# Systematic vitamin D supplementation is associated with improved outcomes and reduced thyroid adverse events in patients with cancer treated with immune checkpoint inhibitors: results from the prospective PROVIDENCE study.

Bersanelli M et al.

## Contents

# Supplementary Methods

## Statistical analysis

Baseline patients' characteristics were reported with descriptive statistics as appropriate. The $\chi 2$ test was used to compare categorical variables.

Considering that cumulative incidence of adverse events during treatment is time-dependent, the probability of experiencing each irAE category between the PROVIDENCE Cohort 1 and the control cohort was compared with multivariable logistic regressions including the interaction term between the cohort and treatment duration (TTF) and estimated through adjusted odd ratios (OR) with 95% confidence intervals (CIs)

Median TTF and overall survival (OS) were evaluated using the Kaplan-Meier method and compared with the log-rank test. Objective response rate (ORR) and disease control rate (DCR) were reported as crude rates with 95%CI. The duration of follow-up was calculated according to the reverse Kaplan-Meier method. Considering the limited sample size of subgroups, an Inverse Probability of Treatment Weighing (IPTW) procedure was used to weigh key baseline characteristics between the PROVIDENCE cohort 1 and the control cohort to fit comparative univariable analysis, with balancing ability estimated through the standardized mean differences (SMD) of the weighted characteristics. Key variables included: primary tumor types (non-small cell lung cancer - NSCLC, melanoma, renal cell carcinoma, urothelial cancers, and others), age ($\geq$ *vs.* < 70 years), biological sex (male *vs.* female), Eastern Cooperative Oncology Group-Performance Status (ECOG-PS) (0 *vs.* 1 *vs.* $\geq$ 2), burden of disease (number of metastatic sites $\leq$ 2 *vs.* > 2), treatment line (first *vs.* second and further lines of therapy).

Cox proportional hazards regression was used to estimating the risk of treatment discontinuation/death and presented through hazard ratios (HR) with 95%CIs. To further mitigate any residual imbalance of key characteristics, we also performed a double adjustment, including variables with post-weighing SMD $\geq$ 0.10 in IPTW-fitted multivariable regression models for the risk of treatment discontinuation (TTF) and death (OS) [14]. Probability of achieving objective response (ORR) and disease control (DCR) were also compared with logistic regression and presented through OR with 95%CI.

Acknowledging that the data source consisted of different institutions, with patients followed by treating physicians in clinical practice, therefore without pre-established monitoring procedures, a clustered-robust correction for participating center was applied to 95%CI from logistic regression and a center-specific conditional interpretation by using frailty models was applied to correct all the 95%CIs from multivariable Cox regressions, whilst a clustered-

robust correction for participating center was applied to 95%CI from multivariable logistic regressions. All P-values were 2-sided, and confidence intervals were set at the 95% level, with significance pre-defined to be at <0.05. Analyses were performed using the R-studio software, R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, and the MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

**Supplementary Table 1:** Summary of dynamic changes in Vitamin D levels over time in cohorts 1 and 2.

| | **Baseline** | **3-months** | **6-months** | **9-months** |
|---|---|---|---|---|
| **Vitamin D** | **N° (%) – 101** | **N° (%) – 71** | **N° (%) – 43** | **N° (%) – 28** |
| **Cohort 1** | | | | |
| **Median ng/ml (range)** | **13 (4 – 73)** | **38 (5 – 95)** | **31 (7 – 106)** | **34 (6 – 59.3**) |
| Adequate (>30) | 6 (5.9) | 50 (70.4) | 26 (60.5) | 18 (64.3) |
| Insufficiency (20 - 30) | 23 (22.8) | 19 (26.8) | 14 (32.6) | 8 (28.6) |
| Deficiency (10 -20) | 39 (38.6) | - | 2 (4.7) | 1 (3.6) |
| Severe deficiency (<10) | 33 (32.7) | 2 (2.8) | 1 (2.3) | 1 (3.6) |
| **Cohort 2** | | | | |
| | **n = 63** | **n = 45** | **n = 40** | **n = 36** |
| **Median (ng/ml) (range)** | **11 (4 – 29)** | **41 (8-125)** | **36 (9-77)** | **33 (10-56)** |
| Adequate (>30) | - | 35 (77.8) | 35 (87.5) | 24 (66.7) |
| Insufficiency (20 - 30) | 12 (19.0) | 6 (13.3) | 1 (2.5) | 8 (22.2) |
| Deficiency (10 -20) | 24 (38.1) | 3 (6.7) | 3 (7.5) | 4 (11.1) |
| Severe deficiency (<10) | 27 (42.9) | 1 (2.2) | 1 (2.5) | - |

**Supplementary Table 2:** Comparison of baseline patients' characteristics between the PROVIDENCE cohort 1 and the control cohort before and after the ITPW procedure. ECOG-PS: eastern cooperative oncology group-performance status; NSCLC: non-small cell lung cancer; SMD: standardized mean difference; IPTW: inverse probability of treatment weighing.

