

Review

Vitamin D Compounds: Activity Against Microbes and Cancer

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Abstract. *Vitamin D₃ is produced in skin and is sequentially metabolized by the liver and kidney to the biologically active form 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]. It is a seco-steroid hormone that regulates calcium homeostasis within the body. The genomic actions of 1,25(OH)₂D₃ are modulated through the vitamin D receptor (VDR) (1). VDR belongs to a superfamily of nuclear receptors that transduce hormonal signals from the immediate environment and transactivate genes in response to these signals. Target genes contain hormone response elements (VDREs) in their promoters to which heterodimers of VDR and retinoid X receptors (RXR) can bind and transactivate expression of the target genes (2). The VDR is expressed in at least 30 different target tissues including bone, kidney, blood, breast, prostate, gut, activated B- and T- lymphocytes, monocytes and keratinocytes (3, 4). Most dividing cell types, normal and malignant, can express VDR and respond to 1,25(OH)₂D₃. Although 1,25(OH)₂D₃ and its analogs (termed deltanoids) are important regulators of calcium and bone metabolism, their non-calcitropic activities that include inhibition of cell proliferation, promotion of cell differentiation and modulation of immune cell function have spurred interest in therapeutic applications in a wide variety of diseases. In this report, the anticancer and newly discovered antimicrobial actions of 1,25(OH)₂D₃ and deltanoids are reviewed.*

Novel Immunoregulatory Properties of Vitamin D₃

Vitamin D receptor, 1,25(OH)₂D₃ and the immune system. The vitamin D receptor (VDR) is expressed in most cells of

the immune system, including macrophages, dendritic cells (DCs) and lymphocytes, suggesting an immunoregulatory role for VDR and its agonists (5, 6). Indeed, VDR agonists modulate T-cell responses including proliferation, cytokine production, and result in decreased Th1 development and enhanced frequency of Th2 and regulatory T-cells. In addition, VDR agonists keep DCs in an immature state with reduced expression of costimulatory molecules, increased IL-10 and reduced IL-12 levels, thus inhibiting T-cell activation (6-8). The ability of VDR agonists to promote tolerance in DCs and T-cells has prompted investigators to explore possible therapeutic treatments for a number of human autoimmune diseases (5, 8-10).

In addition to responding to VDR agonists, macrophages, DCs and T-cells express the enzyme 25(OH)D₃-1- α -hydroxylase that mediates the final step in the production of 1 α ,25(OH)₂D₃, thereby allowing them to produce 1 α ,25(OH)₂D₃ in response to immune signals (11, 12-14). Additionally, the major enzyme that degrades 1 α ,25(OH)₂D₃, 24-hydroxylase, is expressed by these cells (15, 16). The ability of immune system cells to regulate the production of and response to VDR agonists suggests an important biological role for 1 α ,25(OH)₂D₃ in regulating innate and adaptive immunity.

Recently, exciting and novel innate immune system regulatory activities for 1,25(OH)₂D₃ were discovered. The innate immune system of animals provides the capacity to quickly repel assaults by numerous infectious agents including bacteria, viruses, fungi and parasites (17-22). The cationic antimicrobial peptides (AMPs), including defensins and cathelicidins, are a major component of this defense in mammals. Utilizing different approaches, we and two other groups demonstrated that 1 α ,25(OH)₂D₃ and some deltanoids directly induced cationic AMP gene expression in a variety of tissue and cell types (23-25). In a screen of the human genome for VDREs and their corresponding genes, Wang *et al.* identified AMP genes, cathelicidin antimicrobial peptide (CAMP) and human β -defensin 2 (hBD2) and subsequently showed their induction by

