancreatic cancer in two cohort studies. *Cancer Epidemiol Biomarkers Prev* 2006;15(9):1688–95.
20. Chiang KC, Chen TC. Vitamin D for the prevention and treatment of pancreatic cancer. *World J Gastroenterol* 2009;15(27):3349–54.
21. Colston KW, Welsh J. Vitamin D and breast cancer. In: Feldman D, Pike JW, Glorieux FH, editors. *Vitamin D*, vol. 2. San Diego (CA): Elsevier Academic Press; 2005. p. 1663–77.
22. Cross HS. Vitamin D and colon cancer. In: Feldman D, Pike JW, Glorieux FH, editors. *Vitamin D*. vol. 2. San Diego (CA): Elsevier Academic Press; 2005. p. 1709–25.
23. Gombart AF, Luong QT, Koeffler HP. Vitamin D compounds: activity against microbes and cancer. *Anticancer Res* 2006;26(4A):2531–42.
24. Gonzalez-Sancho JM, Larriba MJ, Ordonez-Moran P, et al. Effects of 1 α , 25-dihydroxyvitamin D₃ in human colon cancer cells. *Anticancer Res* 2006;26(4A):2669–81.

25. Gross MD. Vitamin D and calcium in the prevention of prostate and colon cancer: new approaches for the identification of needs. *J Nutr* 2005;135(2):326–31.
26. Krishnan AV, Peehl DM, Feldman D. Inhibition of prostate cancer growth by vitamin D: regulation of target gene expression. *J Cell Biochem* 2003;88(2):363–71.
27. Krishnan AV, Peehl DM, Feldman D. Vitamin D and prostate cancer. In: Feldman D, Pike JW, Glorieux FH, editors. 2nd edition. *Vitamin D*. vol. 2. San Diego (CA): Elsevier Academic Press; 2005. p. 1679–707.
28. Luong QT, Koeffler HP. Vitamin D compounds in leukemia. *J Steroid Biochem Mol Biol* 2005;97(1–2):195–202.
29. Schwartz GG, Skinner HG. Vitamin D status and cancer: new insights. *Curr Opin Clin Nutr Metab Care* 2007;10(1):6–11.
30. Stewart LV, Weigel NL. Vitamin D and prostate cancer. *Exp Biol Med (Maywood)* 2004;229(4):277–84.
31. Welsh J. Targets of vitamin D receptor signaling in the mammary gland. *J Bone Miner Res* 2007;22(Suppl 2):V86–90.
32. Campbell MJ, Elstner E, Holden S, et al. Inhibition of proliferation of prostate cancer cells by a 19-nor-hexafluoride vitamin D3 analogue involves the induction of p21waf1, p27kip1 and E-cadherin. *J Mol Endocrinol* 1997;19(1):15–27.
33. Yang ES, Burnstein KL. Vitamin D inhibits G1 to S progression in LNCaP prostate cancer cells through p27Kip1 stabilization and Cdk2 mislocalization to the cytoplasm. *J Biol Chem* 2003;278(47):46862–8.
34. Blutt SE, Allegretto EA, Pike JW, et al. 1,25-Dihydroxyvitamin D3 and 9-cis-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G1. *Endocrinology* 1997;138(4):1491–7.
35. Jensen SS, Madsen MW, Lukas J, et al. Inhibitory effects of 1alpha,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol Endocrinol* 2001;15(8):1370–80.
36. Ma Y, Yu WD, Hershberger PA, et al. 1alpha,25-Dihydroxyvitamin D3 potentiates cisplatin antitumor activity by p73 induction in a squamous cell carcinoma model. *Mol Cancer Ther* 2008;7(9):3047–55.
37. Boyle BJ, Zhao XY, Cohen P, et al. Insulin-like growth factor binding protein-3 mediates 1 alpha,25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. *J Urol* 2001;165(4):1319–24.
38. Peng L, Malloy PJ, Feldman D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. *Mol Endocrinol* 2004;18:1109–19.
39. Gocek E, Studzinski GP. Vitamin D and differentiation in cancer. *Crit Rev Clin Lab Sci* 2009;46(4):190–209.
40. Blutt SE, McDonnell TJ, Polek TC, et al. Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2. *Endocrinology* 2000;141(1):10–7.
