

The role of vitamin D in cancer prevention

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[ABSTRACT] Vitamin D, also known as cholecalciferol, is the precursor to the active steroid hormone 1, 25-dihydroxyvitamin D₃ (calcitriol; 1, 25(OH)₂D₃). The main physiological role for 1, 25(OH)₂D₃ is to regulate calcium and inorganic phosphate homeostasis for bone health. More recently, vitamin D has been investigated for its effects in the prevention and treatment of a variety of diseases such as cancer, autoimmune disorders, and cardiovascular disease. Preclinical data strongly support a role for vitamin D in the prevention of cancer through its anti-proliferative, pro-apoptotic, and anti-angiogenic effects on cells. Epidemiologic and clinical studies have shown mixed data on the correlation between serum vitamin D levels and cancer risk. This report seeks to outline results from the most recent preclinical and clinical studies investigating the potential role of vitamin D in cancer prevention.

[KEY WORDS] Vitamin D; Calcitriol; Cancer prevention; Cancer prognosis; Vitamin D deficiency

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Background

Vitamin D is an essential precursor to the steroid hormone calcitriol. In humans, vitamin D is synthesized for use by skin exposure to ultraviolet light (mainly UVB, 280–320 nm), or directly ingested in fortified foods or supplements. As early as 1980, it was hypothesized that vitamin D may reduce cancer risk after a study was published that linked a lack of sunlight exposure to an increased risk for colon cancer [1]. Because vitamin D is a naturally occurring substance, found in foods or synthesized in our own bodies, its use as a preventative agent is attractive. Based on earlier evidence that vitamin D may have a role in cancer prevention, extensive preclinical studies have been performed in an attempt to elucidate the mechanism by which vitamin D may reduce cancer risk, and a number of epidemiologic studies and clinical trials have been completed in an attempt to determine the association between vitamin D levels and risk for cancer development. Many comprehensive reviews have been published detailing the history of vitamin D and its use in the prevention of cancer. Krishnan *et al.* and Idman *et al.*

have recently published extensive reviews looking at the effects of vitamin D in both the prevention and treatment of cancer [2–3]. Additionally, Feldman *et al.* have published an entire book looking at all the potential roles vitamin D may play in the human body [4]. Moukayed *et al.* and Deeb *et al.* have published reviews focusing on the molecular mechanisms that vitamin D is involved with [5–6]. The Endocrine Society has also released a report on the non-skeletal effects of vitamin D [7], and Pereira *et al.* has published a review focusing on vitamin D and its uses in prostate cancer [8]. The purpose of this review is to give a general update on the potential use of vitamin D for cancer prevention. In this report we provide a concise summary of the mechanisms by which vitamin D is synthesized in humans, a synopsis of the effects of vitamin D on cell signaling and transcriptional regulation and how these play a role in cancer prevention. Additionally, we will outline the most recent preclinical, genetic and epidemiologic discoveries that describe associations between vitamin D and cancer risk and outcomes. Finally, we will review the vitamin D analogs currently being investigated in clinical trials.

Synthesis of 1, 25-dihydroxyvitamin D₃ in the body

Vitamin D (cholecalciferol) is synthesized from 7-dehydrocholesterol in the skin upon exposure to ultraviolet (UVB) irradiation from the sun (Fig. 1) [6]. It can also be ingested through the diet or supplements; foods high in vitamin D include fatty fish, eggs, and dairy products [9]. Once in the body, vitamin D circulates to the liver where it is hydroxylated by the cytochrome P450 enzyme, CYP2R1 to the intermediate metabolite, 25-hydroxyvitamin D₃ (calciferol; 25(OH)D₃) [6].

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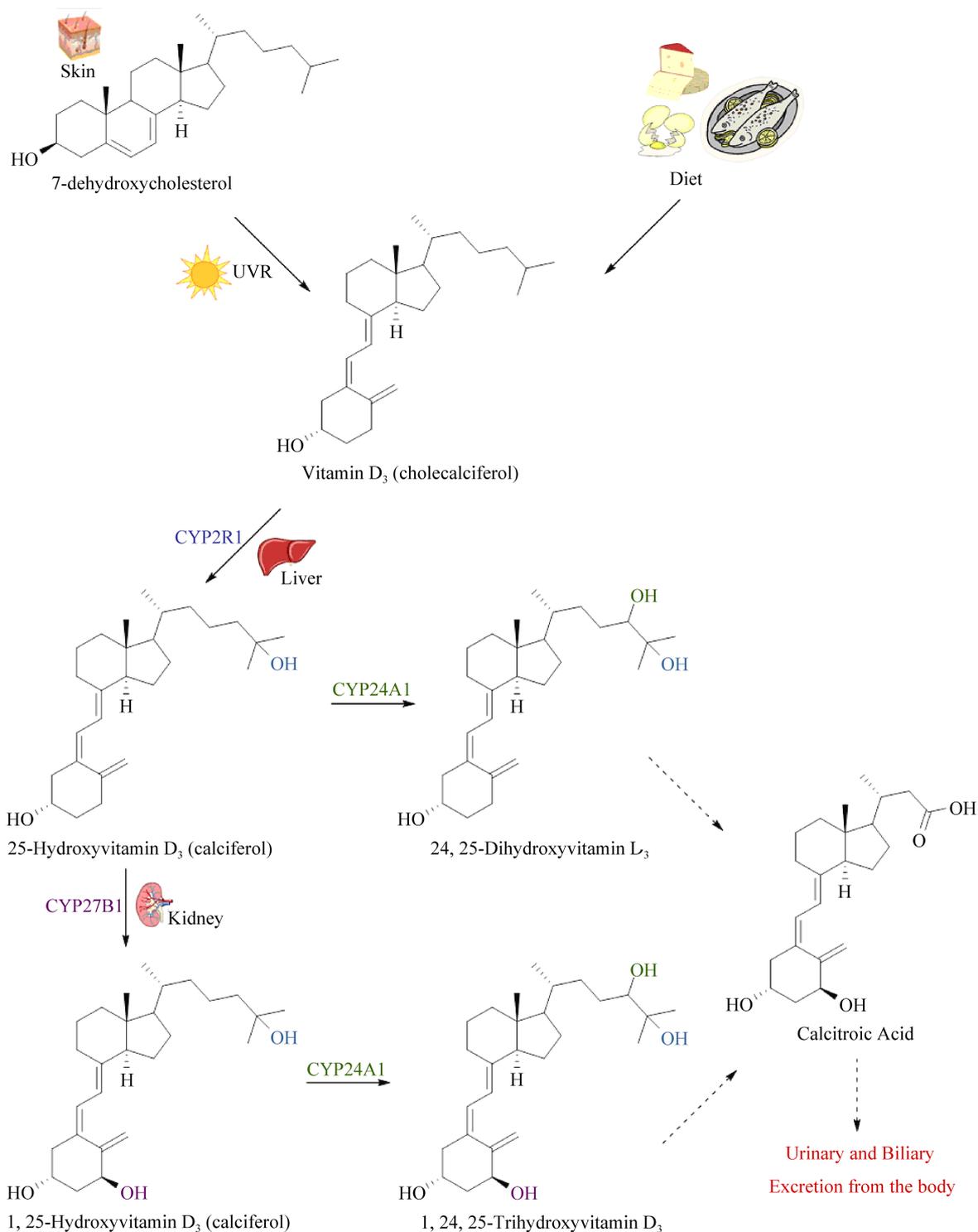


Fig. 1 *In vivo* synthesis of 1, 25(OH)₂D₃. Vitamin D₃ is synthesized in the skin by exposing 7-dehydrocholesterol to UVB light or ingested through dietary foods or supplements. Vitamin D₃ is then converted to 25(OH)D₃ by CYP2R1 in the liver. 25(OH)D₃ circulates in the blood bound to DBP. 25(OH)D₃ can either be converted to the inactive metabolite 24, 25(OH)₂D₃ by CYP24A1 or into the active metabolite 1, 25(OH)₂D₃ by CYP27B1 which is mainly found in the kidneys. 1, 25(OH)₂D₃ exerts its actions throughout the body before it is inactivated by CYP24A1 to the metabolite 1, 24, 25(OH)₃D₃. The two inactive forms, 24, 25(OH)₂D₃ and 1, 24, 25(OH)₃D₃ are then further metabolized to calcitroic acid through multistep processes. Calcitroic acid is then removed from the body through urinary and biliary excretion

25(OH)₂D₃ is the predominant form of vitamin D in the circulation. Because of this, when determining the vitamin D status in an individual, the serum concentration of the 25(OH)₂D₃ metabolite is the value that is quantified. The Institute of Medicine (IOM) defines vitamin D deficiency as a serum 25(OH)₂D₃ level ≤ 20 ng·mL⁻¹, while the Endocrine Society defines vitamin D deficiency as ≤ 30 ng·mL⁻¹ [10-11]. Circulating 25(OH)₂D₃ then migrates to the kidneys where it is converted to the active form of vitamin D, 1, 25-dihydroxyvitamin D₃ (calcitriol; 1, 25(OH)₂D₃) by CYP27B1 [6]. CYP27B1 has also been found to be expressed in extrarenal sites, particularly cancer cells [12]. In the kidneys, 1, 25(OH)₂D₃ exerts its main physiologic effects to regulate mineral homeostasis [13]. The proposed effects of 1, 25(OH)₂D₃ on cancer cells are discussed below. Circulating 25(OH)₂D₃ and 1, 25(OH)₂D₃ both bind to the vitamin D binding protein (DBP) in the blood and both can be inactivated via 24-hydroxylation by CYP24A1 and ultimately catabolized (Fig. 1) [3].

