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Baseline vitamin D status and clinical outcomes in advanced non-small cell lung cancer patients treated with nivolumab

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Authors' Contributions

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All authors contributed to the interpretation of the results, critically revised the manuscript for important intellectual content, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethics Approval:
All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committe of Van Training and Research Hospital (dated 16 January 2026; Approval No: 2026-01-17).

Baseline Vitamin D Status and Clinical Outcomes in Advanced Non-Small Cell Lung Cancer Patients Treated With Nivolumab

Background: Immune checkpoint inhibitors such as nivolumab have brought meaningful benefits to patients with advanced non-small cell lung cancer (NSCLC), yet many patients still experience limited responses. Vitamin D is a key modulator of immune function, and deficiency is common in individuals with cancer. Given its potential role in shaping immunotherapy response, we retrospectively assessed whether baseline vitamin D levels were associated with clinical outcomes in advanced NSCLC patients treated with nivolumab.

Methods: We retrospectively analyzed patients with stage IV NSCLC treated with nivolumab across multiple centers, predominantly as second-line therapy. Prior to nivolumab initiation, all patients were confirmed to be negative for common driver mutations, including EGFR, ALK, ROS1, and other actionable genomic alterations. Baseline serum 25-hydroxyvitamin D levels were measured prior to nivolumab initiation, and patients were categorized as having severe deficiency (<10 ng/mL), deficiency (10-20 ng/mL), or sufficient levels (≥20 ng/mL). The presence of liver metastases was recorded at baseline. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared with log-rank tests. Multivariable Cox proportional hazards models adjusted for age, sex, ECOG performance status, liver metastasis, and other metastatic sites were used to evaluate the independent prognostic value of vitamin D status. Objective response rates (ORR) were assessed in patients with available radiologic data.

Results: A total of 890 patients were screened, and after excluding those with missing baseline vitamin D data, 305 patients were included in the analysis. The median age was 64 years (range 34-85), and 68% of patients were male. Vitamin D levels were <10 ng/mL in 30%, 10-20 ng/mL in 44%, and ≥20 ng/mL in 26% of patients. Patients with severe vitamin D deficiency were numerically older (median age 65 vs. 63 years) and had higher rates of ECOG PS ≥2 (15.1% vs. 7.5%) and squamous histology (37.6% vs. 27.5%), though these differences were not statistically significant (p>0.05 for all). Liver metastases were present in 54 patients (17.7%), and the distribution was similar across vitamin D groups (p=0.92). Median PFS was shortest in patients with severe vitamin D deficiency (3.4 months), intermediate in the 10-20 ng/mL group (5.2 months), and longest in patients with sufficient levels (8.1 months). Median OS ranged from 8.6 months in the <10 ng/mL group to 15.8 months in the ≥20 ng/mL group. Patients with liver metastases had significantly shorter OS compared to those without (median OS 9.8 vs. 13.6 months, HR 1.48, p=0.006). Vitamin D deficiency was associated with significantly worse PFS and OS on univariate analysis. In multivariable analysis, vitamin D levels <20 ng/mL remained

independently associated with poorer OS. ORR was numerically higher in patients with sufficient vitamin D levels (30% vs. 14%).

Conclusions: Lower baseline vitamin D levels were associated with inferior clinical outcomes in advanced NSCLC patients treated with nivolumab. Patients with vitamin D levels ≥20 ng/mL demonstrated longer PFS and OS and a trend toward higher response rates. These findings support a prognostic role for vitamin D status in immunotherapy-treated NSCLC; however, prospective studies are required to determine whether vitamin D optimization can causally enhance immunotherapy efficacy.

Keywords: Non-small cell lung cancer; nivolumab; vitamin D deficiency; immunotherapy; prognosis; survival; liver metastasis.

Introduction

Nivolumab and other PD-1/PD-L1 inhibitors have revolutionized the treatment of advanced non-small cell lung cancer (NSCLC), yielding improved survival in both squamous and non-squamous histologies after chemotherapy [1-4]. However, a substantial fraction of patients derive no durable benefit from immunotherapy, underscoring the need to identify host- and tumor-related factors influencing treatment response.

Vitamin D, a secosteroid hormone, has pleiotropic effects on immune regulation and the tumor microenvironment [5,6]. Preclinical studies have demonstrated that the active form of vitamin D, calcitriol, can modulate anti-tumor immunity by enhancing cytotoxic T-cell activity and regulating dendritic cell function, thereby reducing immunosuppressive signals within the tumor milieu [7,8].

Vitamin D deficiency is highly prevalent among cancer patients, including those with lung cancer, due to inadequate sunlight exposure, nutritional factors, and cancer-related metabolic alterations [9-11]. Emerging clinical evidence suggests that low vitamin D status may be associated with inferior outcomes in patients receiving immune checkpoint inhibitors. In melanoma, patients with sufficient vitamin D levels during anti-PD-1 therapy exhibited higher response rates and longer progression-free survival compared with vitamin D-deficient patients [12]. A prospective Chinese cohort study in NSCLC (n=77) also reported that vitamin D sufficiency was associated with improved outcomes in patients receiving anti-PD-1 therapy [13]. Similarly, prospective and retrospective studies in lung cancer have reported improved outcomes in patients with adequate vitamin D status treated with immune checkpoint inhibitors [14-16]. A large database analysis including lung cancer patients further supported this association [17].

Among metastatic sites in NSCLC, liver metastasis is recognized as one of the most potent negative prognostic factors [18]. Recent studies have confirmed that NSCLC patients with liver metastases derive less benefit

from immunotherapy, with hazard ratios for mortality ranging from 1.4 to 2.0 compared to patients without liver involvement [18]. Therefore, careful assessment of liver metastasis status is essential when evaluating prognostic factors in immunotherapy-treated NSCLC.

In the era of precision oncology, molecular selection has become critical for optimizing immunotherapy outcomes in NSCLC. Patients with NSCLC harboring activating EGFR mutations or ALK/ROS1 rearrangements typically derive limited benefit from immune checkpoint inhibitors and are often treated with targeted therapies instead [19,20]. Therefore, studies evaluating immunotherapy outcomes in patients with NSCLC should ideally be conducted in molecularly mutation-negative populations to avoid confounding by these established predictive factors.