41. Narvaez CJ, Welsh J. Role of mitochondria and caspases in vitamin D-mediated apoptosis of MCF-7 breast cancer cells. *J Biol Chem* 2001;276(12):9101–7.
42. Ylikomi T, Laaksi I, Lou YR, et al. Antiproliferative action of vitamin D. *Vitam Horm* 2002;64:357–406.
43. Park WH, Seol JG, Kim ES, et al. Induction of apoptosis by vitamin D3 analogue EB1089 in NCI-H929 myeloma cells via activation of caspase 3 and p38 MAP kinase. *Br J Haematol* 2000;109(3):576–83.
44. Posner GH, Crawford KR, Peleg S, et al. A non-calcemic sulfone version of the vitamin D(3) analogue seocalcitol (EB 1089): chemical synthesis, biological

- evaluation and potency enhancement of the anticancer drug adriamycin. *Bioorg Med Chem* 2001;9(9):2365–71.
45. Sundaram S, Sea A, Feldman S, et al. The combination of a potent vitamin D3 analog, EB 1089, with ionizing radiation reduces tumor growth and induces apoptosis of MCF-7 breast tumor xenografts in nude mice. *Clin Cancer Res* 2003;9(6):2350–6.
 46. Getzenberg RH, Light BW, Lapco PE, et al. Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system. *Urology* 1997;50(6):999–1006.
 47. Nakagawa K, Kawaura A, Kato S, et al. 1 alpha,25-Dihydroxyvitamin D(3) is a preventive factor in the metastasis of lung cancer. *Carcinogenesis* 2005;26(2):429–40.
 48. Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. *Cell Growth Differ* 2000;11(4):221–9.
 49. Gonzalez-Sancho JM, Alvarez-Dolado M, Munoz A. 1,25-Dihydroxyvitamin D3 inhibits tenascin-C expression in mammary epithelial cells. *FEBS Lett* 1998;426(2):225–8.
 50. Sung V, Feldman D. 1,25-Dihydroxyvitamin D3 decreases human prostate cancer cell adhesion and migration. *Mol Cell Endocrinol* 2000;164(1–2):133–43.
 51. Bao BY, Yeh SD, Lee YF. 1alpha,25-dihydroxyvitamin D3 inhibits prostate cancer cell invasion via modulation of selective proteases. *Carcinogenesis* 2006;27(1):32–42.
 52. Allavena P, Garlanda C, Borrello MG, et al. Pathways connecting inflammation and cancer. *Curr Opin Genet Dev* 2008;18(1):3–10.
 53. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 2007;7(4):256–69.
 54. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. *Nature* 2008;454(7203):436–44.
 55. Lucia MS, Torkko KC. Inflammation as a target for prostate cancer chemoprevention: pathological and laboratory rationale. *J Urol* 2004;171(2 Pt 2):S30–4 [discussion: S35].
 56. Mantovani A, Pierotti MA. Cancer and inflammation: a complex relationship. *Cancer Lett* 2008;267(2):180–1.
 57. Angelo LS, Kurzrock R. Vascular endothelial growth factor and its relationship to inflammatory mediators. *Clin Cancer Res* 2007;13(10):2825–30.
 58. Kundu JK, Surh YJ. Inflammation: gearing the journey to cancer. *Mutat Res* 2008;659(1–2):15–30.
 59. Moran EM. Epidemiological and clinical aspects of nonsteroidal anti-inflammatory drugs and cancer risks. *J Environ Pathol Toxicol Oncol* 2002;21(2):193–201.
 60. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anti-cancer agents: mechanistic, pharmacologic, and clinical issues. *J Natl Cancer Inst* 2002;94(4):252–66.
 61. Krishnan AV, Shinghal R, Raghavachari N, et al. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. *Prostate* 2004;59(3):243–51.
 62. Peehl DM, Shinghal R, Nonn L, et al. Molecular activity of 1,25-dihydroxyvitamin D3 in primary cultures of human prostatic epithelial cells revealed by cDNA microarray analysis. *J Steroid Biochem Mol Biol* 2004;92(3):131–41.

