Vitamins in Pancreatic Cancer: A Review of Underlying Mechanisms and Future Applications\textsuperscript{1,2}

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ABSTRACT

Although there is increasing evidence that vitamins influence pancreatic adenocarcinoma biology and carcinogenesis, a comprehensive review is lacking. In this study, we performed a PubMed literature search to review the anticancer mechanisms and the preclinical and clinical studies that support the development of the bioactive vitamins A, C, D, E, and K in pancreatic cancer intervention. Preclinical studies have shown promising results for vitamin A in pancreatic cancer prevention, with clinical trials showing intriguing responses in combination with immunotherapy. For vitamin C, preclinical studies have shown slower tumor growth rates and/or increased survival when used alone or in combination with gemcitabine, with clinical trials with this combination revealing decreased primary tumor sizes and improved performance status. Preclinical studies with vitamin D analogues have shown potent antiproliferative effects and repression of migration and invasion of pancreatic cancer cells, with a clinical trial showing increased time to progression when calciferol was added to docetaxel. For vitamin E, preclinical studies have shown that \textit{d}-tocotrienol and \textit{g}-tocotrienol inhibited tumor cell growth and survival and augmented gemcitabine activity. Early-phase clinical trials with \textit{d}-tocotrienol are ongoing. Vitamin K demonstrates activation of apoptosis and inhibition of cellular growth in pancreatic tumor cells; however, there are no clinical studies available for further evaluation. Although preclinical and clinical studies are encouraging, randomized controlled trials with endpoints based on insights gained from mechanistic and preclinical studies and early-phase clinical trials are required to determine the efficacy of bioactive vitamin interventions in pancreatic cancer.

Introduction

Pancreatic cancer is the fourth-leading cause of cancer-related death, with an estimated 46,420 new cases in 2014 in the United States and 39,590 deaths resulting from this formidable health care problem (1). Only 6.7\% of patients will survive \text{\geq}5\text{yr}, with 33\% initially diagnosed at a stage with advanced cancer metastasis (1). Conventional treatment approaches such as surgery, chemotherapy, and/or radiation have generally had little impact on the course of this aggressive cancer despite efforts over the past several years. Although patients with metastatic pancreatic cancer have shown improved outcomes with combinations of cytotoxic chemotherapy agents, not all patients can receive these regimens, and such treatment has not yet been adopted in the adjuvant setting because of severe intolerable toxicities (2). Therefore, gentler alternative approaches are highly sought, and the strategy of investigating the less toxic cancer bioactive vitamins to augment cytotoxic therapy and for metastasis prevention is very attractive. Indeed, it is widely documented that the majority of patients with cancer take vitamin supplements, in part to decrease their risk of cancer relapse (3). In a systematic review of 32 studies performed between 1999 and 2006, 64–81\% of cancer patients and cancer survivors used vitamin and mineral supplements (4). In addition to conventional treatment for cancer, many patients seek advice on the use of dietary supplements. They receive varied advice about what supplements to take, how much to take, and how long to take them. Unfortunately, this advice is often unreliable and not yet empirically supported (5, 6). Despite the widespread use of these supplements, there are currently no established recommendations on the use of bioactive vitamins to augment conventional treatment and to prevent recurrence or metastasis in pancreatic cancer.

In this review, we explore the evidence regarding bioactive vitamins, including vitamins A, C, D, E, and K, and their role in pancreatic cancer, with the goal of assisting with informed decision making for pancreatic cancer survivors. We will also discuss the potential mechanisms of these bioactive vitamins in pancreatic cancer by focusing on the results of

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mechanistic studies. In conclusion, recommendations and potential future research strategies for pancreatic cancer intervention with the use of bioactive vitamins will be proposed.

**Methods**

A PubMed search was performed and included all publications that used the keywords “vitamin A and pancreatic cancer,” “vitamin C and pancreatic cancer,” “vitamin D and pancreatic cancer,” “vitamin E and pancreatic cancer,” “vitamin K and pancreatic cancer,” and “vitamins and pancreatic cancer,” without restrictions on year. No language restriction was applied. Reference lists from studies selected by the electronic search were manually searched to identify further relevant studies. Reference lists from all available review articles, primary studies, and proceedings of major meetings were also considered. Vitamins A, C, D, E, and K were considered to be the most relevant vitamins based on the amount of evidence available. We focused on all of the relevant in vivo and in vitro studies, as well as on clinical trials that were conducted with these vitamins.

**Vitamin A**

Vitamin A is required by the body for cell growth and differentiation of epithelial tissue and is only obtained through the diet in the form of retinol, retinyl ester, or β-carotene. Vitamin A is typically found in liver, fish oils, dairy products, and fortified cereals in the United States. A plasma retinol concentration lower than 0.70 μM reflects inadequacy and concentrations of 0.70–1.05 μM could be marginal in some people (7). Retinoic acid (RA)3, an active metabolite of retinol, regulates a wide spectrum of biological processes, such as cellular development, differentiation, proliferation, and apoptosis (8). Retinoids have been studied as chemopreventive agents in clinical trials, with many endpoints focusing on safety and tolerability, as well as the antitumor activity these supplements may have alone or in combination with other agents. They result in the inhibition of growth, induction of cellular differentiation, and decreased adhesion to certain components of the extracellular matrix, which are all features compatible with a less malignant phenotype. We will review current preclinical and clinical research on the role of vitamin A in pancreatic cancer intervention and the mechanisms describing it.

**Mechanisms of action of vitamin A in pancreatic cancer**

**Retinoic acid receptor modulation.** RA has been demonstrated to inhibit cell proliferation and induce cell differentiation in many malignant tissues. RA exerts its pleiotropic effects on cellular growth and differentiation through nuclear receptors, retinoic acid receptors (RARs), and retinoid X receptors (RXRs). There are 3 RARs: RAR-α, RAR-β, and RAR-γ, encoded by the RARA, RARB, and RARG genes, respectively, as well as 3 RXRs: RXR-α, RXR-β, and RXR-γ, encoded by the RXRA, RXRB, and RXRG genes, respectively. RA forms a heterodimer with RXR, increasing its affinity for binding to a hormone response element. In the absence of a ligand, it becomes complexed with a corepressor protein, causing transcriptional repression through histone deacetylation with histone deacetylases (HDACs) (8). When an agonist ligand such as RA binds RAR on the RAR/RXR heterodimer, it results in dissociation of the corepressor and recruitment of a coactivator protein, promoting histone acetylation with histone acetyltransferases and activation of mRNA transcription (8).

Prolonged stable disease in pancreatic cancer has been demonstrated by using a combination of the HDAC inhibitor entinostat with 13-cis RA. The proposed mechanism is that RAR-β gene expression is silenced by histone deacetylation, which blocks the recruitment of the transcription-activating complex and makes the transcriptional site inaccessible to retinoid ligands (9). When an HDAC inhibitor such as entinostat is added, histone acetylation and transcription-activating complex binding occur, allowing transcription of the RAR-β gene to occur (9). This combination was found to be safe and have the potential to induce clinical benefits through prolonged stable disease in this phase I clinical trial. Another study observed that approximately one-third of all pancreatic tumors completely lose expression of RAR-β when compared with nontransformed counterparts, and, furthermore, the remaining tumors expressed significantly less RAR-β mRNA transcripts than adjacent normal pancreatic ductal cells (10). There was also a tight correlation between the loss of RAR-β expression and degree of cellular de-differentiation. By creating overexpression of RAR-β in the pancreatic tumor cell line DAN-G by stable transfection, inhibition of cellular proliferation in vitro and in vivo occurred (10). In addition, increased RAR-β expression led to induction of cellular differentiation, as evidenced by increased tumor cell expression of markers such as carcinobromyric antigen, cancer antigen 19-9, and cytokeratin 7 (10). This suggests that RAR-β may play a key role in the maintenance of the malignant phenotype in pancreatic adenocarcinoma and may represent a novel target for experimental strategies in treatment.

RAR-γ expression has been found to occur in all retinoid-sensitive pancreatic ductal tumor cell lines, and lack of expression was found in the retinoid-resistant pancreatic amphiicrine cell line AR42J (11, 12). This receptor isoform has not been observed in any other gastrointestinal tissue and was previously thought to be restricted to keratinocytes of the skin. This observation suggests that RAR-γ might play a key role in retinoid action on human pancreatic carcinogenic cells. This is further supported by experiments that use target gene disruption of both RAR-γ alleles, resulting in these RAR-γ-deficient cells losing their
responsiveness to retinoids and not exhibiting a differentiated phenotype upon retinoid treatment (13). Given this evidence, RAR-γ is thought to confer retinoid sensitivity to human pancreatic ductal carcinoma cells, and the lack of RAR-γ expression is responsible for retinoid resistance (11). Another study examined the rat pancreatic carcinoma cell line DSL-6A/C1 and demonstrated that treatment with retinoids results in time- and dose-dependent inhibition of cell growth predominantly mediated by stimulating the RAR-α subtype (14).

**Interaction with protein kinase C.** Retinoids also have been shown to interfere at the level of other intracellular signaling pathways for the regulation of cellular growth, such as with protein kinase C (PKC), a phospholipid-dependent serine/threonine kinase implicated in the regulation of gene expression, cellular proliferation, and cellular differentiation. PKC normally acts as a receptor for mitogenic phorbol esters such as diacylglycerol and arachidonic acid, which act as tumor promoters. They are generated as second messengers upon cellular binding of hormones, growth factors, and other extracellular ligands. RA was found to exert opposite effects on anchorage-independent growth in 2 human pancreatic adenocarcinoma cell lines derived from identical histologic origins (15). RA treatment of PKC in AsPc1 cell lines resulted in induction of PKC-α expression along with a dose-dependent stimulation of anchorage-independent growth. Selective downregulation of PKC-α would also subsequently block growth stimulation by RA in AsPc2 cells (15). However, when Capan 2 cell lines were observed, RA decreased PKC-α expression and inhibited cell growth. Both of these cell lines expressed identical patterns of RARs and RXRs (15). These data suggest that the differential regulation of PKC-α expression plays a central role in determining the bidirectional effects of retinoids in pancreatic carcinoma cell growth.

**Inhibition of cellular adhesion.** Retinoid treatment results in decreased adhesion to certain components of the extracellular matrix, thus inhibiting the metastatic potential of pancreatic carcinoma cells. During the process of metastasis, tumor cells traverse the vascular basement membrane during their excursion from the circulation to their target organ. The initial step in this cascade is adhesion to the basement membrane. Accordingly, the ability to metastasize has been correlated with the extent of tumor cell adhesion to basement membrane components (16). Type IV collagen, heparin sulfate proteoglycan, nidogen/entactin, and laminin are major components of the basement membrane. Laminin has been shown to modulate tumor cell adhesion, growth, and differentiation, and is of particular interest, given that the ability of tumors to interact with laminin has been shown to correlate with their level of tumorigenicity and metastatic potential (17–19). Laminin binding proteins are referred to as integrins, which mediate tumor cell interactions. Through a yet unidentified mechanism involving alteration of the α6β1-integrin receptor function, retinoids have been shown to decrease pancreatic carcinoma cell adhesion to laminin (20). This decreased adhesion parallels growth inhibition and induction of differentiation induced by retinoids (20).

**Downregulation of IL-6.** RA has been shown to inhibit pancreatic carcinoma cell migration and epithelial-mesenchymal transition (EMT) of tumor cells through the downregulation of IL-6 in cancer-associated fibroblast cells (CAFs). IL-6 is a potent pleiotropic cytokine that regulates cell growth and differentiation, playing an important role in the immune response. IL-6 has been shown to be related to tumorigenicity, proliferation, migration, metastasis, and chemotherapy resistance in many types of malignancy (21). Inhibition of IL-6 expression by RA has been shown in many cells, such as astrocytes, macrophages, and cardiomyocytes (22–24). It is known that RA inhibits pancreatic cancer cell migration, and recent evidence may have elucidated part of the underlying mechanism (21). CAFs are a cell type found in the stroma of pancreatic cancer. They support the proliferation, migration, metastasis, and chemotherapeutic resistance of tumor cells by producing extracellular matrix, secreting cytokines, and activating signaling pathways in tumor cells (25–29). Treating CAFs with RA caused these cells to become static because of the low expression of α-smooth muscle actin, fibroblast activation protein, and IL-6, and decreased production of extracellular matrix. It was also verified that low secretion of IL-6 from CAFs was related to RA-induced inhibition of migration and EMT of tumor cells (21). However, RA could not inhibit the migration and EMT of tumor cells directly. The addition of IL-6 also restored the migration and EMT of tumor cells. In conclusion, one of the therapeutic effects of RA on tumor cells is through its modulation of CAFs in the tumor microenvironment (21).

**Preclinical studies of vitamin A in pancreatic oncogenesis**

Several studies have established an effect from retinoids on many types of cancer cells, most notably pancreatic cancer. One main focus is on the stimulation of RAR, specifically the RAR-β subtype, in which decreased expression of RAR-β plays a key role in the maintenance of a malignant phenotype in human pancreatic adenocarcinoma, representing a novel target for treatment (10). In animal studies, stimulation of the RAR-α subtype by retinoids resulted in a time- and dose-dependent inhibition of cell growth (14). Retinoids also play a role in other intracellular signaling pathways. They have been shown to be involved in the differential regulation of PKC-α, which plays a central role in pancreatic carcinoma cell growth (15). They have also been implicated in decreasing pancreatic carcinoma cell adhesion to laminin, a component of the basement membrane, during infiltrative growth and metastasis (20). Retinoids also inhibit pancreatic carcinoma cell migration and EMT of tumor cells through downregulation of IL-6 in CAFs (21). Collectively, these data provided biologically plausible
Clinical studies of vitamin A in pancreatic oncogenesis

Several studies have searched for a link between the administration of RA and the prevention of metastasis and recurrence in pancreatic cancer. Although no objective responses were observed, prolonged stable disease occurred in pancreatic cancer patients in a phase I study of the HDAC inhibitor entinostat in combination with 13-cis-RA (9) Another very interesting study used the concept of maintenance immunotherapy consisting of IL-2 and 13-cis-RA after treatment for stage III pancreatic cancer (30). The study subjects were progression-free stage III patients who had received cisplatin and gemcitabine therapy consolidated with radiotherapy with concurrent capcitabine. Patients treated with the maintenance immunotherapy had a median progression-free survival of 16.2 mo, with overall survival still pending after an average of >24 mo. These outcomes were superior over historical controls of standard cytotoxic chemotherapy with chemoradiation therapy for stage III pancreatic cancer. A phase II trial of 13-cis-RA combined with INF-α in locally advanced pancreatic cancer was noted to be well tolerated but with no improvement in the response rate (31). A phase II pilot trial of 13-cis-RA and INF-α in patients with advanced cancer also noted tolerance of the therapy but no substantial response rates (32). There are also examples of the successful development of bioactive vitamins to prevent cancer relapse. One example is the use of 13-cis-RA to prevent relapse in high-risk patients with neuroblastoma after bone marrow transplant (33–35). There are currently no active or recruiting clinical trials evaluating Vitamin A in conjunction with pancreatic cancer.

Conclusion

Vitamin A has many different preclinical and clinical studies available to evaluate its efficacy as a therapeutic agent for pancreatic cancer treatment. Its antitumor effects occur through RAR modulation, interaction with PKC, inhibition of cellular adhesion, or downregulation of IL-6 (Table 1). Preclinical studies display the potential that RA has for treatment (Table 2). Animal studies display a time- and dose-dependent inhibition of cell growth upon stimulation of the RAR-α subtype by retinoids along with other studies showing RA's role in the inhibition of pancreatic carcinoma cell migration, adhesion, and cell growth. Although tolerable, phase II clinical trials have shown no objective responses with RA in combination with chemotherapeutic agents for treatment; however, its use as maintenance immunotherapy had a median progression-free survival of 16.2 mo, with overall survival results still pending (Table 3). These outcomes are superior compared with historical controls of standard cytotoxic chemotherapy with chemoradiation for stage III pancreatic cancer, showing promising results. Vitamin A may prove useful as a maintenance therapy for pancreatic cancer and not as an adjunct for treatment; however, further clinical trials are needed to support this notion.