| | **PROVIDENCE Cohort 1** | **Control cohort** | | **PROVIDENCE Cohort 1 Weighted** | **Control cohort Weighted** | |
|---|---|---|---|---|---|---|
| | **N° (%) – 101** | **N° (%) – 238** | **p-value - SMD** | **%** | **%** | **p-value - SMD** |
| **Age, (years)** | | | | | | |
| Non-elderly | 46 (45.5) | 127 (53.4) | 0.231 – 0.15 | 48.2 | 50.4 | 0.817 – 0.04 |
| Elderly (≥70 years) | 55 (54.5) | 111 (46.6) | | 51.8 | 49.6 | |
| **Sex** | | | | | | |
| Female | 24 (23.8) | 81 (44.0) | 0.081 – 0.22 | 31.0 | 32.1 | 0.895 – 0.02 |
| Male | 77 (76.2) | 157 (66.0) | | 69.0 | 67.9 | |
| **ECOG-PS** | | | | | | |
| 0 | 46 (45.5) | 78 (32.8) | 0.014 – 0.36 | 36.2 | 37.0 | 0.379 – 0.25 |
| 1 | 44 (43.6) | 105 (44.1) | | 34.0 | 43.4 | |
| ≥ 2 | 11 (10.9) | 55 (23.1) | | 29.7 | 19.7 | |
| **Primary Tumor** | | | | | | |
| NSCLC | 50 (49.5) | 48 (20.2) | <0.001 – 1.02 | 27.4 | 29.6 | 0.653 – 0.20 |
| Melanoma | 27 (26.7) | 37 (15.5) | | 13.6 | 17.2 | |
| Renal cell carcinoma | 13 (12.9) | 125 (52.5) | | 50.2 | 41.0 | |
| Urothelial | 4 (4.0) | 18 (7.6) | | 3.9 | 6.4 | |
| Others | 7 (6.9) | 10 (4.2) | | 4.9 | 5.7 | |
| **No. of metastatic sites** | | | | | | |
| ≤ 2 | 66 (65.3) | 112 (47.1) | 0.003 – 0.37 | 45.6 | 52.6 | 0.424 – 0.14 |
| > 2 | 35 (34.7) | 126 (52.9) | | 54.4 | 47.4 | |
| **Treatment line of Immunotherapy** | | | | | | |
| First | 47 (46.5) | 49 (22.1) | <0.001 – 0.57 | 24.6 | 28.4 | 0.548 – 0.08 |
| Non-First | 54 (53.5) | 189 (79.4) | | 75.4 | 71.6 | |

**Supplementary Table 3:** IPTW-fitted multivariable analysis for the risk of treatment discontinuation and risk of death including variables with SMD ≥ 0.1. A centre-specific conditional interpretation by using frailty models was applied to correct all the 95%CIs for HR and a clustered robust correction for participating center was applied to correct all the 95%CI for OR. HR: hazard ratio; NSCLC: non-small cell lung cancer; ECOG-PS: eastern cooperative oncology group performance status; IPTW: inverse probability of treatment weighing; SMD: standardized mean difference.

| | **Multivariate Analysis** | | | |
|---|---|---|---|---|
| | **Risk of Treatment discontinuation** | **Risk of death** | **Probability of achieving tumour response** | **Probability of achieving disease control** |
| **VARIABLE** | **HR (95% CI)** | **HR (95%CI)** | **OR (95% CI)** | **OR (95%CI)** |
| **Cohort** | | | | |
| Control | 1 | 1 | 1 | 1 |
| PROVIDENCE cohort 1 | **0.61 (0.40-0.91)** | **0.55 (0.34-0.90)** | 0.89 (0.40-2.00) | 1.95 (0.84-4.31) |
| **ECOG-PS** | | | | |
| 0 | 1 | 1 | 1 | 1 |
| 1 | 1.98 (1.39-2.82) | 2.34 (1.47-3.73) | 0.82 (0.37-1.82) | 0.85 (0.50-1.41) |
| ≥2 | 3.68 (2.11-6.41) | 3.03 (1.32-6.98) | 1.15 (0.44-2.98) | 1.61 (0.43-5.91) |
| **Primary Tumour** | | | | |
| NSCLC | 1 | 1 | 1 | 1 |
| Melanoma | 0.95 (0.64-1.40) | 0.81 (0.51-1.26) | 1.06 (0.43-2.62) | 1.57 (0.88-2.81) |
| Kidney | 0.66 (0.43-1.02) | 0.61 (0.35-1.04) | 0.43 (0.03-5.84) | 1.61 (0.55-4.69) |
| Urothelial | 0.69 (0.37-1.27) | 0.68 (0.33-1.42) | 0.45 (0.03-8.61) | 0.85 (0.18-3.98) |
| Others | 0.68 (0.30-1.52) | 0.74 (0.29-1.89) | 2.35 (0.74-7.40) | 2.45 (0.82-7.28) |
| **Number of metastatic sites** | | | | |
| ≤ 2 | 1 | 1 | 1 | 1 |
| >2 | 1.35 (1.03-1.75) | 1.37 (0.90-2.10) | 0.79 (0.47-1.31) | 0.56 (0.28-1.09) |

