Vitamin D and Breast Cancer - Chemoprevention or Therapy?

Gruber-Bzura BM*
Department of Biochemistry and Biopharmaceuticals, National Medicines Institute, Chełmska, Poland

Abstract
Most evidence studies have concluded that there is an inverse association between blood levels of calcidiol and cancer incidence and survival. These findings especially apply in colorectal and breast cancer (BC). The phenomenon of the multidirectional activity of vitamin D is possibly due to the presence of the vitamin D receptor (VDR) in most non-skeletal human cells including cancer cells. The crucial is that a wide range of the genes regulated by VDR are related with cell proliferation, differentiation, angiogenesis and metastasis. The aim of this paper was to present recent data on the possible role of vitamin D as an chemopreventive or therapeutic agent against BC through considering the anticancer mechanisms induced by this vitamin as well as presenting the results of clinical studies on the impact of vitamin D status on BC incidence, survival and response to therapy. This review is based on an electronic search of articles in the PubMed database, including papers published mostly within last five years in that field and selected according to the following criteria: well commented studies concerning the association between vitamin D status and BC risk, response to therapy or survival, with the vitamin D supplementation outline and statistical data.

Introduction
Now we know that because of pleiotropic actions, bone homeostasis and mineralization is only one of the effects of vitamin D. Most evidence studies have concluded that there is an inverse association between blood levels of calcidiol and cancer incidence and survival. These findings especially apply in colorectal cancer and BC [1].

The phenomenon of the multidirectional activity of vitamin D is possibly due to the presence of VDR in most non-skeletal human cells including cancer cells [2,3]. VDR is a ligand-dependent transcription factor. When bound to its ligand, calcitriol, VDR dimerizes with the retinoid X receptor that allows the heterodimer to translocate into the nucleus and next to bind to vitamin D response elements in promoter regions inducing transcriptional regulation of target genes [1,3,4]. The crucial is that a wide range of the genes regulated by VDR are related with cell proliferation, apoptosis, differentiation, angiogenesis and metastasis [3].

The aim of this paper was to present recent data on the possible role of vitamin D as an chemopreventive or therapeutic agent against BC through considering the anticancer mechanisms induced by this vitamin as well as presenting the results of clinical studies on the impact of vitamin D supplementation on BC incidence and survival and response to anticancer therapy.

Literature Review
This review is based on an electronic search of articles in the PubMed database, including papers published mostly in the last five years up until 2018 in that field. The relevant papers are included. All research articles were found with a combination of the following keywords: vitamin D and breast cancer, vitamin D and chemoprevention and breast cancer, vitamin D supplementation and breast cancer risk, vitamin D and anticancer therapy. Published articles were selected according to the following criteria: published in English, concerning the association between vitamin D status and BC risk, response to therapy or survival, with vitamin D supplementation outline, and statistical data. Studies with insufficient data were excluded.

Anticancer properties of vitamin D
The discovery that epithelial breast cells possess the same enzyme system as the kidney, where mainly is generated calcitriol, suggests the impact of vitamin D on BC cells. As was given by de La Puente-Yagüe et al. [5], human mammary cells cultured from normal breast tissue express VDR, 1a hydroxylase, CYP27B1 and the megalin-cubulin complex. This complex, among others, promotes the binding of 25(OH)D to vitamin D binding protein (VBP) [5]. VDR is a member of the nuclear family of receptors that also includes: estrogen and progesterone receptors (ER and PR), the androgen receptor and the T4/T3 receptor [1].

VDR has been implicated in cell cycle arrest, apoptosis and promotion of differentiation. This receptor affects cell proliferation, among others, via direct induction of growth arrest, indirect impact on the proteins, which affect G0/G1 cell cycle arrest or via suppression of human epidermal growth factor receptor 2 (HER2) as well as via the regulation of protooncogenes, such as c-Myc and c-Fos [4,6,7]. In BC cells, calcitriol induces apoptosis by stimulating Ca" release from intracellular stores [6]. According to Santos et al. [8], apoptosis induction by vitamin D in BC cells is associated also with decreased expression of mammalian target of rapamycin, which regulates glycolysis and cancer survival.

The growth factors and hormones up regulate cell proliferation and growth and in this way play a role in BC progression. According to Duffy et al. [1], VDR-mediated inhibition of ER° BC cells may be at least partly effected by downregulation of ER. In contrast, treatment with calcitriol was reported to induce ER expression in the ER- cells, SUM-229PE. This ability of calcitriol to convert ER- breast cancer cells to an ER° status, a potentially endocrine-sensitive would have major implications for the treatment of BC.