Vitamin C

Vitamin C, also known as ascorbic acid, acts as an antioxidant, and, in the presence of catalytic metals, acts as a pro-oxidant. It is typically obtained from fruits and vegetables, particularly citrus fruits, broccoli, spinach, cauliflower, and sweet and white potatoes. The usual plasma ascorbic acid concentrations are 0.010–0.080 mM and are dependent upon dietary and supplement intake. Even if consumed in large quantities of many grams daily taken every few hours, plasma ascorbic acid concentrations in people do not exceed 0.25 mM (83). Ascorbic acid is an essential nutrient and co-factor in many enzymatic reactions, including synthesis of collagen, l-carnitine, and certain neurotransmitters. Ongoing research is currently examining whether vitamin C plays a role in cancer prevention, with many endpoints evaluating the safety, efficacy, tolerability, and response of vitamin C in combination with chemotherapeutic agents. We will review the proposed mechanisms and clinical and experimental research investigating the role of vitamin C in pancreatic cancer.

Mechanisms of vitamin C in pancreatic cancer

Creation of reactive oxygen species. The mechanism of high-dose intravenous vitamin C is distinct from orally administered vitamin C. Intravenous ascorbate in pharmacologic doses can produce peak plasma concentrations that are several-hundredfold higher than those from maximal oral doses. Plasma ascorbate concentrations obtained from oral intake of foods rich in vitamin C, such as fruits and vegetables, are usually around 0.1 mM, and those from intake of supplements are <0.15 mM (80). In contrast, when intravenous doses are administered, concentrations approaching 30 mM can be attained (38, 83). At this range, ascorbate can readily diffuse into the extracellular fluid. The combination of hydrogen peroxide and ascorbate in the extracellular fluid has been shown to result in the formation of reactive oxygen species (ROS), which selectively kills pancreatic cancer cells and not normal tissue cells (36). This allows vitamin C to act as a prodrug for the delivery of hydrogen peroxide to tumors, but without hydrogen peroxide accumulation in the blood. The same pharmacologic concentrations of ascorbate in whole blood generated little detectable ascorbate radicals and no detectable hydrogen peroxide (36). This is likely secondary to the redundant hydrogen peroxide catabolic pathways in whole blood, such as catalase and glutathione peroxide.

Depletion of intracellular adenosine triphosphate. Hydrogen peroxide created from pharmacologic ascorbic acid concentrations may cause tumor cell death via adenosine triphosphate (ATP) depletion. Hydrogen peroxide may cause tumor cell death through DNA single-strand breaks, requiring repair by polyADP-ribose polymerase (PARP). This enhanced PARP activity catabolizes NAD+, depleting substrate for NAD(H) formation and subsequent ATP synthesis (38). A second method may occur through concurrent oxidation of glutathione via glutathione peroxidase to create glutathione disulfide. NAD(P)H is required by glutathione reductase to reduce glutathione disulfide back to glutathione. NAD(P)H
TABLE 1  Common mechanisms underlying the anticancer effect in pancreatic cancer

Vitamin D

- RAR-β gene expression is silenced by histone deacetylation, blocking the recruitment of the TAC and making the transcriptional site inaccessible to retinoid ligands (9). When an HDAC inhibitor such as entinostat is added, histone acetylation and TAC binding occurs, allowing transcription of the RAR-β gene to occur (9).
- By creating overexpression of RAR-β in pancreatic tumor cell line DAnG by stable transfection, inhibition of cellular proliferation in vitro and in vivo occurred (10).
- Increased RAR-β expression leads to induction of cellular differentiation, as evidenced by increased tumor cell expression of markers such as CEA, CA19-9, and cytoketin 7 (10).
- RAR-γ lack of expression was found in the retinoid-resistant pancreatic amphicrine cell line AR42J (11, 12). RAR-γ is thought to confer retinoid sensitivity to human pancreatic ductal carcinoma cells, and the lack of RAR-γ expression is responsible for retinoid resistance (11).

Interaction with PKC

- Induction of PKC-α expression along with dose-dependent stimulation of anchorage-independent growth. Selective downregulation of PKC-α also subsequently blocked growth stimulation by retinoic acid in AsPC-2 cells (15). Decreases PKC-α expression and inhibits cell growth.

Inhibition of cellular adhesion

- Alteration of the αβ3-integrin receptor function decreases pancreatic cancer cell adhesion to laminin (20).

Downregulation of IL-6

- Inhibits pancreatic carcinoma cell migration and epithelial-mesenchymal transition of tumor cells through the downregulation of IL-6 in CAFs.
- CAFs become static because of the low expression of α-SMA, FAP, and IL-6 and decreased production of extracellular matrix.
- Low secretion of IL-6 from CAFs was related to retinoic acid-induced inhibition of migration and EMT of tumor cells (21).

Vitamin A

Creation of ROS

- Hydrogen peroxide and ascorbate in the extracellular fluid result in the formation of ROS, which selectively kills pancreatic cancer cells and not normal tissue cells (36).

Autophagy

- Formation of a double membrane organelle that engulfs cellular proteins and cytoplasmic organelles, and subsequently fuses with lysosomes to facilitate their degradation (37).
- The role of autophagy induced cell may also require ROS to occur (38).

Depletion of intracellular ATP

- Hydrogen peroxide may cause tumor cell death through DNA single-strand breaks, requiring repair by PARP. This enhanced PARP activity catalyzes NAD+, depleting substrate for NADH formation and subsequent ATP synthesis (38).
- NADPH is required by glutathione reductase to reduce glutathione disulfide back to glutathione. NADPH is provided from glucose via the pentose phosphate pathway. Glucose used to reduce NADP+ to NADPH cannot be used for glycolysis or NAD production, subsequently causing decreased ATP generation (38).
- Hydrogen peroxide may also directly damage mitochondria, decreasing ATP production (38).
- Cancer cells rely on glycolysis rather than oxidative phosphorylation via the Warburg effect. The loss of glucose to the pentose phosphate pathway may also result in decreased ATP, leading to cell death.

Vitamin D

VDR agonist

- Upon binding of 1α,25(OH)2D3 to a VDR in target tissues, 2 independent sites are formed. One facilitates association with a heterodimer, an RXR, required for specific DNA binding to a VDRE, and a second is essential for recruitment of coregulatory proteins that modulate gene expression (39).
- 1α,25(OH)2D3 exhibits antiproliferative, prodifferentiating, anti-inflammatory, and proapoptotic properties via VDR.

AMPK-dependent mechanisms

- AICAR acts as an AMPK agonist and inhibits the growth of several cancer cell lines via arrest in the S phase and activation of cell cycle proteins p21, p27, and p53, as well as through activation of AMPK and inhibition of the PI3K/Akt pathway (40).
- When AICAR was combined with 1α,25(OH)2D3, 3-BE, there was an increase in cell growth inhibition of pancreatic cancer cells from 60% to 85%; however, when used alone, AICAR was too low in potency to reduce the growth of BxPC-3 cells (41).

Inhibition of PI3K/Akt pathway

- Akt regulates tumorigenesis and cell proliferation, growth, and survival, and is activated by the binding of the lipid kinase PI3K, which generates PIP3 at the plasma membrane (42). Akt binds PIP3, resulting in its translocation to the plasma membrane. It is activated by a dual phosphorylation mechanism.
- AICAR also works via inhibition of the PI3K/Akt phosphorylation pathway to cause pancreatic cancer cell growth inhibition.

Regulation of the cell cycle

- 1α,25(OH)2D3 dephosphorylates Rb, which permits binding to E2F-1 and halts the progression of the cell cycle (43). p21 and p27, CDKIs, also play a role in cell cycle progression as tumor repressors responsible for G1 cell cycle arrest and withdrawal from the cell cycle. They contain VDRE within the cell membrane regions, and are targets of RAR-α, RAR-β/γ, and RAR-γ/β, respectively, all of which are expressed in many cell types (43, 44).
- Vitamin D analogues upregulate p21 and p27 as an early event to induce growth inhibition of pancreatic cancer cell lines (45).
- MART-10 acts by downregulation of cyclin D3, CDK4, and CDK6 in BxPC-3 cells to inhibit cell growth via cell cycle arrest at G0/G1 (46).
- Downregulation of several cyclins, upregulation of CDK (p19, 21, 27), upregulation of TGFβ and IGF-BP3 and their signaling pathways, and the downregulation of epidermal growth factor receptors, c-myc, jun, and fos (43).
- 25(OH)D3 also inhibited the growth of 3 of 4 pancreatic cell lines, which correlated with the level of induction of p21 and p27 and with the induction of cell cycle arrest at G1/S checkpoint (47).

Decreased cellular migration and invasion

- MART-10 and 1α,25(OH)2D3 inhibited cellular migration and invasion of BxPC-3 with wild type k-ras and PANC with mutant k-ras, with MART-10 much more potent than 1α,25(OH)2D3 (46).
- MART-10 and 1α,25(OH)2D3 inhibited EMT in BxPC-3 and PANC cells via downregulation of Snail and Slug, transcription factors known to trigger EMT, leading to the repression of the mesenchymal cell marker Vimentin (46).

(Continued)
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Table 1 (Continued)

• In BxPC-3 cells, MART-10 and 1-α25(OH)2D3 also inhibited MMP-2 and MMP-9 expression in extracellular components as determined by Western blot and zymography (46).
• MART-10 and 1-α25(OH)2D3 increased E-cadherin expression in BxPC3 and PANC cells, with only MART-10 decreasing N-cadherin expression in BxPC-3 cells, potentially inhibiting the metastatic potential of pancreatic cancer cells (46).

Vitamin E

Inhibition of NF-κB activity
• NF-κB is constitutively active in 67% of human pancreatic cancers, but not in normal pancreatic tissues (48).
• Δ- and γ-tocotrienol inhibit NF-κB activity, cell growth, cell survival, and tumor growth in nude mice. α-tocotrienol and α-tocopherol have no effect on NF-κB activity (49).
• β2, γ2, and δ-tocotrienol also significantly decreased p65 (RelA), a NF-κB family member, to DNA in the cytosol, whereas γ2- and δ-tocotrienol significantly decreased p65 binding to DNA in the nucleus (50).
• NF-κB inhibition can enhance gemcitabine activity in pancreatic cancer cells (49, 51–54).

Ras-Raf-MEK-ERK pathway
• This pathway promotes cellular proliferation and prevents apoptosis. With inhibition of this pathway, it promotes apoptosis via caspase-6, -8, and -9 activation (53).
• Tocotrienols can cause a reduction in total ERK and phospho-ERK in pancreatic cancer cell lines MiaPaCa-2 and Panc-28 at concentrations of 80μM for 24 h. However, the same did not occur with tocopherols (56).
• δ-tocotrienol is related to inhibition of the Raf-MEK-ERK pathway, however, the mechanism by which δ-tocotrienol inhibits Ras activation signaling is poorly understood.
• A previous study implicated δ-tocotrienol inhibition of Ras by its suppression of HMG-CoA activity, however, this was not demonstrated in other studies (57).

Regulation of the cell cycle
• Induction of p27kip1 is a crucial event in δ-tocotrienol-induced G1 cycle arrest and inhibition of cell proliferation in MiaPaCa-2 pancreatic cancer cells.
• p27kip1 was shown to also function as a tumor suppressor in the G1-to-S transition checkpoint through binding and inhibiting the cyclin E-CDK2 complex in the nucleus (58).
• In the cytoplasm, it supports the assembly and nuclear import of cyclin D-CDK4/6 and promotes cellular proliferation. Cytosplasmic mislocalization of p27kip1 may contribute to the progression of many cancers by promoting increased cellular motility and metastasis (59).
• δ-tocotrienol induced an increase in p27kip1 in the nucleus of pancreatic cancer cells in vitro and in vivo, favoring the tumor-suppressor function of p27kip1 (58).
• Ras activation was also found to indirectly cause cytoplasmic localization of p27kip1 via activation of Raf-MEK-ERK, favoring p27kip1’s role as a cytoplasmic oncopogene.
• VES has also been implicated as having a role in cell cycle arrest. In PANC-1 cells, G2 checkpoint arrest and inhibition of CDC-2 occurred with treatment of VES (60). p21, a target protein of p53, was induced without any change in expression or phosphorylation of p53 with VES treatment (60).

Induction of apoptosis
• Treatment of pancreatic cancer cells, COLO-357 and PANC-1 cells, with VES resulted in cleavage of PARP and caspase-3.
• Survivin is a possible target for VES given that inhibition of survivin potentiates VES-induced apoptosis in PANC-1 cells (60).
• VES induces apoptosis by blocking Bak BH3 binding to Bcl-extra large and Bcl-2, which leads to destabilization of mitochondria and the release of cytochrome c into the cytosol (61, 62). Cytochrome c then acts an initiator caspase, activating caspase-3, subsequently committing the cell to apoptosis.
• Bcl-2 plays a role in pancreatic cancer cell apoptosis by protecting cells from VES-dependent apoptosis (63).
• VES inhibition of cell growth was much less pronounced when Bax and Bak were knocked out (63).
• Tocotrienol can induce apoptosis in pancreatic cancer cells through the suppression of vital cell survival and proliferative signaling pathways like the PI3-kinase/AKT and ERK/MAP kinases via downregulation of Her2/ErbB2 expression (56).

Vitamin K

Induction of apoptosis
• VK-1 and VK-2 showed a time-dependent increase in cleaved caspase-3 and the proapoptotic Bcl-2 member BAX (64).
• VK-1 induces apoptosis through mediation of wild-type p53, intracellular calcium, and ROS (65).
• In combination with sorafenib, VKI inhibited cell growth and caused apoptosis via activation of JNKs/c-Jun and inhibition of the MEK-ERK pathway, 2 proposed pathways ultimately leading to caspase activation.

JNK MAP signaling pathway
• Sorafenib and VK-1 cause cell growth inhibition and apoptosis through the activation of JNK, which increases phosphorylation of c-Jun and decreases FasL expression, activating the extrinsic apoptosis pathway (66).

Ras–Raf–MEK–ERK pathway
• Sorafenib or VK-1 alone can induce apoptosis through inhibition of phospho-ERK and phospho-ERK concentrations at high concentrations. At low concentrations, VK-1 added to sorafenib mediated inhibition of the MEK-ERK pathway and induction of apoptosis via the extrinsic pathway (66).
• In contrast, a dose-dependent increase in phospho-ERK occurred in VK-1- and VK-2–treated pancreatic cancer cells (64).
• VK-3 jounces into pancreatic tumor tissues also resulted in ERK phosphorylation and growth inhibition (67).