63. McCarty MF. Targeting multiple signaling pathways as a strategy for managing prostate cancer: multifocal signal modulation therapy. *Integr Cancer Ther* 2004; 3(4):349–80.
64. Maeda S, Omata M. Inflammation and cancer: role of nuclear factor-kappaB activation. *Cancer Sci* 2008;99(5):836–42.
65. Moreno J, Krishnan AV, Swami S, et al. Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. *Cancer Res* 2005;65(17):7917–25.
66. Srinivas S, Feldman D. A phase II trial of calcitriol and naproxen in recurrent prostate cancer. *Anticancer Res* 2009;29(9):3605–10.
67. Nonn L, Peng L, Feldman D, et al. 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(8):4516–24.
68. Culig Z, Steiner H, Bartsch G, et al. Interleukin-6 regulation of prostate cancer cell growth. *J Cell Biochem* 2005;95(3):497–505.
69. Finley DS, Calvert VS, Inokuchi J, et al. Periprostatic adipose tissue as a modulator of prostate cancer aggressiveness. *J Urol* 2009;182(4):1621–7.
70. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol* 2002;3(3):221–7.
71. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; 109(Suppl):S81–96.
72. Palayoor ST, Youmell MY, Calderwood SK, et al. Constitutive activation of I-kappaB kinase alpha and NF-kappaB in prostate cancer cells is inhibited by ibuprofen. *Oncogene* 1999;18(51):7389–94.
73. Sovak MA, Bellas RE, Kim DW, et al. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. *J Clin Invest* 1997; 100(12):2952–60.
74. Yu XP, Bellido T, Manolagas SC. Down-regulation of NF-kappa B protein levels in activated human lymphocytes by 1,25-dihydroxyvitamin D3. *Proc Natl Acad Sci U S A* 1995;92(24):10990–4.
75. Harant H, Wolff B, Lindley IJ. 1Alpha,25-dihydroxyvitamin D3 decreases DNA binding of nuclear factor-kappaB in human fibroblasts. *FEBS Lett* 1998; 436(3):329–34.
76. Stio M, Martinesi M, Bruni S, et al. The vitamin D analogue TX 527 blocks NF-kappaB activation in peripheral blood mononuclear cells of patients with Crohn's disease. *J Steroid Biochem Mol Biol* 2007;103(1):51–60.
77. Sun J, Kong J, Duan Y, et al. Increased NF-kappaB activity in fibroblasts lacking the vitamin D receptor. *Am J Physiol Endocrinol Metab* 2006;291(2): E315–22.
78. Schwab M, Reynders V, Loitsch S, et al. Involvement of different nuclear hormone receptors in butyrate-mediated inhibition of inducible NF kappa B signalling. *Mol Immunol* 2007;44(15):3625–32.
79. Bao BY, Yao J, Lee YF. 1alpha, 25-Dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. *Carcinogenesis* 2006;27(9): 1883–93.
80. Jogie-Brahim S, Feldman D, Oh Y. Unraveling IGFBP-3 actions in human disease. *Endocr Rev* 2009;30(5):417–37.
81. Criswell T, Leskov K, Miyamoto S, et al. Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. *Oncogene* 2003;22(37):5813–27.

82. Kimura K, Bowen C, Spiegel S, et al. Tumor necrosis factor- α sensitizes prostate cancer cells to gamma-irradiation-induced apoptosis. *Cancer Res* 1999;59(7):1606–14.
83. Xu Y, Fang F, St Clair DK, et al. Suppression of RelB-mediated manganese superoxide dismutase expression reveals a primary mechanism for radiosensitization effect of 1 α ,25-dihydroxyvitamin D(3) in prostate cancer cells. *Mol Cancer Ther* 2007;6(7):2048–56.
84. De Marzo AM, DeWeese TL, Platz EA, et al. Pathological and molecular mechanisms of prostate carcinogenesis: implications for diagnosis, detection, prevention, and treatment. *J Cell Biochem* 2004;91(3):459–77.
