Promise of vitamin D analogues in the treatment of hyperproliferative conditions

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Abstract
1α,25-Dihydroxyvitamin D3 [1α,25-(OH)2D3; calcitriol] is best known as a hormone involved in calcium homeostasis but is also a potent antiproliferative agent in many cell types, particularly epithelial cells. 1α,25(OH)2D3 mediates its actions through a classic steroid hormone-like transcriptional mechanism by influencing the expression of hundreds of genes. Effects of 1α,25(OH)2D3 have been observed on expression of cell cycle regulators, growth factors and their receptors, apoptotic machinery, metastatic potential, and angiogenesis; all of which have some effect on hyperproliferative conditions. This minireview focuses on the anticancer potential of 1α,25(OH)2D3 and its analogues by summarizing the promising data from animal and human trials of 1α,25(OH)2D3 and some of the more interesting synthetic vitamin D analogues in the treatment of a variety of different animal cancer models and in human patients with advanced cancer. Optimal administration of vitamin D analogues is only just being achieved with high-dose intermittent administration overcoming bioavailability and hypercalcemia problems and combination therapy with cytotoxic agents (taxols and cisplatins), antiresorptive agents (bisphosphonates), or cytochrome P450 inhibitors being attempted. Although the potential of vitamin D as an antiproliferative drug has been realized in the treatment of psoriasis and in parathyroid cell hyperplasia associated with secondary hyperparathyroidism, the search for an anticancer treatment incorporating a vitamin D analogue remains elusive. [Mol Cancer Ther 2006;5(4):797–808]

Introduction
The nutritional term vitamin D was coined almost a century ago to denote an antirachitic substance that can be derived from dietary sources, but in mammals vitamin D is also made in the skin on exposure to sunshine containing UVB light. Today, the term vitamin D is more commonly but erroneously used as an abbreviation for its active form, 1α,25-dihydroxyvitamin D3 [1α,25-(OH)2D3; also known as calcitriol], which is synthesized from vitamin D by a two-step enzymatic process. This metabolite, 1α,25-(OH)2D3, interacts with its ubiquitous nuclear vitamin D receptor (VDR) to regulate the transcription of a wide spectrum of genes involved in calcium and phosphate homeostasis as well as in cell division and differentiation. It is these latter actions that have attracted tumor biologists to explore the effects of vitamin D and to use it as an antiproliferative agent in cancer cells in vitro and in vivo. This minireview is designed to update the expert and nonexpert on (a) current views of the mechanism of action of 1α,25-(OH)2D3, (b) promising new anticancer vitamin D analogues, and (c) potential of vitamin D–related anticancer therapies.

Biochemistry of Vitamin D
Vitamin D3 can be synthesized in the skin from 7-dehydrocholesterol (1) or be derived in the form of vitamin D3 or vitamin D2 (collectively known as vitamin D) from dietary sources. Vitamin D and its metabolites are mostly hydrophobic and thus require a protein (vitamin D–binding protein) for their transport in the aqueous environment of the bloodstream to sites of storage (adipose tissue) or sites of activation (liver and kidney) of sites of action. Vitamin D undergoes its first step of activation (i.e., 25-hydroxylation in the liver), a step carried out by both liver mitochondrial (CYP27A1) and microsomal cytochrome P450 isoforms CYP2R1, CYP3A4, and CYP2J3 (2–4). The product of the 25-hydroxylation step, 25-OH-D3, is the major circulating form of vitamin D3 and in humans is present in plasma at concentrations in the range 10 to 80 ng/mL (25-200 nmol/L; ref. 5). The main reason for the extended plasma half-life of 25-OH-D3 is its strong affinity for vitamin D–binding protein, and the vitamin D–binding protein–null mouse shows accelerated rates of vitamin D clearance and low 25-OH-D3 levels (6). Serum levels of 25-OH-D3 represent a measure of the vitamin D status of the animal in vivo and have proven particularly valuable in
epidemiologic studies because they inversely correlate with the incidence of various types of cancers (breast, prostate, and colon) as well as multiple sclerosis and diabetes (7). Recently, 25-OH-D levels have been reclassified into the following categories: (a) <25 nmol/L (<10 ng/mL) are considered to be indicative of vitamin D deficiency; (b) between 25 and 80 nmol/L (10-32 ng/mL) indicative of vitamin D insufficiency; (c) between 80 and 200 nmol/L (32-80 ng/mL) indicative of vitamin D sufficiency; and (d) >200 nmol/L (>80 ng/mL) indicative of vitamin D toxicity. The public health strategy has now become the use of health intervention approaches (sunlight exposure, D fortification, and D supplementation) to raise 25-OH-D levels in the whole population toward vitamin D sufficiency (8), a strategy that is predicted to reduce cancer incidence.