Mechanisms of vitamin D action

Vitamin D exerts its actions on the cell both directly, by regulating transcription, and indirectly, by affecting genomic regulation of cell signaling pathways (Fig. 2). Multiple extensive reviews detailing the proposed mechanisms of vitamin D's anticancer effects have been written [5-6, 14]. Briefly, upon entering the cell 1, 25(OH)₂D₃ binds to the vitamin D Receptor (VDR). This allows translocation of VDR to the nucleus where it heterodimerizes with Retinoic X Receptor (RXR). The VDR-RXR-dimer binds to vitamin D response elements (VDREs) in the promoter region of target genes where it recruits the necessary comodulators and RNA polymerase II to activate transcription of the target genes [6]. Transcriptional regulation by vitamin D has a variety of effects including: (1) anti-proliferation, (2) induction of apoptosis, (3) stimulation of differentiation, (4) reduced inflammation, (5) inhibition of invasion and metastasis, and (6) inhibition of angiogenesis [3]. Because most cell lines available for *in vitro* studies are cancer cell lines, studies into the mechanistic effects of vitamin D are all based on the effects 1, 25(OH)₂D₃ or its analogs exert on cancer cells, making it difficult to determine the exact cellular mechanisms vitamin D may have for preventing cancer. However, while the reported inhibition of invasion, metastasis, and angiogenesis are more specific for how vitamin D may play a role in cancer treatment, it may be postulated that the other effects may also play a role in both cancer treatment and in prevention of cancer development. Specific cellular pathways involved in each of these effects are described in Box 1.

The indirect genomic regulation by 1, 25(OH)₂D₃ is not as well understood as are direct transcriptional effects. The effects on cell signaling pathways of 1, 25(OH)₂D₃ are thought to begin at a non-classical membrane associated VDR receptor (memVDR) and a 1, 25(OH)₂D₃-membrane-associated rapid-response steroid binding protein (1, 25(OH)₂D₃-MARRS) [15-16].

It appears that binding of memVDR and classical VDR leads to the activation of a store operated calcium (SOC) channel. SOC channel activation leads to the influx of Ca²⁺ into the cell and subsequent activation of Protein Kinase C (PKC) followed by phosphorylation of rat sarcoma (Ras) proteins [16].

Binding of 1, 25(OH)₂D₃-MARRS by 1, 25(OH)₂D₃ activates phospholipase C γ (PLC γ) which also activates PKC leading to Ras [47]. Phosphorylation of Ras triggers the traditional cascade by activating the protein kinase activity of the rapidly accelerated fibrosarcoma (RAF) protein. RAF kinase phosphorylates and activates mitogen/extracellular signal-related kinase (MEK) 1/2, which in turn phosphorylates and activates extracellular signal-related kinase (ERK)-mitogen-activated protein kinase (MAPK) 1/2. ERK-MAPK1/2 activation influences genomic regulation, leading to many of the same effects as the direct transcriptional regulation of 1, 25(OH)₂D₃-VDR complex (Fig. 2).

Two recent studies of the roles of vitamin D on transcription evaluated effects in breast cancer cell lines, as there is an interest for better understanding the genome-wide effects of vitamin D on this cancer type. In the first of these studies Goeman *et al.* [48] used RNA-Seq technology to create a profile of the primary transcriptional targets of 1, 25(OH)₂D₃. After 3 hours of treatment with 1, 25(OH)₂D₃, 88 genes were significantly upregulated and 23 were down regulated. Early transcription targets were involved in adhesion, growth regulation, angiogenesis, actin cytoskeleton regulation, hexose transport, inflammation and immunomodulation, apoptosis, endocytosis, and signaling. Other transcriptional factors were also found to be regulated which would further diversify (indirectly) the transcriptional effects of 1, 25(OH)₂D₃. A second study, completed by Beaudin *et al.* [49] compared the transcriptional output of three non-tumorigenic breast epithelial cell lines and three breast cancer cell lines after treatment with 1, 25(OH)₂D₃. The effects of 1, 25(OH)₂D₃ on the various cell lines was highly variable and more investigation into the effects on cancer versus non-cancer cell lines is necessary to further understand how and if 1, 25(OH)₂D₃ influences these cancer cells.

Preclinical studies

Preclinical studies have recently supported the role of vitamin D as a synergistic agent in inducing apoptosis and suppressing tumor cell growth in ovarian and mammary cancer cell lines. In one study, Zhang *et al.* [50] evaluated the effects of 1, 25(OH)₂D₃ alone or adding 10 nmol·L⁻¹ of 1, 25(OH)₂D₃ to carboplatin to induce apoptosis in ovarian cancer SKOV-3 cells. They found that the addition of 1, 25(OH)₂D₃ to cell culture had no effect alone, but that it decreased the IC₅₀ of carboplatin by more than two fold. These authors hypothesize that the addition of 1, 25(OH)₂D₃ induced cell cycle arrest at the G₁/S and G₂/M checkpoints, enhancing the effects of carboplatin to result in apoptosis, reactive oxygen species (ROS) production and reducing mitochondrial membrane potential. In another study, Linnewiel-

Box 1 Anti-cancer effects of vitamin D**1. Anti-proliferative effects**

- Treatment of MCF-7 breast cancer cell line with 1, 25(OH)₂D₃ results in suppression of the proto-oncogene c-Myc and increased expression of MAD1/MXD1, a transcriptional repressor of c-Myc. This effect is further mediated by F-box protein (FBW7), a tumor suppressor protein [17-18].
- Treatment of colorectal cancer cells with 1, 25(OH)₂D₃ suppresses c-Fos and c-Myc gene expression through β -catenin signaling [19]. This suppression is also observed in prostate cancer cells and is independent of retinoblastoma protein [20].
- Treatment of ovarian cancer cells with 1, 25(OH)₂D₃ causes p27 (Kip1) stabilization and downregulation of cyclin E/cyclin-dependent kinase 2 and Skp1-Cullin-F-box protein/Skp2 ubiquitin ligase resulting in G₁ arrest [21].
- Treatment of human head and neck squamous cell carcinoma cells SCC25 with vitamin D causes upregulation of GADD45 α , a growth-arrest DNA damage repair factor, induction of cyclin-dependent kinase inhibitor p21, and arrest of cell proliferation at the G₀/G₁ phase [22].
- Treatment of prostate cancer cells with 1, 25(OH)₂D₃ leads to inhibition of cyclin-dependent kinase 2 activity and G₀/G₁ cell cycle arrest. Androgen receptors are required for this growth inhibition effects of vitamin D in prostate cancer cells indicating signaling cross-talk is required between 1, 25(OH)₂D₃ and androgens for this protective effect [23].

2. Induction of apoptosis

- Treatment of MCF-1 breast cancer cells with 1, 25(OH)₂D₃ or its analog MART-10 induces expression of the proapoptotic proteins, BAX and BCL, and initiation of apoptosis through cytochrome C release from the mitochondria [24].
- Treatment of adenoma and carcinoma colorectal cells with 1, 25(OH)₂D₃ or its analog EB1089 resulted in upregulation of the proapoptotic protein, Bak, induction of apoptosis (independent of p53 signaling), and increased number of cells in the G₁ phase [25].
- Treatment of the C6.9 cell line (rat glioma cells) with 1, 25(OH)₂D₃ results in DNA fragmentation, upregulation of p53 and GADD45, and ultimately induction of apoptosis [78].
- Treatment of Caco-2 (colon cancer cells) with 1, 25(OH)₂D₃ sensitizes the cells to the proapoptotic effects of transforming growth factor β 1 (TGF β 1) [95].