1ACAR, 5-amino-midazole-4-carboxamide ribonucleoside; Akt, protein kinase B; AMPK, AMP-activated protein kinase; Bcl-xL, Bcl-extra large; CA19-9, cancer antigen 19-9; CAF, cancer-associated fibroblast; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; CDQ, cyclin-dependent kinase 2, C2A, carcinoembryonic antigen; D-CDDP/4, cyclin D-cyclin dependent kinase 4/6; E-CDK2, cyclin E-cyclin dependent kinase 2; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FAP, fibroblast activation protein; HDAC, histone deacetylase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IGF-BP3, insulin-like growth factor binding protein 3; JNK, c-Jun-terminial protein kinase; MAP, mitogen-activated protein; MART-10, 19-nor-2α-(3-hydroxypropoxy)-1α,25(OH)2D3; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; MMP, matrix metalloproteinase; PARP, poly(ADP-ribose) polymerase; Pikk, phosphatidylinositol 3-kinase; PI3K, phosphatidylinositol 3,4,5 trisphosphate; PKC, protein kinase C; RA, retinoic acid; RAR, retinoic acid receptor; Rb, retinoblastoma protein; ROS, reactive oxygen species; RXR, retinoid X receptor; TAC, transcription activating complex; VDR, vitamin D receptor; VDR, vitamin D response element; VES, vitamin E succinate; VK, vitamin K; α-SMA, α-smooth muscle actin; α2,25(OH)D3, 1,25-dihydroxycholecalciferol; α2,25(OH)2D3, 1,25-dihydroxycholecalciferol; 25(OH)D3, 25-hydroxycholecalciferol.
TABLE 2 Preclinical studies of vitamins in pancreatic cancer

<table>
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<tr>
<th>Study (reference)</th>
<th>Nutrient</th>
<th>Chemical form</th>
<th>Concentration</th>
<th>Biomarker/endpoint studied</th>
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<td>Retinoic acid receptor β regulates growth and differentiation in human pancreatic carcinoma cells, Kaiser et al. (11)</td>
<td>Vitamin A</td>
<td>RARβ</td>
<td>n/a</td>
<td>The role of RARβ expression in propagation of a malignant phenotype in human pancreatic carcinoma cells</td>
<td>Overexpression of RARβ in DAN-G cells inhibited cellular proliferation in vitro and in vivo (P &lt; 0.05) and resulted in induction of cellular differentiation in xenografted tumors as evidenced by a pronounced increase in the tumor cell expression of duct cell differentiation markers carcinoembryonic antigen, CA19-9, and cytokeratin 7.</td>
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<tr>
<td>Retinoic acid receptor α mediates growth inhibition by retinoids in rat pancreatic carcinoma DSL-6A/C1 cell, Brembeck et al. (14)</td>
<td>Vitamin A</td>
<td>RARα</td>
<td>10 μM ATRA (concentration achieved after oral uptake of this substance at maximum and similar nontoxic plasma concentrations in humans)</td>
<td>To examine the effects of retinoids on cell growth in DSL-6A/C1 cells and to characterize further the molecular mechanisms underlying the antiproliferative actions of retinoids.</td>
<td>After incubation with ATRA, a significant time-dependent growth inhibition in DSL-6A/C1 cells occurred in as early as 2 d (P &lt; 0.05). Antiproliferative effects of ATRA are dose dependent, with half-maximal growth inhibition observed at 100 nM (P &lt; 0.05). ATRA-mediated growth inhibition can be blocked by the RARα-specific antagonist Ro 40–6658 in a dose-dependent manner, with complete growth inhibition observed at an antagonist concentration of 10 μM (P &lt; 0.05).</td>
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<td>Differential growth regulation by all-trans retinoic acid is determined by protein kinase C α in human pancreatic carcinoma cells, Rosewicz et al. (15)</td>
<td>Vitamin A</td>
<td>ATRA</td>
<td>1 μM ATRA</td>
<td>To investigate the role of PKC isoenzymes in the differential growth regulation of human pancreatic carcinoma cell lines by ATRA.</td>
<td>Differential growth regulation by ATRA is demonstrated by a dose-dependent growth stimulation of AsPc1 cells compared with controls with maximal stimulation occurring at 1 μM ATRA (P &lt; 0.05), and, in contrast, 10 μM ATRA demonstrated a dose-dependent decrease of anchorage-independent growth in AsPc1 cells.</td>
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<td>Retinoids inhibit adhesion to laminin in human pancreatic carcinoma cells via the α6 β 1-integrin receptor, Rosewicz et al. (20)</td>
<td>Vitamin A</td>
<td>RA</td>
<td>10 μM ATRA, 13-cis RA, or 9-cis RA</td>
<td>To investigate the effects of retinoids as an experimental therapy on human pancreatic carcinoma cell adhesion to baseline membrane components, with a focus on tumor cell adhesion to laminin.</td>
<td>Pretreatment with ATRA and 9-cis RA resulted in significant decreases of DAN-G cell adhesion to fibronectin and laminin, whereas 13-cis RA had no significant effect. Most pronounced were the effects of 9-cis RA on DAN-G cell adhesion to laminin (8% ± 3% vs. 52% ± 4% of control, n = 4; P &lt; 0.01). Overall, there was a decrease in pancreatic carcinoma cell adhesion to laminin via the α6 β 1-integrin receptor during infiltrative growth and metastasis. Tumor cells were treated with the conditioned media from CAFs. ATRA and 9-cis RA in the pretreated group showed a reduction in the migration distances of tumor cells in comparison with control (P &lt; 0.01), along with a decrease in the expression of IL-6. Tumor cells directly treated with conditioned media supplemented with RA showed no significant reduction in migration distances of the cells. Overall, there was an inhibition of pancreatic carcinoma cell migration and epithelial-mesenchymal transition of tumor cells through the inhibitory effects of RA on CAFs, rather than through its direct effects on tumor cells. This is thought to be via the downregulation of IL-6 in cancer-associated fibroblast cells.</td>
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<tr>
<td>Retinoic acid inhibits pancreatic cancer cell migration and EMT through the downregulation of IL-6 in cancer-associated fibroblast cells, Guan et al. (21)</td>
<td>Vitamin A</td>
<td>RA</td>
<td>10 μM ATRA and 9-cis RA</td>
<td>Through inhibition of CAFs, RA can decrease the support that the CAFs provide to tumor cells.</td>
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<td>Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo, Chen et al. (38)</td>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
<td>Administration of par-enteral or oral ascorbate in typical human pharmacologic doses (~0.25–0.5 mg/g body weight).</td>
<td>To determine if pharmacologic ascorbate is a prodrug for preferential steady-state formation of Asc· and H2O2 in the extracellular space compared with blood.</td>
<td>After intravenous injection, ascorbate baseline concentrations of 50–100 µM in blood and extracellular fluid increased to peaks of &gt;8 mM. After intraperitoneal injection, peaks approached 3 mM in both fluids. In blood, Asc· concentrations measured by EPR were undetectable with oral administration and always &lt;50 µM with parenteral administration, even when corresponding ascorbate concentrations were &gt;8 mM. After parenteral dosing, Asc· concentrations in extracellular fluid were 4–12-fold higher than those in blood, were as high as 250 nM, and were a function of ascorbate concentrations. Overall, this provides a foundation for pursuing ascorbate as a pro-oxidant therapeutic agent.</td>
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<td>Mechanisms of ascorbate-induced cytotoxicity in pancreatic cancer, Du et al. (37)</td>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
<td>Ascorbate was used at physiologic (0.1 mM) and pharmacologic (0.3–20 mM) concentrations with the use of 1 h incubations to mimic clinical intravenous use.</td>
<td>To test whether ascorbate killed cancer cells selectively, and, if so, to determine mechanisms with the use of clinically relevant conditions.</td>
<td>Ascorbate induced concentration-dependent cell death at nearly 100% at 2 mM. As ascorbate concentration increased, the pattern of death changed from apoptosis to pyknosis necrosis, a pattern suggestive of H2O2-mediated cell death. Apoptosis occurred by 6 h after exposure, and cell death by pyknosis was ~90% at 14 h after exposure. Generation of ROS via formation with hydrogen in a dose-dependent manner selectively killed some cells, but not normal cells. On day 21 of the experiment, the control group had a mean tumor volume of 472 mm³, whereas the ascorbate group had a mean tumor volume of 138 mm³. The mixed linear regression analysis of the tumor growth curves demonstrated that their rate of growth differed significantly between the groups (P &lt; 0.001). The log-rank analyses of survival demonstrated that the animals that received ascorbate had increased survival compared with controls (68 d vs. 78 d) (P &lt; 0.0001). Ascorbate administered in doses achievable in humans decreased viability in all pancreatic cancer cell lines via an H2O2-mediated mechanism. Treatment with pharmacologic ascorbate induced a noncaspase-mediated cell death consistent with autophagy. The effective concentration that decreased survival by 50% was 10 mM for 75% of tumor cells tested, whereas cytotoxicity was not evident in normal cells with 20 mM ascorbate. The addition of catalase to the medium ameliorated the death of pancreatic carcinoma exposed to 10 mM ascorbate for 1 h, indicating cytotoxicity was mediated by H2O2. There was a significant decrease in tumor growth rate with ascorbate treatment in comparison with control (P &lt; 0.005) in pancreatic tumor cells.</td>
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<tr>
<td>Pharmacologic doses of ascorbate act as a pro-oxidant and decrease growth of aggressive tumor xenografts in mice, Chen et al. (68)</td>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
<td>4 g ascorbate/kg body weight vs. control group of osmotically equivalent saline</td>
<td>To examine the efficacy of parenteral ascorbate administration on tumor growth in vivo by using dose-toxicity relations of ascorbate in numerous types of cancer cells in vitro.</td>
<td>Pancreatic cancer cells treated with ascorbate at doses of 0, 5, and 10 mM for 1 h, then delivered subcutaneously into nude mice, which were then randomly assigned to receive either 4 g ascorbate/kg body weight or osmotically equivalent saline for 2 wk. To determine whether ascorbate concentrations achievable with intravenous dosing would be cytotoxic in pancreatic cancer.</td>
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<td>Pharmacologic ascorbate synergizes with gemcitabine in preclinical models of pancreatic cancer, Espey et al. (69)</td>
<td>Vitamin C</td>
<td>Ascorbic acid + gemcitabine</td>
<td>4 g ascorbate/kg body weight daily with either 30 mg or 60 mg gemcitabine/kg body weight every 4 d vs. control group receiving daily saline solution osmotically equivalent to ascorbate.</td>
<td>To optimize and improve the efficacy of gemcitabine monotherapy through combination with ascorbic acid in pancreatic carcinoma cell lines.</td>
<td>After 21 d of using gemcitabine-sensitive PAN-2 xenografts, a paired 2-tailed t test showed a significant difference between 30 mg/kg gemcitabine (74.5% decrease in tumor volume) and 30 mg/kg gemcitabine + ascorbate treatment (84% decrease in tumor volume) ( P = 0.038 ), as well as between 60 mg/kg gemcitabine (84% decrease in tumor volume) and 60 mg/kg gemcitabine + ascorbate treatment (90% decrease in tumor volume) ( P = 0.021 ). After 33 d in gemcitabine-resistant PANC-1 xenografts, paired 2-tailed t test showed a significant difference between 30 mg/kg gemcitabine + ascorbate treatment, with a 52% decrease in tumor volume compared with only a 4% decrease in the gemcitabine group ( P = 0.003 ). 60 mg/kg gemcitabine + ascorbate treatment resulted in a 55% decrease in tumor volume compared with only 10% with gemcitabine alone ( P = 0.002 ). 1α-hydroxylase is expressed in normal and malignant pancreatic tissue and detected in 4 pancreatic cancer cell lines. 25(OH)D3 caused a dose-dependent inhibition of the growth of 3 of the 4 pancreatic cell lines, and inhibited the growth of cell lines either stably transfected with a mutant Ki-ras allele or with an endogenous Ki-ras activating mutation. The ( IC_{50} ) was 10–18 nM for 1,25(OH)2D3 and 800–900 nM for 25(OH)D3. 1,25(OH)2D3–3-BE inhibits pancreatic tumor cell growth in several cell lines to a greater extent than does 1α,25(OH)2D3 ( P &lt; 0.0001 ). Cell growth inhibition increased from 60% to 85% with the addition of AICAR.</td>
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<td>Pancreatic cancer cells express 25-hydroxyvitamin D-1α-hydroxylase and their proliferation is inhibited by the prohormone 25-hydroxyvitamin D3, Schwartz et al. (47)</td>
<td>Vitamin D</td>
<td>1α-hydroxylase</td>
<td>Cells were treated with increasing doses of 1,25(OH)2D3 (10, 25, 50, and 100 nM) or 25(OH)D3 (100, 250, 500, and 1000 nM) vs. ethanol (control) evaluating antiproliferative effects. Doses of 0.3 μM 1,25(OH)2D3–3-BE were used in combination with 30 μM AICAR.</td>
<td>To study the expression pattern of 1α-hydroxylase in various normal and cancerous tissues, including the pancreas, with the use of a polyclonal antibody.</td>
<td>1α-hydroxylase is expressed in normal and malignant pancreatic tissue and detected in 4 pancreatic cancer cell lines. 25(OH)D3 caused a dose-dependent inhibition of the growth of 3 of the 4 pancreatic cell lines, and inhibited the growth of cell lines either stably transfected with a mutant Ki-ras allele or with an endogenous Ki-ras activating mutation. The ( IC_{50} ) was 10–18 nM for 1,25(OH)2D3 and 800–900 nM for 25(OH)D3. 1,25(OH)2D3–3-BE inhibits pancreatic tumor cell growth in several cell lines to a greater extent than does 1α,25(OH)2D3 ( P &lt; 0.0001 ). Cell growth inhibition increased from 60% to 85% with the addition of AICAR.</td>
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<td>Anti-growth effect of 1,25-dihydroxyvitamin D3–3-bromooacetate alone or in combination with 5-amino-imidazole-4-carboxamide-1-β-4-ribofuranoside in pancreatic cancer cells, Persons et al. (41)</td>
<td>Vitamin D</td>
<td>1,25-dihydroxyvitamin D3–3-BE</td>
<td>100 nM, 1 μM, and 1 μM 1,25(OH)2D3 and 1,25(OH)2D3–3-BE vs. ethanol (control) evaluating antiproliferative effects. Doses of 0.3 μM 1,25(OH)2D3–3-BE were used in combination with 30 μM AICAR.</td>
<td>To determine the antitumor growth effect of 1,25(OH)2D3–3-BE alone or in combination with AICAR in pancreatic cancer cells.</td>
<td>1α-hydroxylase is expressed in normal and malignant pancreatic tissue and detected in 4 pancreatic cancer cell lines. 25(OH)D3 caused a dose-dependent inhibition of the growth of 3 of the 4 pancreatic cell lines, and inhibited the growth of cell lines either stably transfected with a mutant Ki-ras allele or with an endogenous Ki-ras activating mutation. The ( IC_{50} ) was 10–18 nM for 1,25(OH)2D3 and 800–900 nM for 25(OH)D3. 1,25(OH)2D3–3-BE inhibits pancreatic tumor cell growth in several cell lines to a greater extent than does 1α,25(OH)2D3 ( P &lt; 0.0001 ). Cell growth inhibition increased from 60% to 85% with the addition of AICAR.</td>
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<td>Evaluation of the potential therapeutic role of a new generation of vitamin D analogue, MART-10, in human pancreatic cancer cells in vitro and in vivo, Chiang et al. (70)</td>
<td>Vitamin D</td>
<td>MART-10</td>
<td>Concentrations of 1α,25(OH)2D3 (10 nM–10 μM) or MART-10 (0.1 nM–1 μM) for 20 h, and 1α,25(OH)2D3 (0.1 nM–10 μM) or MART-10 (10 μM–1 μM) for 7 d.</td>
<td>To investigate the effectiveness and safety of a new-generation less calcemic analogue of 1α,25(OH)2D3, MART-10, in BxPC-3 human pancreatic carcinoma cells in vitro and in vivo.</td>
<td>After 20 h of treatment, 1α,25(OH)2D3 caused 50% ± 2%, 79% ± 3%, and 76% ± 2% inhibitions at concentrations of 100 nM, 100 μM, and 10 μM, respectively, reaching plateau at 1 μM, and MART-10 at 1, 10, and 100 nM repressed BxPC-3 cell growth by 46% ± 2%, 68% ± 3%, 83% ± 2%, respectively ( P &lt; 0.001 ). A dose-dependent inhibition occurred with different doses of 1α,25(OH)2D3 or MART-10 at 7 d as well ( P &lt; 0.05 ) and ( P &lt; 0.001 ), respectively, vs. control. MART-10 demonstrated that it is 100-fold more potent than 1α,25(OH)2D3, inhibiting BxPC-3 cell proliferation in a time- and dose-dependent manner without causing hypercalcemia.</td>
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<td>The vitamin D analogue, MART-10, represses metastasis potential via downregulation of epithelial-mesenchymal transition in pancreatic cancer cells, Chiang et al. (46)</td>
<td>Vitamin D</td>
<td>MART-10</td>
<td>d-Tocotrienol, 20–40 μM</td>
<td>Because the minimum effective antigrowth concentration of 1-a, 25(OH)_2D_3 against BxPC-3 cells is around 10 nM, our treating dose was between 0.1 μM and 1 nM.</td>
<td>Because metastasis is the major cause of pancreatic cancer-related death, we therefore investigated the antimitastasis effect of MART-10 on pancreatic cancer. 1-a,25(OH)_2D_3 at 0.1 μM inhibited the migration of BxPC-3 cells by 63% ± 5%, whereas MART-10 decreased the migration ability of BxPC-3 cells by 89% ± 4% and 85% ± 7% at 10 nM and 0.1 μM, respectively, indicating that MART-10 is much more potent than 1-a,25(OH)_2D_3 in inhibiting BxPC-3 cell migration (P &lt; 0.01). Inhibitions of 29% ± 12% and 55% ± 11% for BxPC-3 invasion were observed as treated by 10 nM (P &lt; 0.05) and 0.1 μM (P &lt; 0.01) 1-a,25(OH)_2D_3. In terms of MART-10, 55% ± 6% and 72% ± 3% inhibition for BxPC-3 invasion were observed at 10 nM and 0.1 μM (both P &lt; 0.01). Both 1-a,25(OH)_2D_3 and MART-10 repressed migration and invasion of BxPC-3 and PANC cells, with MART-10 displaying much greater potency.</td>
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<td>Vitamin E d-Tocotrienol-mediated suppression of the proliferation of human PANC-1, MIA PaCa-2, and BxPC-3 pancreatic carcinoma cells, Hussein et al. (57, 71)</td>
<td>Vitamin E</td>
<td>d-Tocotrienol</td>
<td>100 mg · kg⁻¹ · d⁻¹</td>
<td>To evaluate the impact of d-Tocotrienol, the most potent vitamin E isomer, on human MIA PaCa-2 and PANC-1 pancreatic carcinoma cells and BxPC-3 pancreatic ductal adenocarcinoma cells.</td>
<td>In BxPC-1 cells, after a 72 h incubation with 0, 20, and 40 μM d-Tocotrienol, the percentages of viable cells were 89.9% ± 0.6%, 83.3% ± 2.9%, and 77.4% ± 4.8%, respectively (P &lt; 0.05). Concomitantly, the percentages of early and late apoptotic cells increased from control cells (0.6% ± 0.1% and 7.6% ± 0.6%, respectively) to cells treated with 20 μM (0.8% ± 0.1% and 11.4% ± 2.6%) and 40 μM (2.1% ± 0.6% and 16.5% ± 3.1%) (P &lt; 0.05) of d-Tocotrienol. A concentration-dependent impact was also seen in PANC-1 cells. The concentrations of d-Tocotrienol required to totally block the increase in the numbers of MIA PaCa-2, PANC-1, and BxPC-3 cells, are 40, 80, and 80 μM, respectively, which are estimated from the effective concentration that decreased survival 50% plots. After a single oral administration of Tocotrienol at 100 mg/kg, the peak plasma concentration was 57 ± 5 M. When mice were fed Tocotrienol for 6 wk, the concentration in tumor tissue was 41 ± 3.5 nmol/g and the concentration of Tocotrienol was significantly increased in the pancreas (P &lt; 0.001) and tumor (P &lt; 0.05) compared with vehicle. This concentration was observed with the oral dose (100 mg/kg) of Tocotrienol, which inhibited tumor growth by 80% in our previous studies. In summary, the oral administration of 100 mg · kg⁻¹ · d⁻¹ of Tocotrienol to mice resulted in concentrations that were 10 times higher in the pancreas than in subcutaneously implanted tumor tissue, suggesting that these compounds will have reasonable bioavailability for pancreatic tumor intervention. No toxicity was observed from Tocotrienol, with mice gaining normal weight with no histopathologic changes in tissues.</td>
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<td>Vitamin E δ-Tocotrienol Augments the Anti-tumor Activity of Gemcitabine and Suppresses Constitutive NF-κB Activation in Pancreatic Cancer, Husain et al. (49)</td>
<td>Vitamin E δ-tocotrienol</td>
<td>Tocotrienols (α-, β-, γ-, and δ-) were administered at 200 mg/kg oral gavage twice daily for 4 wk. Gemcitabine-treated were given 100 mg/kg intraperitoneally twice a week for 4 wk; δ-tocotrienol + gemcitabine-treated were given 200 mg/kg oral gavage daily plus gemcitabine at 100 mg/kg intraperitoneally twice a week for 4 wk.</td>
<td>To investigate the potential of the 4 natural tocotrienols to inhibit pancreatic cancer and NF-κB activation in vitro and in vivo. In addition, we investigated the potential of the most bioactive tocotrienol to augment gemcitabine activity in vitro and in vivo.</td>
<td>δ-Tocotrienol and γ-tocotrienol exhibited the most significant inhibitory effects (cell viability reduced up to 40% with 50 μM) and β-tocotrienol had a moderate inhibitory effect (cell viability reduced up to 40% with 50 μM). Both α-tocotrienol and saturated vitamin E (α-tocopherol) did not show any significant inhibitory effects. δ-Tocotrienol significantly inhibited malignant transformation compared with vehicle (P &lt; 0.001) and β- or γ-tocotrienol treatment (P &lt; 0.002). In contrast, no effect was observed with α-tocotrienol or α-tocopherol treatment. Gemcitabine (20 μM) alone inhibited colony formation by 84% (P &lt; 0.01); δ-tocotrienol (50 μM) alone inhibited colony formation by 67% (P &lt; 0.01), and gemcitabine (20 μM) + δ-tocotrienol (50 μM) resulted in 99% inhibition of anchorage-independent growth (P &lt; 0.001).</td>
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<td>Prolonged survival and delayed progression of pancreatic intraepithelial neoplasia in LSL-KrasG12D;Pdx-1-Cre mice by vitamin E δ-tocotrienol, Husain et al. (73)</td>
<td>Vitamin E δ-tocotrienol</td>
<td>δ-tocotrienol (200 mg/kg × 2 times/d, orally)</td>
<td>To test the chemopreventive activity of δ-tocotrienol in the LSL-KrasG12D;Pdx-1-Cre pancreatic cancer mouse model</td>
<td>δ-tocotrienol showed increased median survival from the onset of treatment (11.1 mo) compared with vehicle-treated mice (9.7 mo) and nontreated mice (8.5 mo) (P &lt; 0.0025). Furthermore, δ-tocotrienol treatment also resulted in significant suppression of mouse pancreatic intraepithelial neoplasia progression compared with vehicle treated and nontreated mice, including mPanIN-1: 47–50% (P &lt; 0.09); mPanIN-2: 6–11% (P &lt; 0.01); mPanIN-3: 3–15% (P &lt; 0.001); and invasive cancer: 0–10% (P &lt; 0.001).</td>
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In an orthotopic nude mouse model of human pancreatic cancer, γ-tocotrienol almost completely suppressed proliferation with a 50 μM dose and enhanced the antitumor properties of gemcitabine at 1 μM. With the use of bioluminescence imaging, the tumor volume in the combination group was significantly lower than in the group treated with tocotrienol or gemcitabine alone, as well as in the vehicle-treated control group (P < 0.001 vs. gemcitabine; P < 0.001 vs. control). The decreased tumor volume was confirmed in mice that were killed (P < 0.001 vs. control). Overall, γ-tocotrienol potentiates the antitumor activity of gemcitabine by inhibiting NF-κB and NF-κB-regulated gene products, leading to the inhibition of proliferation, angiogenesis, and invasion.
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<td>Vitamin E δ-tocotrienol prolongs survival in LSL-Kras(^{G12D/+;Pdx-1-Cre}) transgenic mouse model of pancreatic cancer, Husain et al. (74)</td>
<td>Vitamin E</td>
<td>δ-tocotrienol + gemcitabine</td>
<td>200 mg/kg orally twice a day</td>
<td>Previous work has shown δ-tocotrienol prolongs survival and delays progression of pancreatic cancer in the LSL-Kras(^{G12D/+;Pdx-1-Cre}) mouse model of pancreatic cancer. Here, we studied the effects of δ-tocotrienol alone and combination of δ-tocotrienol and gemcitabine in these mice.</td>
<td>At 16 wk, survival was 10% in the vehicle group, 30% in the gemcitabine group, 70% in the δ-tocotrienol group, and 90% in the δ-tocotrienol combined with gemcitabine group (P &lt; 0.05). Mice treated with gemcitabine and δ-tocotrienol alone and in combination had significantly reduced tumor weights of 32% (P &lt; 0.05), 51% (P &lt; 0.001), and 69% (P &lt; 0.001), respectively. Gemcitabine, VEDT, and the combination of the 2 drugs significantly elicited apoptotic cell death in the circulating tumor cells (CK18) in the blood by 45% (P &lt; 0.01), 71% (P &lt; 0.001), and 84% (P &lt; 0.001), respectively, compared with vehicle. Overall, there was prolonged survival and delayed progression of pancreatic intraepithelial neoplasia in LSL-Kras(^{G12D/+;Pdx-1-Cre}) mice. The oral intake of δ-tocotrienol alone and in combination with gemcitabine prolonged survival in the LSL-Kras(^{G12D/+;Pdx-1-Cre}) mouse model. Only the PSN1 pancreatic cancer cell line with a SMAD4/DPC4 homozygous deletion was sensitive to nontoxic 5-fluorouracil and δ-tocopheryl succinate combination. A 20 μM dose of δ-tocopheryl succinate inhibited mouse embryonic fibroblast wild-type cells, but not mouse embryonic fibroblast double knockout growth. Only PSN1 cells were sensitive to nontoxic 5-fluorouracil and δ-tocopheryl succinate combination. SMAD4/DPC4 transfection restored PSN1 resistance to the effects of combined 5-fluorouracil and δ-tocopheryl succinate effects. The overall effect of VES was minimal in pancreatic cancer cells, with any sensitivity appearing to be correlated with SMAD4/DPC4 homozygous deletion and Bax/Bak expression.</td>
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Analogs of vitamin E epitomized by α-Tocopherol succinate for pancreatic cancer treatment: in vitro results induce caution for in vivo applications, Greco et al. (63)