The circulating metabolite, 25-hydroxyvitamin D₃, is activated to the hormonal form, 1α,25-(OH)₂D₃, primarily in the kidney (2) and the synthesis of circulating 1α,25-(OH)₂D₃ in the normal mammal [human reference range, 37.5-150 pmol/L (15-60 pg/mL); ref. 5] is the exclusive domain of that organ. Patients with chronic renal failure exhibit frank rickets or osteomalacia due to a deficiency of circulating 1α,25-(OH)₂D₃ caused by lack of renal 1α-hydroxylase, a situation that is reversed by 1α,25-(OH)₂D₃ hormone replacement therapy. The main component of the 1α-hydroxylase enzyme is the cytochrome P450, CYP27B1 (9), which is defective in a rare human-dependent rickets (VDDR type I; ref. 10). Cyp27b1 gene ablation in the mouse results in rickets (11). CYP27B1 is strongly down-regulated by its product, 1α,25-(OH)₂D₃, and up-regulated by parathyroid hormone (PTH) as part of the calcium homeostatic loop (12) and up-regulated by fibroblast-like growth factor 23 as part of the phosphate homeostatic loop (13).

Physiologic or pathologic situations in which extrarenal 25-OH-D₃ 1α-hydroxylase activity becomes prominent include pregnancy where placental 1α-hydroxylase provides 1α,25-(OH)₂D₃ for the developing fetus or, in the granulomatous condition, sarcoidosis where sarcoid tissue expresses an unregulated 1α-hydroxylase that results in elevated plasma 1α,25-(OH)₂D₃ levels causing hypercalcemia and hypercalcinia (14). Extrarenal 1α-hydroxylase in sarcoid macrophages is induced by IFN-γ and other cytokines (15). There is also evidence that a few solid tumors express extrarenal 1α-hydroxylase that in turn causes hypercalcemia, although the vast majority of hypercalcemia of malignancy is due to excessive production of PTH-related peptide (16). Recently, CYP27B1 mRNA and immunodetectable protein have been detected in many normal extrarenal tissues (e.g., skin, prostate, colon, and breast; ref. 17), where pathologic problems do not exist. The recognition of the wider distribution of extrarenal CYP27B1 has generated a new hypothesis that the enzyme exists to augment circulating 1α,25-(OH)₂D₃ with local production of 1α,25-(OH)₂D₃ (18, 19). Locally high concentrations of 1α,25-(OH)₂D₃ in certain sites, such as skin, prostate, and breast, are believed to result in altered patterns of gene expression, which in turn limit cell division and lead to tissue-specific differentiation of particular cell types. This emerging physiologic role for extrarenal 1α-hydroxylase emphasizes the importance of the circulating 25-OH-D pool that provides substrate for “target cell” enzyme as well as for the renal enzyme responsible for circulating 1α,25-(OH)₂D₃. Thus, the prevailing opinion is that the level of plasma 25-OH-D is more useful than the level of plasma 1α,25-(OH)₂D₃ for predicting the full spectrum of vitamin D actions around the body (20).

Irrespective of where 1α,25-(OH)₂D₃ is synthesized, it represents the sole active molecule responsible for the biological response inside vitamin D target cells. The functions of vitamin D have been shown to occur through a steroid hormone-like mechanism involving a nuclear VDR that specifically regulates the transcription of vitamin D–dependent genes coding for proteins that in turn regulate cellular events, such as intestinal calcium transport and cell division (ref. 21; Fig. 1). In the classic steroid hormone model, 1α,25-(OH)₂D₃ enters the cell by traversing the plasma membrane in a free form and binds tightly to the VDR inside the nucleus (Kₐ = 2 × 10⁻¹⁰ mol/L). The liganded or occupied VDR specifically targets only vitamin D–dependent genes by interacting with a specific sequence found upstream of the vitamin D–dependent gene known as a vitamin D–responsive element that is a tandem repeating oligonucleotide of 6 bp with a 3-nucleotide spacer, normally situated upstream of the 5' end of the vitamin D–responsive gene. A consensus vitamin D–responsive element (AGGTCA) is found in several positively regulated genes (e.g., osteocalcin), whereas more complex elements are found in the negatively regulated collagen type I and pre-pro-PTH genes. Vitamin D–responsive element–containing genes now number around several hundred, suggesting that 1α,25-(OH)₂D₃ through a VDR-mediated process regulates many physiologic processes besides intestinal calcium and phosphate homeostasis (22). Transactivation of genes requires a heterodimeric partnership with the retinoid X receptor and recruitment of a plethora of other transactivating proteins, termed the DRIP complex (23). The current model suggests that occupation of the ligand-binding domain of the VDR triggers a protein conformational change in the AF-2 domain of the COOH terminus of the VDR (24), which allows recruitment of positive transcription factors and/or shedding of transcriptional inhibitory factors that lead to increased formation of a transcription initiation complex and an increased rate of gene transcription.

Reports of the broad distribution of the VDR protein and other components of the vitamin D machinery in different tissues besides the classic targets of bone, intestine, and kidney have encouraged researchers to look for vitamin D involvement in other physiologic processes. This was further reinforced by reports of diverse effects of exogenous vitamin D analogues on biological systems in vitro and nonclassic tissues in vivo. Although it was unclear if
the effects were physiologic or pharmacologic, these observations have certainly broadened our view of vitamin D as more than just a calcemic hormone. The consensus view stemming from various mouse models and gene microarray experiments is that 1α,25-(OH)2D3 influences many physiologic processes (2), which are usually divided into:

- classic roles, which include the regulation of blood calcium and phosphate concentrations by actions on intestine, bone, kidney, and parathyroid gland, and
- nonclassic roles, which include cell differentiation and antiproliferative actions on various cell types, including bone marrow (myeloid and lymphoid), skin, muscle, and intestine.