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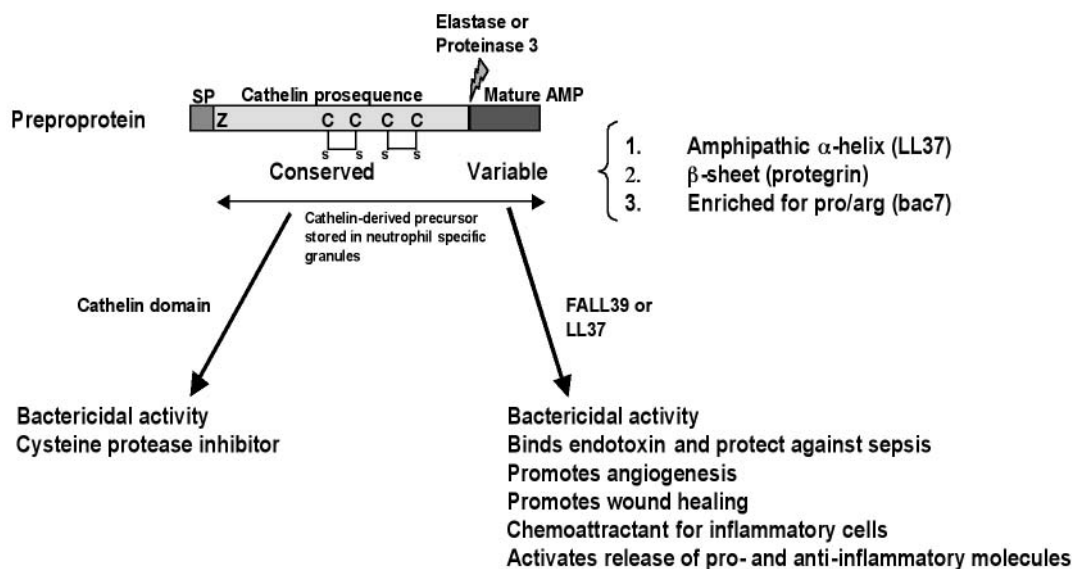


Figure 1. Structure of the human CAMP protein (FALL39/LL37/hCAP18). Mammalian cathelicidins share the same structure. The highly variable antimicrobial domain possesses structurally very different effectors. The pro-protein begins with an N-terminal pyrrolidone (Z) and contains four cysteines that participate in disulfide bonds. Cleavage (lightening strike symbol) of the pro-protein by elastase or proteinase 3 activates the C-terminal AMP that potentially executes a wide range of biological effects. The conserved cathelin domain might participate in important immune functions as well.

1,25(OH)₂D₃ (23, 26). Studying hCAP18 expression in skin, Weber *et al.* examined agents that affect skin proliferation and differentiation and identified 1,25(OH)₂D₃ and the analog MC903 as inducers (25). Our own group was screening various compounds on acute myeloid leukemia cell lines in an attempt to induce CAMP expression and identified 1 α ,25(OH)₂D₃, KH1060 and EB1089 as potent inducers (24).

Cationic AMPs of the innate immune system. The cathelicidins are a family of proteins consisting of a C-terminal cationic AMP domain that is activated by cleavage from the N-terminal cathelin portion of the propeptide. While species such as pigs, cows and sheep express numerous cathelicidins, humans and mice possess a single cathelicidin gene (27, 28). In humans and mice, the official gene name of the shared cathelicidin gene is CAMP; however, its aliases include hCAP18/LL37/FALL39 (humans) and CRAMP/CNLP/MCLP (mice). All cathelicidins are synthesized as a pre-propeptide consisting of an N-terminal signal peptide, a conserved prosequence (cathelin domain) and a highly variable C-terminal cationic AMP (Figure 1) (29). The evolutionarily conserved cathelin domain is named for its homology to cathelin, a porcine neutrophil protein that inhibits cathepsin L (29, 30). The hCAP18/CRAMP protein is synthesized and secreted in significant amounts by those tissues that are exposed to environmental microbes. This includes the squamous epithelia of the mouth, tongue, esophagus, lungs, intestine, cervix and

vagina (31, 32). In addition, it is produced by salivary and sweat glands (33, 34), epididymis and testis (35-37) resulting in the secretion of the peptide in wounds (38), sweat (34), airway surface fluids (32) and seminal plasma (39). Various white blood cell populations express hCAP18 including natural killer cells, $\gamma\delta$ T cells, B-cells, monocytes (40), mast cells (41) and immature neutrophils (42); however, the majority of the propeptides are stored in the granules of neutrophils (42) from which they can be released at sites of microbial attack. In addition, hCAP18 is secreted into the blood and significant levels are found in the plasma (43).

