Successful Replacement Therapy After a Confirmed Vitamin D Deficiency Correlates with Improved DFS in the Curative Intent Treatment of HER2+ Breast Cancer

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Article

Successful Replacement Therapy After a Confirmed Vitamin D Deficiency Correlates with Improved DFS in the Curative Intent Treatment of HER2+ Breast Cancer

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Abstract

Background. Vitamin D has recognized immunomodulatory, anti-proliferative, and differentiation-regulating effects primarily mediated through its genomic effects via the vitamin D receptor (VDR). Observational studies have suggested associations between vitamin D deficiency and more aggressive breast cancer phenotypes, including estrogen receptor–negative disease and higher-grade tumors. Recent randomized trials have also reported improved pathological complete response (pCR) rates with vitamin D supplementation during neoadjuvant chemotherapy, although these studies included heterogeneous breast cancer subtypes and greater effects were mostly seen in the ER- subtypes. Because HER2-targeted therapies have dramatically improved outcomes in HER2-positive breast cancer, it was hypothesized that successful correction of vitamin D deficiency would be associated with improved disease-free survival (DFS) in patients with early-stage HER2-positive breast cancer treated with curative-intent therapy. Methods. We conducted a retrospective cohort study of patients with HER2-positive breast cancer treated at Cancer Treatment Centers of America Midwestern Regional Medical Center between 2008 and 2014. Eligible patients had early-stage HER2-positive disease, received trastuzumab-based therapy with curative intent (± pertuzumab), continued follow-up at the institution for at least 12 months, and had baseline vitamin D deficiency defined as serum 25-hydroxyvitamin D (D25) < 30 ng/mL. Vitamin D levels were routinely measured at baseline and serially during treatment as part of institutional standard practice. Patients received vitamin D3 supplementation, typically ranging from 2,000–10,000 IU/day, with dose adjustments guided by follow-up D25 levels. Patients were classified as responders if their mean D25 level during the first year of follow-up reached ≥30 ng/mL and non-responders if levels remained < 30 ng/mL. Responders were further stratified into low (30–40 ng/mL), medium (40–50 ng/mL), and high (>50 ng/mL) categories to explore potential dose-response relationships. The primary endpoint was disease-free survival (DFS), defined as time from initiation of neoadjuvant therapy or surgery to recurrence, metastasis, or death. Kaplan–Meier methods and Cox proportional hazards models were used to evaluate associations between vitamin D response and DFS while adjusting for relevant clinical covariates. Results. Among 196 eligible patients, 129 (65.8%) were vitamin D deficient at baseline. Of these, 76 (60.3%) achieved adequate vitamin D repletion and were classified as responders, while 50 (39.7%) remained deficient. Over the follow-up period, 31 DFS events (15.8%) occurred. The mean DFS for the cohort was 10.2 years (95% CI 9.58–10.83), and the estimated 3-year DFS rate was 88%. Responders demonstrated numerically improved outcomes compared with non-responders, with 3-year DFS rates of 90% versus 85%, respectively. Kaplan–Meier curves suggested a potential dose-response relationship, with progressively improved DFS among patients achieving higher mean D25 levels, particularly those exceeding 50 ng/mL. In Cox regression analyses, vitamin D non-responders demonstrated a 1.7-fold higher hazard of recurrence compared with responders, although this did not reach statistical significance. Conclusions. In this retrospective cohort of patients with HER2-

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positive breast cancer, failure to correct baseline vitamin D deficiency was associated with a numerically higher risk of disease recurrence. The observed separation of Kaplan–Meier curves across increasing vitamin D levels suggests a possible dose-response relationship supporting a biologically meaningful effect. Although the study is limited by retrospective design and modest sample size, the findings are consistent with emerging randomized trial data suggesting improved treatment responses with vitamin D supplementation. Prospective studies specifically targeting correction of vitamin D deficiency in HER2-positive breast cancer are warranted to determine whether optimized vitamin D status can improve oncologic outcomes.

Keywords: HER2+ breast cancer; vitamin D

1. Introduction

Breast cancer is the most common cancer among women in the United States, with an estimated 321,910 new invasive cases and 42,140 deaths projected in 2026, accounting for nearly one-third of all new cancer diagnoses in women [1]. Breast cancer is a biologically and clinically heterogeneous disease, encompassing multiple tumor subtypes with distinct cellular and molecular features that contribute to variability in prognosis and optimal treatment options(Gradishar et al., 2024).