In routine clinical practice, molecular testing for EGFR, ALK, and ROS1 is recommended primarily for patients with non-squamous histology, based on the higher prevalence of these drivers in adenocarcinoma, and can also be considered for patients with squamous histology for NSCLC [19,20].

This study aimed to evaluate the prognostic significance of baseline vitamin D levels in patients with advanced NSCLC treated with nivolumab in a real-world clinical setting. Specifically, we investigated the association between pretreatment vitamin D status and clinical outcomes, including progression-free survival and overall survival. In addition, the analysis accounted for key clinical factors known to influence prognosis, such as the presence of liver metastasis, in order to better clarify the potential role of vitamin D as a prognostic biomarker in advanced NSCLC receiving immune checkpoint inhibitor therapy.

Methods

Study Design

We conducted a retrospective, multicenter cohort study including patients with metastatic non-small cell lung cancer (NSCLC) treated with nivolumab as second-line therapy following chemotherapy failure. Patients were screened across participating tertiary oncology centers. Those with missing baseline serum 25-hydroxyvitamin D measurements were excluded. In this study cohort, all patients with NSCLC underwent molecular testing for major driver alterations, including EGFR mutations and ALK and ROS1 rearrangements, prior to nivolumab initiation. Molecular analysis confirmed the absence of these driver mutations in all patients included in the study. Baseline vitamin D levels were assessed prior to nivolumab initiation.

Patients were stratified into three groups according to baseline serum 25-hydroxyvitamin D levels: severe deficiency (<10 ng/mL, n = 93), deficiency (10-20 ng/mL, n = 132), and sufficient levels (≥20 ng/mL, n = 80). A flow diagram illustrating patient screening, exclusion, and stratification is presented in Figure 1.

Baseline patient characteristics (age, sex, histology, Eastern Cooperative Oncology Group performance status [ECOG PS], smoking history, presence of baseline liver metastases, brain metastases, and bone metastases) were collected from medical records, and only patients with negative molecular testing results for EGFR, ALK, ROS1, and other relevant driver mutations were included in the study. Data on baseline comorbidities including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and body mass index (BMI) were collected when available. However, these data were not systematically recorded for all patients; COPD and CVD status were documented in approximately 60% of patients, and BMI was available in approximately 45% of patients. Therefore, these variables were not included in the primary multivariable models, but their potential confounding effect is addressed in the limitations and through sensitivity analyses. Serum 25(OH) vitamin D was measured by standard chemiluminescent immunoassay within 4 weeks prior to starting nivolumab. For analysis, patients were stratified into three vitamin D categories based on conventional cut-offs: severe deficiency <10 ng/mL, moderate deficiency 10-20 ng/mL, and sufficient ≥20 ng/mL. These thresholds reflect clinically relevant levels (with <10 ng/mL indicating profound deficiency).

Treatment and Follow-up

All patients received nivolumab 3 mg/kg IV every 2 weeks (standard dosing during the study period) until disease progression or unacceptable toxicity. Radiographic tumor response was assessed by CT imaging approximately every 8-12 weeks and evaluated per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 in routine practice. Patients were followed for survival outcomes with periodic clinic visits. The data cut-off for survival analysis was January 1, 2025.

Outcome Measures

Progression-free survival (PFS) was defined as the time from nivolumab start to the first documented disease progression or death, whichever occurred first. Patients without progression were censored at the date of last disease evaluation. Overall survival (OS) was defined from nivolumab initiation to death from any cause, with living patients censored at last follow-up. Objective response rate (ORR) was the proportion of patients achieving a best response of complete or partial response. ORR was evaluated in the subset with available RECIST-assessments; stable disease and progression were considered non-responders.

The primary objective was to compare PFS and OS across the three vitamin D groups. Secondary objectives included comparing ORR between groups and determining if vitamin D level was an independent predictor of PFS/OS after controlling for other factors.

Statistical Analysis

Baseline clinical variables were summarized by vitamin D group and compared using appropriate tests (Chi-square for categorical variables, ANOVA or Kruskal-Wallis for continuous variables) to detect any imbalances. Survival functions for PFS and OS were estimated by the Kaplan-Meier method for each vitamin D category. Kaplan-Meier curves were generated to visualize differences. The log-rank test was used to compare survival across groups overall and pairwise. Survival outcomes were also compared between patients with and without liver metastases, given the established poor prognosis associated with hepatic involvement **.** Median PFS and OS with 95% confidence intervals (CIs) were reported for each group. We also calculated 1-year and 2-year survival rates by group. In addition to the overall cohort analysis, we performed pre-specified subgroup analyses restricted to patients with ECOG PS 0-1, and within that subgroup, further restricted to patients with adenocarcinoma histology, to evaluate the consistency of the vitamin D effect in more prognostically favorable populations.

To assess the potential impact of comorbidities on the observed associations, we performed sensitivity analyses in the subset of patients with available data. For COPD and CVD, we compared the distribution of these comorbidities across vitamin D groups using Chi-square tests. We also examined whether the association between vitamin D status and survival persisted after adjusting for these comorbidities in the available subset. A multivariable Cox proportional hazards regression was performed to evaluate the association of vitamin D status with PFS and OS while adjusting for potential confounders. Covariates in the Cox models included: age (continuous), sex (male vs female), ECOG performance status (dichotomized as 0-1 vs ≥2), histology (squamous vs non-squamous), liver metastasis (yes vs no) , brain metastasis (yes vs no), and baseline bone metastasis (yes vs no). PD-L1 status was not included in the primary multivariable model due to a high proportion of missing data, but its potential confounding effect is addressed in the limitations. Vitamin D was entered as categorical (with ≥20 ng/mL as reference category). Hazard ratios (HR) with 95% CI and p-values were obtained. The proportional hazards assumption was checked by inspecting log(-log) survival plots.

For ORR, proportions were compared using Chi-square or Fisher's exact test as appropriate. A two-sided $p<0.05$ was considered statistically significant for all analyses. Statistical analyses were performed using SPSS (v26) and R (v4.1) software.