85. De Marzo AM, Marchi VL, Epstein JI, et al. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 1999;155(6):1985–92.
86. Zha S, Gage WR, Sauvageot J, et al. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res* 2001;61(24):8617–23.
87. Herszenyi L, Miheller P, Tulassay Z. Carcinogenesis in inflammatory bowel disease. *Dig Dis* 2007;25(3):267–9.
88. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004;287(1):G7–17.
89. Seril DN, Liao J, Yang GY, et al. Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* 2003;24(3):353–62.
90. Whittemore AS, Cirillo PM, Feldman D, et al. Prostate specific antigen levels in young adulthood predict prostate cancer risk: results from a cohort of black and white Americans. *J Urol* 2005;174(3):872–6 [discussion: 876].
91. Gupta S, Adhami VM, Subbarayan M, et al. Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. *Cancer Res* 2004;64(9):3334–43.
92. Alagbala A, Moser MT, Johnson CS, et al. Prevention of prostate cancer progression with vitamin D compounds in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Presented at the 4th Annual AACR International Conference on Frontiers in Cancer Prevention Research. Baltimore (MD), October 30 to November 2, 2005.
93. Alagbala AA, Moser MT, Johnson CS, et al. 1 α ,25-Dihydroxyvitamin D₃ and its analog (QW-1624F2-2) prevent prostate cancer progression. Presented at 13th Workshop on Vitamin D. Victoria, BC (Canada), April 8–12, 2006.
94. Banach-Petrosky W, Ouyang X, Gao H, et al. Vitamin D inhibits the formation of prostatic intraepithelial neoplasia in Nkx3.1;Pten mutant mice. *Clin Cancer Res* 2006;12(19):5895–901.
95. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1(1):27–31.
96. Sakamoto S, Ryan AJ, Kyprianou N. Targeting vasculature in urologic tumors: mechanistic and therapeutic significance. *J Cell Biochem* 2008;103(3):691–708.
97. Giaccia A, Siim BG, Johnson RS. HIF-1 as a target for drug development. *Nat Rev Drug Discov* 2003;2(10):803–11.
98. Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ* 2008;15(4):678–85.

99. Majewski S, Skopinska M, Marczak M, et al. Vitamin D3 is a potent inhibitor of tumor cell-induced angiogenesis. *J Investig Dermatol Symp Proc* 1996;1(1): 97–101.
100. Mantell DJ, Owens PE, Bundred NJ, et al. 1 alpha,25-Dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circ Res* 2000;87(3):214–20.
101. Ben-Shoshan M, Amir S, Dang DT, et al. 1alpha,25-Dihydroxyvitamin D3 (calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. *Mol Cancer Ther* 2007;6(4):1433–9.
102. Bernardi RJ, Johnson CS, Modzelewski RA, et al. Antiproliferative effects of 1alpha,25-dihydroxyvitamin D(3) and vitamin D analogs on tumor-derived endothelial cells. *Endocrinology* 2002;143(7):2508–14.
103. Chung I, Han G, Seshadri M, 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(3):967–75.
104. Fukuda R, Kelly B, Semenza GL. Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. *Cancer Res* 2003;63(9):2330–4.
105. McElwain MC, Dettelbach MA, Modzelewski RA, et al. Antiproliferative effects in vitro and in vivo of 1,25-dihydroxyvitamin D3 and a vitamin D3 analog in a squamous cell carcinoma model system. *Mol Cell Differ* 1995;3(1):31–50.
106. Zhang X, Jiang F, Li P, et al. Growth suppression of ovarian cancer xenografts in nude mice by vitamin D analogue EB1089. *Clin Cancer Res* 2005; 11(1):323–8.
107. Colston KW, Chander SK, Mackay AG, et al. Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. *Biochem Pharmacol* 1992;44(4):693–702.
108. Welsh J, Wietzke JA, Zinser GM, et al. Vitamin D-3 receptor as a target for breast cancer prevention. *J Nutr* 2003;133(Suppl 7):2425S–33S.
109. Ma Y, Yu WD, Trump DL, et al. 1,25D3 enhances antitumor activity of gemcitabine and cisplatin in human bladder cancer models. *Cancer* 2010, in press.