3. Stimulation of differentiation

- Treatment of human myeloid leukemia cells with 1, 25(OH)₂D₃ induces terminal differentiation into monocytes and macrophages [26].
- Treatment of breast, prostate, and colon cancer cells with 1, 25(OH)₂D₃ increases differentiation markers. For breast cancer cells these include casein, lipid droplets, and adhesion proteins [27]. For prostate cancer cells, prostate specific antigen (PSA), E-cadherin, and bone morphogenetic protein 6 (BMP6) are upregulated, and for colon cancer, colonic epithelial cell differentiation markers are increased [28].
- Proposed pro-differentiation markers are cell-type specific, but some include: regulation of β -catenin, JUN N-terminal kinase, PI3K, and NF κ B signaling pathways, and regulation of transcription factors such as the activator protein 1 complex and CCAAT/enhancer-binding protein (C/EBP) [6, 28].

4. Reduced inflammation

- Treatment of macrophages with 1, 25(OH)₂D₃ blocks production of interleukin-1 β (IL-1 β), a key proinflammatory cytokine [29]. Suppression of IL-1 β in colon cancer epithelial cells blocks activation of Wnt signaling which is required for the progression of colon tumors [30].
- 1, 25(OH)₂D₃ treatment has been shown to decrease levels of IL-1 β , IL-6, IL-7, and NF κ B in inflammation associated with breast and prostate cancer cells [31-33].

5. Inhibition of invasion and metastasis

- Mice xenografted with renal carcinoma cells while simultaneously being treated with vitamin D do not develop tumors; this effect is hypothesized to be due to inhibition of the Sonic Hedgehog (SHH) signaling cascade [34].
- Treatment of prostate cancer cells with 1, 25(OH)₂D₃ results in upregulation of Insulin-like growth factor binding protein 3 (IGFBP3) [35]. Typically, IGFBP3 protein levels decrease when prostate cancer cells transform from benign to malignant metastasis [36-37]. IGFBP3 and IGFBP5 are also upregulated in malignant breast cancer cell lines after treatment with 1, 25(OH)₂D₃ [38-39].
- Treatment of breast, colorectal, and prostate cancer cells with 1, 25(OH)₂D₃ has been shown to result in a downregulation of Wnt signaling through VDR interaction with β -catenin [14, 27, 31, 39-43]. Downregulation of this signaling may prevent or decrease the metastatic transformation associated with Wnt-activated tumor cells [31]. Treatment of cells with 1, 25(OH)₂D₃ also upregulates DKK-1, a Wnt antagonist, further suppressing Wnt-associated cellular transformation [43-44].

6. Inhibition of angiogenesis

- Treatment of colon and breast cancer cells with 1, 25(OH)₂D₃ leads to downregulation of hypoxia-inducible factor 1 (HIF-1 α). This results in inhibition of secretion of vascular endothelial growth factor (VEGF) and downregulation of endothelin 1 (ET-1) and glucose transporter 1 (Glut-10), all proteins essential for inducing angiogenesis [45].
- Loss of VDR in mouse tumor-derived endothelial cells results in increased levels of HIF-1 α , VEGF, angiopoietin 1, and platelet-derived growth factor. *In vivo*, VDR-knockout mice show enlarged blood vessels in tumor lesions [46].

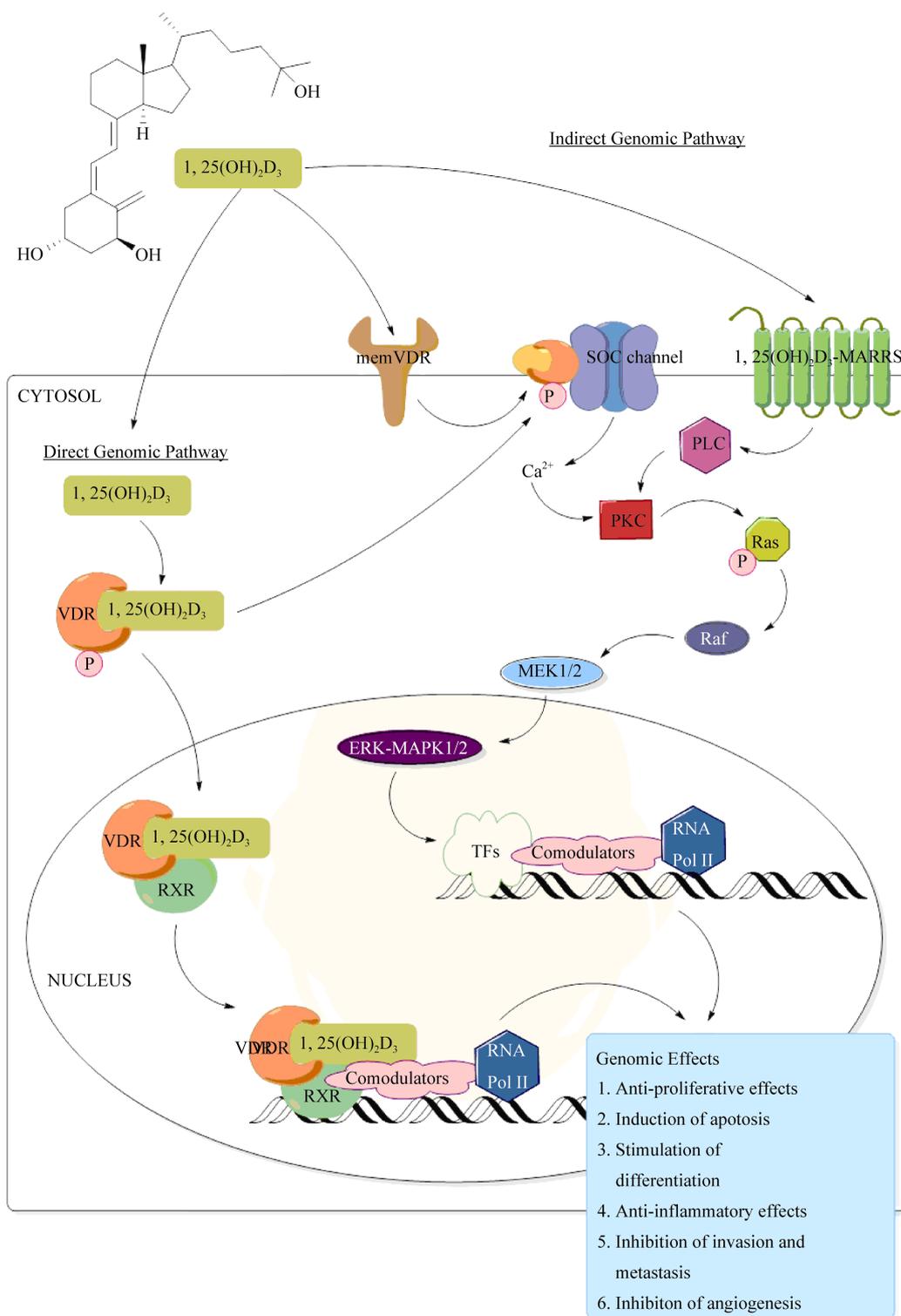


Fig. 2 Direct and indirect genomic effects of 1, 25(OH)₂D₃. Direct genomic effects: 1, 25(OH)₂D₃ enters the cell and binds VDR. This leads to phosphorylation of VDR and translocation to the nucleus. In the nucleus the VDR-1, 25(OH)₂D₃ complex heterodimerizes with RXR. This complex then binds to various VDREs to recruit comodulators and activate transcription. Indirect genomic effects: Binding of 1, 25(OH)₂D₃ to cytoplasmic VDR or to a non-canonical membrane VDR leads to activation of the SOC channel. This leads to an influx of Ca²⁺ which activates PKC. Binding of 1, 25(OH)₂D₃ to 1, 25(OH)₂-MARRS activates PLC which also causes activation of PKC. Once PKC is activated, it triggers a phosphorylation cascade through Ras, Raf, and MEK1/2 leading to ERK-MAPK1/2 activation and transcriptional modulation. Both direct and indirect transcriptional modulation lead to anti-proliferative effects, induction of apoptosis, stimulation of differentiation, anti-inflammatory effects, inhibition of invasion and metastasis, and inhibition of angiogenesis

Hermoni *et al.* [51] evaluated the ability of combining 1, 25(OH)₂D₃ and astaxanthin, a carotenoid, to inhibit growth in MCF-7 cells (a mammary cancer cell line). They found that either 100 nmol·L⁻¹ of 1, 25(OH)₂D₃ or 5 μmol·L⁻¹ of astaxanthin were required to inhibit cell growth when administered alone, but that when administered together only 1 nmol·L⁻¹ of 1, 25(OH)₂D₃ and 0.3 μmol·L⁻¹ of astaxanthin were required to inhibit cell growth.

