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Vitamin D: A Multi-Faceted Role for the Prevention, Management, Survivorship and Palliative Care of Breast Cancer

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Abstract

Breast cancer is the most common cause of cancer-related death in women worldwide. Conventional treatments for breast cancer are associated with a number of side effects in the cancer patient. There is therefore an urgent need to investigate the anti-cancer potency of natural compounds which are efficacious and affordable and may be used in the chemoprevention and/or the pharmacological management of breast cancer. In the past few decades, the role of Vitamin D in the prevention and management of cancer has been the focus of many research studies. Evidence in the literature supports the potency of $1,25(OH)_2D_3$ and Vitamin D synthetic derivatives to induce cell cycle block and caspase-dependent programmed cell death (i.e. classical apoptosis) and to inhibit angiogenesis in breast cancer. While the signaling pathways related to the anti-cancer potency of Vitamin D are the focus of current investigation, evidence is emerging on the existence of caspase-independent pathways of programmed cell death. Promising results from cellular and animal studies have also led to the development of clinical trials investigating the role of Vitamin D in breast cancer prevention and pharmacological management. In addition to the possible role of Vitamin D in the chemoprevention and adjuvant therapy of breast cancer, there may be a beneficial use for prescribing Vitamin D to breast cancer survivors and to advanced breast cancer patients receiving palliative care. The scope of this review is to provide evidence supporting a multi-faceted role of Vitamin D in the prevention, management (adjuvant treatment), survivorship and palliative care of breast cancer.

Keywords: Breast cancer; Vitamin D; 1, $25(OH)_2D_3$; Caspase-independent programmed cell death; Prevention; Management; Survivorship; Palliative care

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Introduction

Breast cancer is the most common cause of cancer-related death in women worldwide [1,2]. The WHO estimated that worldwide over 508,000 women died due to breast cancer in 2011 (WHO, 2019; American Cancer Society, 2019), whereas epidemiological data has shown that in 2015 breast cancer was the most common cancer in women overall, with an estimated 2.4 million incident cases [3-5]. Interestingly, an observed increase in the prevalence of breast cancer has been observed worldwide. This increase has been reported in post-menopausal women and in countries where the incidence in the past had been low such as Southern and Eastern Europe, Japan and China (WCRF, AICR, 2007) [6]. In addition, there is a large variation in breast cancer survival between high income and low income countries. The 5 year survival in low income countries is estimated to be less than 40% compared to 80% in high income countries [7]. Ethnic background seems also to be important in terms of disease progression. Evidence in the literature supports those African American women with breast cancer have the highest mortality rates compared to other ethnic groups and across all age groups [8].

Of all breast cancer cases, only 5% to 10% are because of genetic defects such as the inherited mutations in the BRCA1 and BRCA2 genes [9,10]. Therefore, 90% to 95% of all breast cancer cases are attributed to environmental and lifestyle factors such as diet which contributes to 30% to 35% [11-14]. In addition to unhealthy nutrition, risk factors for breast cancer include early menarche, late menopause, no or late childbearing (>30 years), inflammation, smoking, alcohol, obesity, low physical activity and lack or short-term duration of breastfeeding [15-21].

Conventional treatments for breast cancer include surgery, radiation, chemotherapy,

immunotherapy and hormonal therapy and the type of breast cancer and stage determines the treatment protocol to be followed [22]. Unfortunately, many of the therapies in current use are associated with a number of side effects in cancer patients [23]. Currently, there is growing evidence for a role for dietary factors in breast cancer path physiology [1,13]. Studies have shown that several dietary natural products and their bioactive components may inhibit breast cancer by preventing the expression and activity of Estrogen Receptor (ERα), activating the cell cycle block and apoptosis and inhibiting metastasis and angiogenesis [24-29]. There is therefore an urgent need to investigate the anti-cancer potency of natural compounds which are efficacious and affordable and which could be used in the chemoprevention or the pharmacological management of breast cancer [30].

In the past few years' research focused on investigating the use of the fat-soluble secosteroid Vitamin D for the chemoprevention and management of breast cancer. These studies were initiated when a number of observational studies provided evidence that Vitamin D deficiency is associated with increased risk of breast cancer [31-38]. In addition, numerous studies have provided evidence that treatment of breast cancer cells with Vitamin D and synthetic analogues leads to the activation of cell cycle block, apoptosis, autophagy and differentiation and the inhibition of angiogenesis, invasion and metastasis [14,36-40]. Animal studies supported the anti-tumour potency of Vitamin D [41-43]. The findings from the animal studies facilitated the development of large clinical trials designed to investigate the effectiveness of Vitamin D for the chemoprevention and management of breast cancer [44-47]. In addition to investigating the anti-cancer potency of the natural form of Vitamin D, due to the calcemic activity of the latter, synthetic analogues have also been developed and assessed in cell and animal studies and discussed here in this review [30].

Vitamin D Metabolism, Vitamin D Receptor and Expression in Mammary Cells

Vitamin D is a group of fat-soluble secosteroids involved in maintaining calcium and phosphorous homeostasis, bone metabolism, neuromuscular function and immunity [48]. The two major forms of Vitamin D are Vitamin D_2 (ergocalciferol) and Vitamin D_3 (also known as cholecalciferol). Vitamin D_2 (ergocalciferol) is synthesised by plants and is not produced by the human body. Therefore, humans ingest D_2 from their diet and from supplements. Vitamin D_3 (cholecalciferol) is produced in the skin when sunlight strikes bare skin but can also be ingested from animal and fish sources [49-51]. Nevertheless, Vitamin D cannot easily be obtained through diet since it is present mainly in the liver of fatty fish, fortified milk and egg yolk [14]. Whether it is absorbed through unprotected skin or ingested and then absorbed by the intestines, Vitamin D is bound to a binding protein (both albumin and Vitamin D binding protein) and carried to the liver where it undergoes two hydroxylation processes. In the liver it is transformed into 25(OH)D (calcidiol), which is the most commonly measured form in serum. 25(OH)D is transformed in the kidneys to 1,25 dihydroxy-Vitamin D_3 (1,25$(OH)_2D_3$-calcitriol), which is the biologically active form of Vitamin D [52].