As was shown, calcitriol also is a potent inhibitor of tumor cell-induced angiogenesis in experimental models [9]. It inhibits vascular endothelial growth factor-induced endothelial cells tube formation in vitro and decreases tumor vascularization in vivo in mice bearing xenografts of BC cells over-expressing vascular endothelial growth factor. It can also directly inhibit the proliferation of endothelial cells leading to inhibition of angiogenesis [3,9]. Acting indirectly, calcitriol...
suppresses the expression of the proangiogenic factor interleukin 8 [9].

Chronic inflammation has been recognized as a risk factor for cancer development and many of the pro-inflammatory mediators which are then over expressed activate angiogenic processes and thereby promote tumor progression, metastasis and invasion [9]. Calcitriol, among others, suppresses the activation and signalling of nuclear factor κB, regulating the genes involved in inflammatory and immune responses and cellular proliferation [3]. Another mechanism of calcitriol that limit pro-inflammatory events is the increase of mitogen-activated protein kinase phosphatase-5 which indirectly decreases the pro-inflammatory cytokines, such as interleukin-6 [9].

The mechanisms underlying reduction of the invasive and metastatic potential of many malignant cells by calcitriol include a modulation of the expression of different surface proteins, such as induction of N-cadherin switching to E-cadherin, whose expression is inversely correlated to metastatic potential, downregulation of metalloproteinase-9 or an increase in tissue inhibitor of metalloproteinase-1 [4,9]. According to Santos et al. [8], significant reduction of cell migration and increased cell stiffness is probably a consequence of reversal of the epithelial to mesenchymal transition resulting in the increased E-cadherin and F-actin and reduced cell migration and increased cell stiffness is probably a consequence of reversal of the epithelial to mesenchymal transition that regulates the key molecules involved in these processes, such as components of the plasminogen activator system or tenascin-C that promotes growth, invasion and angiogenesis [9].

Although many studies were taken to analyze the effect of vitamin D on the immune system, there are only a few reports on the effect of vitamin D on that system during progression and metastasis of solid tumors. In the light of evidence that calcitriol shows an immunosuppressive effect, it is very important to consider this action which is conflicting with other studies proving antitumor properties of calcitriol [12]. Pawlik et al. [12] who studied the impact of calcitriol and its analogs on T11 mouse mammary gland cancer observed that the most upregulated genes were related to Th2 and Treg cells which are involved in the immunosuppressive response. The authors noticed that the immunomodulating role of calcitriol and its analogs may play different roles at different stages of tumor progression and the cytokine profile activated or not by these compounds is dependent on the cell type. Very interesting role of vitamin D presented Thyer et al. [13]. On the basis of the study on human BC cells MCF-7, the authors demonstrated the interaction between VDR and VBP-derived macrophage activating factor (Gc-MAF) in the macrophage activation. As a result of such interaction, macrophages attacked human BC cells, inducing apoptosis and phagocytizing them. As was given by Saburi et al. [14] human macrophages activated in vitro with Gc-MAF (100 pg/mL) killed 60% and 86% of MCF-7 cells after 4h and 18h of incubation, respectively. Although it has been demonstrated that macrophages activated by Gc-MAF bind to neoplastic cells in vitro, including BC cells, there is lack of clinical evidence about the action of activated macrophages to cancer cells [14]. According to the authors, differential responses to Gc-MAF that have been observed in human monocyes as well as in metastatic BC cells may be the result of individual VDR genotype. The same authors [14] also focus on the role of the VBP Gc-globulin (human group-specific component), the protein which apart from the storage and transport of vitamin D has an important function as a scavenger of extracellular G-actin to increase neutrophil chemotaxis and macrophage activation. By Saburi et al. [14], the modified Gc-globulin affects the activation and fortification of immune cells exhibiting antitumor activity.

**Vitamin D status vs. BC risk and survival**

The epidemiologic studies regarding the association between vitamin D and BC risk are on the one side promising and on the other side there are conflicting or inconclusive. The causes of the controversial results include: the degree of skin pigmentation, ER status, pre- or peri-menopausal period, BMI, potential modifying effect by VDR polymorphism that vary by ethnic groups or the circulating level of 25(OH)D [15].

Kim et al. [16] proved that race is an important factor which can determine the role of vitamin D in BC. The authors conducted a nested case-control study within Multiethnic Cohort Study of five race/ethnic groups between 2001-2006. Pre-diagnostic plasma levels of 25(OH)D were examined among 707 postmenopausal BC cases and matched controls. As was shown, 20 ng/mL increases of plasma 25(OH)D were inversely correlated with BC risk among white women and not among women in other race/ethnic groups (Table 1).