Ethics Approval

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Van Training and Research Hospital (approval date: 16 January 2026; approval number:

2026-01-17). Due to the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee.

Results

Patient Characteristics

A total of 305 patients were included in the final analysis. Baseline characteristics by vitamin D group are summarized in Table 1. The cohort had a median age of 64 years (range 34-85) and was 68% male. Histologic subtypes were 61% adenocarcinoma, 32% squamous cell carcinoma, and 7% other NSCLC. ECOG performance status was 0 in 25%, 1 in 64%, and ≥2 in 11% of patients. Vitamin D deficiency was prevalent: 93 patients (30%) had <10 ng/mL, 132 (43%) had 10-20 ng/mL, and 80 (26%) had ≥20 ng/mL vitamin D. As shown in Table 1, patients with severe deficiency (<10 ng/mL) were numerically older (median age 65 vs. 63 years) and had higher rates of ECOG PS ≥2 (15.1% vs. 7.5% in the sufficient group) and squamous histology (37.6% vs. 27.5%), though these differences were not statistically significant (p>0.05 for all). The incidence of baseline brain or bone metastases was similar across the three groups (brain metastases ~18% in each group; bone metastases ~29-31%).

Liver metastases were present in 54 patients (17.7%), and the distribution of liver metastases was balanced across vitamin D groups: 17 patients (18.3%) in the <10 ng/mL group, 24 patients (18.2%) in the 10-20 ng/mL group, and 13 patients (16.3%) in the ≥20 ng/mL group (p=0.92).

Molecular testing was performed for all patients and had documented negative mutation status for EGFR, ALK, and ROS1 prior to nivolumab initiation. Data on PD-L1 expression were incomplete and are not presented in Table 1; this limitation is addressed in the discussion. No patients were on high-dose vitamin D supplementation at baseline.

Comorbidity data were available for a subset of patients. Among those with available data, the prevalence of COPD was 38% (69/182) and CVD was 29% (53/183). The distribution of COPD and CVD was similar across vitamin D groups (COPD: 41% in <10 ng/mL vs. 37% in 10-20 ng/mL vs. 36% in ≥20 ng/mL, p=0.82; CVD: 31% vs. 28% vs. 27%, p=0.79). BMI data were available for 137 patients (45%), with median BMI of 24.5 kg/m² (range 17.8-34.2), and did not differ significantly across groups (p=0.68). In the subset of patients with available comorbidity data, the association between vitamin D deficiency and poorer survival remained consistent (data not shown), suggesting that these comorbidities are unlikely to fully explain the observed vitamin D effect.

Treatment Exposure and Follow-up

The median duration of nivolumab therapy was 5.3 months. At data cut-off, 24 patients (8%) remained on nivolumab, 251 (82%) had discontinued

due to progression, and 30 (10%) had stopped due to toxicity or other reasons. The median follow-up time for survivors was 14.2 months (95% CI 13-15.5). By the analysis date, 154 patients (50%) had died.

Survival Outcomes by Vitamin D Group

Progression-Free Survival (PFS): Baseline vitamin D level showed a strong association with PFS (log-rank $p<0.001$ for overall comparison among the three groups). Figure 2 illustrates the Kaplan-Meier PFS curves stratified by vitamin D status. Patients with vitamin D <10 ng/mL had the shortest PFS, while those with ≥20 ng/mL had the longest PFS. Median PFS in the <10 ng/mL group was 3.4 months (95% CI 2.8-4.0). In the 10-20 ng/mL group, median PFS was 5.2 months (95% CI 4.5-6.0). By contrast, patients with vitamin D ≥20 ng/mL had a median PFS of 8.1 months (95% CI 6.5-9.7), more than double that of the severely deficient group. At 12 months, progression-free rates were 15% for <10 ng/mL, 20% for 10-20 ng/mL, and 35% for ≥20 ng/mL. Pairwise log-rank tests confirmed that both the <10 and 10-20 groups had significantly worse PFS than the ≥20 ng/mL group ($p<0.01$ for each comparison). The difference between <10 and 10-20 ng/mL groups trended toward significance, suggesting a gradient effect (severe deficiency faring worst). These findings indicate that higher vitamin D levels were associated with prolonged disease control under nivolumab.

Overall Survival (OS): Consistent with PFS, overall survival outcomes were worst in patients with severe vitamin D deficiency (Figure 3). The median OS was 8.6 months (95% CI 6.9-10.3) in the <10 ng/mL group, 12.1 months (95% CI 10.5-13.7) in the 10-20 ng/mL group, and 15.8 months (95% CI 13.2-18.4) in the ≥20 ng/mL group. The OS difference among groups was statistically significant (log-rank $p=0.002$). At one year, overall survival was 37% for <10 ng/mL, 50% for 10-20 ng/mL, and 60% for ≥20 ng/mL. The survival advantage for vitamin D sufficient patients began to emerge by 6 months and widened over time. By 2 years, ~30% of patients in the ≥20 group were alive, double the ~15% 2-year survival in the <10 group.

In pairwise comparisons, the <10 vs ≥20 ng/mL OS difference was most pronounced (HR for death 1.72, $p<0.001$). Patients with 10-20 ng/mL also had poorer OS than ≥20 ng/mL (HR 1.31), although this difference was of borderline significance ($p=0.07$). There was a trend toward worse OS for <10 vs 10-20 ng/mL (HR ~1.3) but with overlapping confidence intervals. Overall, the data indicate vitamin D deficiency (particularly <10 ng/mL) correlates with significantly shorter survival in nivolumab-treated NSCLC.

Subgroup Analyses:

We performed exploratory subgroup analyses to assess the consistency of the observed associations. The PFS/OS advantage of vitamin D sufficiency was observed consistently in both adenocarcinoma and squamous

subpopulations. For example, median OS in adenocarcinoma patients was 16.5 vs 9.0 months for ≥20 vs <10 ng/mL vitamin D; in squamous, 15.0 vs 8.2 months, respectively. We also saw the trend in both sexes. Among never-smokers (n=40), vitamin D ≥20 ng/mL patients had markedly longer PFS/OS than deficient never-smokers, though numbers were small. In the subgroup of patients with ECOG PS 0-1 (n=272), the association between vitamin D status and survival remained robust. Median OS was 9.2 months in the <10 ng/mL group, 13.0 months in the 10-20 ng/mL group, and 16.4 months in the ≥20 ng/mL group (log-rank p=0.004). Within the ECOG 0-1 subgroup, restricting to adenocarcinoma histology (n=166) yielded similar results: median OS 9.5, 13.8, and 17.1 months, respectively (log-rank p=0.01).