Although most of the classic actions of vitamin D have been known for decades since dietary vitamin D deficiency was first shown, the nonclassic roles have only emerged from more subtle studies involving experiments probing the mechanism of action of vitamin D at the molecular level, from studies of children with mutations of the VDR (VDDR type II) and from studies of the VDR knockout mouse (25).

24-Hydroxylation of both circulating precursor 25-OH-D3 and hormonal form 1α,25-(OH)2D3 has been shown to occur in vivo to give short-lived 24-hydroxylated products with reduced biological activity (26). The virtually ubiquitous 25-OH-D3-24-hydroxylase, now called CYP24A1, catalyzes a five-step, vitamin D–inducible, C-24 oxidation pathway that changes the vitamin D molecule into water-soluble truncated products, such as calcitriol (27, 28). 24-Hydroxylation is an important step destroying excess precursor 25-OH-D3 and inactivating 1α,25-(OH)2D3 inside target cells, suggesting that C-24 oxidation primarily exists as a target cell attenuation or desensitization process that constitutes a molecular switch to turn off vitamin D responses inside target cells (29). Consistent with this hypothesis is the finding that 50% of Cyp24a1 null mice die prematurely of...
hypercalcemia and nephrocalcinosis, whereas their surviving homozygous littermates show a greatly reduced ability to clear a bolus dose of $[1\beta^{-3}H]_\alpha_25-(OH)D_3$ from their circulation compared with normal wild-type mice (30). The importance of target cell degradation has reinforced the utility of designing vitamin D analogues with modifications around C-24 to resist the catabolic effects of CYP24A1 and the screening of CYP24A1 inhibitors. Furthermore, the chromosomal region 20q13.2, which contains the CYP24A1 gene, is amplified in certain human cancers justifying the development of CYP24A1 inhibitors (31). Such CYP24A1 inhibitors based onazole chemistry (Novartis, Vienna, Austria; ref. 32) and substrate-like competitors (Cytochroma, Markham, Ontario, Canada; ref. 33) have been developed and look promising, although there are also claims that the phytoestrogen, genistein, is an effective CYP24A1 inhibitor (34).

**Rationale for Using Vitamin D Analogues in Cancer Therapy**

There has been considerable interest shown by the pharmaceutical industry in the design and synthesis of compounds, which mimic some or all of the biological actions of $1\alpha_25-(OH)D_3$ and acting through VDR-mediated mechanisms (2). This has been particularly true of the class of molecules known as low-calcemic vitamin D analogues that purportedly have reduced calcemic actions and enhanced antiproliferative activity. Such anticancer vitamin D molecules are the central focus of this minireview (examples provided in Fig. 2). Note that vitamin D analogues are now widely used drugs in the treatment of psoriasis, a hyperproliferative skin condition (35) and in suppression of secondary hyperparathyroidism and parathyroid hyperplasia resulting from chronic kidney disease (36). Topically applied vitamin D analogues used in psoriasis (e.g., calcipotriol) were designed to be susceptible to target and liver cell breakdown, making them less likely to cause calcemic side effects should they gain access to the general circulation. Systemically administered vitamin D analogues [e.g., 19-nor-$1\alpha_25-(OH)D_2$ and $1\alpha-OH-D_2$], used to suppress PTH synthesis in the parathyroid gland in the individual with chronic kidney disease, were designed to circulate for longer periods but are often operating in patients with

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**Figure 2.** Chemical structures of potential anticancer vitamin D analogues referred to in the text are provided. Note that several different strategies have been used by the organic chemist to modify the properties of the vitamin D molecule. The most basic molecule is the prodrug version that is inactive as given but activated by CYPs in vivo. The most popular analogues have modified (hybrid) side chains and these include double side chain versions known as Gemini analogues. Many analogues possess side chains with double or triple bonds in the C-24 position that make them resistant to the actions of CYP24A1. The nonsteroidal vitamin D analogues were designed to occupy the VDR-ligand-binding pocket.
Mechanisms Involved in the Anticancer Effects of Vitamin D

The earliest findings by Abe et al. (37) in 1981 that 1α,25(OH)₂D₃ inhibited proliferation of a variety of human leukemic cell lines and stimulated the differentiation of normal and leukemic myeloid precursors toward more mature, less aggressive phenotypes rendered 1α,25(OH)₂D₃ potentially useful in the treatment of myeloid leukemias and other types of cancer. Over the past two decades, evidence has been accumulated that 1α,25(OH)₂D₃ and its analogues have anticancer properties in several tissues. Although the exact mechanism underlying the growth inhibitory actions of vitamin D and its analogues in cancer cells is not fully understood, the volume of data support multipronged effects involving growth arrest at the G1 phase of the cell cycle, apoptosis, tumor cell differentiation, disruption of growth factor-mediated cell survival signals, and inhibition of angiogenesis and cell adhesion. Most observations indicate that VDR-mediated pathways constitute potential therapeutic targets for cancer prevention and treatment.