Vitamin E succinate inhibits survivin and induces apoptosis in pancreatic cancer cells, Patacsil et al. (60)

Vitamin E | VES | Concentrations ranging from 25.1 to 51.3 μM to measure IC\(_{50}\) and 40-80 μM to evaluate apoptosis. | To explore the anti-proliferative action of VES and its effects on inhibitors of apoptosis proteins in pancreatic cancer cells. | When inhibition of proliferation in PANC-1 cells was evaluated, varying concentrations of VES treatment for 24, 48, and 72 h resulted in IC\(_{50}\) values of 40.2, 36.6, and 35.06 μM, respectively. Similarly, the IC\(_{50}\) values for COLO-357 and ASPC-1 were 51.3, 39.2, and 25.1 μM and >80, 48.7, and 50 μM for the 24, 48, and 72 h treatments, respectively. Apoptotic cells were observed to have increased from 4% to 8% compared with the control at 40 μM VES, and significantly increased to 25% at 80 μM VES (P < 0.01). In summary, VES inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. | |

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<td>Peptide YY augments growth inhibition by vitamin E succinate of human pancreatic cancer cell growth, Heisler et al. (75)</td>
<td>Vitamin E</td>
<td>VES</td>
<td>40 μL VES or RRR-α-tocopherol succinate was added to wells to create a total concentration in the wells of 1 pg/mL.</td>
<td>To confirm the hypothesis that Peptide YY and VES would inhibit pancreatic tumor cells more effectively in combination than when administered individually.</td>
<td>The combination group treated with peptide YY and vitamin E succinate showed significant inhibition of cell growth compared with the groups treated with each agent individually at the intervals of 24 (P &lt; 0.02), 48 (P &lt; 0.02), and 72 h (P &lt; 0.001). The combination group also showed significant inhibition of viability at 24 h (P &lt; 0.01), 48 h (P &lt; 0.01), and 72 h (P &lt; 0.01). VES and peptide YY both inhibit growth of pancreatic cancer cells in vitro with a significant effect when used in combination.</td>
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<td>Vitamins A and D but not E and K decreased the cell number in human pancreatic cancer cell lines, Ohlsson et al. (76)</td>
<td>Vitamins A, D, E, and K</td>
<td>ATRA, EB1089, VES, VK</td>
<td>Concentrations of 0.1 nM–0.1 mM were used when Vitamin D analogue EB1089 was studied alone and 10 pM–10 μM when used in a combination of EB1089 and retinoids: 0.1 nM–1 μM VES and 0.1 nM–0.1 mM VK was used.</td>
<td>To study the effect of fat-soluble vitamins on pancreatic cancer cells, alone or in combination.</td>
<td>The vitamin A and D analogues decreased the pancreatic cancer cell number when high concentrations of 0.1 mM were administered. A combination of retinoids and the vitamin D analogue EB1089 did not enhance the effect. VES inhibited cell growth to a small extent (maximal 26%) with no effect found in 4 out of 7 cell lines tested, whereas vitamin K increased the pancreatic cancer cell number in 3 of 7 cell lines, with the most potent effects at 0.1 nM–0.1 μM (P &lt; 0.01). VES did not have significant effects on cell growth inhibition in pancreatic cancer cells.</td>
</tr>
<tr>
<td>Menadione induces both necrosis and apoptosis in rat pancreatic acinar AR4–2J cells, Sata et al. (66)</td>
<td>Vitamin K</td>
<td>VK-3</td>
<td>Concentrations of 0, 1, 5, 10, 20, and 100 μM menadione</td>
<td>To evaluate the action of menadione on cell proliferation and integrity of the rat pancreatic acinar cell line, AR4–2J.</td>
<td>A high concentration of 100 μM menadione induced cell death within 4 h, whereas ~80% of cells survived after 24 h of treatment with 20 μM menadione.</td>
</tr>
<tr>
<td>The utility of Vitamin K3 (Menadione) against pancreatic cancer, Osada et al. (77)</td>
<td>Vitamin K</td>
<td>VK-3</td>
<td>50 μM and 100 μM VK-3</td>
<td>To evaluate the efficacy of VK-3 against pancreatic cancer, and the molecular mechanism of VK-3 or gemcitabine-induced inhibition of proliferation.</td>
<td>VK-3 induced rapid phosphorylation of ERK and JNK within 30 min of application. JNK was phosphorylated after treatment of 50 μM VK-3, but the response diminished after 60 min. In response to 100 μM VK-3, ERK phosphorylation was observed after 10 min, and tyrosine phosphorylation was observed after 60 min. 50 μM VK-3 also activated apoptosis, as shown by caspase-3 activation and PARP cleavage of the 112-kDa form within 6 h of treatment. The IC50 for VK-2 was estimated to be 153 μM in MiaPaCa-2 cells and 708 μM with geranylgeraniol during a 4 d incubation.</td>
</tr>
<tr>
<td>Vitamin K2 selectively induced apoptosis in ovarian TYK-nu and pancreatic MIA PaCa-2 cells out of eight solid tumor cell lines through a mechanism different from geranylgeraniol, Shibayama-Imazu et al. (65)</td>
<td>Vitamin K</td>
<td>VK-2</td>
<td>200 μM VK-2</td>
<td>To examine the effects of VK-2, which has a geranylgeranyl side chain, on various lines of cells derived from human solid tumors and compare them with the effects of geranylgeraniol, which is shown to induce apoptosis in solid tumors.</td>
<td>The IC50 for VK-2 was estimated to be 153 μM in Mia PaCa2 cells and 708 μM with geranylgeraniol during a 4 d incubation.</td>
</tr>
<tr>
<td>Naturally occurring K vitamins inhibit pancreatic cancer cell survival through a caspase-dependent pathway. Showalter et al. (78)</td>
<td>Vitamin K</td>
<td>VK-1, VK-2</td>
<td>200 μM VK-1 and 200 μM VK-2 vs. serial dilutions of 5-fluorouracil.</td>
<td>To determine whether VK-1 and VK-2 may be used to decrease pancreatic cancer cell survival.</td>
<td>The IC50 for VK-1 was estimated to be 150 μM and 75 μM for VK2 in the sensitive cell lines MiaPaCa2 and PLS. In comparison, 5-fluorouracil achieved equal and complete death at 1 μM in all 4 cell lines.</td>
</tr>
</tbody>
</table>
Tumor killing by ascorbate may also occur through oxidative phosphorylation via the Warburg effect. The loss of glucose to the pentose phosphate pathway may also result in decreased ATP, leading to cell death.

Autophagy. Tumor killing by ascorbate may also occur through a caspase-independent autophagic pathway (37, 84). Autophagy is a dynamic process characterized by the formation of a double membrane organelle that engulfs cellular proteins and cytoplasmic organelles and subsequently fuses with lysosomes to facilitate their degradation (37). Light chain 3-II (LC3-II) protein is a product of autophagy, which becomes activated through lipidation of LC3. However, experiments showed that, when these cells were pretreated with catalase, they demonstrated a decrease in LC3-II protein, suggesting that the role of autophagy in cells may also require ROS to occur (38).