Vitamin D has also been shown to be effective as a single agent in suppressing development and progression of tumors in murine models of colorectal and ovarian cancer. Meeker *et al.* [52] provided dietary vitamin D supplement one week prior to and then continuously after bacterial inoculation with *Helicobacter bilis* in a mouse model of bacteria driven colitis and colon cancer (129-*Smad3*^{tm1Par/J}). The mice who received the vitamin D supplemented diet (5 IU vitamin D/g diet) were less likely to develop cancer than those who received the standard diet which contains only 1 IU vitamin D/g diet (11% versus 41%, $P = 0.01$). Rebel *et al.* [53], in a study that included mice (Fabp1^{Cre}; *Apc*^{15lox/+}) that develop tumors in the epithelial cells of the distal ileum and colorectum due to mutation of the *Apc* allele, reported that when compared to control mice (neither treatment), those who received either vitamin D or UV radiation from ages 6–22 weeks had reduced tumor load (square mm of intestine covered with tumor: control 202 ± 23; supplement 130 ± 5 ($P = 0.02$), UV 88 ± 9 ($P < 0.001$)), and that those who received UV radiation were less likely than either the control or supplement group to have tumors with a malignant pathology. Finally, a study by Kasiappan *et al.* [54] evaluated the effects of a vitamin D analog (EB1089) in a murine model where a high fat diet, because it stimulates the production of leptin and associated tumor growth, migration, invasion and angiogenesis, has previously been shown to promote an aggressive cancer phenotype [55]. These authors reported that, after 30 days, when compared to mice who received a high fat diet with no EB1089 supplementation, those whose high fat diet was supplemented with EB1089 had about half of the tumor growth. These authors also demonstrated that administration of EB1089 increased expression of miR-498 which interferes with telomerase elongation in replicating cancer cells.

Genetic studies

Due to the inconsistent human data on the association between vitamin D and cancer risk, several studies have looked to genetics to see if underlying genetic differences may help explain some of the conflicting data. Studies have looked at genetic factors impacting serum levels of vitamin D, investigated the effects of polymorphisms in VDR, and conducted analyses looking at vitamin D pathway genes. A summary of the genetic and epidemiologic studies done in humans can be found in Table 1.

In a population-based study conducted by Skaaby *et al.* [56], the effects of loss-of-function mutations in the filaggrin gene (*FLG*) on cancer risk were investigated. *FLG* mutations

result in decreased skin barrier function [57] which results in increased sun absorption and up to 10% higher levels of serum vitamin D [58] suggesting individuals with these loss-of-function mutations may be at a lower risk of cancer. In the 13 376 individuals genotyped for *FLG* mutations, 1 339 incident cancer cases occurred (median follow-up 11.4 years). No significant association was found between the risk of any cancer in *FLG* mutation carriers compared to wild type individuals (HR = 0.95). The only significant association found was that in individuals older than 60 years at baseline, *FLG* mutation carriers had a lower risk of all cancers (HR = 0.62, $P = 0.01$) and non-melanoma skin cancer (NMSC) (HR = 0.32, $P = 0.03$) compared to wild-type controls. A second study by Moy *et al.* [59] investigated the impact of single nucleotide polymorphisms (SNPs) on serum DBP levels. They conducted a genome wide association study (GWAS) in 1 380 men to determine if any SNPs were associated with differing levels of serum DBP. Two independent SNPs were identified in the gene encoding DBP, *GC*, as being significantly associated with serum DBP levels: rs7041 ($P = 1.42 \times 10^{-246}$) and rs705117 ($P = 4.7 \times 10^{-91}$). Both alleles were associated with decreased serum levels of DBP. Unfortunately, the authors did not look into cancer risk associated with these decreased levels of serum DBP; more studies are necessary to see if these genotypes play a role in the potential reduction in cancer risk by vitamin D.

More than 470 polymorphisms have been reported in the vitamin D receptor gene [60], the *TaqI*, *FokI*, *BsmI*, *Apal*, and *Cdx2* are just some of the polymorphisms that have been investigated for their role in vitamin D and cancer risk. A case-control study of Jordanian colorectal cancer patients was conducted by Atoum *et al.* [61] looking at the association between the *TaqI* polymorphism in VDR (rs731236) and colorectal cancer risk. Among the 93 patients and 102 healthy controls, no association was found between *TaqI* genotype and colorectal cancer incidence. However, patients were statistically more likely to be vitamin D deficient (serum levels < 10 ng·mL⁻¹) if they were TT ($P = 0.01$) or Tt ($P = 0.04$) genotype for the *TaqI* polymorphism compared to the healthy controls of the same genotype. The T allele of *TaqI* has previously been shown to be associated with lower serum levels of vitamin D [62], but in this study the cancer itself may be the cause of the decreased vitamin D levels. In contrast, ameta-analysis by Serrano *et al.* [63] was able to demonstrate that the tt genotype resulted in an increased risk of colorectal cancer (SOR 1.43). This same study also investigated the role of the polymorphisms *Apal* (rs797523) and *Cdx2* (rs11568820) in cancer risk. No association was found for *Apal* for any of the cancer types investigated, while the *Cdx2* gg and Gg (versus GG) genotypes showed a small but significant association with increased cancer risk (SOR 1.12 and 1.03 respectively). A second meta-analysis conducted by Gnagnarella *et al.* [64] found that the ff genotype of the *FokI* polymorphism (rs10735810) was significantly associated with increased risk

Table 1 Summary of human studies

Author	Population	Study Design	Outcome
GENETIC STUDIES			
Skaaby <i>et al.</i> ^[56]	Population-based cohort (Denmark)	Cohort, SNP inquiry	No significant association between <i>FLG</i> mutation carriers and overall cancer risk. Individuals with <i>FLG</i> mutation and > 60 years at baseline had lower risk of all cancers and NMSC.
Moy <i>et al.</i> ^[59]	White male smokers (Finland)	GWAS on a study population who participated in a RCT for cancer prevention	Two independent SNPs were associated with decreased serum levels of DBP. Cancer risk was not assessed.
Atoum <i>et al.</i> ^[61]	Colorectal cancer patients with gender and age matched healthy controls (Jordan)	Case-control, SNP inquiry	No association was found between <i>TaqI</i> genotype and colorectal cancer incidence.
Anderson <i>et al.</i> ^[66]	Pancreatic cancer patients with gender and age matched healthy controls	Case-control, SNP inquiry	No findings were significant after adjustment for multiple comparisons.
Serrano <i>et al.</i> ^[63]	All cancer types	Meta-analysis	<i>TaqI</i> tt genotype resulted in increased risk of colorectal cancer. <i>Apal</i> genotype was not associated with cancer risk. <i>Cdx2</i> gg and Gg genotypes were associated with increase cancer risk (all types).
Gnagnarella <i>et al.</i> ^[64]	All cancer types	Meta-analysis	<i>FokI</i> ff genotype was associated with increased risk of any cancer type, in particular ovarian and skin cancers.
Raimondi <i>et al.</i> ^[65]	All cancer types	Meta-analysis	BB and Bb <i>BsmI</i> genotypes increased risk of cancer at any site.
EPIDEMIOLOGIC STUDIES			
Kim <i>et al.</i> ^[67]	Breast cancer	Meta-analysis	No association was found between vitamin D intake or higher 25(OH)D ₃ levels and breast cancer incidence, but higher serum levels of 25(OH)D ₃ were associated with reduced risk for breast cancer mortality.
Wang <i>et al.</i> ^[68]	White, post-menopausal female nurses	Case-control	No association was found between circulation 25(OH)D ₃ or DBP and breast cancer incidence.
Li <i>et al.</i> ^[69]	Breast cancer	Meta-analysis	An inverse association was found between 25(OH)D ₃ serum levels and breast cancer mortality.
Weinstein <i>et al.</i> ^[70]	Colorectal cancer patients with gender, age, and race matched controls	Case-control of participants in a RCT for cancer screening	Higher serum levels of 25(OH)D ₃ were associated with decreased risk for colon cancer. No association was found with serum DPB levels.
Anic <i>et al.</i> ^[71]	White male smokers (Finland)	Case-control of participants in a RCT for cancer prevention	Higher serum levels of 25(OH)D ₃ were associated with decreased risk for colon cancer. No association was found with serum DPB levels.
Zgaga <i>et al.</i> ^[72]	Colorectal cancer patients	Prospective study	Higher serum levels of 25(OH)D ₃ were associated with decreased risk of colon cancer mortality.
Kristal <i>et al.</i> ^[73]	Men age ≥ 55 (≥ 50 if African American)	Case-control of participants in a RCT for cancer prevention	A U-shaped association was found between prostate cancer risk and serum 25(OH)D ₃ levels.
Schenk <i>et al.</i> ^[74]	Men age ≥ 55	Case-control of participants in a RCT for cancer prevention	No association was found between prostate cancer risk, but lower 25(OH)D ₃ levels were associated with higher grade disease.
Cheng <i>et al.</i> ^[75]	Smokers and former asbestos workers	Case-control of participants in a RCT for cancer prevention	Vitamin D intake ≥ 600 IU·d ⁻¹ decreased risk of NSCLC in smokers compared to intake < 200 IU·d ⁻¹ . Only ≥ 400 IU·d ⁻¹ was required to see this benefit if participants also received beta carotene and retinyl palmitate or had vitamin A intake ≥ 1 500 μg·d ⁻¹ .