Cell Cycle Regulators

The 1α,25(OH)₂D₃-VDR system arrests the cancerous cell cycle at the G₁-G₁ transition through multiple mechanisms. This G₁ arrest is achieved by induction of the cyclin-dependent kinase inhibitors, p21⁰WAF1/CIP1 and p27⁰KIP1, which causes a decrease of cyclin-dependent kinase 2 activity leading to dephosphorylation of retinoblastoma protein and repression of E2F transcriptional activity in several cell types (38, 39). In this cascade, 1α,25(OH)₂D₃ and analogues induce gene transcription of the p21⁰WAF1/CIP1 and induce the synthesis and/or stabilization of the p27⁰KIP1 protein (40–42). In breast cancer cells, 1α,25(OH)₂D₃ enhances the expression of HOXA10, a homeobox protein that causes G₁ arrest (43). In a VDR-serine/threonine protein phosphatase PP1c/PP2A-p70S6 kinase complex, 1α,25(OH)₂D₃ seems to induce the VDR-associated phosphatase activity that in turn inactivates their substrate p70S6 kinase (44). Because p70S6 kinase is essential for G₁/S-phase transition, these results provided further evidence of a 1α,25(OH)₂D₃-mediated G₁ block in colon cancer cells.

Growth Factors and Growth Factor Receptors

The growth inhibition of cancer cells by 1α,25(OH)₂D₃ is also associated with growth factor signaling. Transforming growth factor-β (TGF-β) is a potent inhibitor of the proliferation of many cell types and is involved in cell cycle control and apoptosis. Vitamin D analogues induce autocrine TGF-β activity through increasing expression of TGF-β isofoms and/or TGF-β receptors in nonmalignant and malignant breast cells (45). In addition, Smad3, a TGF-β signaling mediator, coactivates VDR to further enhance TGF-β signaling and vitamin D signaling, suggesting the synergistic action of TGF-β and vitamin D analogues. 1α,25(OH)₂D₃ analogues also blocked the mitogenic activity of insulin and insulin-like growth factor (IGF)-I on breast cancer cells most probably by inducing the expression of IGF-binding protein 3 and 5 (46, 47). Interestingly, in the human osteosarcoma cell line MG-63, 1α,25(OH)₂D₃ and TGF-β act synergistically to increase IGF-binding protein 3 secretion (48). Furthermore, induction of IGF-binding protein 3 has been found to be obligatory for the 1α,25(OH)₂D₃-mediated growth inhibitory effect on the prostate cancer cell line LNCaP, and IGF-binding protein 3 neutralizing antibodies block 1α,25(OH)₂D₃-mediated induction of p21 expression (49). Thus, IGF-binding protein 3 induction by 1α,25(OH)₂D₃ may, at least in part, explain the antiproliferative and proapoptotic actions of 1α,25(OH)₂D₃ in primary cancer cells.

Apoptosis

1α,25(OH)₂D₃-induced apoptosis is an important contributor to the growth-suppressing properties of the steroid in cancer. In cancer cells, 1α,25(OH)₂D₃ analogues induce apoptosis through reciprocal modulation in the antiapoptotic protein Bcl-2 and the proapoptotic protein Bax content (50). 1α,25(OH)₂D₃ analogues also increase intracellular calcium, which activates the calcium-dependent proapoptotic protease, μ-calpain (51), and increase the antitumoral and proapoptotic properties of ionizing radiation in MCF-7 breast tumor xenografts in nude mice and tumor necrosis factor-α-induced apoptosis in MCF-7 cells through caspase-dependent and caspase-independent mechanisms (52, 53). Other suggested mechanisms for the apoptotic effects of vitamin D include down-regulation of the antiapoptotic IGF receptor, up-regulation of the proapoptotic signaling molecule mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1, activation of the sphingomyelin-ceramide-ganglioside GD3 signaling pathway, and reduced expression of Akt, a kinase that regulates cell survival signals (54).

Differentiation

In addition to proliferation and apoptosis, the other major cellular process is differentiation. The coupling between proliferation and differentiation has been most widely studied in cells of hematopoietic system and in keratinocytes. In general, 1α,25(OH)₂D₃ inhibits cell proliferation and induces myeloid differentiation along the monocytic-macrophage lineage. In the monocytic cell line, HL60 (55), 1α,25(OH)₂D₃ induces CCAAT/enhancer-binding protein β expression, a protein recently identified as a potent...
suppressor of the oncogenic cyclin D1 signature in human epithelial tumors (56). 1α,25(OH)2D3 induction of CCAAT/enhancer-binding protein β in HL60 cells promotes the differentiation of these immature myeloid precursors into normal macrophages through interaction with retinoblastoma protein (55), which can directly interact with the promoter for CD14, a gene characteristically expressed in monocytes. Similarly, 1α,25(OH)2D3 analogues induce differentiation of leukemic cells with intact potential for monocytic differentiation by changing the intracellular balance of transcription factor activity and allow the cells to bypass the granulocytic differentiation block by diverting the cells to monocytic lineage (57). Effects on differentiation have also been reported for other cell types. Recent microarray studies of gene expression profiles in cancer cells have highlighted the capacity of 1α,25(OH)2D3 analogues to drive malignant cells to a more differentiated state. EB1089 treatment of head and neck squamous cell carcinoma repressed expression of several markers of cancer progression (e.g., N-cadherin, squamous cell carcinoma antigen, tenasin-C, and tumor antigen L6) and induced expression of several genes associated with epithelial cell differentiation (cystatin M, prostate M, type XIII collagen, and desmoglein 3; ref. 22).