The defensins are classified as α - and β -defensins based on their tertiary structure, an $\alpha\beta$ -sheet structure that is determined by the formation of three disulfide bonds involving six cysteine residues (44). In α -defensins cysteine residues 1-6, 2-4 and 3-5 are linked. In β -defensins, residues 1-5, 2-4 and 3-6 are linked. For humans, six α -defensins (human neutrophil peptides [HNP] 1-4) and human defensins [HD]-5 and -6) and four β -defensins (hBD 1-4) have been well characterized. HNPs1-4 are primarily expressed in granulocytes and packaged in neutrophil primary granules, while HD-5 and HD-6 are primarily expressed in intestinal Paneth cells and epithelial cells of the female genital tract. Like cathelicidins, the β -defensins are expressed in various epithelial cells that are exposed to the environment. The human β -defensin 1 (hBD1) is constitutively expressed, whereas hBD3 and hBD2 are transcriptionally induced by inflammatory signals (*e.g.*,

tumor necrosis factor, interleukin-1 β , interferon- γ , phorbol myristate acetate and exposure to bacteria) in various epithelial tissues (keratinocytes, skin, digestive and airway) and monocytes (45).

The induction of human CAMP by 1,25(OH) $_2$ D $_3$ has been described in various epithelial cells including squamous cell carcinoma, lung cancer, colon cancer, primary keratinocytes and hematopoietic cells including monocytes, neutrophils, macrophages, myeloid cell lines and both normal and leukemic bone marrow cells (23-25). hBD2 was induced in the above-mentioned epithelial cell types, but not the hematopoietic cells (23). ELISAs on the medium of treated U937 cells indicated that increased secretion of the protein and medium from the treated SCC25 or Calu-3 cells possessed bactericidal activity against *Escherichia coli* and *Pseudomonas aeruginosa* indicating secretion of a functional peptide from these cells (23, 24). The induction occurred *via* binding of the VDR to a perfect DR3-type consensus sequence (CAMP: 5'-GGTTCAatgGGTTCA-3' and hBD2: 5'-AGGTCAGgcAGGTCA-3') in the promoter of each gene resulting in significant synthesis of the mRNA and protein (23-25). Interestingly, the regulation of the CAMP and hBD2 genes by vitamin D $_3$ is not conserved in other mammals such as mouse, rat and dog (Gombart *et al.*, unpublished observations) (24). For the CAMP gene, the VDRE is carried on a transposable short-interspersed nuclear element (SINE) that is present in primate genomes. We have identified the VDRE in the genomes of the chimp, orangutan and African green monkey (COS-1 cells; Gombart *et al.*, unpublished observations) (24). For the hBD2 gene, the VDRE is present on an approximately 130-bp fragment that is present only in primate genomes (Gombart *et al.*, unpublished observations). Current studies are underway to characterize the functional conservation of the VDREs in both the CAMP and hBD2 promoters in Old World and New World primates. Even though other mammals appear to lack vitamin D-mediated regulation of their CAMP or BD2 genes, it remains to be determined whether other cathelicidin or defensin genes are subject to regulation by VDR and its agonists. Nearly 900 eukaryotic antimicrobial peptides have been described (<http://www.bbcm.units.it/~tossi/pag1.htm>). Some of the genes encoding these peptides may be regulated by VDR and its agonists.

Our group has been most interested in modulating the expression of the CAMP gene and its protein product hCAP18 for therapeutic purposes. Protective effects of CAMP overexpression in respiratory epithelia have been observed in cystic fibrosis models (46). The systemic expression of hCAP18 in mice improved survival rates following intravenous injection of lipopolysaccharide (LPS) (46). LPS is a component of the bacterial cell wall of Gram-negative bacteria such as *E. coli* or *P. aeruginosa*. Massive Gram-negative bacterial infection can result in septic shock

due to the large amounts of LPS present in the blood. Thus, hCAP18 may not only aid in the clearance of bacterial infection, but may protect against sepsis. This protection probably derives from the ability of CAMP to bind to LPS and neutralize it (47-50). The hCAP18 peptide has been shown to inhibit LPS-induced cellular responses such as release of TNF- α , tissue factor and nitric oxide, thus protecting mice and pigs from septic shock (47, 51). *In vitro*, hCAP18 inhibits macrophage activation by LPS and other bacterial components (50). If endogenous hCAP18 levels can be increased by extrinsic manipulation with vitamin D or deltanoids, then it may be feasible to prevent septic shock in conditions that predispose patients to sepsis.