110. Colston KW, James SY, Ofori-Kuragu EA, et al. Vitamin D receptors and antiproliferative effects of vitamin D derivatives in human pancreatic carcinoma cells in vivo and in vitro. *Br J Cancer* 1997;76(8):1017–20.
111. Moore TB, Koeffler HP, Yamashiro JM, et al. Vitamin D3 analogs inhibit growth and induce differentiation in LA-N-5 human neuroblastoma cells. *Clin Exp Metastasis* 1996;14(3):239–45.
112. Chung I, Wong MK, Flynn G, et al. Differential antiproliferative effects of calcitriol on tumor-derived and Matrigel-derived endothelial cells. *Cancer Res* 2006; 66(17):8565–73.
113. Chung I, Karpf AR, Muindi JR, et al. Epigenetic silencing of CYP24 in tumor-derived endothelial cells contributes to selective growth inhibition by calcitriol. *J Biol Chem* 2007;282(12):8704–14.
114. Ly LH, Zhao XY, Holloway L, et al. 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(5):2071–6.
115. Miller GJ, Stapleton GE, Hedlund TE, et al. Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1alpha,25-dihydroxyvitamin D3 in seven human prostatic carcinoma cell lines. *Clin Cancer Res* 1995;1(9): 997–1003.

116. Peehl DM, Seto E, Hsu JY, et al. Preclinical activity of ketoconazole in combination with calcitriol or the vitamin D analogue EB 1089 in prostate cancer cells. *J Urol* 2002;168(4 Pt 1):1583–8.
117. Hershberger PA, McGuire TF, Yu WD, et al. Cisplatin potentiates 1,25-dihydroxyvitamin D₃-induced apoptosis in association with increased mitogen-activated protein kinase kinase 1 (MEKK-1) expression. *Mol Cancer Ther* 2002;1(10):821–9.
118. Moffatt KA, Johannes WU, Miller GJ. 1 α ,25-Dihydroxyvitamin D₃ and platinum drugs act synergistically to inhibit the growth of prostate cancer cell lines. *Clin Cancer Res* 1999;5(3):695–703.
119. Hershberger PA, Yu WD, Modzelewski RA, et al. Calcitriol (1,25-dihydroxycholecalciferol) enhances paclitaxel antitumor activity in vitro and in vivo and accelerates paclitaxel-induced apoptosis. *Clin Cancer Res* 2001;7(4):1043–51.
120. Trump DL, Hershberger PA, Bernardi RJ, et al. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. *J Steroid Biochem Mol Biol* 2004;89–90(1–5):519–26.
121. Muindi JR, Yu WD, Ma Y, et al. CYP24A1 inhibition enhances the antitumor activity of calcitriol. Submitted for publication, 2010.
122. Rochlitz CF, Damon LE, Russi MB, et al. Cytotoxicity of ketoconazole in malignant cell lines. *Cancer Chemother Pharmacol* 1988;21(4):319–22.
123. Kahraman M, Sinishtaj S, Dolan PM, et al. Potent, selective and low-calcemic inhibitors of CYP24 hydroxylase: 24-sulfoximine analogues of the hormone 1 α ,25-dihydroxyvitamin D(3). *J Med Chem* 2004;47(27):6854–63.
124. Lechner D, Manhardt T, Bajna E, et al. A 24-phenylsulfone analog of vitamin D inhibits 1 α ,25-dihydroxyvitamin D(3) degradation in vitamin D metabolism-competent cells. *J Pharmacol Exp Ther* 2007;320(3):1119–26.
125. Gallagher JC. Metabolic effects of synthetic calcitriol (Rocaltrol) in the treatment of postmenopausal osteoporosis. *Metabolism* 1990;39(4 Suppl 1):27–9.
126. Gallagher JC, Goldgar D. Treatment of postmenopausal osteoporosis with high doses of synthetic calcitriol. A randomized controlled study. *Ann Intern Med* 1990;113(9):649–55.
127. Gross C, Stamey T, Hancock S, et al. Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D₃ (calcitriol). *J Urol* 1998;159(6):2035–9 [discussion: 2039–40].