Vitamin D is a fat-soluble secosteroid that is primarily synthesized in the skin following ultraviolet light exposure, making the term “vitamin” (normally defined as a trace dietary constituent) technically a misnomer [3] although it can also be obtained from dietary and/or supplemental sources. Vitamin D serves as a precursor to the active steroid hormone 1,25-dihydroxyvitamin D (calcitriol) which subsequently binds to Vitamin D receptor (VDR) and retinoic X receptor heterodimeric complex and regulates the expression of > 1000 genes [4]. While rickets and the classic bony abnormalities (frontal bossing, bow leggedness) associated with a vitamin D deficiency were first reported during Europe’s industrial revolution in the mid-17th century [5], vitamin D is now recognized for its many extraskeletal functions, including and not limited to modulation of immune responses, attenuation of inflammatory pathways, and regulation of cellular proliferation and differentiation [6].

Retrospective case-control studies focused on the association of serum 25-hydroxyvitamin D (D25), currently the best practical proxy for vitamin D status, with breast cancer subtype risk [7,8]have demonstrated that a vitamin D deficiency is associated with a greater risk of higher-grade tumors and ER negative subtypes. In a meta-analysis limited to prospective cohorts(Visvanathan et al., 2023),no statistically significant associations were found but there was a suggestion of association with triple negative breast cancer and distant metastases. The research connecting vitamin D deficiency with higher risk of overall cancer mortality is much stronger than breast cancer incidence [10] (Kuznia et al., 2023).

Randomized controlled trials (RCTs) on vitamin D supplementation focused on breast cancer specific outcomes are limited and all three have been published 2024-25. Two recent randomized clinical trials (one placebo controlled) that tested vitamin D3 supplementation concurrent with neoadjuvant chemotherapy for breast cancer were positive for improved odds of pathological complete response rate (pCR)(Özkurt et al., 2025). (Omodei et al., 2025) [13] A third study [14] was negative for the endpoint of pCR but was likely underpowered due to enrollment of 76 patients to four arms (placebo, vitamin D and placebo, vitamin D and probiotics, and probiotics and placebo). A weakness of all three studies is that all subtypes of breast cancer were included. Of note, pCR has been shown to be relevant to long term prognosis in triple negative breast cancer and ER-HER2+ breast cancer subtypes [15,16]but not so in ER+ breast cancer, so the clinical significance of these findings is unclear.

With the emergence of HER2-targeted monoclonal antibodies for the treatment of HER2+ breast cancer, it has been previously queried whether vitamin D intake might be clinically relevant in HER2+ breast cancer [17]. In this retrospective analysis of all patients receiving curative intent trastuzumab

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(+/- pertuzumab) at a single academic institution, there was a statistically significant improvement in disease free survival (DFS) after multivariate analysis in those taking vitamin D supplementation (HR 0.36 p=0.03). A weakness of the study was that D25 measurements at baseline and follow-up were not available.

At Cancer Treatment Centers of America, Midwestern Regional Medical Center, (CTCA MRMC) it was standard of care to routinely screen all new patients with a D25 and follow serial measurements to assure adequate replacement was provided. Leveraging this unique clinical practice, we examined whether successful correction of vitamin D deficiency was associated with improved disease-free survival among patients with HER2-positive breast cancer.

2. Materials & Methods

2.1. Study Design and Population

We conducted a retrospective cohort study of patients with HER2+ breast cancer treated at CTCA MRMC (located in the north suburbs of Chicago, i.e. Zion IL) between 2008–2014. The study was approved by the Institutional Review Board of CTCA MRMC.

Patients were selected without risk of bias by beginning with a list of all patients treated with trastuzumab +/- pertuzumab at the institution. Patients were included for final analysis if they had a diagnosis of early-stage HER2+ breast cancer, and continued care at CTCA for a minimum of 12 months from intake, and had a baseline vitamin D deficiency i.e. serum D25 <30 ng/ml. The inclusion criteria were predefined in the IRB protocol before data extraction was initiated.

The study hypothesis was that successful vitamin D supplementation would be associated with an improved disease-free survival (DFS) using Kaplan-Meier methods. A secondary aim was to see if there was any evidence that targeting higher levels i.e. > 50ng/ml might have additional benefit and see if there was a 'dose response' with higher tiers of D25 levels achieved.

2.2. Exposure Assessment and Vitamin D Responder Classification

Baseline vitamin D status was determined from an intake visit D25 (standard of care for all patients seen at CTCA MRMC prior to initial consultation). Vitamin D deficiency was defined as a baseline D25 < 30 ng/ml. Patients who were vitamin D deficient at baseline received vitamin D supplementation as part of routine clinical care through consultation with a naturopath (also standard of care) and the medical oncology team. Although a formal protocol for replacement was not defined by the institution, starting replacement doses ranged from 2,000-10,000 IU/d D3 and were adjusted accordingly based on subsequent D25 measurements.