Patients with liver metastases (n=54, 17.7%) had significantly worse outcomes than those without liver involvement. Median OS was 9.8 months (95% CI 7.6-12.0) in patients with liver metastases versus 13.6 months (95% CI 11.9-15.3) in those without (HR 1.48, 95% CI 1.12-1.95, p=0.006), an effect size comparable to published estimates (HR range 1.4-2.0) . Importantly, the association between vitamin D status and survival remained robust in patients without liver metastases (n=251), with median OS of 9.1, 13.2, and 16.8 months for <10, 10-20, and ≥20 ng/mL groups, respectively (log-rank p=0.001). Among the smaller subgroup with liver metastases (n=54), a similar trend was observed, though statistical power was limited. These subgroup analyses suggest that the prognostic impact of vitamin D is not confined to a particular demographic or disease subset, though residual confounding cannot be excluded.

Tumor Response and Clinical Benefit

Objective response data were available for 280 patients (91% of cohort; others had clinical progression or early death precluding response assessment). The overall response rate (ORR) to nivolumab in our cohort was 21%. When stratified by vitamin D, ORR was 14% in the <10 ng/mL group, 19% in 10-20 ng/mL, and 30% in ≥20 ng/mL. Although these differences in ORR did not reach statistical significance (Chi-square p=0.08, likely due to limited power), a numerical trend is evident. Vitamin D sufficient patients were approximately twice as likely to experience tumor shrinkage than those with severe deficiency. Conversely, the disease control rate (DCR) (ORR plus stable disease) was significantly higher in the ≥20 ng/mL group (64%) vs <10 ng/mL (45%, p=0.01), driven by both higher response and more frequent stable disease.

Patients with liver metastases had a numerically lower ORR compared to those without liver metastases (15% vs. 23%, p=0.18), consistent with the concept of the liver as an immune-tolerant organ that may attenuate immunotherapy efficacy . The disease control rate was also lower in

patients with liver metastases (41% vs. 57%, p=0.04). Furthermore, patients with adequate vitamin D more often achieved durable responses. Among responders, the median response duration was longer in the ≥20 ng/mL group (not reached, with many ongoing responses beyond 1 year) compared to ~8 months in <20 ng/mL groups. This aligns with the prolonged PFS seen in vitamin D sufficient patients.

Multivariable Analysis

To determine if vitamin D is an independent prognostic factor, we built multivariable Cox regression models for PFS and OS (Table 4). The models included vitamin D group and key baseline covariates (age, sex, ECOG PS, liver metastasis, brain metastasis, bone metastasis, and histology). Liver metastasis was included based on extensive evidence demonstrating its independent prognostic significance in NSCLC patients receiving immunotherapy .

In the OS Cox model, vitamin D deficiency remained significantly associated with higher mortality risk after adjustment. Taking the vitamin D ≥20 ng/mL group as reference, the adjusted HR for death was 1.55 (95% CI 1.12-2.15, p=0.009) for <10 ng/mL, and 1.30 (95% CI 0.98-1.73, p=0.069) for 10-20 ng/mL. Thus, severe deficiency (<10) was an independent predictor of worse OS. Moderate deficiency (10-20) showed a strong trend toward worse OS that narrowly missed significance. Liver metastasis was independently associated with significantly worse OS (adjusted HR 1.42, 95% CI 1.06-1.91, p=0.02), consistent with published literature where HRs for liver metastasis typically range from 1.4 to 2.0 . Other significant covariates in the OS model included ECOG ≥2 (HR 1.79, p<0.001) and presence of brain metastases (HR 1.41, p=0.02). Notably, even controlling for ECOG and liver metastasis, low vitamin D had an effect size comparable to these traditional prognostic factors.

For PFS, multivariate results were similar. Vitamin D <10 ng/mL independently predicted shorter PFS (adjusted HR 1.61 vs ≥20, p=0.001). The 10-20 ng/mL group had HR 1.29 vs ≥20 (p=0.048). Liver metastasis was also independently associated with shorter PFS (adjusted HR 1.33, 95% CI 1.01-1.75, p=0.04). Other factors associated with shorter PFS were ECOG ≥2 and brain metastasis.

Discussion

In this study, baseline vitamin D status was strongly associated with survival outcomes in patients with advanced NSCLC treated with nivolumab. Patients with sufficient vitamin D levels experienced significantly longer progression-free and overall survival compared with vitamin D-deficient patients, particularly those with severe deficiency. However, it is important to acknowledge that patients with low vitamin D levels also had a higher prevalence of poor prognostic features such as older age, worse ECOG performance status, and squamous histology, which may have contributed to the observed outcome differences. After

multivariable adjustment for these factors and for liver metastasis status, severe vitamin D deficiency remained independently associated with inferior survival, but residual confounding due to unmeasured factors cannot be ruled out.

The prevalence of liver metastases in our cohort (17.7%) aligns with published rates in advanced NSCLC [18]. Patients with liver metastases had significantly worse outcomes (median OS 9.8 vs. 13.6 months; HR 1.48), consistent with reported HRs of 1.4-2.0 for patients with liver metastases receiving immunotherapy [18].

In our cohort, molecular testing for these key driver alterations was performed in all patients with NSCLC regardless of histologic subtype. All patients were confirmed to be negative for EGFR mutations as well as ALK and ROS1 rearrangements prior to nivolumab initiation. This approach ensured the exclusion of patients with actionable driver alterations who are more likely to benefit from targeted therapies, thereby minimizing potential confounding effects in the evaluation of immunotherapy outcomes. Consequently, the study population represents a molecularly homogeneous cohort with respect to major driver mutations, strengthening the validity of the survival analyses performed.