CAMP expression is up-regulated during cutaneous infection, injury, or inflammation (psoriasis) of the skin (52-54). Interestingly, decreased levels of hCAP18 in the skin of individuals with atopic dermatitis correlated with increased susceptibility of these patients to skin infection as compared to those with psoriasis (53). Mice deficient in CRAMP were much more susceptible to skin infection than wild-type mice (55). CAMP was up-regulated in gastric inflammation caused by *Helicobacter pylori* infection (56). Chronic oral bacterial infections occur in Kostmann syndrome patients who suffer from severe chronic neutropenia. These patients lack expression of hCAP18 in their saliva, plasma and neutrophils (57). Patients suffering from specific granule deficiency (SGD) lack expression of both defensins and hCAP18 and suffer severe, recurrent bacterial infections (58). Most recently, CRAMP was demonstrated to play a role in wound healing by promoting wound neovascularization (pro-angiogenic properties) and re-epithelialization of healing skin (59, 60). The ability to modulate the expression of CAMP in the skin (25) and mucosal surfaces could provide therapeutic benefits in situations where the barrier is compromised. This is particularly true for burn patients who face a severe problem with wound infection (61, 62).

In addition to its antimicrobial and LPS-binding activities, hCAP18 is associated with an increasingly wide range of biological effects (Figure 1). The discovery of additional activities for hCAP18 indicates that it may have a broader role in host defense than previously suspected. *In vitro* studies show hCAP18 induced the migration of human peripheral blood monocytes, neutrophils and CD4 T-cells and rat mast cells (40, 63). In addition, it stimulated histamine release and intracellular Ca $^{2+}$ mobilization in rat mast cells (64). Also, hCAP18 has been shown to alter transcription of both pro- and anti-inflammatory genes in murine macrophage and human epithelial cell lines (50).

Several studies suggested that vitamin D is an evolutionarily ancient system (65-67). Hewison *et al.*, hypothesized that the original function of vitamin D might be to regulate both immune and epithelial cell function to

preserve barrier function (68). They postulated that its endocrine and calciotropic functions are an evolutionarily recent adaptation. The regulation of innate immune proteins by vitamin D is consistent with this hypothesis and holds potential therapeutic promise in the treatment of conditions where this barrier is breached.

Anticancer Activities of 1,25(OH)₂D₃

Normal hematopoiesis and hematological malignancies. Hematopoiesis is the process of the formation of specialized circulating blood cells from pluripotent stem cells from bone marrow that have the ability to either self-replicate or differentiate. The feedback mechanism that regulates these stem cells is affected by depletion of bone marrow, infection, hemorrhage and stress. 'Committed' stem cells proliferate and differentiate down different lineages acquiring specific functional properties such as the ability to fight infection (granulocytes and monocytes), to stop and prevent bleeding (platelets) or to carry oxygen (erythrocytes) (69).

The VDR is expressed in various normal hematopoietic and leukemic cells. It is constitutively expressed in monocytes, subsets of thymocytes, and activated T- and B-lymphocytes (70). VDR knockout mice have normal numbers of white cells (granulocytes, monocytes and lymphocytes), platelets and red cells compared to wild-type mice (71). However, addition of 1,25(OH)₂D₃ to an *in vitro* clonogenic assay dramatically increased the number of committed hematopoietic stem cells of the bone marrow that differentiated to macrophages from the wild-type, but not the knockout mice. This suggests that 1,25(OH)₂D₃ is not required for normal terminal differentiation of hematopoietic cells including macrophages. Therefore, even though 1,25(OH)₂D₃ has a profound effect *in vitro* on macrophage development, the *in vivo* actions remain unclear.

1,25(OH)₂D₃ and deltanoids in leukemia and lymphoma. A brief overview of the use *in vitro* and *in vivo* of vitamin D compounds in cancer is presented in Table I and was recently reviewed by Luong and Koeffler (72). The main consideration for the use of 1,25(OH)₂D₃ is the high doses required to achieve the desired anticancer activities. Such doses could result in life-threatening hypercalcemia. Analogs of 1,25(OH)₂D₃, or deltanoids that are highly potent and have reduced calcemic actions, are currently undergoing clinical trials to determine efficacy and whether they can be used therapeutically in cancer treatments (73-79). Many deltanoids have been tested against multiple cancer models, a few of which are listed in Table I. These studies revealed a number of potential analogs that have 10- to 1000-fold greater growth inhibitory activity *in vitro* than the parental 1,25(OH)₂D₃