The primary exposure of interest was response to vitamin D supplementation. Among patients with baseline vitamin D deficiency, a response was determined from the mean D25 concentration measured after the intake assessment through the end of the first year (to mirror the typical duration of HER2-targeted therapy). Patients were classified as responders if the mean follow-up mean D25 level was ≥ 30 ng/ml and as non-responders if the mean D25 remained <30 ng/ml. Responders were further stratified into low (30–40 ng/ml), medium (40–50 ng/ml), and high (>50 ng/ml) responder categories.

2.3. Outcomes

The primary outcome was disease-free survival (DFS). DFS was defined as the interval from the date of initiation of neoadjuvant therapy or definitive surgery, whichever occurred first, to the earliest documented disease recurrence, development of metastatic disease, or death from any cause. Patients without disease progression were censored at the date of last clinical follow-up.

2.4. Covariates

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Clinical and pathologic covariates of interest included age at diagnosis, body mass index (BMI), estrogen receptor (ER) status, progesterone receptor (PR) status, tumor size, number of metastatic lymph nodes, presence of lymphovascular invasion, type of chemotherapy (neoadjuvant vs. adjuvant), use of pertuzumab, type of definitive surgery, and receipt of radiation therapy.

2.5. Statistical Analysis

Baseline patient characteristics were summarized using frequencies and percentages for categorical variables and appropriate measures of central tendency for continuous variables. Differences across groups were assessed using standard comparative statistical methods, as appropriate.

DFS was estimated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. Univariate Cox proportional hazards regression models were first used to assess the association between individual covariates and DFS. Variables demonstrating clinical or statistical relevance were subsequently included in multivariable Cox proportional hazards models to evaluate independent associations after adjustment for potential confounders. Effect estimates were reported as hazard ratios (HR) with corresponding 95% confidence intervals (CI).

The proportional hazards assumption was evaluated using both graphical methods, including log-minus-log plots for categorical variables, and statistical testing through extended Cox models incorporating time-dependent covariates for continuous variables. A two-sided p value ≤0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 28.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Study Cohort and Baseline Characteristics

A total of 196 patients met the initial eligibility criteria and were then subcategorized into vitamin D sufficiency or deficiency status at baseline. Baseline demographic and clinicopathologic characteristics are summarized in **Table 1**. Of the 196 patients eligible for analysis, 129 patients (65.8%) had deficient D25 levels (<30 ng/ml), while 67 patients (34.2%) had sufficient levels (≥ 30 ng/ml).

Table 1.

Variables.	**N (%)**
Baseline Vitamin D	
<30 ng/ml (inadequate)	129 (65.8)
>=30 ng/ml (adequate)	67 (34.2)
Age at diagnosis	
<50 years	101 (51.5)
>=50 years	95 (48.5)
BMI	
<30 kg/m^2	108 (55.1)
>=30 kg/m^2	88 (44.9)
Race	
White	130 (66.3)
Black	42 (21.4)
Hispanic	15 (7.7)
Others	9 (4.6)

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Tumor size	
<2cm	123 (64.1)
>=2cm	69 (35.9)
Unknown	4
Number of metastatic lymph nodes	
0	108 (55.7)
>=1	86 (44.3)
Unknown	2
Lymphovascular invasion	
No	104 (63.8)
Yes	59 (36.2)
Unknown	33
ER status	
Negative	56 (29.5)
Positive	134 (70.5)
Unknown	6
PR status	
Negative	89 (46.6)
Positive	102 (53.4)
Unknown	5
Type of chemotherapy	
Neoadjuvant	97 (49.5)
Adjuvant	99 (50.5)
Definitive Surgery	
Lumpectomy	84 (43.1)
Mastectomy	111 (56.9)
Unknown	1
Radiation therapy	
No	52 (26.5)
Yes	144 (73.5)
Pertuzumab	
No	98 (50)
Yes	98 (50)
Disease progression	
No	165 (84.2)
Yes	31 (15.8)
Vitamin D response after supplementation*	
Responders	76 (60.3)
low	*27*
medium	*31*
high	*18*
Non-responders	50 (39.7)
Unknown	3

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Nearly half of the patients had a body mass index (BMI) > 30 kg/m² (44.9%). Most patients were White (66.3%), followed by Black (21.4%) and Hispanic (7.7%). Most tumors measured <2 cm (64.1%), and 55.7% of patients had no metastatic lymph node involvement. Lymphovascular invasion was present in 36.2% of cases.