The 1,25$(OH)_2D_3$ receptor, more commonly known as the Vitamin D Receptor (VDR), is a member of the nuclear receptor family of transcription factors [53]. 1,25$(OH)_2D_3$, binds to VDR and forms a heterodimer with the retinoid-X receptor. This complex then binds to hormone response elements on DNA resulting in expression or trans-repression of specific gene products [54]. Colston et al., [55] first showed the expression of the Vitamin D Receptor (VDR) and the biding of the 1,25$(OH)_2D_3$ ligand in normal mouse breast tissue. More recently, cell and animal studies have shown that Vitamin D is expressed and functional during normal breast development [56-58]. Colston et al., [36] have reported the expression of VDR on breast tumours induced in rats. The presence of VDR was also reported in a high proportion of human breast cancer biopsies in many studies [59-61]. Berger et al., [37] have shown that VDR was present in breast carcinomas and those patients whose tumours contained immunocytochemically detectable VDR had a longer disease-free interval than those patients with negative tumours. Evidence therefore supported a correlation between levels of VDR and disease free survival [37,62]. Since VDR expression is around 90% in ER positive tumours compared to only 27% in basal/triple negative tumours, Vitamin D therapy could potentially help a significant number of women suffering with breast cancer who are positive for VDR [63].

Chandler et al., [64] conducted a Mendelian randomisation analysis of the relationship between a Vitamin D genetic risk score (GRS, range 0 to 10), comprised of five Single Nucleotide Polymorphisms (SNPs) of Vitamin D status in the DHCR7, CYP2R1 and GC genes and cancer risk among women. The results of the study showed that SNPs associated with Vitamin D were associated with the risk of breast cancer [65].

Association between Serums Levels of Vitamin D and Risk of Breast Cancer

It is generally accepted that levels of 25(OH)D below 20 ng/ml are considered deficiency, levels above 150 ng/ml are considered toxic and levels between 30 ng/ml to 60 ng/ml are considered optimal [14]. Currently, it is known that hypovitaminosis D affects almost 50% of the population worldwide. The latter can be attributed to reduced exposure to sunlight and the fact that the vitamin is only present in a few nutritional sources such as the liver of fatty fish, fortified milk and egg yolk [14,66]. Several factors contribute to a decrease of Vitamin D in the blood and these include obesity, low physical activity, smoking, age, race, skin type and living at high latitudes [14,67-71]. In addition, Vitamin D synthesis however is further affected by behavioral factors such as time spent in the sun, use of sun protection and clothing practices (i.e. extent of cover). Time spent in the sun has been shown to be very important in inducing cutaneous Vitamin D production. This has been shown in people of all ages [72-74].

There are two systematic reviews that looked at the prevalence of hypovitaminosis/levels of Vitamin D worldwide and also risk factors [75]. More specifically, the study by Hilger et al., [75] reviewed studies which used continuous values of Vitamin D and made comparisons across different subgroups of the population defined by age, gender and geographic region. The studies included in the review were published between 1990 to 2011 and originated from 44 different countries. The mean age of the participants in these studies was 51.7 years whilst 66.7% of them were females. The results of the review showed a great variability in Vitamin D levels between studies. Nonetheless 88.1% of them reported a mean serum Vitamin D value <30 ng/mL, 37.3% reported mean values <20 ng/mL and 6.7% had mean values below 10 ng/mL. Vitamin D values were significantly higher in North America compared to other regions such as Europe, Middle East and Africa, a finding attributed to the routine Vitamin D food fortification in North American countries. Furthermore, there did not seem to be any significant differences in Vitamin D levels by age and gender in most areas of the world. Nonetheless,

children and adolescents in Middle East and Africa were shown to have higher levels than adults whilst women in the same areas had lower levels than men. This was explained by the fact that children in these areas spend more time outdoors for play as compared to adults whilst traditional clothing was suggested to be the impeding factor for Vitamin D production in women. In addition, exploratory analysis showed that newborns and institutionalized elderly were also more likely to have lower Vitamin D levels.

On the other hand, the study by Palacios and Gonzalez published a year later reviewed evidence from studies worldwide reporting Vitamin D status (as a categorical variable) rather than Vitamin D levels (as a continuous variable) [76]. The studies included in this review were published between 2003 to 2013 and reported Vitamin D status in healthy populations only. Vitamin D classification was based on cut off points relevant to bone health i.e. Vitamin D severe deficiency (≤ 12 ng/mL), deficiency (≤ 20 ng/mL) and insufficiency (≤ 30 ng/mL). A total of 103 studies were identified and considered in that review. The findings from these studies were used to produce global maps for each of the following age groups: Infants, children, adolescents, adults, lactating women and elderly. Most of the studies (n=31) reported Vitamin D status in the elderly whilst only 12, 17, 15 from all around the world reported Vitamin D status in infants, children and adolescents respectively. Despite the variability of Vitamin D status around the world, the maps created indicated that the prevalence of low Vitamin D status is high almost in all areas of the world and in all age groups. In adults for example, the prevalence of Vitamin D deficiency has been reported to be between 37% to 42% in USA, 31% in Australia, 34% in Spain, whilst it whilst it was reported to be in the range of 40% to 60% in Northern European countries such as UK, Norway, Denmark, and Germany [33,63,77-82]. Vitamin D deficiency was very high in Middle East and Asian countries such as Israel (41% to 50% in males, 51% to 60% in females), Iran (51%) and Pakistan (58%) [83-85]. In addition, Vitamin D deficiency has been observed in all races, even in hot climates and is particularly high in African-American women [56].

In the 1950's and 1960's, geographical ecological studies reported an inverse correlation between solar UVB dose and breast cancer mortality rate in the US [30,86]. Observational studies subsequently provided more evidence that Vitamin D have a protective effect in preventing the development of breast cancer [1]. Mawer et al., [87] showed that there was an association between Vitamin D and breast cancer disease activity since serum 25(OH)D concentration fell in patients whose disease progressed but remained constant in those who were stable or responded to treatment. Janowsky et al., [31] carried out a study to determine if blood levels of 25(OH)D or its active metabolite $1,25(OH)_2D_3$ are lower in women at the time of first diagnosis of breast cancer than in women without breast cancer. The results of the study are supporting a protective effect of $1,25(OH)_2D_3$ for breast cancer in white women.

In addition, Yao et al., [32] examined serum concentrations of 25(OH)D in relation to breast cancer prognostic characteristics, including histologic grade, ER, PR and HER2, among women with incident breast cancer and controls over a period of 5 years. The results of the study showed that higher serum levels of 25(OH)D were associated with reduced risk of breast cancer. The associations were stronger with poor prognostic characteristics (i.e. high grade, ER negative or triple negative cancers) in premenopausal women. Their findings proposed that Vitamin D supplementation could be used to reduce breast cancer risk, particularly those with poor prognostic characteristics among premenopausal women. Moreover, Tretli et al., [88] investigated the association between serum levels of 25(OH) D and risk of death in Norwegian cancer patients. The results of the study showed that higher circulating serum levels of 25(OH)D were positively associated with the survival for cancers of the breast, colon, lung, and lymphoma.