of any cancer (SOR 1.20), in particular ovarian cancer (SOR 1.08) and skin cancer (SOR 1.24). A third meta-analysis by Raimondi *et al.* ^[65] looked at the *BsmI* polymorphism (rs1544410) and cancer risk. They found that the BB and Bb genotypes had a significant 6% and 7% reduction in cancer risk at any site respectively compared to the bb genotype. They also found a significantly lower risk of skin cancer in

those with a Bb genotype compared to bb (SOR 0.86) and significantly lower risk of colorectal cancer in those with a BB or Bb genotype compared to bb (SOR 0.92). In summary, these meta-analyses suggest that mutations in the *VDR* gene may play a significant role in how vitamin D levels and cancer risk are associated, but more functional studies are necessary to elucidate exactly how these mutations impact

vitamin D and cancer risk.

One recent case-control study has attempted to evaluate genomic effects of vitamin D and cancer risk at a higher level by looking at the association between SNPs in key genes throughout the vitamin D pathway and pancreatic cancer. Anderson *et al.* [66] reported the frequencies of 87 SNPs in key genes among 628 pancreatic cancer patients and compared them to 1 193 age and sex matched controls. Their data suggest that there may be an association between SNPs in the *CYP24A1*, *CYP2R1*, calcium sensing receptor (*CASR*), and megalin (*LRP2*) genes and pancreatic cancer risk. However, none of the findings were significant after adjustment for multiple comparisons suggesting that more studies are necessary to determine the role of these vitamin D pathway genes in cancer risk.

Epidemiologic studies

Epidemiologic studies – cancer risk

Associations between vitamin D intake, serum 25(OH)D₃ levels, and circulating levels of vitamin D binding protein (DBP) and both breast cancer incidence and survival have been reported. Kim *et al.* [67] recently published a meta-analysis including 30 prospective studies that examined the association between vitamin D status (both intake and serum levels) and breast cancer incidence (31 867 cases, 24 studies) and breast cancer mortality (870 deaths among 6 092 patients, 6 studies). When comparing highest (mean > 500 IU·d⁻¹, > 29 ng·mL⁻¹) to lowest (mean < 148 IU·d⁻¹, < 21 ng·mL⁻¹) categories of vitamin D intake or 25(OH)D₃ levels, the pooled relative risks (RR) for incident breast cancer were not significant (intake 0.95, 95% CI 0.88–1.01; serum 0.92, 95% CI 0.83–1.02). However, high serum levels of 25(OH)D₃ were associated with reduced risk for breast cancer mortality (RR 0.58, 95% CI 0.40–0.85). These data are supported by a report from the Nurses' Health Study II [68] where the authors found no associations between circulating 25(OH)D₃ or DBP status and breast cancer incidence, and by another meta-analysis [69] where the authors reported an inverse association between serum 25(OH)D₃ levels and breast cancer mortality with those in the highest quartile at a lower risk for death (RR 0.63, 95% CI 0.51–0.77) than those in the lowest quartile of circulating 25(OH)D₃.

Similar work has been done in large cohorts to evaluate the associations between vitamin D and colorectal cancer. For colorectal cancer, vitamin D status, but not DBP, appears to be associated with both colorectal cancer risk and survival. Weinstein *et al.* [70], used data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial to do a case control study (476 cases, 476 controls matched on age, sex, race and baseline screening date) examining the association between serum 25(OH)D₃, DBP and risk of colorectal cancer. Serum levels of 25(OH)D₃, but not DBP, were associated with decreased risk for colon cancer (OR 0.60, 95% CI 0.38–0.94). This association appears to be modified by smoking status, as another group of investigators [71] reported an association

between higher levels of serum 25(OH)D₃ and colorectal cancer risk (highest versus lowest quartile OR 1.53, 95% CI 1.01–2.32). They also examined the role of DBP, but again, did not find an association. In a large study of prospectively followed colorectal patients (N = 1 598) whose 25(OH)D₃ levels were drawn immediately post-operatively, Zgaga *et al.* [72] reported an inverse association between 25(OH)D₃ and colorectal cancer specific and all-cause mortality. This association was also influenced by specific VDR gene polymorphisms, suggesting that vitamin D may have a causal influence on survival among patients with colorectal cancer.

Vitamin D status has also been evaluated in high risk populations for its association with incident prostate and lung cancers. Results of small studies for prostate cancer have been mixed, some suggesting an inverse association, some a U-shaped association, but most suggesting no association. Recently, Kristal *et al.* [73] examined associations between plasma vitamin D and prostate cancer risk among participants (n = 1 731 cases; 3 203 cohort) in the Selenium and Vitamin E Cancer Prevention Trial. These authors suggest a U shaped association between plasma 25(OH)D₃ and prostate cancer risk, with both the lowest and the highest quintiles conferring an increased risk for any prostate cancer. Associations were strongest for higher stage (Gleason 7–10) disease. Conversely, Schenk *et al.* [74] reported no association between serum 25(OH)D₃ and total prostate cancer risk in the Prostate Cancer Prevention Trial (n = 1 695 cases; 1 682 controls), but suggest a linear decrease in risk for the highest grade disease (Gleason 8–10). Finally, Cheng *et al.* [75] estimated the effect of vitamin D intake and its interaction with vitamin A on incident lung cancer among smokers and former asbestos workers. Their data included 749 incident cases and 679 non-cases from the Carotene and Retinol Efficacy Trial whose intervention group members received 30 mg beta carotene and 25 000 IU retinyl palmitate per day. Vitamin D intake was estimated by food frequency and supplement questionnaires. A beneficial association was found among smokers whose vitamin D intake was ≥ 600 versus < 200 IU·d⁻¹ who had a decreased risk for non-small cell lung cancer and among participants who had vitamin D intake ≥ 400 IU·d⁻¹ and who received the intervention or had total vitamin A intake ≥ 1 500 μg·d⁻¹.

The most convincing evidence for the use of vitamin D in cancer prevention is going to come from randomized prospective clinical trials. Thus far three clinical trials have been completed, only one of which has published data, and eight more trials are currently ongoing. The Vitamin D for Chemoprevention study looked at the required doses of vitamin D to achieve either ≥ 20 ng·mL⁻¹ or ≥ 33 ng·mL⁻¹ serum 25(OH)D₃ in African Americans treated with oral vitamin D for 3 months (1 640 IU·d⁻¹ and 4 000 IU·d⁻¹ respectively) [76]. No information was given on cancer incidence before or after vitamin D treatment in this population, and no significant change was observed in

inflammatory markers [77]. The Modulation of Breast Cancer Risk Biomarker by High Dose Vitamin D study has also been completed [78]. Some study results are listed on ClinicalTrials.gov, but no statistical analyses of the results have been published, making it difficult to draw any conclusions. Of the 30 women who enrolled in the trial, 27 patients

completed the 6 month course of 10 000 IU per week vitamin D, and hypercalcemia was not listed among the side effects observed. The Efficacy of Vitamin D Colorectal Cancer Chemoprevention study has also been completed, but no results are yet published [79]. The ongoing clinical trials are summarized in Table 2.