Kim and Je [15] on the basis of 30 prospective studies (nested case-control or cohort) focused on vitamin D as the intriguing factor which can influences BC survival. The authors found an overall non-significant inverse association between vitamin D intake or 25(OH)D blood levels and BC risk but in BC patients the risk of death from BC decreased by 42% for high vs. low 25(OH)D levels (Table 1). The authors considered strengths as well as weaknesses of their meta-analyses [16]. According to them, in the included studies, vitamin D intake or blood levels of 25(OH)D were measured before BC was diagnosed, so the possibility that cancer status affected vitamin D status was minimised. In the analyses of mortality from BC or overall mortality, the results were reported as compared with the healthy women. Most of the studies included in the meta-analysis used a single measurement at baseline which could lead to an underestimation of risk evaluation. The authors also pointed to the fact that unmeasured or residual confounding may affect the risk estimates in each study and thus pooled estimates in the meta-analyses. Similar observations to these presented by Kim and Je [15] in regard of positive role of vitamin D in BC made Hauser et al. [17] collecting medical records from adult solid tumor patients. Low 25(OH)D levels, i.e., vitamin D deficient and insufficient were highly prevalent in people with solid tumors including BC. The interesting relations in the effects of vitamin D blood levels and BC risk and prognosis revealed also Shirazi et al. [18] during the prospective, population-based cohort study. The significantly lower risk of ER-, PR- and tumor with high expression of Ki67-proliferation biomarker was found only in the group having vitamin D blood level ≥ 77 ≤ 97 nM/L. The groups with vitamin D blood level below or above this range had a relatively high risk of tumors with unfavourable prognosis (Table 1). A limitation of the study was that some tumor groups were relatively small.

According to the authors, these results suggest a U-shaped association between vitamin D blood levels and aggressive BC risk. Jeffreys et al. [19] in the cohort study confirmed that any vitamin D prescription, compared to never having been prescribed one was associated with a better survival from BC, however 3 or more
prescriptions was not (Table 1). Not fully consistent results were obtained by Bidgoli et al. [20] who did not show the differences in 25(OH)D levels between BC patients and healthy women and observed only slight associations between the lack of vitamin D and calcium supplementation or weekly egg consumption and premenopausal BC risk. The most important risk factor for BC incidence was the lack of sunlight exposure (Table 1).

From the studies presented by Cadeau et al. [21] it results the complex interactions between vitamin D intake, BMI and menopausal hormone therapy (MHT) use which affect premenopausal BC risk. Authors found the increased premenopausal BC risk associated with vitamin D supplementation in MHT never users with BMI<25 kg/m², especially below 22 kg/m² than with BMI 22-24.9 kg/m². There was no association in women with higher BMI. Ever vitamin D supplementation was related to decreased BC risk in MHT ever users, regardless of BMI. As a summary, the increased risk associated with vitamin D supplementation in MHT never users was restricted to women with BMI<25 kg/m² (Table 1).

O’Brien et al. [22] confirmed that the beneficial effects of vitamin D supplementation can be related to menopausal status. The prospective cohort study conducted by the authors enrolled healthy women who had a sister with BC. It was found that 25(OH)D levels >38 ng/mL were associated with a 21% lower BC risk. Self-reported vitamin D supplementation ≥ 4 times/week was associated with an 11% lower hazard of BC wherein the inverse association was noted among postmenopausal women and a positive but statistically non-significant association among premenopausal women. Similar effects obtained Brisson et al. [23]—premenopausal female volunteers divided into four groups were supplemented with 1000 IU, 2000 IU or 3000 IU/day vitamin D or unsupplemented. The authors found that only supplementation with 3000 IU/day vitamin D was associated with a slightly smaller decline in breast density compared with placebo (Table 1). On the contrary, Fair et al. [24] in their cross-sectional study revealed the significant trends of decreasing breast density with increasing vitamin D and calcium intake in premenopausal but not among postmenopausal women, after statistical adjustment for age, race and BMI. Surprisingly, there was no association between serum vitamin D and breast density, regardless menopausal status (Table 1). Premenopausal status as more favourable for beneficial effects of vitamin D intake against BC risk found also Estébanez et al. [25] in the meta-analysis on the effects of 25(OH)D blood levels and vitamin D intake, which included sixty eight studies published between 1998-2018, but only in the nested case-control study (Table 1). According to the authors, the observed effects dependent on menopausal status may result from the interaction between insulin-like growth factor-I (IGF-I) and vitamin D. As there is a physiological decline of IGF-I with aging, the interaction between IGF-I and vitamin D is likely to be stronger for pre- than for postmenopausal women.