The study 'Epidemiological Analysis of Vitamin D and Breast Cancer Risk in Saudi Arabian Women (NCT01817231) was an analysis of de-identified data collected from 240 Saudi Arabian women, 120 with breast cancer and 120 control women, to evaluate if Vitamin D status was associated with breast cancer risk. Yousef et al., [89] have reported that breast cancer cases had significantly lower (mean ± SD) serum concentrations of 25(OH)D (9.4 ± 6.4 ng/mL) than did controls (15.4 ± 12.3 ng/mL; P=0.001). Therefore, an inverse association existed between serum 25(OH)D concentrations and breast cancer risk in Saudi Arabian women. A meta-analysis carried out by Wang et al., [34] showed an inverse association between serum 25(OH)D concentration and breast cancer risk. In particular, dose-response analysis showed that every 10 ng/mL increment in serum 25(OH)D concentration was associated with a significant 3.2% reduction in breast cancer risk. Furthermore, Bauer et al. [90] conducted a meta-analysis of 9 prospective studies evaluating the association between circulating 25(OH)D Vitamin D and breast cancer risk. In summary, this dose-response meta-analysis of prospective studies of plasma 25(OH)D suggested that while no association was found in premenopausal women, a nonlinear inverse association was observed among postmenopausal women (an inverse association was observed beyond a threshold of 27 ng/ml, with flattening of effects above 35 ng/ml).

A study by Wu et al., [91] investigated the association of Vitamin D_3 levels with breast cancer risk and progression in African-Americans and Hispanics. About 69.2% of African-Americans and 37.8% of Hispanics had 25(OH)D below 20 ng/ml. The 25(OH)D level below 20 ng/ml was significantly associated with breast cancer in both African-Americans and Hispanics. Furthermore, serum level of 25(OH)D below 20 ng/ml was significantly associated with triple negative breast cancer in African-Americans (but not in Hispanics). The conclusion of this study was that there is an association between the levels of 25(OH)D and the risk of breast cancer. In another study, evaluated the association between baseline serum 25(OH) D levels, supplemental Vitamin D use, and breast cancer incidence over the subsequent 5 years of follow-up. The researchers concluded that of women with elevated risk, high serum 25(OH)D levels and regular Vitamin D supplement use were associated with lower rates of incident, postmenopausal breast cancer over 5 year of follow-up. Straub et al., [92] reinforced the assumption that higher levels of 25(OH)D in pre-menopause women are associated with lower breast density and may reduce the risk of breast cancer. In another study, Vitamin D deficiency had a negative effect on overall and disease-free survival in Egyptian women suffering from breast cancer and low deficiency was related to tumour size, stage, grade, nodal status and HER2/neu receptor expression [93].

The Anti-Cancer Potency of Vitamin D in Breast Cancer Cell Lines

Inhibition of cell proliferation: Numerous studies have supported the potency of $1,25(OH)_2D_3$ and Vitamin D synthetic derivatives to inhibit the cell cycle [94,95]. The anti-proliferative

effect of $1,25(OH)_2D_3$ on human cancer cell lines was first reported in melanoma cells in culture, while Abe et al., [96] reported that $1,25(OH)_2D_3$ could cause the differentiation of mouse cultured myeloid leukemia cells. In subsequent years numerous studies have investigated and reported the potency of $1,25(OH)_2D_3$ to inhibit a variety of different cancer cell types including breast cancer cells including MCF-7 and T47D cells, which express VDR at moderate levels [38,97].

Evidence in the literature supports that $1,25(OH)_2D_3$ and the synthetic analogue EB1089 induce G0/G1 cell cycle block which is associated with accumulation of the hypophosphorylated form of the Retinoblastoma protein (Rb) in MCF7 breast cancer cells [98]. The induction of cell cycle block in breast cancer cells is associated with increased expression of cyclin dependent kinase inhibitors, dephosphorylation of Rb and an increase in the levels of p21 mRNA and protein [99-101]. In addition, an increase in the levels of p27 has also been reported following treatment of breast cancer cells with $1,25(OH)_2D_3$ or the synthetic analogue EB1089 in BT20 and ZR-75-1 and MCF7 breast cancer cells [100,102].

Sensitivity to $1,25(OH)_2D_3$ is higher in cells that express the Estrogen Receptor (ER) than in cells that are ER negative [103]. Two studies have shown that the analogue EB1089 down-regulates the expression of ER in MCF7 cells [104,105]. A subsequent study has also provided evidence for the down regulation of the mRNA expression of ER by $1,25(OH)_2D_3$ and the Vitamin D analogues EB-1089, KH-1060 and Ro27-0574 in the MCF-7 cell line [106]. By down-regulating the levels of ER, there is a decrease in the growth stimulatory effects of 17-β estradiol [106]. In addition, $1,25(OH)_2D_3$ decreases the transcription of aromatase (CYP19) which is the enzyme that catalyses estrogen synthesis in breast cancer cells and the surrounding adipose tissue. As a result there is a decrease in the levels of ER-α [107,108]. In addition, $1,25(OH)_2D_3$ upregulates and down regulates genes involved in cell cycle block in MCF7 cells. The upregulated genes involve cyclin G1 and cyclin I, P21-Activated Kinase-1 (PAK-1), p53, Retinoblastoma like-2 [Rb2 (p130)] and Insulin-like Growth Factor Binding Protein-5 (IGFBP5). The down-regulated genes include ERα, growth factors, cytokines and several kinases [109]. Even though $1,25(OH)_2D_3$ is effective in MCF7 cells, it is not effective in certain triple negative breast cancer cell lines such as MDA-MB-157, MDA-MB-231 and MDA-MB 468 [110]. This could be due to the lack of p53 in these cells which may be necessary in mediating the Vitamin D response [111]. Figure 1 provides an overview of some of the important molecules involved in the induction of cell cycle block by $1,25(OH)_2D_3$ and synthetic derivatives.

Induction of Caspase-Dependent Programmed Cell Death (CD-PCD): Several studies have provided evidence that $1,25(OH)_2D_3$ and synthetic analogues of Vitamin D induce Caspase-Dependent Programmed Cell Death (CD-PCD) i.e., classical apoptosis *in vitro* [39,112]. Welsh et al., [112] have shown that MCF-7 cells treated with $1,25(OH)_2D_3$ exhibit characteristic apoptotic morphology (pyknotic nuclei, chromatin and cytoplasmic condensation, nuclear matrix protein reorganization). The researchers also examined the interactions between $1,25(OH)_2D_3$ and the antiestrogen 4-hydroxytamoxifen (TAM), which also induces apoptosis in MCF-7 cells. The results of the study provided evidence that combined treatment with $1,25(OH)_2D_3$ and TAM enhanced the degree of apoptosis. The researchers also selected a subclone of MCF-7 cells resistant to $1,25(OH)_2D_3$ which expressed VDR but were resistant to treatment with $1,25(OH)_2D_3$. Treatment of both parental and resistant MCF-7 cells with TAM induced apoptosis supporting that apoptosis can be induced either by (a) activation of vitamin-D-mediated signaling or (b) disruption of estrogen-dependent signaling. Therefore, two independent signaling pathways may be activated and may achieve higher levels of cell death in malignant cells and prevent resistance which is commonly observed in tumour cells. This observation is consistent with the induction of a caspase-independent pathway by $1,25(OH)_2D_3$ [103,113].