Our findings are consistent with prior reports in other malignancies. Galus et al. demonstrated that melanoma patients with adequate vitamin D levels during anti-PD-1 therapy achieved significantly higher objective response rates and longer progression-free survival [12]. In lung cancer, a prospective Chinese cohort by Wu et al. (n=77) similarly reported that vitamin D sufficiency was associated with improved efficacy of immune checkpoint inhibitors [13]. A large retrospective database analysis by Braun et al., including patients with various cancers including lung cancer, also found that low vitamin D levels were associated with worse outcomes on immunotherapy [17]. These studies support the generalizability of our observations [12,13,17].

Vitamin D deficiency may partially reflect poorer general health, systemic inflammation, or the presence of comorbid conditions such as COPD or cardiovascular disease. COPD and CVD are common in NSCLC patients, with reported prevalence rates of 30-50% and 20-30%, respectively [21]. These conditions are associated with both vitamin D deficiency and worse cancer outcomes, raising the possibility of confounding. In our sensitivity analysis of patients with available comorbidity data, the distribution of COPD and CVD was similar across vitamin D groups, and the association between vitamin D status and survival remained consistent in this subset. However, these data were incomplete, and we cannot exclude residual confounding from unmeasured or poorly measured comorbidities. Our study lacked comprehensive data on these comorbidities, as well as on BMI, which are known to be associated with both vitamin D levels and cancer outcomes [21]. This represents a significant limitation, as low vitamin D could be a marker of overall poor health rather than a direct mediator of immunotherapy resistance. However, vitamin D status

remained an independent prognostic factor after adjustment for ECOG performance status, metastatic burden, and liver metastasis [9,10]. This suggests a potential biological role beyond its function as a surrogate marker, though definitive proof would require prospective interventional studies.

Mechanistically, vitamin D has been shown to enhance anti-tumor immune responses through modulation of dendritic cell maturation and T-cell activation, promoting a more immunogenic tumor microenvironment [7,8,14,16]. Pharmacokinetic interactions may also contribute to this association. Cusato et al. reported that NSCLC patients with severe vitamin D deficiency had lower nivolumab trough concentrations, which correlated with earlier disease progression [15]. Although we did not assess drug levels in our cohort, these findings raise the hypothesis that vitamin D status could influence nivolumab exposure or immune responsiveness.

Importantly, emerging interventional data suggest that vitamin D supplementation may improve outcomes in patients receiving immune checkpoint inhibitors. The prospective PROVIDENCE study demonstrated significantly improved survival in cancer patients treated with immune checkpoint inhibitors who received systematic vitamin D supplementation [22]. In addition, a randomized controlled trial in NSCLC reported a survival benefit associated with vitamin D supplementation [23]. Together with our results, these findings highlight vitamin D as a potentially modifiable factor that warrants prospective evaluation in immunotherapy-treated NSCLC.

The magnitude of outcome differences observed is notable. However, these findings must be interpreted cautiously given the retrospective design and potential for unmeasured confounding. Vitamin D sufficient patients had a median PFS ~8 months vs ~3-5 months in deficient patients, and a median OS advantage of 4-7 months. These are clinically meaningful gaps, comparable to the improvement seen with some second-line combination strategies. Furthermore, sufficient vitamin D was associated with higher disease control and a trend toward nearly doubling of ORR (30% vs ~15%). While ORR did not reach statistical significance in our cohort, the pattern aligns with other reports. Galus et al. observed a significantly higher ORR (56% vs 36%) and longer median PFS (11.3 vs 5.7 months) in melanoma patients with normal vs low vitamin D during anti-PD-1 therapy [12]. Our study reinforces these observations in a real-world NSCLC population and suggests the prognostic influence of vitamin D is independent of ECOG, metastatic burden, and liver metastasis status [12-14].

Several mechanistic hypotheses could explain these findings. Vitamin D can promote a more effective anti-tumor immune response by enhancing T cell function and reducing immunosuppressive cells (like regulatory T-cells and myeloid-derived suppressors) in the tumor microenvironment. Vitamin D also upregulates genes involved in antigen presentation and

anti-microbial pathways, potentially making tumors more immunogenic. Conversely, deficiency may lead to an immunosuppressive milieu less responsive to PD-1 blockade. Additionally, vitamin D influences PD-L1 expression and has been linked to improved efficacy of PD-1 inhibitors in preclinical models [15,16].

Our study did not measure nivolumab drug levels, so we cannot assess whether vitamin D status influenced drug exposure. However, previous pharmacokinetic research has suggested a potential interaction; for example, Cusato et al. reported that NSCLC patients with vitamin D <10 ng/mL had lower nivolumab trough levels, which correlated with earlier progression [15]. While this raises the hypothesis that vitamin D deficiency might affect nivolumab pharmacokinetics, our data cannot confirm such a mechanism. Any potential impact of vitamin D on drug metabolism or distribution remains speculative and requires dedicated pharmacokinetic studies [13,14].

Vitamin D may also reflect broader nutritional and inflammatory status. Low vitamin D could correlate with elevated IL-6 or CRP and a suppressed immune system. However, even if vitamin D is a marker of poor health, it is one that is readily measurable and potentially correctable. Early evidence hints at a benefit from supplementation. Bersanelli et al. conducted a prospective trial (PROVIDENCE) in which advanced cancer patients on ICIs were given vitamin D supplementation; they reported significantly longer OS in those who received vitamin D repletion versus those who did not [22]. In that study, the cohort with early vitamin D supplementation had mOS ~15.9 months compared to 7.1 months in a non-supplemented cohort. Similarly, a randomized controlled trial by Akiba et al. reported a survival benefit associated with vitamin D supplementation in NSCLC [23]. Our results align remarkably well with those figures: ~8-9 months PFS in sufficient patients vs ~5 months in deficient. While these comparisons should be interpreted cautiously, they bolster the plausibility that vitamin D itself contributes to improved ICI outcomes [10,22,23].

Importantly, our study focuses on baseline vitamin D. It remains to be determined whether correcting a deficiency after starting immunotherapy can recapture the outcomes of initially sufficient patients. Some data (Galus et al.) indicate that patients who are deficient but become sufficient with supplementation have outcomes comparable to those with normal baseline levels [12]. In our practice, a significant portion of patients (>70%) were vitamin D deficient, echoing the "epidemic" of deficiency in cancer reported by others [9-11]. This suggests a substantial subset of NSCLC patients might benefit from vitamin D screening and repletion. Given vitamin D supplementation is inexpensive and safe, this could be a simple intervention to implement alongside immunotherapy [22,23].