Preclinical studies of vitamin C in pancreatic oncogenesis

Laboratory studies have shown that ascorbate-induced cytotoxicity in pancreatic cancer occurs through generation of ROS. Chen et al. (36, 38) observed that pharmacologic doses of ascorbate create toxicity through the generation of ROS via formation with hydrogen in a dose-dependent manner. These findings were further confirmed by Du et al. (37) when they performed experiments showing that pretreatment of MiaPaCa-2 cells with catalase or PEG-catalase in the media prevented ascorbate-induced cytotoxicity. In addition, they showed that ascorbate decreased pancreatic cancer cell survival, and this was reversed by adding cells that scavenged hydrogen peroxide. There was an increased survival compared with controls without treatment ($P < 0.0001$), along with a different slower tumor growth rate ($P < 0.01$) (37). Another study demonstrated concordant results showing ascorbate-treated mice had significantly decreased growth rates of pancreatic tumors as well ($P < 0.05$), and the addition of catalase to the medium ameliorated the death of pancreatic cancer cells exposed to 10 mM ascorbate for 1 h, indicating cytotoxicity was mediated by hydrogen peroxide (68).

Other studies also have evaluated ascorbate-induced cell death through either a caspase-independent method associated with autophagy and/or through decreases in intracellular ATP concentrations. MiaPaCa-2 cells were treated with ascorbate, and an increase in LC3-II proteins was observed, which is in accordance with ascorbate-inducing autophagy. However, when these cells were pretreated with catalase, they demonstrated a decrease in LC3-II protein, suggesting that ROS in addition may play a role (38). One study showed that ROS induced autophagic cell death in transformed and cancer cells but did not induce autophagic cell death in nontransformed cells (85). Du et al. (37) also evaluated whether ascorbate decreases intracellular ATP concentrations. They found that ATP concentrations demonstrated a dose-dependent decline with ascorbate treatment, whereas pretreatment with catalase prevented this decline (37). Overall, however, they felt that loss of ATP may not have a role in ascorbate-induced cytotoxicity after they obtained conflicting results while infecting cells with adenoviral vectors to create intracellular overexpression of catalase (37). They also noticed that NAD(P)^+ : NAD(P)H concentrations only revealed a very minor change after ascorbate treatment and the ratio remained unchanged (37). When evaluating whether ATP depletion via PARP-1 may be a mechanism of ascorbate-induced cell death, as suggested by Chen et al. (37), it was found that experiments that used a specific PARP-1 inhibitor did not reverse the decrease in ATP depletion or cause an ascorbate-induced decrease in clonogenic survival. A study performed on tumor xenografts in mice with the use of gemcitabine and ascorbate demonstrated an effect despite notable differences in tumor xenograft gemcitabine resistance. Ascorbate treatment alone exhibited a 40% inhibitory effect on growth rate in gemcitabine-responsive tumors (PANC-02) and gemcitabine-nonresponsive tumors (PANC-1) (69).
TABLE 3

Completed clinical trials of vitamins in pancreatic cancer

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Nutrient</th>
<th>Chemical form</th>
<th>Concentration</th>
<th>Biomarker/endpoint studied</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I study of the histone deacetylase inhibitor entinostat in combination with 13-cis RA and INF-α in patients with solid tumors, Pili et al. (9)</td>
<td>Vitamin A</td>
<td>CRA + entinostat</td>
<td>Fixed dose of 1 mg CRA/ (kg - d) + starting dose 4 mg/m² entinostat escalated by 1 mg/m² increments until dose-limited toxicity was observed at the second dose amount of 5 mg/m²</td>
<td>To determine the safety, tolerability, a pharmacokinetic/pharmacodynamic profiles of the HDAC inhibitor entinostat in combination with CRA in patients with solid tumors</td>
<td>There was a significant decrease in C_{min} between cycle 1 and cycle 2 (P = 0.004), but no difference within a cycle or by dose amount (P &gt; 0.05). The average C_{min} during cycle 1 and cycle 2 was 1949 ± 103.5 and 1318 ± 793 ng · mL⁻¹, respectively. Overall, although no objective responses were observed, prolonged stable disease occurred in pancreatic cancer patients. Recommended phase II doses are 1 mg CRA/(kg · d) + 4 mg/m² entinostat once weekly.</td>
</tr>
<tr>
<td>Chemoradioimmunotherapy in locally advanced pancreatic and biliary tree adenocarcinoma: a multicenter phase II study, Recchia et al. (30)</td>
<td>Vitamin A</td>
<td>CRA + IL-2</td>
<td>Maintenance immunotherapy of 5 mg CRA/kg orally 2 times/d with food + 1.8 × 10⁶ IU IL-2 3 times/wk for 3 wk per month in responders and patients with stable disease until progression. During the 2nd year, the same therapy was given 2 wk/mo, and then 1 wk/mo during the 3rd year and thereafter.</td>
<td>To evaluate the antitumor activity and toxicity of a multistep treatment in patients with locally advanced, inoperable, or incompletely resected pancreatic and biliary tree adenocarcinomas</td>
<td>In the 14 patients treated with maintenance immunotherapy, the CA19-9 test result decreased from a baseline mean of 31,173 to a mean value of 3878 (P = 0.0001). After 1 yr, VEGF also decreased. There was a median progression-free survival of 16.2 mo with overall survival still pending after an average of &gt;24 mo.</td>
</tr>
<tr>
<td>Pilot phase II trial of 13-cis RA and INF-α combination therapy for advanced pancreatic adenocarcinoma, Moore et al. (31)</td>
<td>Vitamin A</td>
<td>CRA + INF-α</td>
<td>CRA (1 mg · kg⁻¹ · d⁻¹) and INF-α (6 million units/d)</td>
<td>To evaluate antitumor activity and toxic effects of the combination of INFs and retinoids in patients with advanced pancreatic cancer</td>
<td>Well tolerated, but no objective antitumor activity was noted against pancreatic adenocarcinomas at the dose and schedule used. Toxicities were mild and reversible.</td>
</tr>
<tr>
<td>A phase II pilot trial of 13-cis RA and INF-α in patients with advanced pancreatic carcinoma, Brembeck et al. (32)</td>
<td>Vitamin A</td>
<td>CRA + INF-α</td>
<td>CRA (1 mg · kg⁻¹ · d⁻¹) and INF-α (6 million units/d)</td>
<td>To examine the feasibility and tolerability of a combination therapy of CRA and INF-α in patients with advanced unresectable pancreatic cancer</td>
<td>The median survival of all patients included was 7.7 mo (range: 0.9–23.91 mo; 95% CI: 5.6, 9.1). The median survival in patients with stage III carcinoma (n = 55) was 8.7 mo (range: 6.8–23.91 mo; 95% CI: 7.1, 10.2). Seventeen patients with stage IV pancreatic carcinoma had a median survival of 7.4 mo (range: 0.9–19.9 mo; 95% CI: 4.3, 10.4). In summary, treatment was well tolerated, but no objective responses reported.</td>
</tr>
<tr>
<td>Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer, Monti et al. (79)</td>
<td>Vitamin C</td>
<td>Ascorbic acid + gemcitabine and erlotinib</td>
<td>An 8 wk cycle of ascorbic acid infusions at starting dose amounts of 50 g, 75 g, and 100 g, with 3 infusions/wk with the use of a dose escalation design + standard treatment of gemcitabine and erlotinib.</td>
<td>To assess the safety and response to treatment of intravenous ascorbic acid added to gemcitabine and erlotinib in stage IV metastatic pancreatic cancer patients.</td>
<td>Ascorbic acid concentrations were measured immediately after infusion end in the 6 patients receiving the 2 upper dosage tiers of either 75 or 100 g per infusion. The plasma ascorbate concentration was between 25.3 and 31.9 mM in these patients.</td>
</tr>
</tbody>
</table>

(Continued)
Clinical studies of vitamin C in pancreatic oncogenesis

Vitamin C has shown promise as an adjunct to chemotherapy for cancer treatment. Reviews have varied on the efficacy of high-dose ascorbate as a therapeutic agent, with limited human data on the use of pharmacologic ascorbate, despite current wide use by complementary and alternative medicine practitioners. The use of vitamin C in cancer treatment remains largely empirical, with mixed results from clinical trials and preclinical studies.

### Table 3 (Continued)

<table>
<thead>
<tr>
<th>Study (reference)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial, Welsh et al. (80)</td>
<td>Vitamin C</td>
<td>Ascorbic acid + gemcitabine</td>
<td>Twice weekly intravenous ascorbate (50–125 g) for a targeted postinfusion plasma concentration of ≥20 mM with concurrent gemcitabine. Average treatment duration was 6 mo.</td>
<td>To establish safety and tolerability of pharmacological ascorbate combined with gemcitabine in patients with biopsy-proven stage IV pancreatic cancer.</td>
<td>Mean plasma ascorbate trough levels were significantly higher than baseline (83 compared with 44 μM, P &lt; 0.001). Of the 9 patients who completed at least 1 mo of protocol therapy, time to progression was 26 ± 7 wk, whereas overall survival was 13 ± 2 mo. The strategy was also to continue treatment until progression per RECIST. Six of 9 patients maintained or improved their performance status.</td>
</tr>
<tr>
<td>Phase II study of calcitriol-enhanced docetaxel in patients with previously untreated metastatic or locally advanced pancreatic cancer, Blank et al. (81)</td>
<td>Vitamin D</td>
<td>Calciferol</td>
<td>Calciferol 0.5 μg/kg was administered orally over 4 h on day 1 (4 doses divided equally every hour), followed by docetaxel 36 mg/m² intravenously over 15–30 min on day 2. Treatment was administered weekly for 3 consecutive weeks, followed by 1 wk without therapy.</td>
<td>To determine the safety and efficacy of weekly high-dose oral calcitriol and docetaxel given to patients with inoperable, incurable pancreatic cancer. Primary endpoint was time-to-progression.</td>
<td>Three patients (12%) experienced a partial response, 7 (28%) had stable disease, and 9 (36%) frankly progressed. Median time-to-progression was 15 wk, or 3.6 mo (95% CI: 1.9, 5.6). Median overall survival was 24 wk, or 5.6 mo (95% CI: 4.1, 9.8). Modest increase in time to progression when compared with historical findings with the use of single-agent docetaxel.</td>
</tr>
<tr>
<td>A phase II trial of the vitamin D analogue Seocalcitrol (EB1089) in patients with inoperable pancreatic cancer, Evans et al. (50)</td>
<td>Vitamin D</td>
<td>Seocalcitrol (EB1089)</td>
<td>Once daily oral treatment with 20 mg seocalcitrol with dose escalation every 2 wk until hypercalcemia occurred, this being defined as either a fasting albumin-corrected serum calcium of ≥2.80 mM or nonfasting albumin-corrected serum calcium of &gt;3.00 mM, followed by maintenance therapy.</td>
<td>To determine the safety and efficacy of treatment with seocalcitrol, a vitamin D analogue, in patients with inoperable pancreatic cancer. Given that this is a nonrandomized phase II study, time to disease progression is not a valid study endpoint.</td>
<td>The individual maximum tolerated dose for the patient population that received seocalcitrol ranged from 5 to 60 μg/d. No objective responses were observed, with 5 of 14 patients having stable disease with a duration of 62–532 d (median: 168 d). There was no correlation between individual dose and either time-to-treatment failure or overall survival.</td>
</tr>
<tr>
<td>A phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of vitamin E δ-tocotrienol administered to subjects with resectable exocrine neoplasia (abstract), Springett et al. (83)</td>
<td>Vitamin E</td>
<td>δ-Tocotrienol</td>
<td>Oral administration of vitamin E δ-tocotrienol 1600 mg twice daily for 14 ± 2 d before surgery, and 1 dose the day of surgery.</td>
<td>To determine the recommended dose, safety, and tolerability of vitamin E δ-tocotrienol, defined as the biological effective dose that induces significant apoptosis in the pancreatic neoplastic cells of resected tumor specimens in a dose 5.6 times the predicted biological effective dose.</td>
<td>Preliminary findings revealed that δ-tocotrienol had no obvious toxicity at doses up to 3200 mg/d.</td>
</tr>
</tbody>
</table>

1 The minimum or “trough” concentration (Cₘᵋᵣ₉) of a drug observed after its administration and just before the administration of a subsequent dose at steady state (Cₛₛᵣ₉). CA19-9, cancer antigen 19-9; CRA, 13-cis retinoic acid; HDAC, histone deacetylase; RA, retinoic acid; RECIST, Response Evaluation Criteria in Solid Tumors; VEGF, vascular endothelial growth factor.
practitioners. One phase I clinical trial, performed over 8 wk, showed that ascorbic acid may have some efficacy when used in combination with gemcitabine and erlotinib. By Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 criteria, 7 patients had stable disease and 2 had progressive disease (79). Eight of 9 patients had a decrease in the size of their primary tumors (79). Another phase I clinical trial performed with ascorbate and gemcitabine in patients with stage IV pancreatic adenocarcinoma contrasted with this previous one, given that the infused ascorbate dose based on plasma concentrations achieved after infusion was completed to achieve a peak target goal of ascorbate concentrations ≥20 mM (80). The strategy of this trial was also to continue treatment until progression per the RECIST. Six of 9 patients maintained or improved their performance status (80). Although results are limited by a small sample size, a phase II clinical trial with patients randomly assigned to ascorbic acid plus gemcitabine or gemcitabine alone for longer treatment duration is warranted.

Currently, there are 4 active or recruiting clinical trials evaluating ascorbic acid and its role in pancreatic cancer treatment. There are 2 actively recruiting phase II clinical trials. One is a randomized study evaluating the safety and efficacy of intravenous ascorbic acid with gemcitabine, 5-fluorouracil (5FU), leucovorin, irinotecan, and oxaliplatin, followed by gemcitabine, 5FU, leucovorin, irinotecan, and oxaliplatin plus low doses of docetaxel and mitomycin C; the other is using intravenous ascorbic acid in combination with gemcitabine and erlotinib for metastatic pancreatic cancer treatment (Table 4). There also is a phase I clinical trial actively recruiting on the combination of gemcitabine, ascorbate, and radiation therapy for pancreatic cancer (Table 4). A fourth phase I/IIa clinical trial is currently active and is evaluating the safety and efficacy of using high-dose intravenous ascorbic acid with gemcitabine in patients with advanced or metastatic pancreatic cancer not eligible for surgical resection (Table 4). These clinical trials are crucial in providing us with additional information on the efficacy of vitamin C as a treatment option for patients with pancreatic cancer.

**Conclusion**

Vitamin C has great potential as an adjunctive agent in the treatment of pancreatic cancer. It exerts its antitumor effects through either the creation of ROS, autophagy, or intracellular ATP depletion (Table 1). Preclinical studies focused on intravenous-dosed ascorbate-induced cytotoxicity via the formation of ROS, selectively killing cancer cells (Table 2). Studies also showed increased survival and slower tumor growth rates in ascorbate-treated mice, and decreased growth rates in mice on gemcitabine and ascorbate, despite a notable difference in gemcitabine resistance between groups (Table 2). When combined with gemcitabine, 8 of 9 patients in one study had decreases in the size of their primary tumors and 6 of 9 patients in another study demonstrated maintained or improved performance status (Table 3). The use of Vitamin C in combination with gemcitabine shows very promising results and it may be used in the future as an adjunctive agent for pancreatic cancer; however, further and larger clinical trials are needed to confirm its efficacy.

**Vitamin D**

Vitamin D was originally discovered in 1922 as a substance in cod liver oil that cured rickets (87). Around that time, it was also discovered that children with rickets could be cured by exposure to sunlight, thus suggesting a relation between the two (88). Greater than 90% of the vitamin D requirement comes from exposure to sunlight, with the rest from diet (89). Oily fish such as salmon, mackerel, and sardines are excellent sources, with very few foods naturally containing vitamin D. Normal serum plasma concentrations of Vitamin D that are considered adequate for bone and overall health are ≥50 nM (90). However, the clinical application of 1,25-dihydroxycholecalciferol [1-α,25(OH)2D3] is impeded by the lethal side effects of hypercalcemia and hypercalciuria. Therefore, several analogues have been developed that have fewer side effects that are equally as or even more potent than 1-α,25(OH)2D3 in inhibiting tumor growth mediated by cell-cycle arrest, stimulating differentiation, and promoting apoptosis (45, 47, 91–94). Many studies are currently evaluating the safety, efficacy, and antitumor activity of vitamin D analogues alone or in combination with chemotherapy. We will review the proposed mechanisms behind the anticarcinogenic properties of vitamin D, along with ongoing clinical and experimental research evaluating its role in pancreatic cancer intervention.