Simboli-Campbell et al., [39] assessed the role of $1,25(OH)_2D_3$ in inducing apoptosis in MCF-7 cells. Time course studies indicated that $1,25(OH)_2D_3$ reduced MCF-7 cell numbers and morphological assessment demonstrated that MCF-7 cells treated with $1,25(OH)_2D_3$ exhibited characteristic apoptotic features such as cytoplasmic condensation, pyknotic nuclei, condensed chromatin and nuclear matrix re-organisation, DNA fragmentation as well as upregulation of the mRNA and protein levels for TRPM-2/clusterin and cathepsin B. In a follow up study by the same group, Simboli-Campbell et al., [98] treated MCF-7 cells with either by $1,25(OH)_2D_3$ or the synthetic analogue EB1089. The cells exhibited morphological characteristics of apoptosis, upregulated the proteins clusterin and cathepsin B and down regulated the anti-apoptotic protein Bcl-2. These studies demonstrated that, despite its lower calcemic activity *in vivo*, the Vitamin D analog EB1089 induces effects that are indistinguishable from those of $1,25(OH)_2D_3$ on apoptosis in MCF-7 cells *in vitro*. In addition to the work of Simboli-Campbell et al., [98] several other studies have shown that cathepsin B, clusterin and TGFβ are upregulated by $1,25(OH)_2D_3$ and its analogues [39,94,105,114]. Consistent with the evidence from Simboli-Campbell et al., [98], Narvaez and Welsh also reported a decrease in the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xl, an increase in the pro-apoptotic proteins Bak and Bax and an allocation of Bax from the cytosol to mitochondria [103]. $1,25(OH)_2D_3$ has been shown to up-regulate genes involved in apoptosis including caspases [106,109] and Narvaez and Welsh reported that inhibition of cell cycle block is not necessary for the induction of apoptosis by $1,25(OH)_2D_3$ in MCF7 cells. Therefore $1,25(OH)_2D_3$ generates 'distinct pathways' which are independent of cell cycle block and lead to induction of apoptosis in breast cancer cells (Figure 1) [115].

Koçak et al., [116] have investigated the effect of Vitamin D on Cancer Stem Cells (CSC) that sorted from the MCF-7 cell line and on the HEK293 cell line which was used as control. The results showed that treatment with $1,25(OH)_2D_3$ reduced the number of CSCs in the MCF-7 cells but increased the number of CSCs in the HEK293 cells. In addition, gene expression analyses showed that $1,25(OH)_2D_3$ had a significant effect on the induction of genes involved in apoptosis.

Induction of Caspase-Independent Programmed Cell Death (CI-PCD): Mathiasen et al., [117] treated two human breast cancer cell lines i.e. MCF-7 cells, (expressing a wild-type p53 tumour suppressor protein) and T47D cells (lacking a functional p53) with Vitamin D compounds ($1,25(OH)_2D_3$ and the synthetic analogues EB 1089 and CB 1093). Vitamin D compounds induced a growth arrest followed by apoptosis in both cell lines indicating that p53 is not necessary for the growth-inhibitory and apoptotic effects induced by Vitamin D compounds. Interestingly, apoptosis induced by these compounds occurred independently of caspases and inhibition of caspase activation had no effect on the induction of growth arrest or apoptosis by Vitamin D compounds. Moreover, caspase-3

activity was not detected in lysates from apoptotic cells following the treatment with these compounds. However, overexpression of the anti-apoptotic protein Bcl-2 in MCF-7 cells almost completely inhibited the apoptosis induced by Vitamin D compounds. Therefore, the results of this study indicated that Vitamin D compounds induce apoptosis via a novel caspase- and p53- independent pathway that can be inhibited by Bcl-2 [117]. This observation may prove useful in the treatment of tumours that are resistant to therapeutic agents that are dependent on the activation of p53 and/or caspases. Several other studies have also supported that induction of apoptosis in MCF7 cells is independent of caspases and p53 [103,113]. Ceramide and/or cytosolic phospholipase A_2 ($cPLA_2$) may also be involved as downstream effectors in Vitamin D-mediated caspase-independent cell death (Figure 1) [103].

Mathiasen et al., [118] have shown that the treatment of MCF-7 breast cancer cells with $1,25(OH)_2D_3$ or its chemotherapeutic analogue, EB 1089, releases Ca^{2+} from the endoplasmatic reticulum and activation of a calcium-dependent cysteine protease, μ-calpain. Overall, these results suggest that calpains may be the responsible execution protease in CI-PCD induced by Vitamin D compounds. Thus, Vitamin D compounds may prove useful in the treatment of tumours resistant to therapeutic agents dependent on the classical caspase cascade. Further evidence for the induction of caspase independent cell death by $1,25(OH)_2D_3$ has been provided by Narvaez and Welsh [103]. $1,25(OH)_2D_3$ induced apoptosis in MCF-7 cells by disruption of mitochondrial function, which is associated with Bax translocation to mitochondria, cytochrome *c* release, and production of reactive oxygen species. Interestingly, these mitochondrial effects of $1,25(OH)_2D_3$ did not require caspase activation, since they were not blocked by the cell-permeable caspase inhibitor z.VAD.FMK. MCF-7 cells still executed apoptosis in the presence of z.VAD. FMK, indicating that $1,25(OH)_2D_3$ mediated cell death was caspase-independent [103].

Synergistic effects of Vitamin D compounds and chemotherapeutic agents: Numerous studies carried out in breast cancer cells have shown that $1,25(OH)_2D_3$ and other Vitamin D synthetic derivatives enhance the levels of apoptosis induced by numerous anti-cancer agents [94]. These agents include anti-estrogens such as tamoxifen, retinoids, doxorubicin, taxol, carbo/cisplatin, cytokines such as TNFα, and ceramide [113,118-125]. In addition, synergies have been observed with radiation [126,127]. For example, Chaudhry et al., [127] have shown that the Vitamin D analogue ILX-23-7553 enhanced the effects of adriamycin and irradiation in MCF-7 breast cancer cells.