Table I. *A brief history of vitamin D compounds in cancer therapy.*

Compound	Tumor	First used	Ref
1,25(OH) ₂ D ₃	Murine leukemia cell line, M1	1981	(138)
	Human leukemia cell line, HL-60		(139)
	Myeloid leukemia and myelodysplastic syndrome (MDS)		(140) (141)
1,25(OH) ₂ D ₃ analogs	Hematopoietic and solid malignancies (<i>in vitro</i> studies)	1989	(142)
			(143)
OCT (Chugai Pharmaceutical Company)	Rat osteosarcoma; breast cancer (<i>in vitro</i> and <i>in vivo</i>); prostate cancer; colon cancer (<i>in vivo</i>); pancreatic cancer (<i>in vivo</i>); thyroid carcinoma cells (<i>in vitro</i>)	1991	(144)
			(145)
			(123)
			(146) (147) (148)
EB1089 (Leo Pharmaceutical)	Breast and prostate cancers (<i>in vitro</i> and <i>in vivo</i>)	1992	(149)
			(124)
			(150) (151)
Vitamin D ₂ analogs	Breast and colon cancers (phase I study); pancreatic cancer (phase II study)	1998	(121)
		2002	(152)
			(153) (154)
High dose 1,25(OH) ₂ D ₃	Prostate cancer	2003	(156)
Paricalcitol (Abbott Laboratories)	MDS, prostate cancer	2003	(85)
		2004	(74)

compound but, importantly, these deltanoids are less calcemic (77, 80-93) (Table II).

One of the most powerful families of anti-leukemic deltanoids are the 20-epimers of 1,25(OH)₂D₃, but these are also the most potent at causing hypercalcemia. For example, KH 1060 [1,25(OH)₂-20-epi-22-oxa-24,26,27-trishomo-D₃] is a particularly potent deltanoid with a 14,000-fold greater ability to inhibit clonal proliferation of U937 cells yet retains the same VDR binding affinity (82, 83), but was 5-fold more potent at inducing hypercalcemia (Table II). Therefore, deltanoids have been synthesized with two side-chains (Gemini analogs). These compounds,

Table II. Effect of vitamin D compounds on both clonal proliferation of HL-60 cells *in vitro* and calcium levels in mice.

Compound*	ED ₅₀ (*10 ⁻⁹ mol/L)	MTD (mg)
1,25(OH) ₂ D ₃	4-18	0.0625
1,25(OH) ₂ -16-ene-D ₃	0.015	0.125
1,25(OH) ₂ -16-ene-23-yne-D ₃	3	2
1,25(OH) ₂ -16-ene-19-nor-D ₃	0.8	0.5
1,25(OH) ₂ -16-ene-24-oxo-19-nor-D ₃	0.1	6
1,25(OH) ₂ -20-epi-D ₃	0.006	0.00125
1,25(OH) ₂ -20-epi-22-oxa-24,26,27-trishomo-D ₃ (KH 1060)	0.001	0.0125
1,25(OH) ₂ -diene-24,26,27-trihomo-D ₃ (EB 1089)	0.23	0.25
19-nor-1,25(OH) ₂ D ₂ (Paricalcitol)	2.4	0.1

ED₅₀ represents the effective doses achieving 50% growth inhibition of HL-60 cells in soft agar clonogenic growth assay. The maximum tolerated dose (MTD) was determined by injection of increasing amounts of vitamin D compounds intraperitoneally in mice three times per week until hypercalcemia or other noticeable toxicities were produced. These studies were performed in our laboratory (77, 82, 86).

including Gemini [1 α ,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)vitamin D₃] and Gemini-19-nor, may have some of the increased potency of the 20-epimers with somewhat less hypercalcemia (94-96).

An important deltanoid, paricalcitol [19-nor-1,25(OH)₂D₂] has been given approval by the Food and Drug Administration for the clinical treatment of secondary hyperparathyroidism. Paricalcitol showed *in vitro* anticancer activity against prostate cancer cells (97), as well as human leukemic cells, and increased the expression of the cyclin-dependent kinase inhibitors, p21^{WAF1} and p27^{Kip1} (85).

The 1,25(OH)₂-16-ene-24-oxo-19-nor-D₃ compound has an excellent therapeutic profile inhibiting 50% clonal growth of leukemic cell lines at 10⁻¹⁰ M (40-fold more potent than 1,25(OH)₂D₃). Also, it has a maximum tolerated dose (MTD) of 6 μ g in mice (100-fold higher than 1,25(OH)₂D₃); however, this compound has not been tested in humans (77).