**Mechanisms of action of vitamin D in pancreatic cancer**

**Vitamin D receptor agonist.** Vitamin D comes in 2 biologically inactive forms, vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol), which require activation in the liver and kidney. When either cholecalciferol, formed from exposure of ultraviolet irradiation on 7-dehydrocholesterol stored in the basal and suprabasal layers of skin, or dietary ergocalciferol enters the blood stream, it is carried by vitamin D binding proteins (95). Vitamin D is transported first to the liver, where it is enzymatically hydroxylated to 25-hydroxycholecalciferol (calcidiol) by vitamin D-25-hydroxylase (96). Bound to vitamin D binding proteins, 25-hydroxycholecalciferol re-enters the blood stream and is transported to the kidneys and hydroxylated by 25-hydroxyvitamin D-1-α-hydroxylase to form hormonally active 1-α,25(OH)$_2$D$_3$ (96). Upon binding of 1-α,25(OH)$_2$D$_3$ to a vitamin D receptor (VDR) in target tissues, 2 independent sites are formed. One facilitates association with a heterodimer, an RXR, required for specific DNA binding to a vitamin D response element. The second is essential for recruitment of coregulatory proteins that modulate gene expression (39). 1-α,25(OH)$_2$D$_3$ exhibits antiproliferative, prodifferentiating, anti-inflammatory, and proapoptotic properties via VDR activation in target tissues such as the pancreas, prostate, colon, breast, liver, and lung, which express VDR (95). VDR and 1-α-hydroxylase are also expressed in pancreatic cancer cells. Vitamin D analogues act via the same mechanism as vitamin D with fewer side effects.

**Regulation of the cell cycle.** Vitamin D displays antiproliferative effects through alteration of several key players in the cell cycle. This occurs through $G_0/G_1$ cell cycle arrest and is mediated directly or indirectly by either cyclins,
<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Study</th>
<th>Clinicaltrials.gov identification</th>
<th>Endpoint studied</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>No active or recruiting clinical trials</td>
<td></td>
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</tr>
<tr>
<td>Vitamin C</td>
<td>Phase 2 Trial of G-FLIP (Low Doses Gemcitabine, 5-fluorouracil, Leucovorin, Irinotecan &amp; Oxaliplatin), Followed by G-FLIP-DM (G-FLIP + Low Doses Docetaxel &amp; Mitomycin C), When Used in Combination With Vitamin C, in Patients With Advanced Pancreatic Cancer sponsored by Bruckner Oncology</td>
<td>NCT01905150</td>
<td>A randomized study evaluating the safety and efficacy of intravenous infusion of high doses of ascorbic acid and lose doses of several anticancer drugs, including gemcitabine, fluorouracil, leucovorin, irinotecan and oxaliplatin.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Phase II, Open Label Study of Intravenous Ascorbic Acid in Combination With Gemcitabine and Erlotinib in the Treatment of Metastatic Pancreatic Cancer sponsored by Thomas Jefferson University</td>
<td>NCT01555489</td>
<td>The investigators recently completed a phase I study of intravenous ascorbic acid plus standard chemotherapy (gemcitabine and erlotinib) in patients with metastatic pancreatic cancer. It was determined that the target ceiling dose of 100 g ascorbic acid is safe when given with the chemotherapy. This phase II trial is an initial test of efficacy of the 100 g dose of ascorbic acid, which will be given with the same standard chemotherapy. This open label study will recruit up to 35 subjects with metastatic pancreatic cancer who will receive ascorbic acid combined with gemcitabine and erlotinib as frontline treatment. The phase I data suggest that ascorbic acid, when given in combination of vitamin C with gemcitabine and erlotinib, may result in some tumor response, and the goal of this study is to better evaluate the response and confirm initial safety data.</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Translation of In Vitro and In Vivo Ascorbate Research Into a New Treatment Option for Pancreatic Cancer: Phase I/IIa Clinical Trial sponsored by Jeanne Drisko, MD, CNS, FACN</td>
<td>NCT01364805</td>
<td>To determine the safety and efficacy of using high-dose intravenous vitamin C in combination with gemcitabine chemotherapy in patients with locally advanced or metastatic pancreatic cancer not eligible for surgical resection.</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Gemcitabine, Ascorbate, Radiation Therapy for Pancreatic Cancer, Phase I sponsored by Joseph J. Cullen</td>
<td>NCT01852800</td>
<td>A phase I (first in humans) study testing the safety of adding high dose ascorbate to standard radiation and chemotherapy for treatment of pancreatic cancer.</td>
<td>Actively recruiting</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>No active or recruiting clinical trials</td>
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</tr>
<tr>
<td>Vitamin E</td>
<td>A Phase I Dose-Escalation Study of the Safety, Pharmacokinetics, and Pharmacodynamics of Vitamin E δ-tocotrienol Administered to Subjects With Resectable Pancreatic Exocrine Neoplasia sponsored by H. Lee Moffitt Cancer Center and Research Institute</td>
<td>NCT00985777</td>
<td>To determine the safest dose of the study drug vitamin E δ-tocotrienol, how often it should be taken, and how well people with pancreatic tumors tolerate vitamin E δ-tocotrienol.</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>A Phase I Dose-Escalation Study of the Safety and Pharmacokinetics of Vitamin E δ-Tocotrienol Following Multiple Dose Administration in Healthy Subjects sponsored by H. Lee Moffitt Cancer Center and Research Institute</td>
<td>NCT01446952</td>
<td>The principle investigator believes that vitamin E δ-tocotrienol will slow the progression of pancreatic cancer cells. This study will determine the safety and tolerability of vitamin E δ-tocotrienol in healthy participants before administration to cancer patients. The investigators will do this by giving participants a dose of ≤1600 mg twice a day, not to exceed 3200 mg/d total for 14 consecutive days.</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>A Phase I Dose-Escalation Study of the Safety and Pharmacokinetics of Vitamin E δ-Tocotrienol Following Single Dose Administration in Healthy Subjects sponsored by H. Lee Moffitt Cancer Center and Research Institute</td>
<td>NCT01446952</td>
<td>This is a phase I, open-label, nonrandomized study of vitamin E δ-tocotrienol in subjects with resectable pancreatic tumors. The primary objective is to evaluate the safety and tolerability of vitamin E δ-tocotrienol and to determine the minimally effective dose or maximum tolerated dose of vitamin E δ-tocotrienol administered once.</td>
<td>Active, not recruiting</td>
</tr>
</tbody>
</table>

1 Information obtained from clinicaltrials.gov, accessed on 29 June 2015 (86).
cyclin-dependent kinases (CDKs), or cyclin-dependent kinase inhibitors (CDKIs). In the late G1 phase, CDKs phosphorylate the retinoblastoma protein (Rb), thus displacing transcription factor E2F-1 and allowing the activation of the gene expression essential for the cell to enter the S phase for DNA replication. 1-α,25(OH)2D dephosphorylates the Rb, which permits binding to E2F-1 and halts the progression of the cell cycle (43). The CDKs p21 and p27 also play a role in cell cycle progression as tumor repressors that are responsible for G1 cell cycle arrest and withdrawal from the cell cycle. They contain vitamin D response elements within their promoter regions and are targets of the 1-α,25(OH)2D/VDR complex in many cell types (43, 44). It has been demonstrated that vitamin D analogues upregulate p21 and p27 as an early event to induce growth inhibition of pancreatic cancer cell lines (45). 19-Nor-2α-(3-hydroxypropyl)-1-α,25(OH)2D3 (MART-10), a vitamin D analogue, is much more potent than vitamin D in upregulating p21 and p27 and may cause cell cycle arrest to a greater extent (70). This is attributed to the higher binding affinity to VDR and the greater bioavailability of MART-10 (70). Several studies have shown that many cancer cell lines, such as those for prostate, colon, breast, and lung cancers, as well as melanoma, contain VDR and demonstrate growth inhibition when exposed to 1-α,25(OH)2D (89, 97–99). VDR is also expressed in both benign and malignant pancreatic cells. Moreover, there are many upstream effects involved in antiproliferation, such as downregulation of several cyclins, upregulation of CDK (p19, p21, and p27), upregulation of TGF-β and insulin-like growth factor binding protein 3 and their signaling pathways, and downregulation of epidermal growth factor receptors, c-myc, jun, and fos (43). MART-10 also acts via downregulation of cyclin D3, CDK4, and CDK6 in BxPC-3 cells to inhibit cell growth via cell cycle arrest at G0/G1 (70). 25-hydroxycholecalciferol [25(OH)D3] also inhibited the growth of 3 of 4 pancreatic cancer cell lines, which correlated with the level of induction of p21 and p27 and with the induction of cell cycle arrest at the G1/S checkpoint (47). This is secondary to the presence of 25-hydroxyvitamin D-1-α-hydroxylase in both normal and malignant pancreatic tissue (47).

**AMP-activated protein kinase–dependent mechanisms.** AMP-activated protein kinase (AMPK), a serine/threonine protein kinase, is a key energy sensor that regulates many metabolic pathways. It works by switching off biosynthetic pathways as a mechanism to protect against environmental stress. AMPK phosphorylates and downregulates many key biosynthetic enzymes in metabolic pathways such as acetyl-CoA carboxylase (ACC) in FA synthesis, glycogen synthase in glycogen synthesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in isoprenoid/sterol synthesis, and RSC2 and regulatory-associated protein of mTOR to inhibit the mammalian target of rapamycin (mTOR) (100). AMPK is activated with a rise in the AMP/ATP ratio, which occurs with ATP depletion from environmental stressors such as heat shock and hypoxia to prevent biosynthetic processes. 5-Amino-imidazole-4-carboxamide ribonucleoside (AICAR) is metabolized to 5-amino-imidazole-4-carboxamide ribonucleoside monophosphate, which mimics the effect of AMP (100). AICAR acts as an AMPK agonist by activating AMPK and subsequent ACC phosphorylation pathways. Rattan et al. (40) demonstrated that AICAR inhibits the growth of several cancer cell lines via arrest in S phase and activation of cell cycle proteins p21, p27, and p53, as well as through activation of AMPK and inhibition of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway. When AICAR, with no cell-regulatory activity, was combined with 1,25-dihydroxyvitamin D3-3-bromoacetate (1,25(OH)2D3-3-BE), there was an increase in cell growth inhibition of pancreatic cancer cells from 60% to 85%; however, when used alone, AICAR was too low in potency to reduce the growth of BxPC-3 cells (41). These results suggest that 1,25(OH)2D3-3-BE is strongly accentuated by AICAR in BxPC-3 cells.

**Inhibition of the PI3K/Akt pathway.** Akt is also a serine/threonine kinase that is involved in signal transduction through the PI3K/Akt pathway. Akt regulates tumorigenesis, cell proliferation, growth, and survival and is activated by the binding of the lipid kinase PI3K, which generates phosphatidylinositol 3,4,5 trisphosphate at the plasma membrane (42). Akt binds phosphatidylinositol 3,4,5 trisphosphate, resulting in its translocation to the plasma membrane, and it is activated by a dual phosphorylation mechanism. One of Akt’s primary functions is to directly promote cell growth and G1 cell cycle progression through regulation of the mTOR signaling pathway. The mTOR signaling pathway likely represents a major mediator of Ras-driven oncogenesis and plays a pivotal role in the proliferation and survival of pancreatic cancer cells (101). AICAR also works via inhibition of the PI3K/Akt phosphorylation pathway to cause pancreatic cancer cell growth inhibition. Persons et al. (41) found that the combination of AICAR and 1-α,25(OH)2D3-3-BE almost completely eliminated Akt phosphorylation. When analyzed separately, AICAR strongly inhibited Akt phosphorylation, and 1-α,25(OH)2D3-3-BE was considerably weaker (41). This suggests that AICAR in addition causes growth inhibition via the PI3K/Akt pathway.

**Decreased cellular migration and invasion.** MART-10, a vitamin D analogue, was recently demonstrated to display antiproliferative properties on pancreatic cancer via decreased cellular migration and invasion. MART-10 and 1-α,25(OH)2D3 inhibited cellular migration and invasion of BxPC-3 with wild-type K-ras, and PANC with mutant K-ras, with MART-10 much more potent than 1-α,25(OH)2D3 (46). EMT is an important developmental process in embryology that allows epithelial cells to gain mesenchymal cell markers, leading to differentiation into a variety of cell types. It is also an important mechanism in the progression of cancer, allowing malignant cells to gain stem cell–like properties, along with increased invasion, migration, and subsequent metastasis. Whereas RA could not inhibit the migration and EMT of tumor cells directly, as previously discussed regarding the efficacy of vitamin A, MART-10 and 1-α,25(OH)2D3 inhibited EMT in BxPC-3 and PANC cells via downregulation of Snail.
and Slug, transcription factors known to trigger EMT, leading to the repression of the mesenchymal cell marker, vimentin (46). Another mechanism occurs through matrix metalloproteinases (MMPs), which are collagenases that digest the basement membrane, allowing cell transfer from one location to another, and which show increased expression in malignant cells. In BxPC-3 cells, MART-10 and 1-α,25(OH)_{2}D_{3} also inhibited MMP-2 and MMP-9 expression in extracellular components, as determined by Western blot and zymography (46). Vitamin D also may inhibit cellular migration and invasion through cadherins, calcium-dependent cell adhesion molecules that play a vital role in cell and tissue structure maintenance and embryonic development (102). The low expression of E-cadherin, which regulates cell-to-cell adhesion, has been previously shown in multiple studies to correlate with an invasive and undifferentiated phenotype in many carcinomas, including pancreatic cancer (103–108). The overexpression of N-cadherin correlated with invasiveness in breast carcinoma cells and may be associated with more aggressive disease in pancreatic cancer (109, 110). MART-10 and 1-α,25(OH)_{2}D_{3} increased E-cadherin expression in BxPC3 and PANC cells, with only MART-10 decreasing N-cadherin expression in BxPC-3 cells (46). Collectively, these data demonstrate the ability of vitamin D to potentially inhibit the metastatic potential of pancreatic cancer cells.

**Preclinical studies of vitamin D in pancreatic oncogenesis**

Several in vitro studies have established a direct effect from 1-α,25(OH)_{2}D_{3} analogues, with a decrease in pancreatic tumor cell growth. A VDR-alkylating derivative of 1-α,25(OH)_{2}D_{3}, 1,2-dihydroxyvitamin D3–3-bromoacetate (1-α,25(OH)_{2}D_{3}–3-BE), strongly inhibited pancreatic tumor cell growth in several cell lines to a greater extent than 1-α,25(OH)_{2}D_{3} (41). This was further accentuated when combined with AICAR, an activator of AMPK/ACC phosphorylation pathways and an inhibitor of Akt phosphorylation (41). Chiang et al. (70) studied MART-10, a new vitamin D analogue, and demonstrated its potent antiproliferative effects on pancreatic tumor cells in vitro and in vivo without causing hypercalcemia. Because metastasis is the major cause of pancreatic cancer–related death, they performed additional studies to address the utility of MART-10 in the prevention of metastasis. Both 1-α,25(OH)_{2}D_{3} and MART-10 repressed the migration and invasion of BxPC-3 and PANC cells, with MART-10 displaying much greater potency (46). This occurred through inhibition of EMT in pancreatic tumor cells through downregulation of Snail, Slug, and vimentin expression (46). Schwartz et al. (47) suggested that the presence of 1-α-hydroxylase in normal and tumor pancreatic cells may allow for therapeutic and chemopreventive options via 1-α,25(OH)_{2}D_{3} analogues based on observations that pancreatic cells express VDR and 1-α-hydroxylase, that dietary supplementation of rats with cholecalciferol and calcium reduces the proliferation of pancreatic normal epithelial cells, and that 1-α,25(OH)_{2}D_{3} and 1-α,25(OH)_{2}D_{3} analogues inhibit pancreatic cancer cell proliferation in vivo and in vitro. Miller et al. (111) demonstrated that VDR is required for the antiproliferative effect of 1-α,25(OH)_{2}D in cancer cells. The degree of 1-α,25(OH)_{2}D–induced antiproliferation and cytotoxicity was proportional to VDR concentrations within prostate carcinoma cells stably transfected with cDNA-encoding VDR. On the contrary, stable transfection of antisense VDR cDNA to ALVA-31 prostate cancer cells to knock down VDR attenuated the ability of 1-α,25(OH)_{2}D to inhibit cell growth and induce CYP24A1 expression (111). Thus far, experimental trials have provided promising results.