Inhibition of angiogenesis, migration and invasion: In addition to a role in the induction of cell cycle block and apoptosis, Vitamin D compounds inhibit angiogenesis. Mantell et al., [128] have shown that $1,25(OH)_2D_3$ inhibited vascular endothelial growth factor induced endothelial cell sprouting in a 3D collagen gel system. In addition, $1,25(OH)_2D_3$ has been shown to up-regulate genes involved in angiogenesis including matrix metalloproteinases [106,109]. Klopotowska et al., [129] have investigated the effect of $1,25(OH)_2D_3$ and that of the synthetic derivative 1,24-dihydroxyVitamin D_3 (tacalcitol) on the microRNA MiR-125b. miR-125b has been found to promote migration and invasion of MCF-7 cells and to be involved in chemotherapeutic resistance. Therefore, decreasing miR-125b expression is considered to be of therapeutic significance. The researchers have found that treatment with both $1,25(OH)_2D_3$ and 1,24-dihydroxyVitamin D_3 (tacalcitol) caused a decrease in miR-125b expression and an increase in the level of the pro-apoptotic Bak1 protein which is encoded by a target gene of miR-125b.

The Anti Cancer Potency of Vitamin D in Animal Studies

The anti-cancer potency of Vitamin D compounds has been assessed in animals. A study by Jacobson et al., [130] has shown that dietary Vitamin D can inhibit the tumourigenic effects of a high fat diet on the mammary tissue of rats. Anzano et al., [131] have shown that treatment with the Vitamin D analogue Ro24-5531 prevented the development of carcinogen induced mammary tumours and enhanced the ability of tamoxifen to reduce total tumour burden in rats. Furthermore, $1,25(OH)_2D_3$ and the analogue $1alpha(OH)D_5$ inhibited the development of DMBA-induced preneoplastic lesions in mouse mammary glands compared with untreated glands [132].

Koshizuka et al., [41] have shown that the use of $1,25(OH)_2D_3$ and paclitaxel was more effective in inhibiting tumour growth (MCF7 induced lesions) in BNX triple immunodeficient mice when compared to either $1,25(OH)_2D_3$ or paclitaxel alone or with control. In another study, immunocompromised mice bearing MCF-7 breast cancer xenografts showed significant tumour shrinkage (>50%) after ingestion of a Vitamin D_3-supplemented diet (5000 IU/kg) compared with a control diet (1000 IU/kg). Dietary Vitamin D_3 did not increase serum calcium, demonstrating its safety at the concentration tested. Interestingly, both $1,25(OH)_2D_3$ and dietary Vitamin D_3 suppressed estrogen synthesis. These preclinical data supported a role for dietary Vitamin D_3 supplementation in cancer prevention and cancer chemotherapy [42]. In addition, Jeong et al., [43] has shown that following orthotopic implantation of MMTV-Wnt1 mammary tumour cells into the mice. The mice that were injected with $1,25(OH)_2D_3$ or fed on a Vitamin D supplemented diet caused a striking delay in tumour appearance and growth, while a Vitamin D deficient diet promoted tumour appearance and growth.

The anti-angiogenic property of $1,25(OH)_2D_3$ has been reported by a number of *in vivo* studies [94]. Oikawa et al., [133] have shown that the Vitamin D synthetic derivative 22-oxa-$1,25(OH)_2D_3$) can inhibit angiogenesis. Majewski et al., [134] has provided evidence that three retinoids and $1,25(OH)_2D_3$ significantly decreased Tumour-Induced Angiogenesis (TIA) in mice. In a subsequent study Majewski et al., [135] compared the anti-angiogenic activity of $1,25(OH)_2D_3$, retinoids and IL-12 in an experimental tumour cell-induced angiogenesis assay in mice. Treatment of mice with $1,25(OH)_2D_3$ significantly decreased angiogenesis, comparable to the effect of IL-12. Moreover, the combination of $1,25(OH)_2D_3$ and retinoids resulted in a synergistic inhibition of angiogenesis. Mantell et al., [128] investigated the effect of effect of $1,25(OH)_2D_3$ on angiogenesis *in vivo* by using a model in which MCF-7 breast carcinoma cells induced to overexpress VEGF, were xenografted subcutaneously together with MDA-435S breast carcinoma cells into nude mice. Treatment with $1,25(OH)_2D_3$ (12.5 pmol/d for 8 weeks) produced tumours that were less well vascularized than tumours formed in mice treated with vehicle alone. The results of this study supported a role for $1,25(OH)_2D_3$in inhibiting angiogenesis.

Since $1,25(OH)_2D_3$ is hypercalcemic (effective in increasing serum calcium level), it is not suitable for use in cancer prevention or cancer therapy trials and instead Vitamin D analogues have been developed with the hope that this would be more appropriate in terms of anti-

cancer potency [94,132]. Some of the most popular compounds developed are Calicpotriol (MC903), Mexacalcitol (OCT), Seocalcitol (EB1089), 16-ene and other analogues [94]. These analogues have been used in animal models. OCT has been shown to delay the growth of breast tumour xenografts (developed from ER positive MCF7 cells) in athymic mine and to work synergistically with tamoxifen [119,136]. OCT inhibited DMBA induced rat mammary tumours when used alone or in combination with the aromatase inhibitor CGS 16949 [137]. In the NMU-induced tumour model and in MCF7 cell xenografts, treatment with EB1089 led to tumour regression [138,139]. Furthermore, El Abdaimi et al., [140] have shown that EB1089 increased the survival and inhibited the development of bone metastasis following intracardiac inoculation of athymic mice with MDA-MB-231 cells.

A number of studies have evaluated the use of Vitamin D analogues CB1093, EB1089, OCT, Ro025-6760 and Ro-24-5331 with chemotherapeutic treatments such at taxol, cisplatin, tamoxifen and aromatase inhibitors and these results were very promising [41,94,119,131,141-144]. MART-10 is also a new generation analogue which is more potent than $1,25(OH)_2D_3$ in inhibiting cell proliferation and inducing apoptosis in ER+ MCF7 breast cancer cell lines [145].

Clinical Trials Investigating the Efficacy of Vitamin D for the Chemoprevention of Breast Cancer

Following the promising results derived from cellular and animal studies, several clinical studies have been designed to investigate the anti-cancer potency of Vitamin D in humans. Some of these clinical trials have been completed while others are ongoing. This section provides a summary of the most important clinical trials that have been carried out or are still being carried out to investigate the usefulness of Vitamin D for the chemoprevention of breast cancer. Despite the promising results of many studies, there is still conflicting data in the literature with regards to the efficacy of Vitamin D in the chemoprevention of breast cancer. The latter is attributed to the variability in the studies in terms of (a) the study population e.g. the number of subjects involved premenopausal *vs.* post menopausal, high risk women *vs.* population based studies, (b) the dose of Vitamin D supplementation or Vitamin D supplementation in combination with calcium, (c) the outcomes of the study - i.e. incidence of breast cancer *vs.* changes in mammographic density, (d) the duration and follow up of the study.