Table 2 Ongoing clinical trials for vitamin D and cancer prevention

Study name	Sponsor	Patient population	Treatment	Measured outcomes
Vitamin D, Diet, and Activity Study [132]	Fred Hutchison Cancer Research Center	Post-menopausal women undergoing weight loss program	2 000 IU·d ⁻¹ vitamin D vs placebo for 12 months	Primary: impact of vitamin D on weight loss Secondary: impact of vitamin D on blood biomarkers of breast cancer risk, breast epithelial cell cytomorphology, and breast epithelial cell gene expression
Study of Folic Acid, Calcium, and Vitamin D in Preventing Colorectal Polyps and Colorectal Cancer [133]	Shanghai Jiao Tong University School of Medicine	Individuals age 50–80 who have undergone complete colonoscopy with no adenoma found	Folic acid 1 mg·d ⁻¹ + Calcium 1 200 mg·d ⁻¹ + vitamin D 250 IU·d ⁻¹ OR Folic acid 1 mg·d ⁻¹ OR Calcium 1 200 mg·d ⁻¹ + vitamin D 250 IU·d ⁻¹ OR placebo alone for 3 years	Primary: incidence of colorectal adenoma after folic acid and/or calcium and vitamin D intervention
Vitamin D and Omega-3 Trial (VITAL) [134]	Brigham and Women's Hospital	Men and women age 50 and older with no prior history of cancer, heart disease, or stroke	Vitamin D 2 000 IU·d ⁻¹ + Omacor 1 capsule·d ⁻¹ OR Vitamin D 2 000 IU·d ⁻¹ OR Omacor 1 capsule·d ⁻¹ OR placebo for 5 years	Primary: incidence of cancer, heart disease, or stroke after intervention with vitamin D and/or fish oil
Finnish Vitamin D Trial (FIND) [135]	University of Eastern Finland	Men age 60 and older; women age 65 and older with no prior history of cancer or cardiovascular disease	Vitamin D 40 µg·day ⁻¹ OR Vitamin D 80 µg·day ⁻¹ OR placebo for 5 years	Primary: incidence of cancer or cardiovascular disease after intervention with vitamin D
Calcium/Vitamin D, Biomarkers and Colon Polyp Prevention (PPS4B) [136]	Emory University	Individuals age 45–75 with history of ≥ 1 neoplastic polyps, ≥ 2 mm in diameter removed within 4 months of study entry and now documented free of any further polyps	Calcium 1 200 mg·d ⁻¹ + Vitamin D 1 000 IU·d ⁻¹ OR Calcium 1 200 mg·d ⁻¹ OR Vitamin D 1 000 IU·d ⁻¹ OR placebo for 3 or 5 years	Primary: identify changes in molecular phenotype of a panel putative biomarkers of risk for colorectal neoplasms after 1, 3, or 5 years of vitamin D and/or calcium treatment
S0812 High Dose Cholecalciferol in Premenopausal Women at High Risk for Breast Cancer [137]	Southwest Oncology Group	Premenopausal women age 18–50 years with elevated breast cancer risk	Vitamin D 20 000 IU per week + Vitamin D 400 IU·d ⁻¹ OR Vitamin D 400 IU·d ⁻¹ for 1 year	Primary: to assess if mammographic density is reduced in high risk premenopausal women after treatment with high dose cholecalciferol
Study of High Dose Oral Vitamin D for Prevention of Liver Cancer [138]	Johns Hopkins University	Individuals age 18–75 with cirrhosis of any etiology	Vitamin D daily titrated up to maximum tolerated dose for 1 year	Primary: to determine the maximum tolerated dose of vitamin D in patients with cirrhosis Secondary: to assess progression from cirrhosis to hepatocellular carcinoma after vitamin D treatment
Clinical Trial of Vitamin D3 to Reduce Cancer Risk in Postmenopausal Women (CAPS) [139]	Creighton University	Women age 55 and older whose last menstrual period was 4 or more years ago	Calcium 1 200 mg·d ⁻¹ OR Vitamin D 2 000 IU·d ⁻¹	Primary: incidence of cancer after intervention with vitamin D or calcium

Epidemiologic studies – cancer outcomes

Studies in populations of patients with prostate cancer, melanoma, colorectal cancer, acute myeloid leukemia and bladder cancer have demonstrated an association between circulating 25(OH)D₃, cancer stage and cancer prognosis. Murphy *et al.* [80] examined serum vitamin D levels among men with abnormal prostate-specific antigen and/or digital rectal examination and found an association between circulating 25(OH)D₃ and both a positive prostate cancer diagnosis and Gleason score. Shui *et al.* [81], among 1 260

men with prostate cancer, reported that those in the highest quartile of 25(OH)D₃ levels were 57% less likely to die than those in the lowest quartile. Gamblichler *et al.* [82] reported that lower 25(OH)D₃ levels were associated with both tumor thickness and higher American Committee on Cancer 2002 melanoma stage among 764 patients with melanoma. In a retrospective analysis, Bade *et al.* [83] reported an association between survival and circulating 25(OH)D₃ levels among patients with melanoma. In another retrospective analysis of data that included patients with Stage IV colorectal cancer,

Wesa *et al.* [84] reported an association between serum 25(OH)D₃ levels (≥ 30 ng·mL⁻¹ vs < 30 ng·mL⁻¹) and survival (HR 0.61, 95% CI 0.38–0.98). Those whose 25(OH)D₃ levels were in the lowest quartile survived a median of 80 months, while those whose 25(OH)D₃ levels were in the highest quartile survived a median of 195 months ($P = 0.049$). Lee *et al.* [85] evaluated 25(OH)D₃ levels and relapse-free survival among patients newly diagnosed with acute myeloid leukemia and reported those with circulating 25(OH)D₃ < 32 ng·mL⁻¹ had worse relapse free survival than those with 25(OH)D₃ levels 32–100 ng·mL⁻¹. Initial and on therapy monitoring of circulating 25(OH)D₃ were also positively associated with survival in Veterans with bladder cancer diagnosed between 1999 and 2008 in the Southeastern United States [86]. These data are supported by a large meta-analysis [69] that included the results of 25 studies (17 322 cases) where positive associations between circulating 25(OH)D₃ levels at diagnosis and overall survival among colorectal cancer, breast cancer and lymphoma patients were reported. A 10 nmol·L⁻¹ increment in circulating 25(OH)D₃ reduced the risk for death by 4.2%. Higher 25(OH)D₃ levels were also associated with reduced cancer specific mortality for patients with colorectal cancer or lymphoma and with improved disease free survival among patients with breast

cancer or lymphoma.

Clinical trials with vitamin D analogs

Hypercalcemia is arguably the largest issue encountered when trying to use vitamin D as a therapeutic agent [9]. In an attempt to overcome this therapeutic barrier, thousands of vitamin D analogs, both natural and synthetic, have been investigated in an attempt to achieve the same therapeutic effects with less hypercalcemic effects. The most notable natural vitamin D analog that has been investigated is ergocalciferol, also known as vitamin D₂. Ergocalciferol is derived from plant ergosterol and is often used as a vitamin D supplement. A review article detailing why ergocalciferol is not a good substitute for cholecalciferol (vitamin D₃) when trying to supplement vitamin D has been published by Houghton *et al.* [87]. The various synthetic vitamin D analogs have been extensively reviewed elsewhere [88–92]. Here we discuss the vitamin D analogs that have undergone or are currently undergoing clinical trials for their use in the treatment of cancer. Seven vitamin D analogs are currently being investigated for their role in cancer treatment: alfacalcidol (1), calcipotriol (2), doxercalciferol (3), ILX23-7553 (4), inecalcitol (5), paricalcitol (6), and seocalcitol (7). Their structures are shown in Fig. 3.