Studies of vitamin D compounds in lymphoid malignancies have been performed both by our laboratory and others (98, 99). The growth of normal T- and B-lymphocyte progenitors was significantly inhibited by 1,25(OH)₂D₃. In addition, the growth of malignant B-cell lymphoid cells was inhibited. We provided data and hypothesized that 1,25(OH)₂D₃ plays a role as a co-factor in cross-talk between lymphocytes and macrophages (12, 100-104). Upon infection, stimulated macrophages produce IL-1 and other related proteins, that in turn activate T-lymphocytes. These then synthesize lymphokines (such as IFN γ and GM-CSF) that, in turn, activate macrophages. These stimulated macrophages synthesize 1,25(OH)₂-D₃

which acts by dampening the stimulatory interactions of lymphocytes and macrophages. Activated T-lymphocytes express VDR and their lymphokine production is decreased in the presence of 1,25(OH)₂D₃.

Pepper *et al.* showed that the analog, EB 1089, induced apoptosis in B-cell chronic lymphoblastic leukemia (B-CLL) cells *via* a p53-independent pathway involving suppression of ERK activity and activation of the p38 MAP kinase pathway (90). Also, calcipotriol (MC 903), a relatively non-calcemic analog of 1,25(OH)₂D₃, inhibited proliferation of non-Hodgkin's Lymphoma (NHL) cell lines, SU-DHL4 and SU-DUL5, and induced their differentiation (105). These investigators also reported that *in vivo* treatment of follicular NHL in rats with 1 α (OH)D₃ resulted in regression of these tumors, but this was associated with hypercalcemia. Of interest, some reports have found an association between hypercalcemia and rare cases of NHL. These patients had increased 1,25(OH)₂D₃ levels in their plasma (106, 107). Hewison *et al.* suggested that this was probably due to overproduction of 1,25(OH)₂D₃ by macrophages surrounding the malignant lymphocytes (107).

Both 1,25(OH)₂D₃ and paricalcitol were found to be anti-proliferative and pro-apoptotic against myeloma cell lines (85). In other studies, we showed that the EB 1089 analog had potent antiproliferative activity against the myeloma cell line NCI-H929. In these studies, growth inhibition occurred *via* G1-phase arrest and induction of apoptosis (108). The combination of EB 1089 with the cytokine, transforming growth factor beta-1 (TGF β 1), resulted in G1-phase arrest and induction of apoptosis in the NCI-H929 myeloma cell line (109).

The ability of 1,25(OH)₂D₃ and deltanoids to inhibit cell growth is due, in part, to a number of direct target genes that regulate cell growth. Functional VDREs have been found in the promoters of many genes that regulate the cell cycle including p21(110), Rb (96), cyclin-dependent kinases (CDKs) (111), p53 and PTEN (94). 1,25(OH)₂D₃ responsive genes required for differentiation include p38 kinase and protein kinase C (PKC). A few of the other genes that are regulated by 1,25(OH)₂D₃ are BRCA1, early growth response gene-1, MAPK, and insulin-like growth factor-binding protein (84, 112-115). Furthermore, C/EBP β and Rb are up-regulated as HL60 myeloblasts differentiate to macrophages when cultured with 1,25(OH)₂D₃. Recently, we found that 1,25(OH)₂D₃ increased the expression of C/EBP δ in both the MCF-7 and T47D breast cancer cells and LNCaP prostate cancer cell lines (116). Many microarray studies using different tumor cell types have been performed and some of the genes regulated by vitamin D compounds in leukemia and solid tumors are provided in Table III. In general, growth-promoting genes are down-regulated in their expression, while those genes that limit growth and/or enhance differentiation are up-regulated.

Table III. Molecular targets of vitamin D compounds in leukemia and solid tumors.