Clinical trials in post-menopausal women: Lappe et al., [44] carried out a 4 year, population-based, double-blind, randomised placebo-controlled trial. The purpose of this analysis was to determine the efficacy of calcium alone and calcium plus Vitamin D in reducing incident cancer risk of all types. The subjects were 1,179 women randomly selected from a population of healthy postmenopausal women aged >55 years living in Nebraska. Subjects were randomly assigned to receive 1,450 mg/day of calcium or 1,450 mg/d of calcium plus 1,100 IU/day Vitamin D_3, or placebo. The results of this study showed that improving calcium and Vitamin D nutritional status substantially reduced all-cancer risk (including breast) in postmenopausal women. In a subsequent study, Lappe et al., [45] have reported the results of a Vitamin D cancer incidence trial. A total of 2,303 healthy postmenopausal women 55 years or older were randomised, 1,156 to the treatment group and 1,147 to the placebo group. The duration of treatment was 4 years. The trial consisted of a treatment arm with 2,000 IU/day of Vitamin D_3+1,500 mg/day of calcium and a placebo arm. The results have shown that among healthy postmenopausal older women with a mean baseline serum 25(OH)D level of 32.8 ng/ml, supplementation with Vitamin D_3 and calcium compared with placebo did not result in a significantly lower risk of all-type cancers at 4 years.

The Women's Health Initiative (WHI) was a clinical trial (NCT00000611) in which 36,282 postmenopausal women took 1 g/day calcium plus 400 IU/day of Vitamin D or placebo for 7 years. The results of the study showed that for women who were not taking personal calcium or Vitamin D supplements at randomisation, calcium and Vitamin D decreased the risk of total, breast and invasive cancers by 14% to 20% [146].

Clinical trials on the effect of Vitamin D on mammographic density: Crew et al., [147] examined the safety, feasibility, and biomarker effects of high-dose Vitamin D among women at high risk for breast cancer. Forty high-risk women (20 premenopausal and 20 postmenopausal), defined as a 5-year breast cancer risk ≥ 1.67% per the Gail model, were assigned to a 1-year intervention of Vitamin D_3 20,000 IU or 30,000 IU weekly. The results of the study showed that even though 1 year of high-dose Vitamin D_3 was associated with a significant increase in circulating Vitamin D, there was no significant change in Mammographic Density (MD) regardless of menopausal status or dose level.

Brisson et al., [148] conducted a double-blind, placebo-controlled parallel group trial assessed whether oral supplementation with 1,000, 2,000, or 3,000 IU/day Vitamin D_3 over one year reduced percent mammographic breast density in premenopausal women. However, the results of the study showed that at doses of 1,000 to 3,000 IU/day, Vitamin D supplementation could not reduce breast cancer risk through changes in breast density.

One of the ongoing clinical trials with Vitamin D in the US is the 'Vitamin D and Breast Cancer Biomarkers in Female Patients (NCT01224678)' clinical trial. In this clinical trial premenopausal women of age <55 were assigned to receive either 2,000 IU/day of Vitamin D or placebo for 12 months, stratified by Baseline (BL) Vitamin D level (sufficient *vs.* insufficient). Biomarker specimens were collected at baseline and 12 months. The results of the study have shown that even though Vitamin D supplementation resulted in an increase in serum levels of Vitamin D, no significant change in mammographic density was observed and possibly a longer period of exposure to Vitamin D may be required for any effects to be visible [149].

Clinical trials in pre-menopausal women: The 'Modulation of breast cancer risk biomarkers by high dose Vitamin D (NCT01166763)' is a clinical trial developed to investigate (a) if a high dose Vitamin D3 given to premenopausal women at high risk for development of breast cancer (who initially have insufficient levels of 25(OH)D (<30 ng/ml)), would raise 25(OH)D levels above 50 ng/ml and (b) if certain risk biomarkers for the development of breast cancer could be modulated. The 'Vitamin D in Postmenopausal Women at High Risk for Breast Cancer (NCT00859651)' is a phase II study which enrolled 20 postmenopausal women at high risk for breast cancer development. The goal was to determine whether a one year intervention of high-dose Vitamin D at 2 different doses (20,000 IU weekly or 30,000 IU weekly) would increase circulating blood levels of Vitamin D and to obtain preliminary data on the biologic effects of Vitamin D for breast cancer prevention. No results have

been published by either of the two clinical trials yet. The results of the studies are expected to shed light on whether the interventions and durations used were successful in reducing breast cancer risk in the subjects.

Clinical Trials Investigating the Efficacy of Vitamin D for the Management of Breast Cancer

Clinical trials have been designed to investigate the efficacy of Vitamin D for the pharmacological management of breast cancer either on its own or in the presence of other agents such as chemotherapeutic agents. Similar to the studies investigating a possible role of Vitamin D for the chemoprevention of breast cancer there is also variability in the studies carried out to investigate the role of Vitamin D for the pharmacological management of breast cancer. The studies are also variable in terms of (a) the study population e.g. number of subjects involved, premenopausal *vs.* post menopausal, (b) the patients' type of breast cancer (e.g. hormonal *vs.* triple negative), (b) the dose of Vitamin D supplementation or the use of Vitamin D in combination with other agents such as aromatase inhibitors, (c) the outcomes of the study e.g. biological markers, efficacy and safety, effect on side effects of conventional treatments, gene expression and (d) the duration and follow up of the study.

The clinical trial 'Effects of Vitamin D in Patients With Breast Cancer (NCT01948128) was a prospective, randomised, double blind, placebo-controlled phase 2 trial. 80 eligible women (42 in the Vitamin D group and 38 in the control group) took a high dose of Vitamin D (40,000 IU) for at least 2 weeks leading up to the day of surgery. Within the study cohort, 16/80 (64%) were ER positive, 55/80 (55%) were PR positive, 65/80 (61%) were Her2 negative. Pre- and post- 25(OH)D levels, tumour Ki67 index and caspase 3 were analyzed. The results of the study reported that there was no significant difference seen in these markers as a result of Vitamin D intake, despite significantly higher circulating levels of 25(OH)D in the blood [46].