According to Madden et al. [26], time of initiation of vitamin D supplementation is novel and could have significant clinical implications including the immediate prescribing of vitamin D supplements after BC diagnosis. In the large national BC cohort the authors found that de novo vitamin D use within 6 months after diagnosis is associated with 49% reduction in BC-specific mortality and that vitamin D supplementation≥6 months after diagnosis results only in 20% reduction in BC mortality (Table 1). The lack of the data concerning 25(OH)D blood levels at BC diagnosis, of course, makes impossible to highlight the only role of supplements in a reduction of BC mortality but, on the other side, the authors argue that vitamin D healthy blood level of >30 ng/mL is not possible to obtain without supplementation.

On the contrary to the above mentioned, mostly promising results which point the beneficial role of vitamin D supplementation in BC, Manson et al. [27] did not confirm any relationships between vitamin D intake and BC risk. The authors presented the results from randomized, placebo-controlled trial. As was shown, vitamin D intake 2000 IU/day was not associated with a lower cancer risk of any type (Table 1).

According to Grant [28], for BC only case-control studies consistently find inverse correlations between 25(OH)D and BC and 25(OH)D concentration values are only useful for short follow-up times for BC since it develops rapidly. Moukayed and Grant [29] claim that the prospective studies generally fail to find significant inverse correlations between 25(OH)D concentration and BC incidence because, apart from BC develops rapidly, the point is that a single 25(OH)D concentration measurement rapidly loses predictive ability. Grant et al. [30] suggest that the most likely reason for the failure of randomized controlled trials (RCTs) on the relationship between 25(OH)D blood and BC risk is inappropriate design, conduct, analysis and interpretation of the trials and that the most RCTs use principles designed to test pharmaceutical drugs which incorporate the assumption that RCTs is the sole source of the agent and that dose-response relationships are linear. Both assumptions are not true for vitamin D. In turn, the problem of the case-control studies may be that disease state, its treatment or disease-related behavioral changes may affect 25(OH)D concentration leading to reverse causation [22,29]. Bias from reverse causation can be avoided by prospective studies [22]. Welsh et al. [31] point to the fact that BC is heterogeneous and because of that, analysis of VDR actions in specific molecular subtypes of BC should be considered to clarify all conflicting data obtained in the clinical studies. Another point which should be regarded in such studies is the seasonal variations in vitamin D status. It can be speculated that women who are vitamin D deficient in summer are more likely to be deficient year round, enhancing the BC risk relative to those who are deficient only in winter [31].

**Vitamin D and BC therapy**

As was pointed by Madden et al. [26], there are the evidences to suggest that vitamin D supplementation used in conjunction with standard therapies may reduce BC recurrence and improve survival. According to Jacobs et al. [32], the results for prognosis and survival provide a more consistent picture than for vitamin D and BC incidence. As mostly, well-conducted observational studies are controlled for the confounders which may affect the relationship between 25(OH)D and BC survival, as for example, BMI, physical activity or cancer stage at diagnosis.

The topic which is the most often undertaken by the authors is the role of vitamin D in therapy of TNBC, the leading cancer in women [33]. BCs are categorized into three subtypes, ER+, HER2+ and TNBC. Treatment of ER+ and HER2+ BCs has been successful through targeted therapy with anti-estrogen and anti-HER2 drugs. Due to the lack of these targets, neo-adjuvant therapy is used for treatment of TNBC that is associated with aggressive phenotype, poorer prognosis and the high rate of relapse compared to other BC subtypes. Besides, almost half of the ER+ tumors eventually become resistant to anti-estrogens [34,35].