Limitations

Our findings should be interpreted in light of several limitations inherent to the study design. The retrospective nature introduces risks of selection bias and unmeasured confounding, despite multivariate adjustment. Furthermore, baseline vitamin D data were available for only 305 of 890 eligible patients, and we could not compare those included versus excluded, raising the possibility of selection bias. We also lacked data on vitamin D supplementation during therapy, which may attenuate the observed associations.

While the differences in median age across vitamin D groups were not statistically significant, the numerically older age in the severely deficient group could represent a potential source of residual confounding, as age is an established prognostic factor in NSCLC.

While we were able to confirm negative mutation status for EGFR, ALK, and ROS1 in all patients, data on PD-L1 expression were incomplete and could not be included in the multivariable models. PD-L1 status, in particular, is a strong predictor of immunotherapy response, and its absence represents a potential source of residual confounding.

Furthermore, data on important comorbidities such as COPD, cardiovascular disease, and BMI were incomplete, limiting our ability to fully adjust for these potential confounders. While sensitivity analyses in the available subset suggested that these comorbidities were similarly distributed across vitamin D groups and did not fully explain the survival differences, the possibility of residual confounding remains. The prevalence of these comorbidities in our sensitivity analysis was consistent with published literature in NSCLC populations [21], but systematic collection of these variables would be needed for definitive adjustment.

While the difference in objective response rate was clinically notable, it did not reach statistical significance, likely due to limited power and early deaths precluding response assessment. Exploratory subgroup analyses were underpowered and should be considered hypothesis-generating. Finally, conventional vitamin D cut-offs were used; the optimal threshold for immunotherapy response remains unknown, and vitamin D was not analyzed as a continuous variable.

Despite these limitations, the consistency of our survival findings, their biological plausibility, and corroboration from external studies strengthen the validity of our conclusions. Additionally, the inclusion of liver metastasis in our multivariable models, with effect sizes consistent with published literature, enhances the robustness of our findings regarding vitamin D status.

Clinical Implications

If confirmed prospectively, baseline vitamin D could be used as a biomarker to stratify patients or to trigger interventions. Patients entering immunotherapy trials might be screened for 25(OH)D levels, and

those deficient could receive repletion (e.g., high-dose cholecalciferol) before or during therapy. This is a low-cost strategy with potentially high reward. It is also relevant outside of cancer: maintaining adequate vitamin D is beneficial for general health (bone integrity, immune function), so addressing deficiency is advisable regardless. For oncologists, our data provide a rationale to monitor vitamin D levels in NSCLC patients. It is conceivable that in the future, vitamin D status may join other factors like PD-L1, tumor mutational burden, or inflammatory indices as part of an immunotherapy predictive profile. [13,14]

Future Research Directions

Our study prompts further research. Prospective trials are needed to determine if vitamin D supplementation can truly improve immunotherapy outcomes in NSCLC. Ongoing studies in melanoma (e.g., NCT03472874) and planned trials in lung cancer will hopefully address this. It will also be important to investigate the underlying biological interactions - for example, does vitamin D alter the tumor infiltrating lymphocyte composition or cytokine milieu in NSCLC? And is there a threshold effect (e.g., any level >20 ng/mL is sufficient) or is higher always better? In our data, we saw little difference between 30 ng/mL and 20 ng/mL (small numbers), so ensuring patients reach at least the normal range seems adequate. Beyond immunotherapy efficacy, vitamin D might also affect toxicity profiles; some reports show patients on vitamin D had lower rates of immune-related colitis. This dual benefit - enhancing efficacy and reducing toxicity - makes vitamin D an attractive supportive-care measure.

Conclusion

In conclusion, our study demonstrates that low baseline vitamin D levels are associated with significantly poorer response and survival outcomes in advanced NSCLC patients receiving nivolumab. After adjustment for known prognostic factors, including liver metastasis, severe vitamin D deficiency remained an independent predictor of worse outcomes. While we were able to address several potential confounders, the observational design and incomplete data on variables such as PD-L1 status mean these findings should be considered hypothesis-generating rather than definitive. Vitamin D deficient patients had roughly half the PFS/OS of those with sufficient levels, even after multivariable adjustment. Given the high prevalence of deficiency and the ease of intervention, routine screening and correction of vitamin D deficiency could be a simple, low-cost strategy to support better outcomes in NSCLC treatment. Prospective trials are warranted to determine whether vitamin D supplementation can causally enhance immunotherapy efficacy.

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Table 1. Baseline Demographic and Clinical Characteristics of Patients According to Baseline Serum Vitamin D Levels.
Baseline characteristics of metastatic non-small cell lung cancer patients treated with nivolumab are shown according to serum 25-hydroxyvitamin D categories (<10 ng/mL, 10-20 ng/mL, and ≥20 ng/mL). Data are presented as median (range) or number (percentage), as appropriate.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; LM, liver metastasis.

Table 2. Treatment Outcomes According to Baseline Serum Vitamin D Levels.
Progression-free survival (PFS), overall survival (OS), objective response rate (ORR), and disease control rate (DCR) are presented according to baseline serum 25-hydroxyvitamin D categories. Median survival times are reported with 95% confidence intervals. P values were calculated using the log-rank test for survival outcomes and Chi-square test for response rates.

Table 3. Univariate Cox Regression Analysis for PFS and OS.
Univariate Cox regression analysis evaluating the association between baseline clinical variables and progression-free survival (PFS) and overall survival (OS) in metastatic non-small cell lung cancer patients treated with nivolumab. Hazard ratios (HRs) are presented with 95% confidence intervals.

Table 4. Multivariate Cox Regression Analysis for PFS and OS.
Multivariate Cox regression models evaluating independent prognostic factors for progression-free survival (PFS) and overall survival (OS). Variables included in the model were baseline vitamin D level, ECOG performance status, liver metastasis, brain metastasis, bone metastasis, histology, age, and sex. Hazard ratios (HRs) with 95% confidence intervals are shown.