**Clinical studies of vitamin D in pancreatic oncogenesis**

1,25-dihydroxycholecalciferol, the biologically active form of vitamin D, has been demonstrated in numerous studies to display an antitumor effect in many types of cancer. In a vitamin D phase II study of 25 metastatic and locally advanced pancreatic cancer patients given calciferol-enhanced docetaxel, a modest increase in time to progression was shown when compared with historical findings with the use of single-agent docetaxel (81). In another phase II trial performed in 36 advanced pancreatic cancer patients, the vitamin D analogue seocalcitol (EB1089) was well tolerated, although no objective responses were reported (50). Currently, there are no ongoing clinical trials.

**Epidemiology**

Interest in Vitamin D’s role in the prevention of cancer first arose from epidemiologic documentation that individuals living at higher latitudes had an overall greater risk of dying from cancer than populations living in southern states. Apperley observed this phenomena in 1941 (112); however, it was not until the 1980s that a prospective study revealed that if 1-α,25(OH)_{2}D concentrations were <50 nM, there was a 2-fold higher risk of developing colorectal cancer (113). Prostate cancer was also more prevalent, with higher mortality rates among men living at the highest latitudes in the United States; in addition, ~25% of breast cancer mortality rates are accounted for by lack of UVB radiation from sunlight after controlling for diet (114). Cumulative evidence suggests that increasing vitamin D decreases the risk of developing malignancy, chronic diseases, and autoimmune disorders such as multiple sclerosis. VDR is present in most cells and tissues in the body, allowing vitamin D to exert a wide range of effects. Epidemiologic studies have been performed on vitamin D and pancreatic cancer risk with conflicting results. The Vitamin D Pooling Project of Rarer Cancers Consortium examined prediagnostic 1-α,25(OH)_{2}D concentrations in various cancers in 10 studies and found that there was an increased risk of pancreatic cancer in patients with concentrations of ≥16 nM (115). On the contrary, another pooled analysis of 5 separate studies found a 30% decreased risk of pancreatic cancer in those with higher concentrations of 1-α,25(OH)_{2}D than in those who were vitamin D–insufficient (116).

**Genetic variants in vitamin D pathway genes and risk of pancreatic cancer**

Polymorphisms in Vitamin D pathway genes and their association with pancreatic cancer risk are current topics of research. There is emerging evidence that polymorphisms in VDR and the 1α-hydroxylase gene may influence the risk of developing pancreatic cancer. For example, a study of 684 patients with pancreatic cancer and 1,368 controls in the United States found that individuals with the 275G allele of the VDR gene had a 30% reduced risk of developing pancreatic cancer compared to those with the 275A allele (117). Another study of 1,060 pancreatic cancer cases and 1,060 controls in the United States found that individuals with the 1298C allele of the 1α-hydroxylase gene had a 40% increased risk of developing pancreatic cancer compared to those with the 1298T allele (118). These findings suggest that genetic variation in vitamin D pathway genes may influence the risk of developing pancreatic cancer, but further research is needed to confirm these findings and to better understand the mechanisms underlying this association.
interest. Single nucleotide polymorphisms have been found in several vitamin D–related genes and may affect vitamin D concentrations and function. These loci include GC, which encodes vitamin D binding proteins 7-dehydrocholesterol reductase; VDR; and cytochrome P450 2R1 (CYP2R1), which encodes the enzyme vitamin D 25-hydroxylase (117). Single nucleotide polymorphisms in VDR previously have been associated with breast cancer, prostate cancer, and malignant melanoma (118). A recent population-based case-control study in Ontario, Canada evaluated 628 pathology-confirmed pancreatic adenocarcinoma cases. Single nucleotide polymorphisms were found in the CYP24A1, CYP2R1, calcium-sensing receptor, vitamin D binding protein (GC), and RXR-α and megalin genes and were associated with pancreatic cancer risk (119). CYP24A1 was found to be positively associated with pancreatic cancer risk, whereas CYP2R1 was inversely associated (119). However, these findings failed to reach statistical significance at \( P < 0.05 \) after adjustment for multiple comparisons (\( P \) value range: 0.011–0.050). Future studies are warranted to investigate whether vitamin D pathway polymorphisms are associated with pancreatic cancer risk.

Conclusions

Vitamin D analogues have been investigated in pancreatic cancer treatment for their potential antitumor effects. Antitumor effects in pancreatic cancer occur through cell cycle regulation, growth inhibition by AMPK-dependent mechanisms, PI3K/Akt pathways, and decreased cellular migration and invasion (Table 1). Preclinical studies have shown 25(OH)D \(_3\) to inhibit pancreatic cancer cell growth (Table 2). Vitamin D analogues such as MART-10 have shown potent antiproliferative effects and repressed migration and invasion of pancreatic cancer cells. 1,2-dihydroxyvitamin D–3-bromoacetate inhibited pancreatic tumor growth as well, which was further accentuated by AICAR (Table 2). Phase II clinical trials have shown either no objective response or actually increased time to progression when calciferol was added to docetaxel (Table 3).

Mechanisms of action of vitamin E in pancreatic cancer

Inhibition of NF-κB activation. NF-κB, a major transcription factor for inflammatory pathways, plays a significant role in carcinogenesis and is emerging as a link between inflammation and cancer. NF-κB is constitutively active in 67% of human pancreatic cancers but not in normal pancreatic tissues (48). NF-κB is formed by homodimerization or heterodimerization of its 5 family members and is activated by ligands such as TNF-α, IL-1, or pathogen-associated molecular patterns (130). In the canonical pathway, NF-κB activation is dependent on the kinase activity of 2 subunits, IκB kinase-γ (IKKγ) and IκB kinase-β (IKKβ), leading to phosphorylation of inhibitory protein IκBα (130). IκBα subsequently becomes polyubiquitinated and degraded by the proteasome, allowing NF-κB to translocate to the nucleus and exert its function as a transcriptional regulator of genes that encode proinflammatory cytokines (130). δ- and γ-Tocotrienol inhibit NF-κB activity, cell growth, cell survival, and tumor growth in nude mice (49). α-Tocotrienol and α-tocopherol have no effect on NF-κB activity (49). Kunnumakkara et al. (72) also demonstrated that γ-tocotrienol inhibited NF-κB activity in vitro and in vivo. Immunohistochemical analysis indicated a correlation between tumor growth inhibition and reduced expression of Ki-67, COX-2, MMP-9, NF-κB p65, and vascular endothelial growth factor (VEGF) in the tissue. Combination treatment with gemcitabine also downregulated NF-κB activity, as well as NF-κB–regulated gene products such as cyclin D1, c-myc, VEGF, MMP-9, and chemokine receptor type 4 (72). Our group observed that δ-tocotrienol consistently suppressed NF-κB and resulted in a decrease in phosphorylated IκBα expression in pancreatic cancer cells (AsPc-1 and MiaPaCa-2 cells) and tumor tissues by Western blot (49). β-, γ-, and δ-Tocotrienol also significantly decreased the binding of p65 (RelA), an NF-κB family member, to DNA in the cytosol, whereas γ- and δ-tocotrienol significantly decreased p65 binding to DNA in the nucleus (49). Several studies have also demonstrated that NF-κB inhibition can enhance gemcitabine activity in pancreatic cancer cells (49, 51–54). These results suggest that the bioactivity of tocotrienols against pancreatic cancer may be partially from inhibition of the NF-κB transcription factor.

Regulation of the cell cycle. δ-Tocotrienol suppresses pancreatic tumor cell growth through alteration of the cell cycle. A key hallmark of carcinogenesis is the ability to bypass the G\(_1\) phase checkpoint in the cell cycle, committing the cell to DNA replication and permitting tumor cells to have limitless replicative potential. Movement through this checkpoint is mediated directly or indirectly by either cyclin CDKs or CDKIs. In the late-G\(_1\) phase, CDKs phosphorylate the Rb, thus displacing transcription factor E2F-1 and allowing for
the activation of the gene expression essential for the cell to enter the S phase for DNA replication. p27Kip1, a CDKI, inhibits these CDKs involved with the G1 phase, thus subsequently inhibiting Rb phosphorylation, activating E2F-1, and causing inhibition of the G1→S phase checkpoint. δ-Tocotrienol may suppress tumor growth through the induction of p27Kip1 transcription. δ-Tocotrienol promotes E2F-1 function, resulting in E2F-1 binding to the p27Kip1 promoter and creating upregulation of p27Kip1 transcription and protein expression (58). This induction of p27Kip1 by E2F-1 may potentially function as a regulatory feedback mechanism that limits E2F-1 activity (131).

Induction of p27Kip1 is a crucial event in δ-tocotrienol-induced G1 cycle arrest and inhibition of cell proliferation in MiaPaCa-2 pancreatic cancer cells. p27Kip1 also was shown to function as a tumor suppressor in the G1→S transition checkpoint through binding and inhibiting the cyclin E→CDK2 complex in the nucleus (58). In the cytoplasm, it supports the assembly and nuclear import of cyclin D→CDK4/6 and promotes cellular proliferation. Cytoplasmic mislocalization of p27Kip1 may contribute to the progression of many cancers by promoting increased cellular motility and metastasis (59). δ-Tocotrienol induced an increase in p27Kip1 in the nucleus of pancreatic cancer cells in vitro and in vivo, favoring the tumor-suppressor function of p27Kip1 (58). Overall, vitamin E δ-tocotrienol induces p27Kip1-dependent cell cycle arrest in pancreatic cancer cells via an E2F-1-dependent mechanism, serving as a marker of δ-tocotrienol efficacy in pancreatic cancer.

VES has also been implicated in having a role in cell cycle arrest. In PANC-1 cells, G2 checkpoint arrest and inhibition of CDC-2 occurred with treatment of VES (60). This is not surprising, given that VES has previously been reported to cause cell cycle alterations in other cancer cell lines (132–134). p21, a target protein of p53, was induced without any change in expression or phosphorylation of p53 with VES treatment (60). The mechanism behind this is currently under investigation.

**Ras–Raf–mitogen-activated protein kinase/extracellular signal-related kinase (MEK)–extracellular signal-regulated kinase (ERK) pathway.** The antitumor activity of tocotrienols may also be mediated through the modulation of the Ras–Raf–MEK–ERK pathway in pancreatic cancer cells. Ras, a GTP binding protein and the most frequently mutated oncogene in human cancers, is a common upstream protein kinase in several signaling pathways, such as Raf–MEK–ERK and PI3K/Akt. K-Ras mutations encompass ~75–90% of pancreatic adenocarcinomas (56). Upon binding of epidermal growth factor to epidermal growth factor receptor, Ras becomes activated through the exchange of GDP for GTP. Ras subsequently binds and activates Raf kinase, which in turn phosphorylates and activates MEK. MEK then phosphorylates and activates ERK, also known as mitogen-activated protein kinase, in this signaling cascade. ERK activation promotes the upregulation of epidermal growth factor receptor ligand expression, promoting an autocrine growth loop critical for tumor growth (135). This signaling pathway has become an area of intense research because of its important role in human oncogenesis. The Ras–Raf–MEK–ERK cascade is involved in cellular proliferation, differentiation, and survival. In pancreatic cancer cells, it promotes cellular proliferation and prevents apoptosis; however, with inhibition of the pathway, it promotes apoptosis via caspase-6, -8, and -9 activation (55). Shin-Kang et al. (56) demonstrated that tocotrienols can cause a reduction in total ERK and phospho-ERK as demonstrated by Western blot analysis in pancreatic cancer MiaPaCa-2 and Panc28 cells at concentrations of 80 μM for 24 h. However, the same did not occur with tocopherols (56).

Ras activation was also found to indirectly cause cytoplasmic localization of p27Kip via activation of Raf–MEK–ERK, favoring the role of p27Kip as a cytoplasmic oncogene. δ-Tocotrienol is related to inhibition of the Raf–MEK–ERK pathway; however, the mechanism by which δ-tocotrienol inhibits Ras activation signaling is poorly understood. A previous study implicated δ-tocotrienol inhibition of Ras by its suppression of HMG-CoA activity (57). Conversely, our group did not observe any rescue of δ-tocotrienol antiproliferative activity in MiaPaCa-2 cells with mevalonate supplementation, suggesting that δ-tocotrienol inhibition of activated Ras signaling does not occur via inhibition of HMG-CoA reductase (58). Tocotrienols display some promising antitumor activity on the Ras–Raf–MEK–ERK pathway; however, further investigation is required to discover the overall underlying mechanism.

**Induction of apoptosis.** Apoptosis, also known as programmed cell death, is essential for homeostasis. Dysregulation of this system leads to carcinogenesis, autoimmune diseases, and neurodegenerative disorders, and multiple research efforts have been focused on the mechanisms behind its initiation, execution, and regulation (136–141). The main drivers of apoptosis are caspases, which are cysteine proteases that irreversibly commit a cell to death. Initially, they are inactive zymogens, requiring proteolytic activation during apoptosis carried out by an initiator caspase, which is autoactivated (138). This initiator caspase creates a downstream cascade of caspase activation occurring through either an intrinsic or extrinsic pathway by activation of an effector caspase such as caspase-3. The intrinsic pathway occurs in the mitochondria, where several proteins are released from the intermembrane space of the mitochondria into the cytoplasm. The most notorious of these proteins is the proapoptotic protein cytochrome c, which triggers a cascade of caspase activation. The extrinsic pathway is mediated by binding of FasL, an extracellular death ligand, to Fas, a death receptor (138). This binding leads to the recruitment of other factors to initiate an oligomeric death-inducing signaling complex, which leads to the activation of the initiator caspase, caspase-8, to activate the caspase cascade in apoptosis (138). These 2 pathways also have the ability to crosstalk through other various mechanisms.

Pancreatic cancer COLO-357 and PANC-1 cells were treated with VES to evaluate for induction of apoptosis in
an experiment performed by Patacsil et al. (60). Cleavage of PARP and caspase-3, an effector caspase, was observed, and it was also demonstrated that survivin is a possible target for VES, given that inhibition of survivin potentiates VES-induced apoptosis in PANC-1 cells (60). Survivin acts as an inhibitor of apoptosis through inhibition of caspases-3, -7, and -9 in mammals. Another mechanism by which VES induces apoptosis is through its ability to block Bak BH3 binding to Bcl–extra large (Bcl-xl) and Bcl-2, which leads to destabilization of mitochondria and the release of cytochrome c into the cytosol (61, 62). Cytochrome c then acts as an initiator caspase, activating caspase-3 and subsequently committing the cell to apoptosis.

In the intrinsic pathway, the release of mitochondrial proteins such as cytochrome c is mediated by Bak and Bax, which are proapoptotic members of the Bcl-2 protein family (138). Greco et al. (63) postulated that Bcl-2 plays a role in pancreatic cancer cell apoptosis by protecting cells from VES-dependent apoptosis. Bcl-xl is expressed in untreated pancreatic cancer cell lines; when silenced, pancreatic cancer cell sensitivity to the proapoptotic drug gemcitabine is increased. Overexpression of Bcl-xl is associated with resistance that pancreatic cancer cells may acquire toward proapoptotic chemotherapeutic agents. The key role of the mitochondrial proapoptotic proteins Bak and Bax in mediating the antiproliferative effects of VES was shown by displaying that VES inhibition of cell growth was much less pronounced when Bax and Bak were knocked out (63). Tocotrienols can also induce apoptosis in pancreatic cancer cells through the suppression of vital cell survival and proliferative signaling pathways such as the PI3K/Akt and ERK/MAP kinases via downregulation of Her2/ErbB2 expression (56).