Several studies have been carried out to investigate the effect of Vitamin D supplementation in patients with adjuvant or neoadjuvant chemotherapy for breast cancer [150-153]. Khan et al., [151] carried out a study to investigate the prevalence of Vitamin D deficiency in women initiating the adjuvant Aromatase Inhibitor (AI) letrozole for breast cancer and to determine whether supplementation with 50,000 IU of Vitamin D3 weekly could reduce musculoskeletal symptoms and fatigue in women with Vitamin D deficiency. Sixty women about to begin an adjuvant AI were enrolled. Postmenopausal women with early-stage, receptor-positive invasive breast cancer, who were candidates for an adjuvant AI, were eligible. Pre-menopausal women were eligible if they received ovarian suppression with a GnRH analogue. The results of the study showed that Vitamin D3 supplementation with 50,000 IU per week was safe, significantly increased 25(OH)D levels, and could reduce disability from AI-induced arthralgias. A follow up study by the same group was also carried out. Women with stage I to III breast cancer starting adjuvant letrozole and 25(OH)D level ≤ 40 ng/ml were recruited. All subjects received standard daily supplement of 1,200 mg calcium and 600 IU Vitamin D3 and were randomised to 30,000 IU oral Vitamin D_3/week or placebo. Pain, disability, fatigue, quality of life, 25(OH)D levels, and hand grip strength were assessed for a period of up to 24 weeks. The results of the study showed that although 30,000 IU/week of oral Vitamin D_3 was safe and effective in achieving adequate Vitamin D levels, it was not associated with a decrease in Aromatase Inhibitor-associated Musculoskeletal Symptom (AIMSS) events. Post-hoc analysis using a different tool suggested potential benefit of Vitamin D_3 in reducing AIMSS [153].

The 'Vitamin D Supplementation Tailored to Vitamin D Deficiency in Breast Cancer Patients (VITACAL) (NCT01480869)' was a randomised phase III study developed to assess the safety and efficacy of a tailored, high-dose, oral Vitamin D supplementation in restoring a normal 25(OH)D level in Early Breast Cancer (EBC) patients treated with adjuvant or neoadjuvant chemotherapy. A total of 215 patients including 197 patients with Vitamin D deficiency were recruited, and 195 patients were randomized. Patients were stratified according to three baseline Vitamin D deficiency levels [>10 ng/ml or (10 ng/ml to 20 ng/ml) or (20 ng/ml to 30 ng/ml)], within 7 months to 12 months after the first day of chemotherapy [stratification (0 month to 7 months) or (7 months to 12 months)], hormone receptor positivity (yes or no) and menopausal status (peri- and premenopausal or menopausal). Patients received a high dose of 10,000 IU of Vitamin D at specific dates depending on their Vitamin D deficiency status. The results of the study showed that a tailored high-dose oral Vitamin D supplementation safely allows a higher percentage of the serum 25(OH)D level normalization compared with a conventional regimen in chemotherapy-treated EBC patients [152].

Mohseni et al., [154] investigated changes of 25(OH)D in a randomised, double-blind, placebo-controlled clinical trial, according to VDR genotype, after provision of Vitamin D_3 to breast cancer cases for a 2-month period. Participants were assigned to two treatment arms: placebo (n=28) and Vitamin D3 supplementation (n=28). The supplementation group received 50,000 IU of Vitamin D every week for 2 months. Blood samples were collected at baseline and after intervention to measure serum 25(OH)D_3. Vitamin D_3 supplementation increased serum 25(OH)D level in women with breast cancer. Nevertheless, certain genotypes of VDR were more responsive to Vitamin D supplementation than other genotypes.

27-Hydroxycholesterol (27-HC) is an endogenous Selective Estrogen Receptor Modulator (SERM) which drives the growth of Estrogen Receptor-positive (ER+) breast cancer. 1,25$(OH)_2D_3$ inhibits expression of CYP27B1, which is very similar in structure and function to CYP27A1, the synthesizing enzyme of 27HC. Therefore, Going et al., [155] hypothesized that 1,25$(OH)_2$D may also inhibit expression of CYP27A1, thereby reducing 27HC concentrations in tissues that express CYP27A1, including breast cancer tissue. 27HC, 25-hydroxyVitamin D (25OHD) and 1,25$(OH)_2$D were measured in sera from 29 breast cancer patients before and after supplementation with low-dose (400 IU/day) or high-dose (10,000 IU/day) Vitamin D in the interval between biopsy and surgery [156]. The results of this study showed that Vitamin D supplementation decreased the levels of 27HC of breast cancer patients, likely by CYP27A1 inhibition. This suggests a new mechanism by which Vitamin D can inhibit ER+ breast cancer growth but further studies should be carried out to investigate the mechanism in more detail.

The scope of the clinical trial 'Development of Vitamin D as a Therapy for Breast Cancer - Phase 2 - (NCT00656019)' was to assess whether (a) the levels of Vitamin D impact the characteristics of a woman's breast cancer at diagnosis and (b) a short course of Vitamin D in women with low levels of Vitamin D changes the gene expression of their breast cancers. The clinical trial 'Anti-proliferative Effects of Vitamin D and Melatonin in Breast Cancer (MELO-D) (NCT01965522)' investigated if Vitamin D and melatonin (alone or

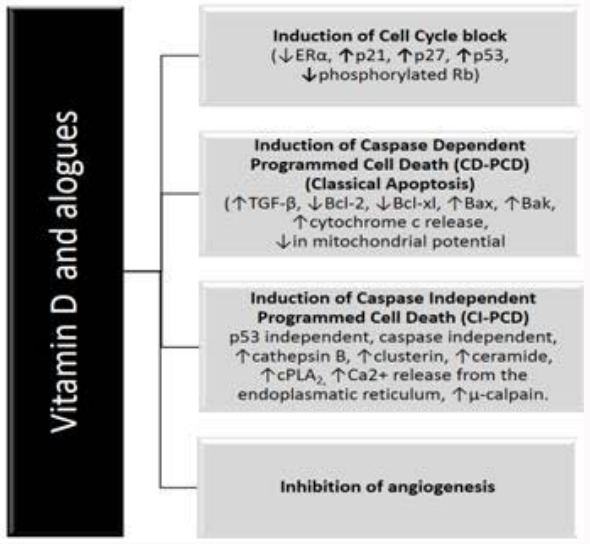

Figure 1: Anti-cancer pathways induced by Vitamin D and analogues.

in combination), can reduce the spread of cancer cells in the tumours of women with breast cancer. This study included women with breast cancer who were planned for surgery, and assessed whether treatment with Vitamin D (dose of 2000 IU per day), or melatonin (dose of 20mg per day) or Vitamin D and melatonin, reduces the spread of cancer cells when compared to a fourth group of women who are treated with sugar pills (control). The results of the studies have not been published yet but are expected to provide important information with regards to the suitability of Vitamin D for the pharmacological management of breast cancer.