**Supplementary Table 4:** Comparison of baseline patients' characteristics between the PROVIDENCE and the control cohort before and after the ITPW procedure. Patients subsequently entered into PROVIDENCE cohort 2 are included in the control cohort. ECOG-PS: eastern cooperative oncology group-performance status; NSCLC: non-small cell lung cancer; SMD: standardized mean difference; IPTW: inverse probability of treatment weighing.

| | PROVIDENCE Cohort 1 | Control cohort | | PROVIDENCE Cohort 1 Weighted | Control cohort Weighted | |
|---|---|---|---|---|---|---|
| | N° (%) – 101 | N° (%) – 263 | p-value - SMD | % | % | p-value - SMD |
| **Age, (years)** | | | | | | |
| Non-elderly | 46 (45.5) | 139 (52.9) | 0.258 – 0.14 | 45.5 | 50.7 | 0.989 – 0.01 |
| Elderly (≥70 years) | 55 (54.5) | 124 (47.1) | | 54.5 | 49.3 | |
| **Sex** | | | | | | |
| Female | 24 (23.8) | 91 (34.6) | 0.062 – 0.24 | 34.1 | 32.3 | 0.826 – 0.03 |
| Male | 77 (76.2) | 172 (65.4) | | 65.9 | 67.7 | |
| **ECOG-PS** | | | | | | |
| 0 | 46 (45.5) | 92 (35.0) | 0.045 – 0.30 | 40.5 | 38.4 | 0.428 – 0.22 |
| 1 | 44 (43.6) | 116 (44.1) | | 33.7 | 43.3 | |
| ≥ 2 | 11 (10.9) | 55 (20.9) | | 25.8 | 18.3 | |
| **Primary Tumor** | | | | | | |
| NSCLC | 50 (49.5) | 60 (22.8) | <0.001 – 0.96 | 29.2 | 30.9 | 0.747 – 0.17 |
| Melanoma | 27 (26.7) | 40 (15.2) | | 13.7 | 17.0 | |
| Renal cell carcinoma | 13 (12.9) | 133 (50.6) | | 48.1 | 40.4 | |
| Urothelial | 4 (4.0) | 20 (7.6) | | 4.4 | 6.6 | |
| Others | 7 (6.9) | 10 (3.8) | | 4.6 | 5.2 | |
| **No. of metastatic sites** | | | | | | |
| ≤ 2 | 66 (65.3) | 130 (49.4) | 0.001 – 0.32 | 49.5 | 54.0 | 0.59 – 0.08 |
| > 2 | 35 (34.7) | 133 (50.6) | | 50.5 | 46.0 | |
| **Treatment line of Immunotherapy** | | | | | | |
| First | 47 (46.5) | 58 (22.1) | <0.001 – 0.53 | 26.0 | 28.9 | 0.639 – 0.06 |
| Non-First | 54 (53.5) | 205 (77.9) | | 74.0 | 71.1 | |

**Supplementary Table 5:** IPTW-fitted multivariable analysis for the risk of treatment discontinuation and risk of death including variables with SMD ≥0.1. Patients subsequently entered into PROVIDENCE cohort 2 are included in the control cohort. A centre-specific conditional interpretation by using frailty models was applied to correct all the 95%CIs. HR: hazard ratio; NSCLC: non-small cell lung cancer; ECOG-PS: eastern cooperative oncology group performance status; IPTW: inverse probability of treatment weighing; SMD: standardized mean difference.

| | Multivariate Analysis | |
|---|---|---|
| | Risk of Treatment discontinuation | Risk of death |
| **VARIABLE** | **HR (95% CI)** | **HR (95%CI)** |
| **Cohort** | | |
| Control | 1 | 1 |
| PROVIDENCE cohort 1 | **0.68 (0.47-0.98)** | **0.62 (0.39-0.98)** |
| **ECOG-PS** | | |
| 0 | 1 | 1 |
| 1 | 2.03 (1.44-2.86) | 2.26 (1.43-3.57) |
| ≥2 | 4.22 (2.38-7.46) | 3.80 (1.73-8.33) |
| **Primary Tumour** | | |
| NSCLC | 1 | 1 |
| Melanoma | 1.18 (0.82-1.71) | 0.88 (0.56-1.37) |
| Kidney | 0.82 (0.53-1.24) | 0.71 (0.41-1.21) |
| Urothelial | 0.79 (0.43-1.45) | 0.79 (0.39-1.56) |
| Others | 0.74 (0.33-1.68) | 0.76 (0.31-1.87) |