Preclinical studies of vitamin E in pancreatic oncogenesis

There have been many laboratory studies on members of the vitamin E family and their potential antitumor activity on pancreatic cancer. The 4 different tocotrienols have different inhibitory effects on the growth and survival of pancreatic cancer cells. δ- and γ-Tocotrienol consistently inhibited pancreatic cancer growth and survival in vitro and in vivo, whereas α- and β-tocotrienol and α-tocopherol did not inhibit growth and survival of pancreatic cancer cells (49). Shin-Kang et al. (56) also indicated that δ- and γ-tocotrienols have potent antiproliferative activity in human pancreatic cancer Panc-28, MiaPaCa-2, Panc-1, and BxPC-3 cells. This was also demonstrated by Hussein and Mo (57), who revealed that δ-tocotrienol suppressed the proliferation of Panc-1, MiaPaCa-2, and BxPC-3 cells. Kunnumakkara et al. (72) also reported that γ-tocotrienol inhibited the growth and survival of Panc-1, MiaPaCa-2, and BxPC-3 cells. It was also demonstrated through immunohistochemical analysis that a correlation exists between tumor growth inhibition and reduced expression of Ki-67, COX-2, MMP-9, NF-κB p65, and VEGF in pancreatic tumor tissue. Combination treatment with gemcitabine also downregulated NF-κB activity, along with the NF-κB-regulated gene products, such as cyclin D1, c-myc, VEGF, MMP-9, and chemokine receptor type 4 (72). Among the 4 isoforms, δ-tocotrienol has previously been demonstrated at our institution to be the most potent (104, 130). The oral administration of 100 mg • kg⁻¹ • d⁻¹ of δ-tocotrienol to mice resulted in concentrations that were 10 times higher in the pancreas than in subcutaneously implanted tumor tissue, suggesting that these compounds will have reasonable bioavailability for pancreatic tumor intervention (71). δ-Tocotrienol prolonged survival and delayed the progression of pancreatic intraepithelial neoplasia in LSL-KrasG12D/+/Pdx-1-Cre mice (73). The oral intake of δ-tocotrienol alone and in combination with gemcitabine prolonged survival in the LSL-KrasG12D/+/LSL-Tp53R172H/+; Pdx-1-Cre mouse model (74). Gemcitabine (20 μM) alone inhibited colony formation by 84%, δ-tocotrienol (50 μM) alone inhibited colony formation by 67%, and gemcitabine (20 μM) plus δ-tocotrienol (50 μM) resulted in 99% inhibition of anchorage-independent growth (49).

There are conflicting results when evaluating the efficacy of VES in pancreatic cancer. The overall effect of VES was minimal in pancreatic cancer cells, with any sensitivity appearing to be correlated with SMAD4/DPC4 homozygous deletion and Bax/Bak expression (63). However, Patacsil et al. (60) showed that VES inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. Another study also revealed that VES and peptide YY both inhibit growth of pancreatic cancer cells in vitro with a significant effect when used in combination (75). On the contrary, another study showed that VES did not have significant effects on cell growth inhibition in pancreatic cancer cells (76). The apoptotic activity of VES has been demonstrated through the promotion of breast cancer tumor dormancy in a mouse model, as well as suppression of melanomas and colon cancer metastasis (124, 126, 127).

Clinical studies of vitamin E in pancreatic oncogenesis

There are 3 active clinical trials occurring right now on Vitamin E and pancreatic cancer. The H. Lee Moffitt Cancer Center and Research Institute is performing a phase I dose-escalation trial, evaluating the safety and tolerability of vitamin E in healthy participants before administering to cancer patients. Preliminary findings reveal that δ-tocotrienol had no obvious toxicity at doses up to 3200 mg/d, which is 5 times the predicted biologically active clinical dosage (Table 4) (82). That institute also is evaluating the safest dose, how often it should be taken, and how well people with pancreatic tumors tolerate δ-tocotrienol. Finally, the institute also is evaluating the safety and tolerability of δ-tocotrienol and determining the minimally effective dose or maximum tolerated dose of vitamin E δ-tocotrienol administered once in patients with resectable pancreatic cancer tumors (Table 4). There have been no clinical studies of tocopherol intervention in pancreatic cancer. Overall, there have been no studies of vitamin E as an adjunctive agent in pancreatic cancer recurrence or metastases.
Conclusions
Vitamin E displays great potential in augmenting pancreatic cancer treatment. Its common mechanisms include NF-κB inhibition, cell cycle regulation, induction of apoptosis, and inhibition of the Ras–Raf–MEK–ERK pathway (Table 1). Multiple preclinical studies have been performed to date. δ-Tocotrienol alone has been demonstrated to suppress pancreatic tumor cell growth. In combination with gemcitabine, it inhibited proliferation and prolonged survival (Table 2). γ-Tocotrienol also inhibited tumor growth and enhanced the efficacy of gemcitabine (Table 2). VES found conflicting results on its ability to inhibit pancreatic cancer cell growth. There is only one ongoing phase I clinical trial on δ-tocotrienol with preliminary results showing no toxicity (Table 3). Although vitamin E shows great potential from its preclinical studies, there are no completed clinical trials available to determine its efficacy in pancreatic cancer intervention. Clinical trials are warranted before any recommendations can be made regarding the efficacy of vitamin E as an adjunctive agent in pancreatic cancer.

Vitamin K
Vitamin K (VK) is a fat-soluble vitamin that plays a principle role in the blood coagulation cascade. It has several forms and is divided into those naturally occurring and synthetic. Naturally occurring VK includes VK-1, or phytanadi-one, which is produced by plants and used to treat human anticoagulation disorders, from which its name was derived from the German word for blood clotting, “koagulation.” Because it is directly involved in photosynthesis, a large source of VK is found in leafy green vegetables. VK-2, or menaquinone, is also naturally occurring. It is the main storage form in animals and is found in the human gut, produced by certain bacteria, and has been investigated for treatment of hepatocellular carcinoma (142, 143). There is also positive evidence for its use as a treatment for osteoporosis (144). Given that it is synthesized by animals, VK-2 is primarily found in meat, eggs, and dairy products. VK-3 and VK-5 make up the category of synthetic Vks. VK-3, or menaquinone, is a short-chain chemically synthesized compound that induces redox cycling, resulting in ROS, and it is toxic to humans. VK-5 is also a short-chain chemically synthesized compound and it has inhibitory actions on both cells and bacteria. Typical plasma serum concentrations of VK are 0.44–7.1 nM (67). Previous studies have revealed VK's value as a potential antitumor agent, given its ability to suppress cancer growth and induce apoptosis and differentiation in cancer cells (64–66, 77, 78). In this review, we will focus on mechanistic studies and preclinical and early clinical trials on the utility and efficacy of VK as an adjunctive treatment for pancreatic cancer.

Mechanisms of action of VK in pancreatic cancer

Induction of apoptosis. Apoptosis is a tightly regulated process of programmed cell death. Dysregulation of this system leads to carcinogenesis, autoimmune diseases, and neurodegenerative disorders. Apoptosis is divided into 2 main pathways: the intrinsic, or mitochondrial, pathway, and the extrinsic, a death-receptor pathway. An initiator caspase, which is autoactivated, creates a downstream cascade of caspase activation occurring through either the intrinsic or extrinsic pathway by activation of an effector caspase such as caspase-3. The intrinsic pathway occurs in the mitochondria, where several proteins are released from the intermembrane space of the mitochondria into the cytoplasm. The most notorious of these proteins is the proapoptotic protein cytochrome c, which triggers a cascade of caspase activation.

The extrinsic pathway is mediated by binding of FasL, an extracellular death ligand, to Fas, a death receptor (137). This binding leads to the recruitment of other factors to initiate an oligomeric death-inducing signaling complex, which leads to the activation of the initiator caspase, caspase-8. This caspase cleaves caspase-3 to directly activate the caspase cascade in apoptosis. These 2 pathways also have the ability to crosstalk through other various mechanisms.

Multiple studies have confirmed that apoptosis plays a role in VK-induced pancreatic cell death. VK-1 and VK-2 showed a time-dependent increase in cleaved caspase-3 and the proapoptotic Bcl-2 member BAX, providing evidence that VK affects pancreatic cancer cell survival through a caspase-dependent pathway (78). Another study evaluating VK-2 also found induction of apoptosis in Mia-PaCa-2 cells (65). VK-3 induces apoptosis through mediation of wild-type p53, intracellular calcium, and ROS and inhibits growth via ERK phosphorylation (66, 77). When VK-1 was combined with sorafenib, it created cell growth inhibition and apoptosis via 2 proposed pathways, ultimately leading to caspase activation. Given that pretreatment with a pan-caspase inhibitor radically blocked induction of apoptosis, this was likely a caspase-dependent reaction (64). This seems to involve activation of c-Jun N-terminal protein kinases (JNKs)/c-Jun and inhibition of the MEK-ERK pathway, both leading to activation of caspase-8, and subsequently apoptosis (64).

Ras–Raf–MEK–ERK pathway. Ras is a GTP-binding protein and is the most frequently mutated oncogene in human cancers, particularly pancreatic cancer. It encompasses ~75–90% of pancreatic adenocarcinomas (56). Ras becomes activated through the exchange of GDP for GTP upon binding epidermal growth factor to epidermal growth factor receptor. Ras subsequently binds and activates Raf kinase, which in turn phosphorylates and activates MEK. MEK then phosphorylates and activates ERK, also known as mitogen-activated protein kinase, in this signaling cascade. ERK activation promotes the upregulation of epidermal growth factor receptor ligand expression, promoting an autocrine growth loop critical for tumor growth (135). The Ras–Raf–MEK–ERK cascade is involved in cellular proliferation, differentiation, and survival. In pancreatic cancer cells, it promotes cellular proliferation and prevents apoptosis; however, with inhibition of the pathway, it promotes apoptosis via caspase-6, -8, and -9 activation (55).

VK has been shown to be directly involved with the MEK-ERK pathway in pancreatic cancer. Wei et al. (66)
demonstrated that sorafenib or VK-1 alone can induce apoptosis through inhibition of phospho-MEK and phospho-ERK concentrations at high concentrations. At low concentrations, VK-1 added to sorafenib mediated inhibition of the MEK-ERK pathway and induction of apoptosis via the extrinsic pathway (66). In contrast, a dose-dependent increase in phospho-ERK occurred in VK-1– and VK-2–treated pancreatic cancer cells, which was reversed when the same medium was treated with an MEK inhibitor (64). Induction of apoptosis also occurred. VK-3 injections into pancreatic tumor tissues also resulted in ERK phosphorylation and growth inhibition (77). Typically, ERK phosphorylation promotes cellular proliferation and inhibits apoptosis, although a previous study demonstrated cell cycle arrest by the MEK-ERK pathway (145).

**JNK MAPK signaling pathway.** JNKs are multifunctional kinases involved in many physiologic processes. They play a major role in apoptosis. JNK is a subfamily of 1 of 3 MAPK pathways. Its name is derived from the discovery that JNK phosphorylates the N-terminal transactivation domain of c-Jun, enhancing its ability to transactivate gene expression (146). Its ability to phosphorylate transcription factors allows the regulation of the expression of several stress-responsive genes. JNK also has been demonstrated to phosphorylate and regulate both pro- and antiapoptotic proteins, such as Bcl-2 and Bcl-xL. FasL has been shown to also be a target gene for c-Jun. It is through these mechanisms that JNK mediates apoptosis (146). Its prolonged activation is implicated in several types of cancers, including breast, gastric, prostate, and ovarian cancers (64).

VK also may act through a JNK-mediated mechanism. Sorafenib and VK-1 have been demonstrated to cause cell growth inhibition and apoptosis when used in combination. One way this occurs is through the activation of JNK, which increases phosphorylation of c-Jun and increases FasL concentrations. When applying a JNK inhibitor, it reduced the phosphorylation concentrations of JNK and c-Jun, decreased FasL concentrations, and partially antagonized apoptosis induced by sorafenib and VK-1 (64). The authors suggest that the mechanism behind sorafenib and VK-1–induced apoptosis involves JNK/c-Jun–dependent upregulation of FasL-mediated apoptosis (64). VK-3 induced rapid phosphorylation of JNK 30 min after application; however, this was not maintained after 12 h or detected in tumor tissue (77).

**Preclinical studies of VK in pancreatic oncogenesis**

Several studies have evaluated VK’s role against pancreatic cancer cell oncogenesis, with all studies having a common theme of apoptosis. Earlier studies demonstrated that VK-1, VK-2, and VK-3 cause apoptosis of pancreatic cancer cells through either caspase-dependent mechanisms and induction of ERK phosphorylation, or via intracellular calcium, ROS, and wild-type p53, respectively (65, 66, 78). VK-3 also has been found to inhibit cellular proliferation via rapid phosphorylation of ERK (77). Wei et al. (64) evaluated VK in combination with sorafenib. They found that when VK-1, VK-2, and VK-5 were combined with sorafenib, the dose of sorafenib required for growth inhibition and induction of apoptosis was subsequently reduced. When used alone, either treatment was ineffective at the same dose. In addition, VK-1 appears to be additive with sorafenib in activating apoptosis. This is believed to be via 2 separate pathways by inhibiting the MEK-ERK pathway and activating caspase activity, and also by JNK/c-Jun–dependent upregulation of FasL-mediated apoptosis.

**Clinical studies of VK in pancreatic oncogenesis**

To our knowledge, are no active or completed clinical studies to date evaluating the role of VK in pancreatic cancer treatment.

**Conclusions**

VK has been evaluated as an adjunctive agent for pancreatic cancer treatment. Mechanistic studies demonstrate that this occurs primarily through apoptosis, along with the Ras–Raf–MEK–ERK pathway and JNK MAPK signaling pathways (Table 1). Preclinical studies evaluate the use of VK alone and with the use of an antitumor agent, sorafenib (Table 2). VK-1, VK-2, and VK-3 have been shown to induce apoptosis through caspase-dependent pathways and with wild-type p53, intracellular calcium, and ROS. In combination with sorafenib, VK causes activation of the JNK MAP signaling pathway and inhibition of the MERK/ERK pathway, resulting in apoptosis; however, alone, VK-1 and VK-2 were demonstrated to cause phosphorylation of ERK, resulting in activation of apoptosis. VK-3 also inhibited cellular growth via phosphorylation of ERK. These results conflict with research showing the MERK/ERK pathway to be a proliferative process and they argue for additional studies. There are no completed or pending clinical trials on VK and pancreatic cancer treatment. More preclinical trials need to be completed and there need to be clinical trials available before any recommendation to use VK as an adjunctive treatment for pancreatic cancer can be made.

**Conclusions and Perspectives**

Upon review of the bioactive vitamins and their potential role in pancreatic cancer intervention, there is a wealth of scientific knowledge available. The present data are encouraging and advocate for an important future role for these 5 vitamins to augment conventional therapy in pancreatic cancer recurrence and metastasis. The biochemical pathways outline the mechanisms behind their antitumor effect, specifically in pancreatic cancer signaling pathways, and provide strong scientific evidence to support their role in oncogenesis prevention. The preclinical studies mostly support these findings; however, there is not yet enough available evidence through clinical trials to fully support the role of these vitamins in pancreatic cancer intervention. Further studies are needed to draw more solid conclusions and clarify their role in patients with an established diagnosis of pancreatic cancer. RA may be useful as a maintenance
immunotherapy. It has not displayed any objective responses in phase II clinical trials as an adjunctive treatment agent and has been well tolerated. Ascorbic acid shows promising results, with 2 phase I clinical trials revealing decreased primary tumor sizes with gemcitabine and improved performance status. For vitamin D, phase II clinical trials either showed no objective response or actually increased time to progression when calciferol was added to docetaxel. Vitamin E studies indicated that δ-tocotrienol and γ-tocotrienol inhibited tumor cell growth and survival, as well as augmenting gemcitabine activity. There is also an ongoing phase I clinical trial with preliminary results revealing no obvious toxicity. Research on VK demonstrates antiproliferative activity through activation of apoptosis and inhibition of cellular growth; however, there are no clinical studies offering further investigation.

Overall, there is a need for large randomized controlled trials for all of the bioactive vitamins, which can also serve to identify duration of treatment and optimal dosing, and determine whether some subgroups of patients may respond better than others, given different genetic backgrounds, lifestyle, and nutrition. These trials need to be carefully designed to address appropriate endpoints based on insights gained from mechanistic studies as well as the preclinical studies and the findings from early-phase clinical trials.

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References


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