As was demonstrated by Chiang et al. [10], calcitriol and another vitamin D analog MART-10 (19-nor-2a-(3-hydroxypropyl)-1a, 25(OH)D) could effectively repress TNBC cells migration and invasion with analog more effective. These compounds induced cadherin switching and down regulated P-cadherin expression in MDA-MB-231 cells as well as repressed lipocalpin 2, one of BC...
<table>
<thead>
<tr>
<th>Author and year</th>
<th>Type of study/Country</th>
<th>Population</th>
<th>Vitamin D supplementation</th>
<th>25(OH)D serum concentration</th>
<th>Results</th>
<th>OR, CI, p-value</th>
<th>Adjustment factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al., 2014 [16]</td>
<td>Nested case-control cohort study/Hawaii, Los Angeles</td>
<td>n=215 000/707 postmenopausal BC cases and matched controls/five race/ethnic groups</td>
<td>-</td>
<td>Measure of pre-diagnostic plasma levels of 25(OH)D</td>
<td>20 ng/mL increases of plasma 25(OH)D levels were inversely associated with BC risk among white women; for women other than non-Hispanic whites such association was not significant</td>
<td>OR=0.28, 95% CI: 0.14-0.56</td>
<td>BMI, multivitamin or calcium supplements, number of live births, family history of BC, season, sunburn and engagement in strenuous sport</td>
</tr>
<tr>
<td>Kim et al., Je, 2014 [15]</td>
<td>prospective nested case-control or cohort studies/South Korea</td>
<td>n=31 867 BC incidences</td>
<td>&gt;500 IU/day vs. &lt;148 IU/day</td>
<td>-</td>
<td>Not significant risk of BC incidence</td>
<td>RR=0.95, 95% CI:0.88-1.01 p=0.09 RR=0.92, 95% CI:0.83-1.02 RR=0.58, 95% CI:0.40-0.85 RR=0.61, 95% CI:0.48-0.79</td>
<td>Menopausal status, BMI, physical activity</td>
</tr>
<tr>
<td>Bidgoli, Azarshab, 2014 [20]</td>
<td>Case-control study/Iran</td>
<td>n=60 BC premenopausal newly diagnosed patients/n=116 controls</td>
<td>Egg consumption &gt;3/week in control group vs. egg consumption &lt;3/week in BC premenopausal patients group</td>
<td>Un-supplemented BC premenopausal patients (1.67% supplemented with vitamin D (18.46%) and calcium (18.1%)) vs. control group</td>
<td>Both groups vitamin D deficient</td>
<td>No significant differences in 25(OH)D blood levels between BC Premenopausal patients and control group</td>
<td>-</td>
</tr>
<tr>
<td>Jeffreys et al., 2015 [19]</td>
<td>Retrospective cohort study/UK</td>
<td>n=21 932 women ≥ 55 y old, at least 5 y with the first diagnosis of BC, colorectal, lung, ovarian or uterine cancer and at least 5 y prior to diagnosis</td>
<td>Pre-diagnostic vitamin D supplementation, 3 prescriptions/dose unknown vs. 1 or 2 prescriptions</td>
<td>Pre-diagnostic vitamin D supplementation, any prescriptions/dose unknown vs. no prescription</td>
<td>Both groups vitamin D deficient</td>
<td>No association with survival from any of the cancers studied</td>
<td>-</td>
</tr>
<tr>
<td>Fair et al., 2015 [24]</td>
<td>Cross-sectional study/USA</td>
<td>n=57 premenopausal women / n=106 postmenopausal women</td>
<td>Premenopausal women total vitamin D intake &lt;191.56 IU/day/n=12 191.56-568.8 IU/day/n=24 ≥ 568.9 IU/day/n=21 Postmenopausal women total vitamin D intake &lt;191.56 IU/day/n=36 191.56-568.8 IU/day/n=36 ≥ 568.9 IU/day/n=34</td>
<td>-</td>
<td>-</td>
<td>Better survival from BC</td>
<td>HR=0.78, 95% CI:0.70-0.88</td>
</tr>
</tbody>
</table>

**Note:** The table above summarizes the results of various studies examining the relationship between vitamin D supplementation and breast cancer risk. The studies vary in their design, population size, and specific vitamin D measurement, which influences the interpretation of the results. It's important to consider the methodological differences and adjust for other factors that might influence the risk of breast cancer when assessing these findings.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Country</th>
<th>Sample Size</th>
<th>Menopausal Status</th>
<th>Vitamin D Levels</th>
<th>Methodology</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shirazi et al., 2016 [18]</td>
<td>Nested case-control study based on prospective, cohort study / Sweden</td>
<td>n=17035 / n=764 BC cases</td>
<td>Premenopausal women</td>
<td>&lt;17.55 ng/mL / n=20 17.56-28.6 ng/mL / n=16 ≥ 28.7 ng/mL / n=21 Postmenopausal women &lt; 17.55 ng/mL / n=35 17.56-28.6 ng/mL / n=38 ≥ 29.7 ng/mL / n=33</td>
<td></td>
<td></td>
<td>High risk of tumors with unfavorable prognosis, lower risk of ER- tumors, lower risk of PR- tumors, high expression Ki67 tumors</td>
</tr>
<tr>
<td>Cadeau et al., 2016 [21]</td>
<td>Prospective cohort study / France</td>
<td>n=57,403 postmenopausal women / n=2482 BC cases</td>
<td></td>
<td></td>
<td></td>
<td>Decreased postmenopausal BC risk in MHT ever users with BMI &lt;25 kg/m², Decreased postmenopausal BC risk in MHT BMI ≥ 25 kg/m², Increased postmenopausal BC risk in MHT never users with BMI &lt;25 kg/m² and stronger with BMI &lt;22 kg/m² than with BMI = 22-24.9 kg/m²</td>
<td></td>
</tr>
<tr>
<td>O'Brien et al., 2017 [22]</td>
<td>Prospective cohort study / USA</td>
<td>n=50,884 healthy women / n=35,074, 35-74 y old who had a sister diagnosed with BC/84% non-Hispanic white/85% well educated</td>
<td>&gt; 38 ng/mL</td>
<td>Regular supplement use / dose unknown (≥ 4 times/week)</td>
<td></td>
<td>21% lower BC hazard, 11% lower BC hazard</td>
<td>20% lower BC hazard adjusted HR=0.79, 95% CI:0.63-0.98, HR=0.89, CI:0.81-0.99, sunlight-related variables, exogenous hormone use, history of osteoporosis, education, BMI</td>
</tr>
</tbody>
</table>
Table 1. The selected human studies on the associations between 25(OH)D serum concentration or vitamin D supplementation and BC risk and survival published between years 2014-2019.