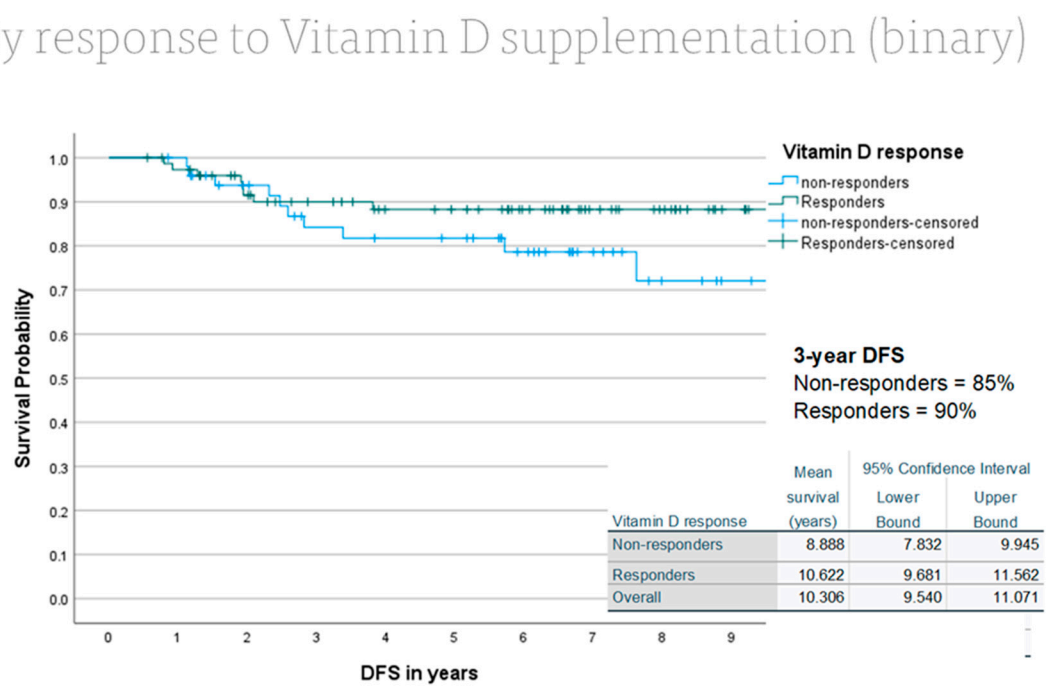
Hormone receptor positivity was common, with estrogen receptor (ER) positivity in 70.5% and progesterone receptor (PR) positivity in 53.4% of patients. Approximately half of the cohort received neoadjuvant chemotherapy (49.5%), while the rest received adjuvant chemotherapy (50.5%). Definitive surgery consisted predominantly of mastectomy (56.9%), and most patients received adjuvant radiation therapy (73.5%). Pertuzumab was used in 50% of patients.

3.2. Baseline Vitamin D Status and Response to Supplementation

Among the 129 patients who were vitamin D–deficient at baseline, 76 patients (60.3%) achieved adequate vitamin D repletion (defined by mean D25 > 30ng/ml in the first year of follow-up) and were classified as responders, while 50 patients (39.7%) remained below the target threshold and were classified as non-responders. Vitamin D response status could not be determined for 3 patients due to incomplete follow-up laboratory data and were not included for analysis. Among the 76 responders, 27 patients were classified as low-responders (mean follow-up D25 level 30–40 ng/ml), 31 medium responders (40–50 ng/ml), and 18 high responders (>50 ng/ml).

3.3. Disease-Free Survival in the Overall Cohort

During follow-up, 31 of 196 patients (15.8%) experienced a disease-free survival (DFS) event. No deaths were recorded, so overall survival analysis was not possible. Owing to the relatively low number of disease recurrence events, the median DFS was not reached. The mean DFS for the overall cohort was 10.2 years (95% confidence interval [CI], 9.58–10.83). The estimated 3-year DFS rate for the entire cohort was 88%, with non-responders having an 85% 3 yr DFS and responders having a 90% 3 yr DFS as shown in **Figure 1A**.



Vitamin D response	Mean survival (years)	95% Confidence Interval Lower Bound	Upper Bound
Non-responders	8.888	7.832	9.945
Responders	10.622	9.681	11.562
Overall	10.306	9.540	11.071