Figure 1. Flow Diagram of Patient Selection and Vitamin D Stratification.

A total of 890 metastatic non-small cell lung cancer patients received nivolumab as second-line therapy after progression on chemotherapy. Of these, 585 patients were excluded due to missing baseline serum 25-hydroxyvitamin D measurements. All patients (n=305) had confirmed negative mutation status for EGFR, ALK, and ROS1. The final analysis included 305 patients with available baseline vitamin D levels, who were subsequently stratified into three groups according to serum vitamin D status.

Figure 2. Kaplan-Meier Curves for Overall Survival According to Baseline Serum Vitamin D Levels.
Overall survival (OS) of metastatic non-small cell lung cancer patients treated with nivolumab is shown according to baseline serum 25-hydroxyvitamin D categories (<10 ng/mL, 10-20 ng/mL, and ≥20 ng/mL). Differences between groups were assessed using the log-rank test.

Figure 3. Kaplan-Meier Curves for Progression-Free Survival According to Baseline Serum Vitamin D Levels.
Progression-free survival (PFS) of metastatic non-small cell lung cancer patients treated with nivolumab is shown according to baseline serum 25-hydroxyvitamin D categories (<10 ng/mL, 10-20 ng/mL, and ≥20 ng/mL). Differences between groups were evaluated using the log-rank test.

Table 1. Baseline Characteristics According to Baseline Vitamin D Levels

Variable	<10 ng/mL (n=93)	10–20 ng/mL (n=132)	≥20 ng/mL (n=80)	p value
Age, median (range)	65 (38–85)	64 (34–82)	63 (36–81)	0.41
Gender, n (%)				0.72
Male	66 (71.0)	88 (66.7)	54 (67.5)	
Female	27 (29.0)	44 (33.3)	26 (32.5)	
ECOG PS ≥2, n (%)	14 (15.1)	12 (9.1)	6 (7.5)	0.09
Histology, n (%)				0.48
Adenocarcinoma	52 (55.9)	84 (63.6)	52 (65.0)	
Squamous cell	35 (37.6)	39 (29.5)	22 (27.5)	
Other	6 (6.5)	9 (6.9)	6 (7.5)	
Brain metastasis, n (%)	17 (18.3)	25 (18.9)	14 (17.5)	0.97
Bone metastasis, n (%)	29 (31.2)	39 (29.5)	21 (26.3)	0.78
Smoking history, n (%)				0.66
Current/former	78 (83.9)	106 (80.3)	62 (77.5)	
Never	15 (16.1)	26 (19.7)	18 (22.5)	
Liver metastasis, n (%)	17 (18.3)	24 (18.2)	13 (16.3)	0.92
EGFR/ALK/ROS1 negative, n (%)	93 (100)	132 (100)	80 (100)	

Abbreviations: *ECOG, Eastern Cooperative Oncology Group; PS, performance status.*

Table 2. Treatment Outcomes According to Baseline Vitamin D Levels

Outcome	<10 ng/mL	10–20 ng/mL	≥20 ng/mL	p value
Median PFS, months (95% CI)	3.4 (2.8–4.0)	5.2 (4.5–6.0)	8.1 (6.5–9.7)	**<0.001**
12-month PFS rate, %	15	20	35	
Median OS, months (95% CI)	8.6 (6.9–10.3)	12.1 (10.5–13.7)	15.8 (13.2–18.4)	**0.002**

Outcome	<10 ng/mL	10–20 ng/mL	≥20 ng/mL	p value
12-month OS rate, %	37	50	60	
Objective response rate (ORR), %	14	19	30	**0.08**
Disease control rate (DCR), %	45	54	64	**0.01**

Table 3. Univariate Cox Regression Analysis for PFS and OS

Variable	PFS HR (95% CI)	p value	OS HR (95% CI)	p value
Vitamin D <10 vs ≥20 ng/Ml	1.72 (1.32–2.23)	<0.001	1.68 (1.26–2.24)	**<0.001**
Vitamin D 10–20 vs ≥20 ng/mL	1.34 (1.04–1.73)	0.02	1.31 (1.01–1.71)	**0.04**
ECOG PS ≥2	1.89 (1.39–2.58)	<0.001	1.82 (1.32–2.51)	**<0.001**
Brain metastasis	1.41 (1.08–1.84)	0.01	1.44 (1.09–1.91)	**0.01**
Bone metastasis	1.29 (1.01–1.66)	0.04	1.21 (0.93–1.57)	0.15
Liver metastasis	1.35 (1.04-1.75)	0.02	1.48 (1.12-1.95)	**0.006**

Table 4. Multivariate Cox Regression Analysis for PFS and OS

Variable	HR	95% CI	p value
Progression-Free Survival			
Vitamin D <10 ng/Ml	**1.61**	1.21–2.13	**0.001**
Vitamin D 10–20 ng/Ml	1.29	1.00–1.67	0.048
ECOG PS ≥2	1.74	1.27–2.39	<0.001
Brain metastasis	1.36	1.03–1.80	0.03
Liver metastasis	1.33	1.01-1.75	0.04
Overall Survival			
Vitamin D <10 ng/Ml	**1.55**	1.12–2.15	**0.009**
Vitamin D 10–20 ng/Ml	1.30	0.98–1.73	0.069
ECOG PS ≥2	1.79	1.28–2.50	<0.001
Brain metastasis	1.41	1.05–1.90	0.02