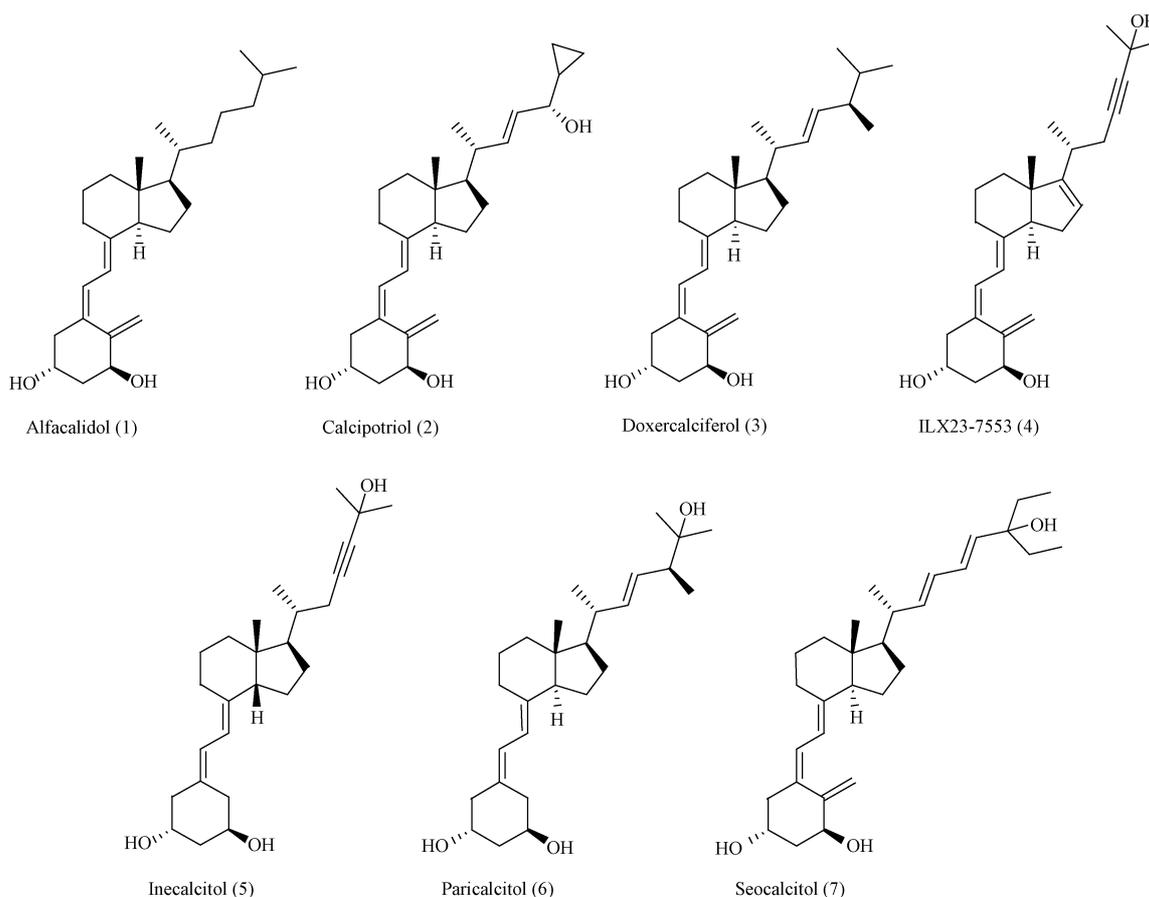


Fig. 3 Vitamin D analogs in clinical trials

Alfacalcidol (**1**) has been used in humans as early as 1980, looking at its effects in treating calcium malabsorption in patients who are post-small bowel resection [93]. Thus far results from seven clinical trials using alfacalcidol in the treatment of various types of cancer have been published. In 1990, Hellstrom *et al.* [94] conducted a study in 63 patients with myelodysplastic syndromes (MDS) and 15 patients with acute myelogenous leukemia (AML). Patients were randomized between two treatment arms. Arm A consisted of low dose cytarabine, while arm B consisted of low dose cytarabine in combination with 13-*cis*-retinoic acid and alfacalcidol. No difference was seen in survival, remission rates, or duration of remissions between the two treatment arms, however, greater side effects were observed in patients randomized to treatment arm B. Three more studies were conducted in patients with hematologic malignancies in 1991. Motomura *et al.* [95] investigated the leukemic transformation free survival time among 30 patients with MDS randomized to 4–6 $\mu\text{g}\cdot\text{d}^{-1}$ alfacalcidol for a median of 17 months or no treatment. Seven cases of acute leukemia developed among the no treatment group, while only one case developed among the patients who were treated with alfacalcidol ($P < 0.001$). These data suggest that alfacalcidol may prevent the progression of MDS to overt leukemia. Raina *et al.* studied the effects of 1 $\mu\text{g}\cdot\text{d}^{-1}$ alfacalcidol among 34 patients with progressive low grade non-Hodgkin's lymphoma. Complete response was observed in four patients, four more patients had partial responses, and only one patient experienced hypercalcemic side effects. A second uncontrolled trial was conducted by Petrini *et al.* [96] in patients with acute non lymphoid leukemia. Patients were treated with low dose cytarabine in combination with alfacalcidol. 17% of patients achieved complete remission and 45% had partial remission of disease. Additionally, immunocytochemical studies showed a monocytic/monoblastic shift and increased expression of VDR in the blast cells, supporting the differentiating role alfacalcidol has been shown to have *in vitro*. These results were further supported by a follow up study done by the same group [97] in which they discovered that patients with AML treated with alfacalcidol had increased numbers of P-170 (a marker of monoblast differentiation) positive blast cells. No further studies were conducted until 2001 when Trouillas *et al.* [98] investigated the effects of alfacalcidol on ten glioblastomas and one anaplastic astrocytoma. Patients were given 0.04 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ alfacalcidol. Two of the patients with glioblastomas and one patient with an anaplastic astrocytoma exhibited clinical remission for 7, 5, and 4 years respectively. No patients exhibited any hypercalcemic effects. The most recent study of alfacalcidol in cancer treatment was conducted by Obara *et al.* [99]. In this prospective study, 16 patients with metastatic renal cell carcinoma were treated with 1 $\mu\text{g}\cdot\text{d}^{-1}$ alfacalcidol and INF- α 3 million units three times weekly. Four patients exhibited partial response, ten exhibit no change, two patients had progressive disease, and one patient had to discontinue alfacalcidol due to hypercalcemia. Based on these

clinical studies it seems that alfacalcidol may be able to induce differentiation in some types of cancer, although no studies have yet been completed with a large enough patient population to draw any conclusive results.

Calcipotriol (**2**), originally termed MC903, was discovered in 1987 and found to be a potent inducer of cell differentiation, inhibit cell proliferation, and was 100 times less hypercalcemic than 1, 25(OH) $_2$ D $_3$ when given orally to rats [100]. Since then it has been used extensively in the topical treatment of psoriasis, and two clinical trials have investigated the potential of topical calcipotriol in the treatment of locally advanced or cutaneously metastatic breast cancer. In the first of these trials, Bower *et al.* [101] found that three of 19 patients had a 50% reduction in the size of their treated lesions and two patients became hypercalcemic. In the second trial, O'Brien *et al.* [102] found that no patients responded to topical treatment with calcipotriol. Since this second study occurred in 1993, no further clinical trials of calcipotriol use in cancer patients have been conducted.

Doxercalciferol (**3**), trade name HECTORAL, was FDA approved in 2000 for the treatment of elevated parathyroid hormone due to secondary hyperparathyroidism in patients undergoing chronic renal dialysis. Four clinical trials have investigated the use of doxercalciferol in prostate cancer, while a fifth has investigated its use in MDS and chronic myeloid leukemia (CML). The first clinical trial was a phase I study of patients with hormone refractory prostate cancer conducted by Liu *et al.* [103]. Patients were given 5–15 $\mu\text{g}\cdot\text{d}^{-1}$ for a minimum of 8 weeks. Of the 25 patients enrolled, two showed a partial response and five achieved stable disease for ≥ 6 months. The main side effects observed were hypercalcemia and renal insufficiency. In this phase I study they were able to determine a phase II dose of 12.5 $\mu\text{g}\cdot\text{d}^{-1}$. The phase II study, coordinated by the same group, was completed in 2003 [104]. Of the 26 patients with hormone refractory prostate cancer, six patients experienced stable disease for > 6 months. The authors recommended that these results suggest possible cytostatic activity for doxercalciferol and that it should be investigated in combination with chemotherapy agents. This suggestion was carried out by Attia *et al.* in 2008 [105] in a double-blind randomized phase II trial. Seventy chemotherapy naïve patients with metastatic, androgen-independent prostate cancer were treated with docetaxel alone or docetaxel in combination with 10 $\mu\text{g}\cdot\text{d}^{-1}$ doxercalciferol. Combination with doxercalciferol was not found to enhance prostatic specific antigen response rate or improve survival compared docetaxel treatment alone. A final study in prostate cancer patients was conducted by Gee *et al.* [106]. Patients were randomized to 28 days of doxercalciferol or placebo prior to radical prostatectomy. Doxercalciferol was well-tolerated and no hypercalcemic effects were observed during the treatment period, however no beneficial effects were observed in serum or tissue markers. A phase II study of doxercalciferol in the treatment of MDS has also been conducted [107]. Among the

15 patients treated with $12.5 \mu\text{g}\cdot\text{d}^{-1}$ doxercalciferol for 12 weeks, six patients had stable disease, none had partial or complete response, and one was removed from the study due to hypercalcemia. A phase I study of doxercalciferol in pediatric solid tumors was also opened in 2007, but as of 2012 the study had been terminated due to slow accrual rates^[108]. Together these trials suggest that doxercalciferol has little to no efficacy in the treatment of prostate cancer and possibly other cancers as well.