Despite some promising results from clinical trials, researchers have reported concerns with regards to the design of some of the clinical trials. The main concern was that these clinical trials have been designed and conducted in a similar manner as that used for pharmaceutical drugs rather than nutrients [157]. Clinical trials for pharmaceutical drugs assume that the only source of the agent is through the trial and that there is a linear dose-response relationship. However, in addition to receiving Vitamin D through the clinical trial, people can obtain Vitamin D by UVB exposure, diet and supplements. Furthermore, due to differences in body mass indices and different baseline 25(OH)D concentrations, supplementation with the same amount of Vitamin D can produce different levels in different individuals [157-159]. Robert Heany has published some important guidelines that should be followed with regards to the design of clinical trials involving Vitamin D. These guidelines are the following: 1. Start with an understanding of the 25(OH)D concentration- health outcome relationship. 2. Measure 25 (OH) D concentrations of prospective participants and try to enrol those with concentrations near the low end of the relationship. 3. Give Vitamin D3 doses high enough to raise 25(OH)D concentrations to the upper part of the relationship. 4. Measure 25(OH)D concentration again during the trial to determine the success of the dosing and assess compliance' [160]. The guidelines to be followed in the design of future clinical trials such as measuring and adjusting the concentration of 25(OH) D during the study have been addressed by two recent papers [161,162].

The Possible Role of Vitamin D for Breast Cancer Survivors

Cancer survivors are often highly motivated to seek information about food and dietary supplements to improve their treatment outcomes, quality of life, and overall survival. Evidence in the literature supports the requirement for guiding cancer survivors with regards to the nutrition they should follow during the post-treatment phase [163]. Despite the availability of international guidelines for nutritional care in cancer patients who have been recently updated, many cancer survivors still do not receive adequate nutritional support [163,164]. Given the anti-cancer potency of Vitamin D, it is very important to investigate the significance of supplementing breast cancer survivors with Vitamin D [165]. Nevertheless, only a limited number of studies have addressed this important issue.

A study by Yao et al., [166] assessed Vitamin D status in relation to breast cancer survival. The results of the study showed that women with higher levels of serum 25(OH)D had better overall survival and there were inverse associations of 25(OH)D with tumour stage and tumour grade. Furthermore, 25(OH)D concentrations were lowest in women with triple negative breast cancer. Therefore, there is potentially an important role for supplementing breast cancer survivors with Vitamin D. Andersen et al., [23] carried out a study in which Vitamin D supplementation was used by 553 breast cancer patient/survivors (193 who used a Naturopathic Oncology [NO] provider and 360 who did not) participating in a matched cohort study of breast cancer. The results of the study showed that Vitamin D supplementation by breast cancer patients is common both during and after treatment for breast cancer, but deficiency may also be common. NO and conventional providers may be able to promote Vitamin D sufficiency through Vitamin D supplementation and by encouraging healthy solar exposure. Further studies should be undertaken examining whether Vitamin D supplementation and higher blood levels might improve HRQOL among women with breast cancer in early survivorship.

Therefore, current results in the literature support that Vitamin D supplementation may be very beneficial to breast cancer survivors. Vitamin D may therefore an important role to play in preventing any metastasis and therefore cancers relapse. In addition, breast cancer survivors may derive additional, non-cancer-related benefits from adequate Vitamin D levels, including improvements in bone mineral density, quality of life, and mood [167]. These benefits are of equal significance since conventional treatments used have a number of side effects.

The Possible Role of Vitamin D in Palliative Care

In addition to a possible role in chemoprevention and management of breast cancer, emerging evidence in the literature is supporting a role for Vitamin D in the palliative care of cancer. In addition to the fact that Vitamin D supplementation would likely be beneficial both with regard to the cancer itself, but also it could improve the quality of life of the patient due to its positive effects on bone mineral density, mood and the immune system. In addition, Vitamin D is safe when used with chemotherapeutic and other agents [167-169].

Dev et al., [170] carried out a retrospective review of 100 consecutive cancer patients with loss appetite or fatigue. The results of the study showed that low levels of Vitamin D were prevalent in advanced cancer patients with cachexia and fatigue. Trivanovic et al., [168] carried out a study in which 69 Vitamin D deficient patients were supplemented with 2,000 IU of Vitamin D per day. In addition to improved serum levels of Vitamin D, the patients' fatigue

Figure 2: The different stages of potential intervention with Vitamin D compounds in breast cancer.

had improved. Even though very few studies have been performed in palliative care patients, the results from the few studies are promising. Bazzan et al., [167] have therefore proposed that Vitamin D supplementation should be provided to patients to achieve a serum level of at least 50 ng/ml and consideration should be given to include cofactors such as calcium and phosphate.

Bjorkhem-Bergman and Bergman performed an observational study in 100 patients with palliative cancer in Sweden [169]. The results of the study showed that low Vitamin D levels were associated with higher opioid dose i.e. more pain. The researchers also described a case report where Vitamin D supplementation resulted in radically decreased opioid dose, less pain and better well-being. Helde-Frankling et al., [171] reported that Vitamin D supplementation to palliative cancer patients was safe and led to improvement in pain management as early as one month after treatment and decreased infections 3 months after Vitamin D treatment. The results from this pilot-study have been used for the power-calculation of a future randomised, placebo-controlled, double-blind study called "Palliative-D" that started in November 2017 and includes 254 palliative cancer patients (Figure 2) [172].

Vitamin D supplementation does not cause any adverse side effects and is easy to administrate. Thus, the supplementation of patients with palliative cancer with Vitamin D may improve their well-being, decrease pain and reduce susceptibility to infections. However, more clinical studies will need to be carried out to assess the suitability of Vitamin D at this stage of cancer [169].

Conclusions

The scope of this review was to report evidence supporting a multi-faceted role of Vitamin D for the prevention and stages of management, survival and palliative care of breast cancer. The current review has provided evidence for a role of the active metabolite $1,25(OH)_2D_3$ and other Vitamin D synthetic derivatives in the induction of cell cycle block, Caspase-Dependent Programmed Cell Death (CD-PCD) (i.e. classical apoptosis), Caspase-Independent Programmed Cell Death (CI-PCD) and the inhibition of angiogenesis in breast cancer. Future research should focus on unraveling the molecular players involved in CI-PCD induced by Vitamin D compounds. These players are expected to be of equal significance to those involved in the classical pathway of apoptosis and could be targets of future chemotherapeutic treatments. Vitamin D compounds and conventional therapeutic agents could be used to activate distinct pathways of programmed cell death (CD-PCD and CI-PCD). Activation of independent pathways of cell death should achieve lower viability and prevent resistance which is commonly observed in tumour cells. In the near future, the results of ongoing clinical trials should be analysed carefully. Future clinical trials should be designed to address (a) the suitability of Vitamin D and (b) the challenges associated with using the optimal levels of Vitamin D not only for the prevention of breast cancer but also for supplementation during the stages of management, survival and palliative care of breast cancer.

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