Variable	HR	95% CI	p value
Liver metastasis	1.42	1.06-1.91	0.02

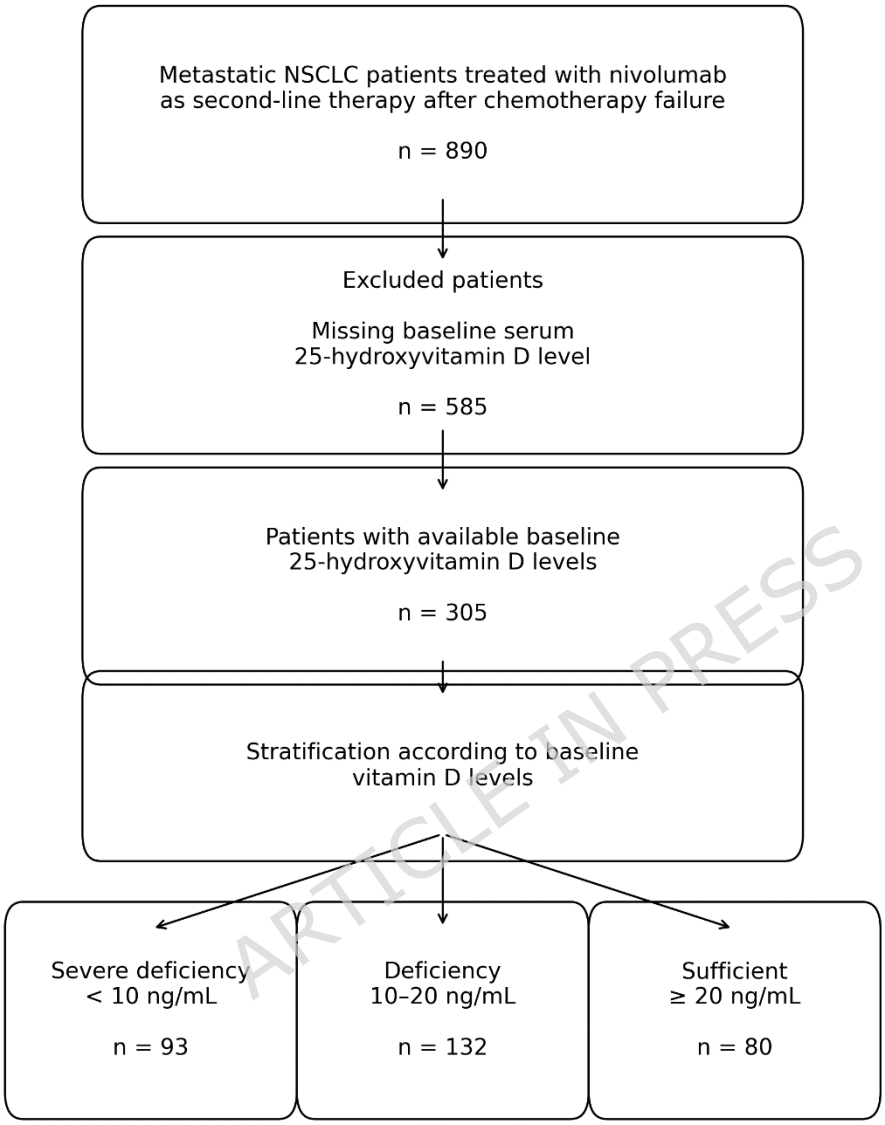

Figure 1. Flow diagram of patient selection and vitamin D stratification.

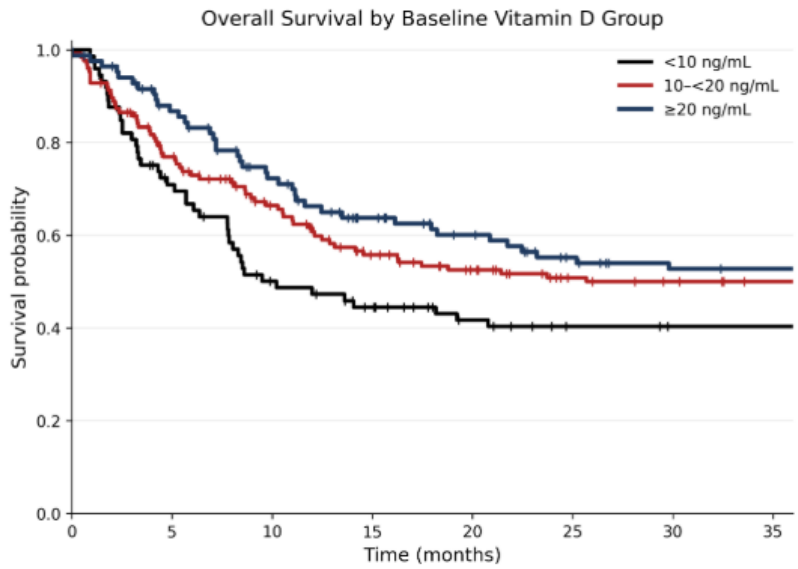

Figure 2: Kaplan–Meier curves for overall survival stratified by baseline vitamin D category.

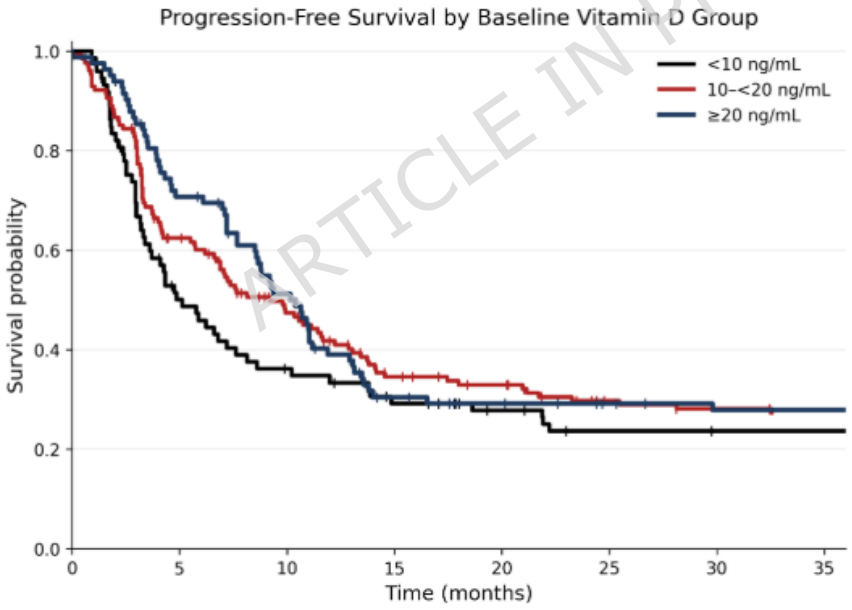

Figure 3. Kaplan–Meier curves for progression-free survival stratified by baseline vitamin D category.