ILX23–7553 (4) was first synthesized and tested for its anticancer effects in 1989^[109]. ILX23–7553 was found to be four-fold more potent than 1, 25(OH)₂D₃ at inhibiting clonal growth of HL-60 cells and induced significantly less hypercalcemia than 1, 25(OH)₂D₃. Since then two phase I clinical trials have been conducted using ILX23–7553. The first investigated the pharmacokinetics and safety of ILX23–7553 in 42 patients with a variety of advanced malignancies that had previously been treated^[110]. Patients were treated with five daily oral treatments over a 14-day cycle at 15 dose levels ranging from $1.3\text{--}45 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Wieder *et al.* found that no grade 3 or 4 toxicities were observed, but that the pharmacokinetics showed that *in vivo* concentrations were not reaching the ED₅₀'s discovered *in vitro* necessary for antitumor effects. In the second study, sixteen patients with advanced solid tumors were treated with ILX23–7552 for three consecutive days in 7-day cycles with 10 different doses ranging from $1.7\text{--}37.3 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ^[111]. Jain *et al.* found that the vitamin D analog was well-tolerated at the doses given and no evidence of hypercalcemia was observed. However, like the original study, the authors found that plasma concentration were significantly lower than those required to achieve tumor growth inhibition *in vitro*. Together these two studies suggest that unless higher concentrations of ILX23–7553 can be tolerated, its use in the treatment of cancer is extremely limited.

Incalcitol (5), originally termed TX522, was first described in 2000^[112]. Incalcitol was found to be 10 times more potent at inhibiting *in vitro* cell proliferation of human breast cancer cells and have much lower hypercalcemic effects when tested in a mouse model of breast cancer compared to 1, 25(OH)₂D₃. In 2014, results of a Phase I safety and pharmacodynamics trial of incalcitol in androgen-resistant cancer patients were published^[113]. Eight dose levels ranging from $40\text{--}8\ 000 \mu\text{g}\cdot\text{d}^{-1}$ were evaluated in 54 patients. The maximum tolerated dose was determined to be $4\ 000 \mu\text{g}\cdot\text{d}^{-1}$ with the dose limiting toxicity being hypercalcemia. Additionally, 85% of patients had $\geq 30\%$ prostate specific antigen decline within 3 months. In Europe, a phase II study of ilecalcitol use in CML patients has begun, but no results have yet been published^[114].

Paricalcitol (6), trade name Zemiplar, was approved in 1998 for treatment of secondary hyperparathyroidism associated with chronic kidney disease. It was originally tested for its anti-proliferative effects in 2000 by Chen *et al.*^[115]; the

authors found that paricalcitol had similar growth inhibitory effects as 1, 25(OH)₂D₃ in both primary prostate cancer cells and the LNCaP cell line. The first clinical trial of paricalcitol in cancer treatment was conducted by Schwartz *et al.*^[116] in 18 patients with androgen-independent prostate cancer. Patients received 5–25 μg paricalcitol IV three times per week. One patient exhibited significant hypercalcemia, but no responses were seen (as measured by prostate specific antigen decline). A second clinical trial has been conducted in women with metastatic breast cancer by Lawrence *et al.*^[117]. Twenty out of 24 women received 8 weeks of continuous daily oral paricalcitol, and no patients experienced hypercalcemia grade 2 or higher. No data on anticancer efficacy was given since the purpose of this trial was only to determine safety. While paricalcitol seems to be safe for use in cancer patients, more studies need to be done to determine actual efficacy.

Seocalcitol (7), originally termed EB1089, is arguably the most investigated vitamin D for its use in cancer treatment. In 1992, seocalcitol was shown to inhibit MCF-7 cell proliferation *in vitro* and oral treatment was shown to inhibit tumor growth with no hypercalcemic effects in a rat mammary tumor model^[118]. In 1998, a phase I study was conducted in 36 patients with advanced breast or colorectal cancer^[119]. Patients were given $0.15\text{--}17 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. No patients had partial or complete response to therapy, but six patients showed stabilization of disease after 90 days. All patients receiving $17 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ experienced hypercalcemia and the maximum tolerated dose was determined to be $7 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. This was followed by a phase II study in patients with inoperable pancreatic cancer^[120]. Among the 36 patients enrolled, 22 were withdrawn prior to completing 8 weeks of treatment, 20 of which were due to clinical deterioration. Five of the 14 evaluable patients had stable disease, but none had partial or complete response. A second phase II study was conducted in 56 patients with inoperable hepatocellular carcinoma^[121]. Two patients had complete response and 12 had stable disease. The main side effect was hypercalcemia, with most patients tolerating a dose of $10 \mu\text{g}\cdot\text{d}^{-1}$. Two further studies investigating the effects of seocalcitol in the treatment of hepatocellular carcinoma were begun, but both trials have since been terminated^[122–123]. Further studies will be necessary to determine if seocalcitol is effective and safe in the treatment of cancer.

Conclusion and future directions

Studies of the effects of vitamin D and 1, 25(OH)₂D₃ on cellular pathways and in various mouse models of cancer clearly suggest a role for vitamin D in both the prevention and treatment of cancer. However, results of studies in the human population thus far have been inconsistent. Attempts to explain these inconsistencies through genetic differences in the vitamin D pathway have shown that various polymorphisms in the VDR may contribute to cancer risk, but mechanisms for this increased risk have yet to be elucidated. Clinical studies so far seem to indicate that there is no clear

association between vitamin D and overall risk of breast cancer, but vitamin D deficiency may increase mortality among breast cancer patients. In contrast, multiple studies have demonstrated a correlation between low vitamin D levels and colorectal cancer risk. The data concerning prostate cancer risk and vitamin D is less consistent, although low vitamin D does seem to be associated with worse disease prognosis in those with prostate cancer. In totality, current data is not sufficient to support vitamin D supplementation in all people for the prevention of cancer. However, it is reasonable to recommend testing for vitamin D deficiency and supplementation in those found to have low serum vitamin D levels. Unfortunately, current recommendations from the IOM and Endocrine Society on what constitutes a vitamin D deficiency are based on the minimum vitamin D level necessary to prevent osteoporosis and improve bone health. It is possible that serum vitamin D levels necessary to prevent cancer may be higher; further clinical trials are necessary to determine this number. Vitamin D supplementation is not without its risks however, in particular hypercalcemia. If serum vitamin D levels sufficient to cause hypercalcemia are necessary to have a significant impact on cancer risk, studies investigating the impact vitamin D analogs with less hypercalcemic effects could prove interesting.

Overall, preclinical studies do indicate a role for vitamin D in the prevention of cancer, but clinical studies thus far have not consistently shown this association. This may be because the association is relatively weak, the association only exists for certain cancer types (i.e. colorectal cancer) or the vitamin D levels necessary to decrease cancer risk in humans is not being reached. Further investigations to determine which of these scenarios is most likely are necessary. Finally, studies of emerging novel vitamin D analogs ^[124-131] that can be given in higher doses with less side effects could provide more definitive information about the correlation between vitamin D levels. Thus far no vitamin D analogs have demonstrated particularly convincing data for their use in the treatment of cancer. However, all of the clinical trials have been conducted in patients with advanced disease. It is possible these analogs may be more beneficial in either early stages of cancer or in the prevention of cancer. More studies are necessary to determine the best place in cancer prevention/therapy for these vitamin D analogs.

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