<table>
<thead>
<tr>
<th>Study Authors</th>
<th>Study Design</th>
<th>Study Population</th>
<th>Vitamin D Intake</th>
<th>Primary Endpoints</th>
<th>Secondary Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brison et al., 2017 [23]</td>
<td>Double-blind, placebo-controlled parallel group trial/ Canada</td>
<td>n=306 premenopausal women supplemented/n=99 placebo</td>
<td>1000 IU / day for one year</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000 IU / day for one year</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3000 IU / day for one year</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Madden et al., 2018 [26]</td>
<td>Cohort study/ Ireland</td>
<td>n=5417 women 50-80 y old with invasive BC not supplemented before diagnosis</td>
<td>&gt; 400 IU after 6 months following diagnosis</td>
<td>20% reduction in BC-specific mortality in de novo users vs. non-users</td>
<td>HR=0.80, 95% CI:0.64-0.99, p=0.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 400 IU within 6 months following diagnosis</td>
<td>49% reduction in BC-specific mortality in de novo user’s vs. non-users</td>
<td>HR=0.51, 95% CI:0.34-0.74, p=0.001</td>
<td></td>
</tr>
<tr>
<td>Manson et al., 2019 [27]</td>
<td>Randomized, placebo-controlled study/ USA</td>
<td>n=25,671 healthy black and white participants including women ≥ 55 y old</td>
<td>2000 IU/day vs. placebo</td>
<td>Site-specific cancer incidence</td>
<td>HR=0.96, 95% CI:0.88-1.06, p=0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BC incidence</td>
<td>HR=1.02, 95% CI:0.79-1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death from cancer</td>
<td>HR=0.83, 95% CI:0.67-1.02</td>
</tr>
</tbody>
</table>

BC-breast cancer; OR-odd ratio; CI-confidence interval; BMI-body mass index; RR-relative risk; HR-hazard ratio; ER-estrogen receptor; PR-progesterone receptor; MHT-menopausal hormone therapy; HER-human epidermal growth factor receptor.

metastasis stimulator. Furthermore, MART-10 downregulated matrix metalloproteinase-9 activity and attenuated F-actin as well as calcitriol. According to Maaty et al. [36], in TNBC cells MDA-MB-231, MDA-MB-468 and HCC-1143 calcitriol regulates energy metabolism. On the opposite, one year earlier, Richards et al. [37] found that vitamin D at the high concentrations inhibited BC cell line MCF-7 but not the TNBC cell lines. As regards BC ER-, Santos-Martinez et al. [38] found that calcitriol is able to induce the expression of a functional ERα that is mediated through VDR. Calcitriol-induced ERα restored the response to anti-estrogens by inhibiting cell proliferation and the calcitriol-treated cells in the presence of anti-estrogen IC-182,780 resulted in a significant reduction of some cell proliferation regulators [38].

Neoadjuvant chemotherapy (NAC) has become a standard of care in locally advanced BC, especially for patients with large tumor size, mammographic breast density, HR and HER2 status, surgery after diagnosis, receipt of chemotherapy.