Class	Genes	Regulation
Cell cycle/ apoptosis	Bax-mitochondrial translocation	↑
	Bcl-2	↓
	Cdk-2, -4, -6	↓
	Cyclin D1, A, E	↓
	Hypophosphorylated Rb	↑
	p21 ^{Waf1} , p27 ^{Kip1}	↑
	Telomerase	↓
Inflammatory	Cox-2	↓
	TNF α	↑
Kinases	PI3-K, p38, ERK activities	↑
	PKC levels	↑
Oncogenes	c-myc	↓
	Dek1	↓
	Fli	↓
Transcription factors	C/EBP β , C/EBP δ	↑
Tumor suppressors	PTEN	↑
	p53	↑

Clinical trials of vitamin D₃ compounds in cancer. Even though *in vitro* studies by our laboratory and others have demonstrated that 1,25(OH)₂D₃ and its analogs show promise against cancer cells, clinical trials of vitamin D compounds in leukemia and myelodysplastic syndrome (MDS) have so far yielded mediocre results (117-119). The data from some of these trials is presented in Luong and Koeffler (72) and outlined here. One of the first trials of 1,25(OH)₂D₃ to treat MDS was performed by our group (117). The data revealed no significant improvement in either hematopoietic parameters or survival. Hypercalcemia was seen in nine out of 18 (50%) patients included in the trial. We recently performed a trial of oral paricalcitol for the treatment of MDS in twelve elderly patients who had poor prognosis at the beginning of the study (74). Although this therapy was not toxic, overall it was not very effective with only one patient showing a partial response with his platelet count rising from 50,000/ μ L of blood to the normal range.

With prostate cancer being the second leading cause of cancer-related deaths in US men and breast cancer being the most frequent malignancy in women in the Western world, new therapies are being sought to treat patients who do not respond to conventional therapy including surgery, radiation therapy and chemotherapy. The antitumor activities of vitamin D compounds have made it an interesting and novel alternative to these therapies. Both 1,25(OH)₂D₃ and several analogs have been tested against

breast (79, 81, 89, 93, 112, 113, 120, 121) and prostate cancer cells (80, 97, 112, 122-125). In these studies, anti-proliferative and pro-apoptotic actions were observed *in vitro* and *in vivo*, against cell lines and primary cells. Again, up-regulation of cell cycle inhibitors, p21^{WAF1} and p27^{Kip1}, are often associated with the action of the hormone. Treatment of RWPE-1 or RWPE2-W99 human prostate cells with cholecalciferol or 1,25(OH)₂D₃ resulted in decreased motility and invasion, as well as increased expression of VDR and other nuclear hormone receptors (126, 127).

Future directions. Clinical trials, combining promising vitamin D₃ analogs and other agents, is a logical next step. Many *in vitro* and animal studies have highlighted the potential use of 1,25(OH)₂D₃ and its analogs as potentiating agents, whereby vitamin D compounds may be combined with other agents that have different toxicities thereby increasing overall tumor cell death. Some of these agents include chemotherapeutic drugs such as doxorubicin and cisplatin (128), vitamin K2 (129), Dexamethasone (130), retinoic acids (4, 83, 131, 132), histone deacetylase inhibitors (133), arsenic trioxide (As₂O₃) (134, 135) and ionising radiation (125). For example, the analog, KH 1060, combined with retinoids had markedly enhanced antiproliferative and pro-apoptotic activities in HL-60 and NB4 AML cells (136). Dunlap *et al.* (125) tested 1,25(OH)₂D₃ and paricalcitol against LNCaP and PC-3 prostate cancer cell lines and primary prostate cancer cells. These investigators observed synergistic effects when ionising radiation was combined with vitamin D compounds. This suggests that vitamin D compounds can potentiate radiation therapy for the treatment of tumors that otherwise would not normally respond. Furthermore, drugs that block the metabolism of 1,25(OH)₂D₃, such as ketoconazole (137), resulted in higher intracellular levels of the vitamin D compounds. This may enhance tumor cell death. Also, some tumors have high levels of 1 α -hydroxylase and investigators have suggested that increasing the blood levels of 25(OH)D₃ may have a therapeutic effect because this can be metabolized to the active, antiproliferative 1,25(OH)₂D₃ in the target cells. Levels of 25(OH)D₃, however, are already fairly high and this approach is unproven.

In summary, treatment of leukemia with vitamin D compounds is unlikely, by itself, to be successful; but when given either in the maintenance phase of therapy or combined with other agents, these agents may be useful therapeutically. Finally, in light of recent findings that vitamin D compounds can induce the significant expression of antimicrobial peptides, the potential of these compounds to protect cancer patients from life-threatening infections during treatment should be evaluated.

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