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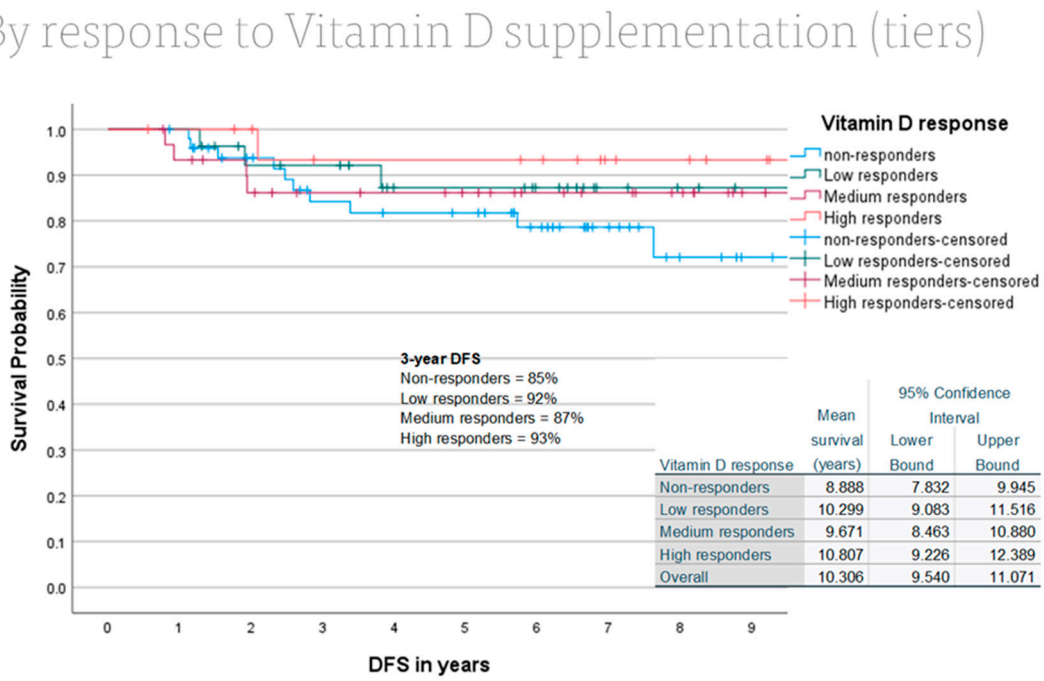

Vitamin D response	Mean survival (years)	95% Confidence Interval Lower Bound	Upper Bound
Non-responders	8.888	7.832	9.945
Low responders	10.299	9.083	11.516
Medium responders	9.671	8.463	10.880
High responders	10.807	9.226	12.389
Overall	10.306	9.540	11.071

Figure 1. FIGURE 1A AND 1B.

In **Figure 1B**, an apparent 'dose response' separation of the curves can be seen for those who were non-responders, low, medium, and high responders to vitamin D replacement therapy with the best outcomes associated with those who achieved mean D25 >50ng/ml.

3.4. Cox Proportional Hazards Analysis

Univariate and multivariable Cox proportional hazards regression analyses were performed to identify factors associated with DFS. See **Table 2** for Univariate Cox Proportional Hazard Analysis**.** In univariate analyses, BMI, lymphovascular invasion, treatment sequence, and pertuzumab use were significantly associated with DFS. Patients with BMI ≥30 kg/m² surprisingly demonstrated a lower hazard of disease progression compared with those with BMI < 30 kg/m² (HR 0.41 p=.03). The presence of lymphovascular invasion was associated with a higher hazard of disease progression (HR 2.30, p=0.04). Patients treated in the neoadjuvant setting versus adjuvant only exhibited a higher hazard of disease progression (HR 3.80 p=0.001) as well as those who received pertuzumab (HR 2.50 p=0.02), likely due to selection bias of more advanced stage presentations.

DFS did not differ significantly by age at diagnosis i.e. ≥ 50 years versus <50 (HR 1.1 p = 0.7), race i.e. non-whites versus whites (HR 1.3, p = 0.3), tumor size i.e. ≥ 2 cm vs < 2 cm (HR 1.3 p = 0.3), nodal status i.e. ≥ 1 metastatic versus 0 lymph nodes (HR 1.4 p = 0.3), estrogen receptor status i.e. positive versus negative (HR 0.79 p = 0.5), progesterone receptor status i.e. positive versus negative (HR 0.81 p= 0.6), nor baseline vitamin D25 status i.e. < 30 ng/ml versus > 30 ng/ml (HR 0.76 p=0.45).

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Table 2. Univariate Cox Proportional Hazard Analysis.

Variables	Hazard Ratio	95% CI	P-value
Vitamin D response after supplementation (2 categories)			
Responders (reference)			
Non-responders	1.7	0.71 – 4.3	0.23
Vitamin D response after supplementation (4 categories)			
High responders (reference)			
Medium responders	1.4	0.26 – 7.8	0.69
Low responders	1.1	0.19 – 6.8	0.89
Non-responders	2.1	0.46 – 9.6	0.34
Baseline Vitamin D			
>=30 ng/ml (reference)			
<30 ng/ml	0.76	0.37 – 1.6	0.45
Age at diagnosis			
<50 years (reference)			
>=50 years	1.1	0.56 – 2.3	0.73
BMI			
<30 kg/m^2 (reference)			
>=30 kg/m^2	0.41	0.18 – 0.91	**0.03***
Race			
White (reference)			
Others	1.3	0.62 – 2.6	0.50
Tumor size			
<2cm (reference)			
>=2cm	1.3	0.64 – 2.8	0.44
Number of metastatic lymph nodes			
0 (reference)			
>=1	1.4	0.69 – 2.9	0.33
Lymphovascular invasion			
No (reference)			
Yes	2.3	1.03 – 5.3	**0.04***
ER status			
Negative (reference)			
Positive	0.79	0.37 – 1.7	0.53
PR status			
Negative (reference)			
Positive	0.81	0.40 – 1.7	0.57
Type of chemotherapy			
Adjuvant (reference)			
Neoadjuvant	3.8	1.7 – 8.7	**0.001***
Definitive Surgery			
Lumpectomy (reference)			
Mastectomy	1.5	0.72 – 3.2	0.28
Radiation therapy			
No (reference)			
Yes	1.4	0.58 – 3.5	0.44
Pertuzumab			
No (reference)			
Yes	2.5	1.2 – 5.5	**0.02***
Nuclear grade			
1 or 2 (reference)			
3	1.4	0.59 – 3.5	0.43