As was given by Thakkar et al. [34], approximately two-thirds of TNBCs express VDR and/or androgen receptor (AR) and it is possible that TNBCs co-expressing AR and VDR could be treated by targeting both of these hormone receptors. The authors provided that treatment of 15 BC cell lines including the cell lines which expressed AR and VDR receptors with AR or VDR agonists inhibited cell viability in a receptor-dependent manner and their combination appeared to inhibit cell viability. Apart from the agonists induced differentiation and inhibited cancer stem cells [34]. Interestingly, cell viability was further decreased when AR/VDR agonists were combined with chemotherapeutic drugs. Among others, the authors showed that combination of calcitriol and Taxol resulted in an additive or synergistic decrease in cell proliferation and viability in two different TNBC cell lines, positive for VDR [34]. Similar results were obtained for TNBC cells positive for AR and VDR. The combination of AR- and VDR- targeted therapy with Taxol or cisplatin had an additive effect in reducing cell viability [34].

Some associations between vitamin D and tumor subtypes as hormone receptor (HR)-/HER2+ and TNBC were noted by Viala et al. [39]. The authors conducted a retrospective, observational, multicenter study which included 327 women treated with NAC with adjunction of therapies for HER2+ subtype. As was shown, vitamin D deficiency (measured as <20 ng/mL) was associated with the odds of not attaining pathologic complete response (pCR). The 5-year date of relapse (PFS) was 92 and 79% in the vitamin D deficient and in the sufficient group, respectively (p=0.3). According to the authors, the lack of statistical significance could be explained by the relatively small number of patients in the TNBC cohort (n=90).

The promising studies were presented by Zeichner et al. [7] who performed a retrospective review of patients who received vitamin D supplementation during trastuzumab-based chemotherapy for HER2+ non metastatic BC and patients who were unsupplemented. As was found, vitamin D intake was associated with improved disease-free survival (DFS). Larger tumor size was associated with worse DFS and there was no overall survival based on any of the categories, including vitamin D supplementation, age at diagnosis or lymphovascular invasion (Table 2).
Charehbili et al. [40] during NEOZOTAC phase III trial studied the relationship between vitamin D serum level that was measured at baseline and before the last cycle of chemotherapy and pCR or pathological good response (defined as >90% decrease in tumor cellularity or total absence of invasive tumor cells in the breast only) in breast and lymph nodes. Admittedly, as was found, there was no association between baseline vitamin D levels and pCR, even including the season in which the baseline vitamin D was measured whereas the positive changes in vitamin D levels were significantly associated with pathological good response and the expected response was observed more often in women with end of treatment vitamin D levels >50.99 nM/L than in those with vitamin D level <50.99 nM/L [40] (Table 2).

Similar results obtained Chiba et al. [41] in retrospective cohort study with patients with operable BC. The authors, after adjusting for the effects of cohort clinical stage and receptor status showed that vitamin D deficiency (defined as <20 ng/mL serum levels measured before NAC) increased the odds of not attaining a pCR (Table 2).

On the contrary, Kim et al. [42] investigating the changes in serum 25(OH)D levels before and after NAC and the associations of this level with pCR and survival in 374 BC patients found that the patients with either pre- or post-NAC sufficient 25(OH)D levels accounted for 23.8% and the overall pCR was 25.9%. Most patients showed 25(OH)D deficiency at diagnosis and 65.8% showed decreased serum 25(OH)D levels after NAC (as was obtained also in other studies) [39,43]. As was concluded, the changes in calcidiol status were associated with postmenopausal status, molecular phenotype, baseline summer examination and rural residence but not with pCR. No association between survival and calcidiol status was found [42]. Similarly, Clark et al. [44] did not prove that vitamin D improves response to NAC. The authors during a retrospective cohort study showed that pre-treatment vitamin D levels had no impact on tumor response to NAC or short-term prognosis. Vitamin D level was not associated with attaining pathologic residual cancer burden 0/1 after NAC with anthracycline and taxane and was not associated with a 3-year relapse-free survival (RFS). However, surprisingly, the lower level of 25(OH)D correlated with higher tumor Ki67 proliferation biomarker adjusting for race (Table 2). As was given by the authors, no correlation between vitamin D and NAC response can be linked to the absence of HER2+ patients in the study. By Clark et al. [43], suggestion that vitamin D higher levels may suppress proliferation of BC is speculative as well as ascertainment that insufficient or deficient vitamin D levels do not impair or predict the efficacy of NAC in BC patients. However, the authors pointed to the fact that their study regarded vitamin D cut off level 30 ng/mL and they would not be able to identify the potential association with BC risk at a higher serum concentrations of 25(OH)D to optimize response to NAC [44]. Furthermore, in these studies potentially important confounding variable, such as smoking status, physical activity, diabetes mellitus 2 status or vitamin D supplements were not regarded and the vitamin D status was not known at the time of NAC. The results described above comply with these shown by Lohmann et al. [45] one year later. The authors during randomized clinical trial with BC patients treated with NAC did not find the evidence that vitamin D blood level is associated with RFS, BC-specific survival (BCSS) and overall survival (Table 2). The authors pointed to the strengths of the study such as the large number of patients, the high quality of data collection, the long follow-up (5 years) and that this was a multicenter international trial. According to the authors, the inverse association between vitamin D blood levels and the improved survival of BC patients treated with NAC which is shown in the observational studies may result from methodology. Besides, it is crucial to consider all confounds which can affect vitamin D blood levels and which are associated with BC outcomes, such as age, BMI, physical activity or good overall health.