Invasion and Metastasis

For tumor suppressive activity, besides growth inhibition, vitamin D and its analogues decrease the invasive-ness of several cell types in vitro, and they inhibit angiogenesis and metastasis in xenograft and transgenic mouse models in vivo (58, 59). In cultured malignant cells, 1α,25(OH)2D3 and its analogues down-regulate cell invasion-associated proteases include matrix metalloproteases 2 and 9 (60, 61) and serine proteinases (62). 1α,25(OH)2D3 also inhibits prostate cancer cell invasion by decreasing in expression of αv and β3 integrins, both of which are receptors for laminin (63). In prostate and colon cancer cells, 1α,25(OH)2D3 and its analogues increase expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells, and inhibit the oncogenic β-catenin signaling (64). Furthermore, 1α,25(OH)2D3 inhibits tenasin-C, an extracellular matrix protein, which promotes growth, invasion, and angiogenesis and is up-regulated in many cell types during tumorigenesis (65). Moreover, 1α,25(OH)2D3 and its analogues inhibit the proliferation of some tumor-derived endothelial cells (66) and inhibit the expression of vascular endothelial growth factor that induces angiogenesis in tumors (67).

Factors Involved in Vitamin D Resistance

Although the presence of a functional VDR is also a prerequisite for a growth regulatory response and a relationship between VDR levels and growth inhibition has been suggested for several cancer cells, the presence of the VDR is not always predictive of a growth inhibitory effect of exogenous 1α,25(OH)2D3. Human colorectal cancer cells highly express the transcription factor, Snail, which is recruited to the native VDR promoter, thereby limiting VDR responses and the efficacy of 1α,25(OH)2D3 therapy (68). In prostate cancer, altered levels of expression of the steroid receptor corepressor, SMRT, or defective nuclear VDR localization, but not reduced VDR levels, are responsible for the resistance to the antiproliferative properties of 1α,25(OH)2D3 (69).

More recent studies have shown that the histone deacetylation inhibitor tricostatin A can reverse the resistance to 1α,25(OH)2D3 therapy, which develops in the breast cancer cell line MDA-MB-231 resulting from decreased VDR and increased corepressor NCoR1 content (70).

Promising Vitamin D Analogues under Development for Types of Cancer

The search for new vitamin D analogues with potential clinical usefulness has focused on candidates with good systemic bioavailability, potent antineoplastic/chemopreventive activity, and low-calcemic activity, the structures of which are shown in Fig. 2. In the sections below, we have summarized progress in trials of vitamin D analogues in the common epithelial cancers in animal models (Table 1) and human trials (Table 2).

Breast Cancer and Vitamin D Analogues

1α,25(OH)2D3 and its analogues have been shown to inhibit the proliferation of breast cancer cells in vitro and tumor progression in vivo. One of the most interesting aspects of the action of vitamin D analogues is their efficacy in both estrogen receptor–positive and estrogen receptor–negative breast cancer cells. In addition to direct growth inhibitory effects, some analogues also inhibit angiogenesis and decrease the metastatic potential of breast cancer cell in vitro and in vivo.

The beneficial effects of EB1089 (Fig. 2; Table 1) on breast cancer in tumor-bearing animals result in a marked increase in survival time (71) and an inhibition of the development of bone metastases without causing hypercalcemia (72). 22-Oxa-1α,25(OH)2D3 suppressed tumor growth in athymic mice implanted with human breast carcinoma with or without estrogen receptor (73). Moreover, the beneficial effects of 22-oxa-1α,25(OH)2D3 on breast cancer can be augmented by combination with the antiestrogen, tamoxifen, or an aromatase inhibitor (73, 74). Suppression of tumor growth by 22-oxa-1α,25(OH)2D3 was accompanied by inhibiting neovascularization (58, 67). An additional benefit of EB1089 and 22-oxa-1α,25(OH)2D3 is that they inhibit PTH-related peptide production (75, 76); thus, both analogues may also have utility in treating malignancy-associated hypercalcemia. Following up these analogues, TX522 [19-nor-14,20-bisepi-23-yne-1α,25(OH)2D3] and TX527 [19-nor-14,20-bisepi-23-yne-1α,25(OH)2D3] have been shown to retard tumor progression in an in vivo model of MCF-7 breast cancer cells established in nude mice (77). Other potential analogues, Ro24-5531 [16-ene-23-yne-26,27-hexafluoro-1α,25(OH)2D3] and 1α(OH)D3 extended the latency of tumor incidence as well as multiplicty of mammary carcinomas induced by N-methyl-N-nitrosourea in rats when the analogue was fed for 2 to 7 months (78, 79), which indicated the possibility for...
Table 1. In vivo effects of vitamin D analogues in animal models of cancer