Although a binary analysis of white vs non-white ethnicity was not significant, blacks continued to have the worst 3yr DFS of 79%, compared to 89% for whites and 100% for hispanics.

When using vitamin D responders as reference, vitamin D non-responders had a HR of 1.7 for DFS although p value was not significant p=0.23.

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Multivariable Cox proportional hazards regression (**Table 3**) was subsequently performed to evaluate independent associations with DFS after adjustment for BMI, lymphovascular invasion, treatment sequence, and pertuzumab use. In the adjusted model, lymphovascular invasion remained independently associated with worse DFS (HR 2.3 p=0.05). Treatment sequence also remained independently associated with DFS, with neoadjuvant therapy associated with a higher hazard of disease progression compared with adjuvant therapy (HR 4.0 p=0.008). After adjustment, BMI ≥ 30 kg/m² continued to demonstrate a sizable lower hazard of disease progression compared with BMI < 30 kg/m² with HR 0.46; however, it lost its statistical significance (p=0.09). Pertuzumab use was not independently associated with DFS (HR 1.20 p=0.69).

Table 3. Multivariate Cox Regression Analysis.

Variables	Hazard Ratio	95% CI	P-value
BMI			
<30 kg/m² (reference)			
≥30 kg/m²	**0.46**	**0.18-1.1**	**0.09**
Lymphovascular invasion			
No (reference)			
Yes	**2.3**	**1.01-5.4**	**0.05***
Type of chemotherapy			
Adjuvant (reference)			
Neoadjuvant	**4.0**	**1.4-11.3**	**0.008***
Pertuzumab			
No (reference)			
Yes	**1.2**	**0.45-3.4**	**0.07**

4. Conclusions

This is the first clinical cohort focused on women with HER2+ breast cancer with baseline and serial D25 measurements where all patients were given replacement therapy if deficient and doses were adjusted over time based on follow-up D25. The results suggest that not successfully fixing the vitamin D deficiency with appropriate vitamin D dosing could lead to a 1.7-fold higher risk of recurrence in patients with HER2+ breast cancer.

While the primary weakness of the cohort is that it is retrospective and underpowered and caution is needed prior to assuming causation not just correlation, the suggestion of a dose-response on Kaplan Meier DFS curves supports vitamin D having a positive biological effect when given concurrently with curative intent chemotherapy and HER2+ targeted therapy. Similar effect sizes on DFS were observed in a previously reported institutional cohort of patients treated with trastuzumab based chemotherapy for HER2+ breast cancer where vitamin D intake (mean 10,472 IU/week) was associated with a HR of 0.36 (p=0.03) after multivariate analysis [17]

These findings are further supported by two recent randomized controlled trials [12,13]which showed fixed vitamin D3 supplementation agnostic of baseline D25 levels led to a statistically significant improvement in pCR amongst patients receiving neoadjuvant chemotherapy for breast cancer.

In the Omodei study (n=80), patients received 2000 IU/d of D3 versus placebo, and mean baseline D25 was 19.6 and 21.0 ng/ml in the vitamin D and placebo groups respectively, with confirmed difference in D25 levels at end of study 28.0 vs 20.2 ng/ml. The pCR rate was 43% vs 24% p=0.04. Those who had higher D25 levels achieved were more likely to have pCR. Subset analysis suggested the benefit was mainly seen in the ER-/PR- subtypes (i.e. ER-HER2+ and triple negative).

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In the Ozkurt 2025 study (n=227), patients were randomized to either D3 50,000 IU/week or no vitamin D supplementation. Patients similarly were vitamin D deficient at baseline 23ng/ml in both arms, and 57% (average 71 ng/ml) achieved sufficiency in the intervention arm versus 18.4% in the control. The pCR rate was 24.3% versus 10.6% p=0.012, and subset analysis indicated the benefit was mostly seen in the ER-/PR- subtypes as well.