Yao et al. [46] proved the importance of menopausal status to reveal the role of vitamin D in BC therapy. During prospective cohort study, the authors investigated a serum biomarker of 25(OH)D status measured at the time of BC diagnosis, with prognosis and found that serum 25(OH)D concentrations were lower in women with advanced stage tumors and the lowest in premenopausal women with TNBC. The calcidiol levels were inversely associated with hazards of disease progression and death, even after adjustment for clinical prognostic factors. Among premenopausal women, there were also associations with overall survival, BCSS and IDFS (invasive disease-free survival) (Table 2).

What about the “optimal” dose of vitamin D as beneficial for BC patients?

Despite of the equivocal results concerning the beneficial impact of vitamin D on BC prevention or therapeutic efficacy, which were presented in this article, the common conclusion is that the sufficient serum level of 25(OH)D is one of the factors which define our self-defence against neoplastic changes and successful therapy when it is needed. The additional benefit of vitamin D supplementation is to maintain the required serum concentration of 25(OH)D during chemotherapy which can decrease its level [39].

The key points are: “if there is the correlation between the beneficial effects of vitamin D and the vitamin D intake?” and “if are the optimal doses for chemoprevention or BC therapy support?” According to Kim et al. [16] it is plausible that the association of serum 25(OH)D with BC risk is non-linear, and a minimum threshold is needed for vitamin D to exert a protective effect. On the opposite, according to de La Puente-Yagüe et al. [5], the concentrations of calcidiol necessary to mediate the anti-cancerogenic effects are well above the physiological range and are associated with undesirable effects in vivo. According to Crew et al. [47], data from observational studies suggest that optimal levels for BC prevention exceed 40-50 ng/mL. As was highlighted by Kim and Je [15] for every 100 IU of vitamin D, blood 25(OH)D levels increase by 1 ng/mL while a healthy level >30 ng/mL of calcidiol is difficult to achieve without supplementation >1000 IU per day.

Some studies, presented above, showed that the relationship between vitamin D status health outcomes is a U-shaped and, that, for example, chemopreventive benefits from vitamin D supplementation are associated with rather lower vitamin D intake, such as less than 1000 IU per day, at least in premenopausal women [23]. These results prove that there is the one, common daily dose of vitamin D having chemopreventive and anticancer effects. Variability in response to vitamin D intake may be due to other factors, such as menopausal status, BC phenotype, VDR signalling and heterogeneity, BMI or race [48]. The other point is the time of initiation of vitamin D supplements that could have significant clinical implications, especially in the case of applied chemotherapy [26]. Truly, the sufficient evidences on the benefits of different times of initiation of other adjuvant interventions including vitamin supplementation are lacking [26].

As was presented in this article, many studies found the association between vitamin D status and lower risk of BC incidence or between vitamin D supplementation used in conjunction with standard therapies and lowered BC recurrence or improved survival. The most important, phenomenical and promising is that because CYP27B1 was found in skin, colon, prostate and a breast cancer that allows calcitriol act in an autocrine or paracrine manner against cancerous transformation.
Furthermore, still new data attain to prove the anticancer effects of vitamin D, such as its potential role in sensitizing of drug-resistant cancer cells [49].

**Conclusion**

On the basis of the studies to date, it seems to be undoubtedly that vitamin D is a potentially modifiable risk factor to target as a strategy for BC prevention and treatment. Of course, it does not mean that more is always better. According to Kim and Je the current evidences do not support use of high dose vitamin D regimens to gain benefits for BC survival and more large randomised clinical trials should be conducted to provide evidence having implications for clinical practice.

Consistently with the last supplementation guideline, obtaining and maintaining higher 25(OH)D concentrations than 30-50 ng/mL is not advisable. This range is recommended to ensure the balanced extra-skeletal effects of vitamin D.

**Conflict of Interest**

The author declares no conflict of interest.

**References**


44. Clark AS, Chen J, Kapoor S, Friedman C, Mies C, et al. (2014) Pretreatment vitamin D level and response to neoadjuvant chemotherapy in women with...