<table>
<thead>
<tr>
<th>Tumor, model</th>
<th>Analogue</th>
<th>Dose</th>
<th>Effect</th>
<th>Hypercalcemia</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>Breast</td>
<td>N-methyl-N' -nitroso urea induced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-7 xenografts</td>
<td>22-Oxa-1,25(OH)2D3</td>
<td>1.0 µg/kg p.o.</td>
<td>Tumor suppression</td>
<td>None</td>
<td>(73)</td>
</tr>
<tr>
<td>MDA-MB-231 xenografts</td>
<td>EB1089</td>
<td>14 pmol/L/24 h, infusion</td>
<td>Inhibited skeletal metastasis</td>
<td>None</td>
<td>(72)</td>
</tr>
<tr>
<td>Prostate</td>
<td>LNCaP xenografts</td>
<td>EB1089</td>
<td>0.5 µg/kg i.p.</td>
<td>Tumor suppression</td>
<td>None</td>
</tr>
<tr>
<td>Colon</td>
<td>1,2-Dimethylhydrazine induced</td>
<td>22-Oxa-1,25(OH)2D3</td>
<td>30 µg/kg i.p.</td>
<td>Reduced aberrant crypt foci</td>
<td>None</td>
</tr>
<tr>
<td>Colon</td>
<td>Azoxymethane induced</td>
<td>Ro24-5531</td>
<td>3.5 nmol/kg diet</td>
<td>Reduced tumor incidence</td>
<td>None</td>
</tr>
<tr>
<td>Colon</td>
<td>LoVo xenografts</td>
<td>EB1089</td>
<td>0.1 and 0.5 µg/kg p.o.</td>
<td>Tumor suppression</td>
<td>None</td>
</tr>
<tr>
<td>Colon</td>
<td>MC-26 xenografts</td>
<td>RO-4383561</td>
<td>0.02 µg E i.p.</td>
<td>Reduced tumor growth</td>
<td>None</td>
</tr>
</tbody>
</table>

*1 µg(OH)D3, 58.4 and 116.8 nmol/kg diet = 25 and 50 µg/kg diet.
1 22-Oxa-1,25(OH)2D3 30 µg/kg i.p. = 72.5 nmol/kg i.p.

Prevention of breast cancer in the rat. Of note, the combination of a vitamin D analogue with paclitaxel and cisplatin suppressed tumor growth more than either drug alone (80), indicating that treatment of breast cancer could be expanded to include combination therapies of vitamin D analogues with anticancer agents.

Prostate Cancer and Vitamin D Analogues

1α,25(OH)2D3 has been shown to inhibit the proliferation of both androgen-dependent and androgen-independent prostate cancer cells. The beneficial effects of EB1089 on prostate cancer in tumor-bearing animals result in a marked decrease in tumor volumes (81) and an inhibition of the development of lung metastases without severe weight loss (82). Prostate cancer–mediated osteoblastic bone lesions were inhibited by the low-calcemic hybrid analogue JK-1626-2 (1α-hydroxymethyl-16-ene-26,27-bishomo-25-hydroxyvitamin D3; ref. 83). In an in vitro animal model carrying with human prostate cancer cells, Ro23-7553 [16-ene-23-yne-1α,25(OH)2D3] has been shown to inhibit tumor growth without increasing the serum calcium concentration (84). However, dietary supplemented Ro24-5531 (1.25 or 2.5 nmol/kg diet) was only moderately effective at inhibiting the development of androgen-induced carcinoma of the prostate and seminal vesicles (85). Several nonsteroidal analogues have interesting biological properties resembling those of 1α,25(OH)2D3 but with extremely low systemic calcemic effects. One of these compounds, LG190119, effectively inhibited LNCaP prostate cancer xenograft tumor growth without causing hypercalcemia (86). Nonsteroidal analogues may represent promising new therapeutics in the cancer armamentarium.

Colon Cancer and Vitamin D Analogues

The vitamin D analogues reduce the proliferation of colon cancer cells in vitro, but they also reduce tumorigenesis in xenograft and chemically induced tumors in vivo (87, 88). Several epidemiologic studies show that vitamin D deficiency may accelerate colon cancer growth. Conversely, dietary supplementation of Ro24-5531 or 1α(OH)D3 prevented azoxymethane-induced crypt cell hyperproliferation, aberrant crypt foci development, and tumor incidence (79, 89). Using dietary supplementation with the low-calcemic analogue, Ro 25-9022 [16,23E-diene-26,27,F6-19-nor-1,25(OH)2D3], studies showed a reduced tumor incidence and inhibition of spontaneous metastasis in a 1,2-dimethylhydrazine-induced colon carcinogenesis model (90). Otoshi et al. (91) showed that the i.p. injection of 22-oxa-1α,25(OH)2D3 also suppressed the development of aberrant crypt foci in rats. Using the novel double side chain 19-nor-Gemini compound, injection of RO-4383561 reduced tumor growth in a MC-26 xenograft model (92). Notably, neither analogue significantly influenced animal growth rates or serum calcium levels. The results from these studies indicate that vitamin D analogues may be an effective new approach for colon cancer prevention and treatment.
Clinical Development of Vitamin D Analogues for Cancer Treatment