Our results might be additionally underpowered since the majority of the HER2+ breast cancer patients were not of the ER-HER2+ subtype (70.5% were ER+); however, we included this subset because unlike the neoadjuvant studies above, our study period was not limited to neoadjuvant therapy and was inclusive of adjuvant therapy when patients were receiving antiestrogen therapy with trastuzumab +/- pertuzumab. Research also suggests triple positive breast cancer has a better prognosis and has outcomes approaching luminal tumors unlike ER-HER2+ breast cancer [18].

A strength of our retrospective cohort is that the patient population queried was more diverse and representative of a typical American population compared to those enrolled in previous RCTs, with a greater proportion of obese patients (44.9% > 30kg/m2) and ethnic minorities including African Americans (21.4%) and Hispanics (7.7%).

A surprising finding in our cohort was that a high BMI emerged as a favorable prognostic factor. Although not statistically significant in multivariate analysis, the HR for disease recurrence for high BMI patients > 30 kg/m^2 was 0.46 which is contrary to what was expected based on previous publications associating high BMI with worse outcomes in curative intent treatment of ER-HER2+ breast cancer [19,20]in the post-trastuzumab era, and in the pre-trastuzumab era [21].

It is our hypothesis that this reversal in the fortunes of high BMI patients could represent a clinical signal that a high BMI in part is a negative prognostic factor in HER2+ breast cancer through the mechanism of serving as a "vitamin D physiological trap" [22–24]. Vitamin 1,25D has also been shown to inhibit leptin and IL-6 production by adipocytes [25,26] It is possible a high BMI in this cohort selected out patients who were more likely to benefit from a D25-guided replacement therapy approach where patients required doses as high as 25,000 IU/d to achieve normal levels.

4.1. Review of Mechanisms of Vitamin D Bioactivity in HER2+ Breast Cancer

Vitamin D is a steroid prohormone and requires sequential hydroxylation to become biologically active, with both renal and extrarenal tissues, including breast epithelium, capable of activating circulating 25-hydroxyvitamin D to 1,25-hydroxyvitamin D or calcitriol [27]. Calcitriol signaling through the vitamin D receptor (VDR) influences multiple cancer-relevant pathways, including inhibition of cellular proliferation via cell-cycle arrest, induction of apoptosis through modulation of Bcl-2 family proteins, inhibition of matrix metalloproteinases involved in tumor progression, downregulation of COX-2 expression and associated inflammation, and suppression of additional invasive and angiogenic processes [6,27]. Recent evidence shows that vitamin D stimulation of intestinal VDR induces an improved microbiome and subsequent anti-tumor immunity in mouse models [28].

Vitamin D has been shown to enhance NK cell activity providing a mechanism by which vitamin D might be particularly beneficial in HER2+ breast cancer treated in the post-trastuzumab era [29]. Preclinical models looking at another monoclonal antibody rituximab for lymphoma identified that maximal NK cell activity was observed at 65 ng/ml, supporting our findings of a dose response with higher D25 levels > 50 ng/ml having the best nominal DFS. Preclinical studies suggest vitamin D analogues may inhibit HER2-associated downstream signaling pathways, including the ERK and PI3K/AKT cascades [30,31]

Another potential mechanism for biological activity of vitamin D in HER2+ breast cancer is the known cross-talk between TP53 and VDR [32]. HER2+ breast cancer has a high prevalence of TP53 mutations, 55-75% [33] and patients with germline TP53 mutations are more likely to develop HER2+ breast cancer (67%) versus other subtypes [34]. In gastric cancer cell lines, 1,25(OH) vitamin D requires VDR and mutated p53 to suppress cell growth through induction of p21 and suppressed CDK2 expression [35]. In a subset analysis of TP53 mutated gastrointestinal cancers (using p53

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immunoreactivity as a proxy) in the AMATERASU study [36] vitamin D supplementation led to 81% 5-year relapse free survival versus 31% in the placebo group (HR 0.27 95% CI 0.11-0.61) suggesting TP53 mutations might be an important biomarker for cancers more likely to benefit from vitamin D supplementation [37].

Of note, and consistent with the two recent RCTs showing improved pCR with vitamin D supplementation [12,13] particularly for ER- breast cancer subtypes, TP53 mutations are much more prevalent in ER- breast cancer 74-88% [38] versus ER+ subtypes, 10-30%. In a cohort of 1420 primary breast cancers, 65.0% of basal-like and 53.4% HER2-enriched subtypes had TP53 mutations versus 24.8% in Luminal B and 9.3% of Luminal A tumors [39] and TP53 mutations were associated with worse overall survival particularly in luminal B and HER2+ breast cancer (HR 1.66 p=0.007 and HR 1.69 p=0.03 respectively). Similar prevalence of TP53 mutations in non-metastatic triple negative versus HER2+ breast cancer was seen in a cohort of 450 patients receiving neoadjuvant chemotherapy (74.8% versus 55.4% respectively) [40].