128. Osborn JL, Schwartz GG, Smith DC, et al. 1,25-Dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer. *Urol Oncol* 1995;1(5):195–8.
129. Muindi JR, Peng Y, Potter DM, et al. Pharmacokinetics of high-dose oral calcitriol: results from a phase 1 trial of calcitriol and paclitaxel. *Clin Pharmacol Ther* 2002;72(6):648–59.
130. Muindi JR, Potter DM, Peng Y, et al. Pharmacokinetics of liquid calcitriol formulation in advanced solid tumor patients: comparison with caplet formulation. *Cancer Chemother Pharmacol* 2005;56(5):492–6.
131. 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(12):2431–9.
132. Beer TM, Javle M, Lam GN, et al. Pharmacokinetics and tolerability of a single dose of DN-101, a new formulation of calcitriol, in patients with cancer. *Clin Cancer Res* 2005;11(21):7794–9.
133. Beer TM, Javle MM, Ryan CW, et al. Phase I study of weekly DN-101, a new formulation of calcitriol, in patients with cancer. *Cancer Chemother Pharmacol* 2007;59(5):581–7.

134. Fakih MG, Trump DL, Muindi JR, 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(4):1216–23.
135. Muindi JR, Johnson CS, Trump DL, 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(1):33–40.
136. Muindi JR, Modzelewski RA, Peng Y, et al. Pharmacokinetics of 1alpha,25-dihydroxyvitamin D3 in normal mice after systemic exposure to effective and safe antitumor doses. *Oncology* 2004;66(1):62–6.
137. Dalhoff K, Dancey J, Astrup L, et al. A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma. *Br J Cancer* 2003;89(2):252–7.
138. Evans TR, Colston KW, Lofts FJ, et al. A phase II trial of the vitamin D analogue Seocalcitol (EB1089) in patients with inoperable pancreatic cancer. *Br J Cancer* 2002;86(5):680–5.
139. Gulliford T, English J, Colston KW, et al. A phase I study of the vitamin D analogue EB 1089 in patients with advanced breast and colorectal cancer. *Br J Cancer* 1998;78(1):6–13.
140. Attia S, Eickhoff J, Wilding G, et al. Randomized, double-blinded phase II evaluation of docetaxel with or without doxercaliferol in patients with metastatic, androgen-independent prostate cancer. *Clin Cancer Res* 2008;14(8):2437–43.
141. Liu G, Oettel K, Ripple G, et al. Phase I trial of 1alpha-hydroxyvitamin D(2) in patients with hormone refractory prostate cancer. *Clin Cancer Res* 2002;8(9):2820–7.
142. Liu G, Wilding G, Staab MJ, et al. Phase II study of 1alpha-hydroxyvitamin D(2) in the treatment of advanced androgen-independent prostate cancer. *Clin Cancer Res* 2003;9(11):4077–83.
143. Medioni J, Eickhoff J, Wilding G, et al. Dose finding and safety analysis of inecalcitol in combination with a docetaxel-prednisone regimen in hormone-refractory prostate cancer (HRPC) patients. *J Clin Oncol* 2009;27:15s[Suppl abstr: 151].
144. Schwartz GG, Hall MC, Stindt D, et al. Phase I/II study of 19-nor-1alpha-25-dihydroxyvitamin D2 (paricalcitol) in advanced, androgen-insensitive prostate cancer. *Clin Cancer Res* 2005;11(24 Pt 1):8680–5.
145. Beer TM, Eilers KM, Garzotto M, et al. Weekly high-dose calcitriol and docetaxel in metastatic androgen-independent prostate cancer. *J Clin Oncol* 2003;21(1):123–8.
146. Beer TM, Ryan CW, Venner PM, 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(6):669–74.
147. Beer TM, Garzotto M, Katovic NM. High-dose calcitriol and carboplatin in metastatic androgen-independent prostate cancer. *Am J Clin Oncol* 2004;27(5):535–41.
148. Flaig TW, Barqawi A, Miller G, et al. A phase II trial of dexamethasone, vitamin D, and carboplatin in patients with hormone-refractory prostate cancer. *Cancer* 2006;107(2):266–74.