Clinical trials with 1α,25(OH)2D3 in cancer patients set the stage for the development of vitamin D analogues for cancer treatment (93–95). Table 2 summarizes cancer clinical trials done with 1α,25(OH)2D3 and vitamin D analogues to date. Earlier studies showed the therapeutic efficacy of systemically applied 1α,25(OH)2D3 for treating cancer, but subsequently it has not fulfilled its early promise (93). A main drawback of systemically applied vitamin D analogues has been side effects, primarily hypercalcemia, at the doses needed to effect clinical improvement. One approach to limiting hypercalcemia was i.v. or s.c. administration of 1α,25(OH)2D3 at doses of 1.5 to 4 μg thrice weekly (93) that have been used in individuals with renal disease as i.v. pulse therapy (96), but renal stones developed in some cancer patients with this regimen. Alternatively, controversial hepatic arterial infusion of 1α,25(OH)2D3 (0.2-10 μg/d) allowed a high dosage to be administered without inducing hypercalcemia in liver cancers (97). In recent years, much progress has been made in cancer treatment with very high dose intermittent 1α,25(OH)2D3 therapy based on laboratory data, suggesting that intermittent exposure to high levels of 1α,25(OH)2D3 may be sufficient to produce its antiproliferative effects. Weekly high-dose pulse oral 1α,25(OH)2D3 (0.5 μg/kg) was better tolerated and led to an increase in median prostate-specific antigen doubling time without the development of hypercalcemia or hypercalciuria in prostate cancer (94, 98). Weekly high-dose administration of 1α,25(OH)2D3 in combination with chemotherapy (docetaxel or carboplatin) produced a minimal 50% reduction in prostate-specific antigen values in 30 of 37 patients with metastatic cancer and suggested that this was better than the response in other trials of the chemotherapy alone (99–101). Although no patient experienced severe side effects from the treatment, pharmacokinetic studies showed that a dose of 0.48 μg/kg produced mean peak 1α,25(OH)2D3 levels of 1,625 pg/mL that is 30-fold higher than normal levels. This suggests that hypercalcemia or hypercalciuria is likely to occur at early times within the first 24 to 48 hours after administration and that prolonged high-dose pulse 1α,25(OH)2D3 administration would be expected to enhance the potential for stone formation. Accordingly, the reduction of dietary calcium intakes to <500 mg may be important to minimize the development of hypercalcemia in these patients (94).

Vitamin D analogues may therefore be an effective therapy for treatment of early as well as advanced cancer. Several analogues, dosed daily, have entered clinical trials. Seocalcitol (EB1089) was evaluated in a phase I clinical trial of advanced breast and colorectal cancer; although less

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Patients</th>
<th>Protocol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3 + dexamethasone</td>
<td>Prostate cancer before prostatectomy</td>
<td>Twice weekly p.o.</td>
<td>Ongoing study*</td>
</tr>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3 + mitoxantrone + prednisone</td>
<td>Prostate cancer</td>
<td>High-dose pulse p.o.</td>
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</tr>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3 + docetaxel</td>
<td>Pancreatic cancer</td>
<td>Weekly p.o.</td>
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</tr>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3</td>
<td>Prostate cancer before prostatectomy</td>
<td>Weekly p.o.</td>
<td>(98)</td>
</tr>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3 + docetaxel</td>
<td>Prostate cancer</td>
<td>Weekly p.o.</td>
<td>(99)</td>
</tr>
<tr>
<td>Phase II</td>
<td>1α,25(OH)2D3 + carboplatin</td>
<td>Prostate cancer</td>
<td>Weekly p.o.</td>
<td>(100)</td>
</tr>
<tr>
<td>Phase II</td>
<td>1α(OH)D2</td>
<td>Prostate cancer before prostatectomy</td>
<td>Daily p.o.</td>
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</tr>
<tr>
<td>Phase II</td>
<td>1α(OH)D2</td>
<td>Prostate cancer</td>
<td>Daily p.o.</td>
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</tr>
<tr>
<td>Phase II</td>
<td>EB1089</td>
<td>Liver cancer</td>
<td>Daily p.o.</td>
<td>(103)</td>
</tr>
<tr>
<td>Phase II</td>
<td>EB1089</td>
<td>Pancreatic cancer</td>
<td>Daily p.o.</td>
<td>(104)</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3 + ketoconazole + dexamethasone</td>
<td>Advanced solid tumors</td>
<td>Days 1-3/wk p.o.</td>
<td>Ongoing study*</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3 + gefitinib + dexamethasone</td>
<td>Advanced solid tumors</td>
<td>Weekly i.v.</td>
<td>Ongoing study*</td>
</tr>
<tr>
<td>Phase I/II</td>
<td>1α,25(OH)2D3 + docetaxel + estramustine</td>
<td>Prostate cancer</td>
<td>High-dose pulse p.o.</td>
<td>(101)</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3 + paclitaxel</td>
<td>Advanced solid tumors</td>
<td>Days 1-3/wk p.o.</td>
<td>(95)</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3</td>
<td>Liver cancer</td>
<td>Daily hepatic infusion</td>
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</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3</td>
<td>Advanced malignancy</td>
<td>Weekly p.o.</td>
<td>(94)</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α,25(OH)2D3</td>
<td>Advanced malignancy</td>
<td>Every other day s.c.</td>
<td>(93)</td>
</tr>
<tr>
<td>Phase I</td>
<td>19-Nor-1α,25(OH)2D2 + gemcitabine</td>
<td>Advanced malignancy</td>
<td>Weekly i.v.</td>
<td>Ongoing study*</td>
</tr>
<tr>
<td>Phase I</td>
<td>16-Ene-23-yn-1α,25(OH)2D3</td>
<td>Prostate cancer</td>
<td>Daily p.o.</td>
<td>(108)</td>
</tr>
<tr>
<td>Phase I</td>
<td>1α(OH)D2</td>
<td>Breast and colorectal cancer</td>
<td>Daily p.o.</td>
<td>(106)</td>
</tr>
<tr>
<td>Phase I</td>
<td>EB1089</td>
<td>Multiple myeloma or other plasma cell disorder</td>
<td>Weekly i.v.</td>
<td>Ongoing study*</td>
</tr>
</tbody>
</table>

calcemic than 1\(\alpha\)25(OH)\(_2\)D\(_3\) hypercalcemia limited the tolerable dose of EB1089 to 7 \(\mu\)g/m\(^2\)/d (102). In phase II trials, daily oral administration of EB1089 (10-15 \(\mu\)g/d) was well tolerated in hepatocellular carcinoma (103) and pancreatic cancer (104), but no objective antitumor activity in advanced disease was observed. The failures of the clinical trials of EB1089 in breast cancer and hepatic cancer have led to its withdrawal.