In luminal breast cancer, vitamin D has been shown to decrease aromatase and COX-2 expression [41] increase endogenous CDK inhibitor expression [42,43]and reduce metastatic potential of breast cancer cells in part by increasing surface E-cadherin [44,45]. Paradoxically, vitamin D can both downregulate ER expression in luminal cancers and reduce subsequent estrogen signaling [46,47] and induce ER expression and restore anti-estrogen sensitivity in triple negative cancers [48]. We hypothesize that these mechanisms would be less relevant in the neoadjuvant setting when patients with HER2+ breast cancer are given chemotherapy, but more relevant in the adjuvant setting when patients typically begin their anti-estrogen therapy and likewise would require longer term follow-up to detect impact in clinical trials.

4.2. Concluding Remarks

Prior to the two recent positive RCTs discussed above, broader clinical research evaluating vitamin D supplementation and cancer-specific endpoints has produced mixed results in part because many studies enrolled participants who were not vitamin D–deficient at baseline and/or used low fixed-dose strategies that would be likely insufficient for achieving physiological repletion in the higher-risk groups such as those with obesity [49,50]. Confirming the importance of proper dosing based on weight, a secondary analysis of the VITAL study [51] showed that 2000 IU/d of D3 supplementation only reduced risk of metastatic or fatal cancer in those with a normal BMI < 25 kg/m^2 (HR 0.62 95% CI 0.45-0.86) but not in those who had BMI 25-30 kg/m^2 (HR 0.89 95% CI 0.68-1.17) nor > 30 kg/m^2 (95% CI 0.74-1.49).

The inefficiency of vitamin D repletion (60.3%) in our cohort emphasizes that a fixed dose replacement strategy, even with adjustments based on serial D25 measurements, does not reliably translate into adequate repletion in real-world practice particularly for high BMI patients [52–54]. To resolve this underpowering issue in future prospective studies, it would be prudent that future clinical trials focus on interventions utilizing scientifically proven dosing formulas based on patient weight and baseline D25 [55]. Also, ethical questions should be raised about randomizing patients with a known vitamin D deficiency to placebo, and implementation studies with analysis based on achieved D25 levels may be a more ethical and biologically appropriate for future studies. Given current practices, randomizing patients to delayed or immediate replenishment or randomizing to fixed low dose supplementation versus weight-based dosing might be another more ethically-sound approach.

As of 2026, it is currently not on ASCO guidelines to screen and address a vitamin D deficiency in patients newly diagnosed with cancer unless they are about to start a bone weakening agent [56]; however, it is NCCN guideline-supported to screen for and treat any nutritional deficiencies concurrent with cancer treatment [57]. 70.3% of Americans have a vitamin D deficiency (<30 ng/ml) [58]. Given the high pretest odds particularly for African Americans (94.5%) and Hispanics (86.5%) and obese individuals (80.1%) [58], and that vitamin D deficiency is commonly associated with

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quality-of-life issues particularly fatigue [59,60] routine screening and replacement therapy should be standard of care.

43% of patients newly diagnosed with cancer report fatigue before even starting their conventional anti-cancer therapies [61], and fatigue is a bidirectional predictor of poor lifestyle choices and willingness to engage with exercise or nutritional interventions [62–65]. The clinical evidence suggests that unresolved pre-treatment fatigue predicts a >3-fold risk of having more severe post-treatment fatigue [66,67], yet fatigue due to a vitamin D deficiency is one of the easiest causes of fatigue to treat [68,69]. One of the mechanisms of fatigue amelioration from vitamin D is enhanced skeletal muscle mitochondrial function [70–72]. Of note, many patients who were treated for vitamin D deficiency in our cohort only recognized their chronic fatigue symptoms, after experiencing the energy improvement with their replacement therapy as many had adapted to the deficient state for decades.

Lastly, vitamin D sufficiency likely has a protective effect on some of the toxicities associated with chemotherapy such as paclitaxel-associated neuropathy [73,74], risk of diabetes [75,76]and impaired cognitive function [77–79]. Vitamin D replacement therapy is extremely well tolerated with most experts agreeing risk of hypercalcemia in healthy children and adults is negligible with D25 levels <100 ng/ml [80–82].

Given the current state of research evidence and the biological plausibility of vitamin D having both these wellness and anti-cancer effects without any evidence of harm, it should be standard of care, until RCT evidence suggests otherwise, to screen for and treat a vitamin D deficiency in all patients with breast cancer, particularly the ER-/PR- subtypes.

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Abbreviations

ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor 2, D25 serum 25-hydroxyvitamin D, RCTs randomized controlled trials, pCR pathological complete response, DFS disease free survival, CTCA MRMC Cancer Treatment Centers of America Midwestern Regional Medical Center, IRB institutional review board, BMI body mass index, HR hazard ratio, CI confidence interval, NK natural killer, VDR vitamin D receptor

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