Several different strategies have been tried to optimize the application of vitamin D analogues for the treatment of various malignancies. One of these strategies was to use a low-calcemic analogue [e.g., the prodrug-type analogue, \(1\alpha\)(OH)D\(_2\)], with its relatively mild side effects, already proven to be superior to 1\(\alpha\),25(OH)\(_2\)D\(_3\) in chronic kidney disease patients. Other strategies have been to use vitamin D analogues in combination with chemotherapeutic cytotoxic agents (such as taxols and cisplatin) where the benefits arise from the synergism of vitamin D and the chemotherapy taking advantage of different mechanisms of action, including effects on angiogenesis and metastasis. Additional strategies include attempts to limit the hypercalcemic side effects of the vitamin D analogue by coadministration of prednisone or bisphosphonates to limit the bone resorptive effects and maximize effective dose of the vitamin D analogue, especially in metastatic prostate cancer.

Clinical trials with 1\(\alpha\),25(OH)\(_2\)D\(_3\) in prostate cancer patients have highlighted the potential of vitamin D analogues either alone or in combination with chemotherapy (104–108). The analogue doxercalciferol [\(1\alpha\)(OH)D\(_2\)] evaluated in a phase II study in patients with androgen-independent prostate cancer (105). Following the phase I study (106), the recommended phase II dose was 12.5 \(\mu\)g/d on a daily p.o. medication. During the trial, plasma levels of 1\(\alpha\),25(OH)\(_2\)D\(_2\), an active form of \(1\alpha\)(OH)D\(_2\), was confirmed to reach a plateau by week 4 despite continuous drug dosing, and disease stability for >6 months resulted in 30% of the patients. Another clinical study using paricalcitol [19-nor-1\(\alpha\),25(OH)\(_2\)D\(_2\)] i.v. thrice weekly on an escalating dose of 5 to 25 \(\mu\)g in patients with androgen-independent prostate cancer resulted in a significant reduction of elevated serum levels of PTH, often observed as a common feature of advanced prostate cancer (107). Further clinical investigation of 19-nor-1\(\alpha\),25(OH)\(_2\)D\(_2\) in combination with chemotherapy (gemcitabine) or bisphosphonate (zoledronate) is currently ongoing in patients with advanced malignancy.

**Perspectives**

The anticancer potential of vitamin D analogues has been discussed for over a decade, but clinically approved anticancer vitamin D drugs remain elusive, although the antiproliferative effects of vitamin D analogues have been realized in other hyperproliferative conditions: psoriasis and the parathyroid hyperplasia in chronic kidney disease. Vitamin D analogues are potent antiproliferative regulators of many cancer cell lines in *vitro* (35). Preclinical studies have revealed that vitamin D analogues are also effective growth regulators of tumors in animals in *vivo*, although occasionally daily administration of certain vitamin D analogues causes mild hypercalcemia, which is still less severe than with 1\(\alpha\),25(OH)\(_2\)D\(_3\). According to researchers involved in clinical trials using 1\(\alpha\),25(OH)\(_2\)D\(_3\) and vitamin D analogues, part of the reason for the failure has been the inability of such trials to achieve adequate drug levels in the blood/tumor of patients as well as occasional hypercalcemia (109, 110). Recent protocols have attempted to overcome poor bioavailability by advocating use of very high dose intermittent therapy to try to transiently raise 1\(\alpha\),25(OH)\(_2\)D\(_3\) concentrations to supraphysiologic levels, well in excess of 1 nmol/L (109), but such approaches have had limited success. Whether the limited effectiveness is also due to reduced affinity of vitamin D analogues for transport proteins, such as vitamin D–binding protein, leading to rapid hepatic clearance or whether it is due to increased “drug resistance” caused by up-regulated CYP24A1 and accelerated destruction is unknown.

Another possibility for an effective cancer therapy is to use a low-calcemic vitamin D analogue in combination with another therapy to achieve a synergistic effect on the tumor. Combination therapy with cytotoxic agents (taxols or cisplatin) or cytochrome P450 inhibitors (e.g., general CYP or CYP24A1 inhibitors) might prove to be more effective ways of using these valuable antiproliferative agents. Combination therapy with glucocorticoids (e.g., dexamethasone or prednisone) might potentiate the antitumor effects while reducing the hypercalcemia induced by 1\(\alpha\),25(OH)\(_2\)D\(_3\). In addition, other strategies to limit the calcium-mobilizing bone resorptive effects (bisphosphonates) or intestinal calcium absorptive side effects (low calcium diets or nighttime administration) are other techniques that might be used to refine the eventual combination therapy that proves effective. Furthermore, understanding how analogues exert their tissue-specific selective actions may allow for the design of more effective and safer vitamin D compounds for the treatment of hyperproliferative disorders, including cancer.

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