Esiste un ruolo immunomodulatorio ed antitumorale della vitamina D? Focus sul tumore polmonare

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Vitamin D...

Vitamin D (VD) is a group of pro-hormones.

5 different liposoluble forms:

- vitamin D_1 : mixed composed by 1:1 of calciferol and lumisterol$_2$
- vitamin D_2 : ergocalciferol
- vitamin D_3 : cholecalciferol
- vitamin D_4 : dihydroergocalciferol
- vitamin D_5 : sitocalciferol

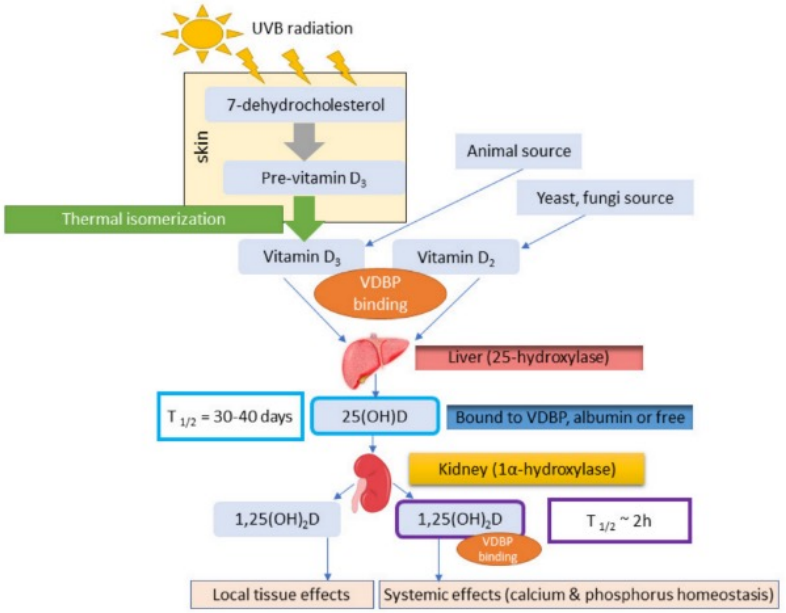
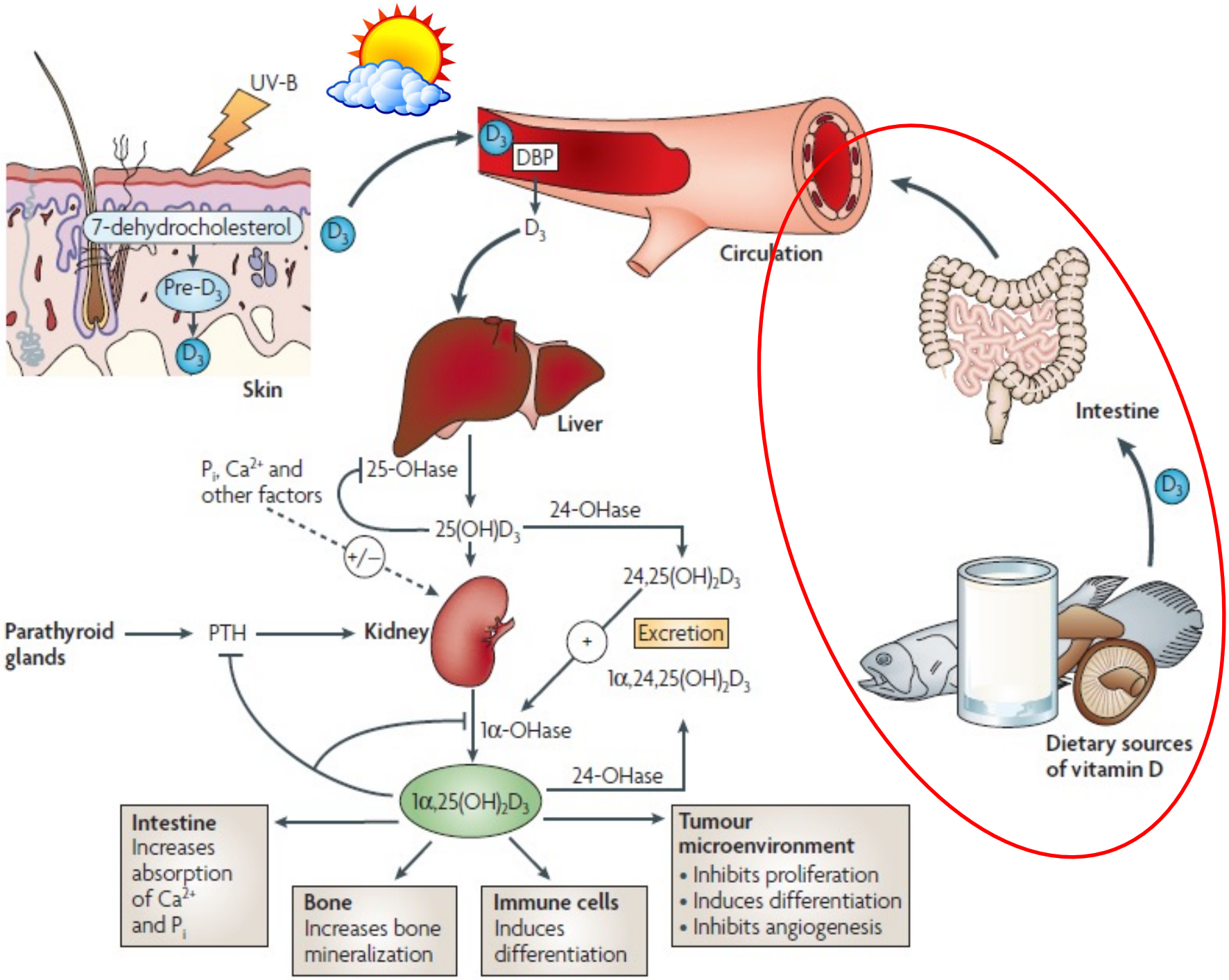
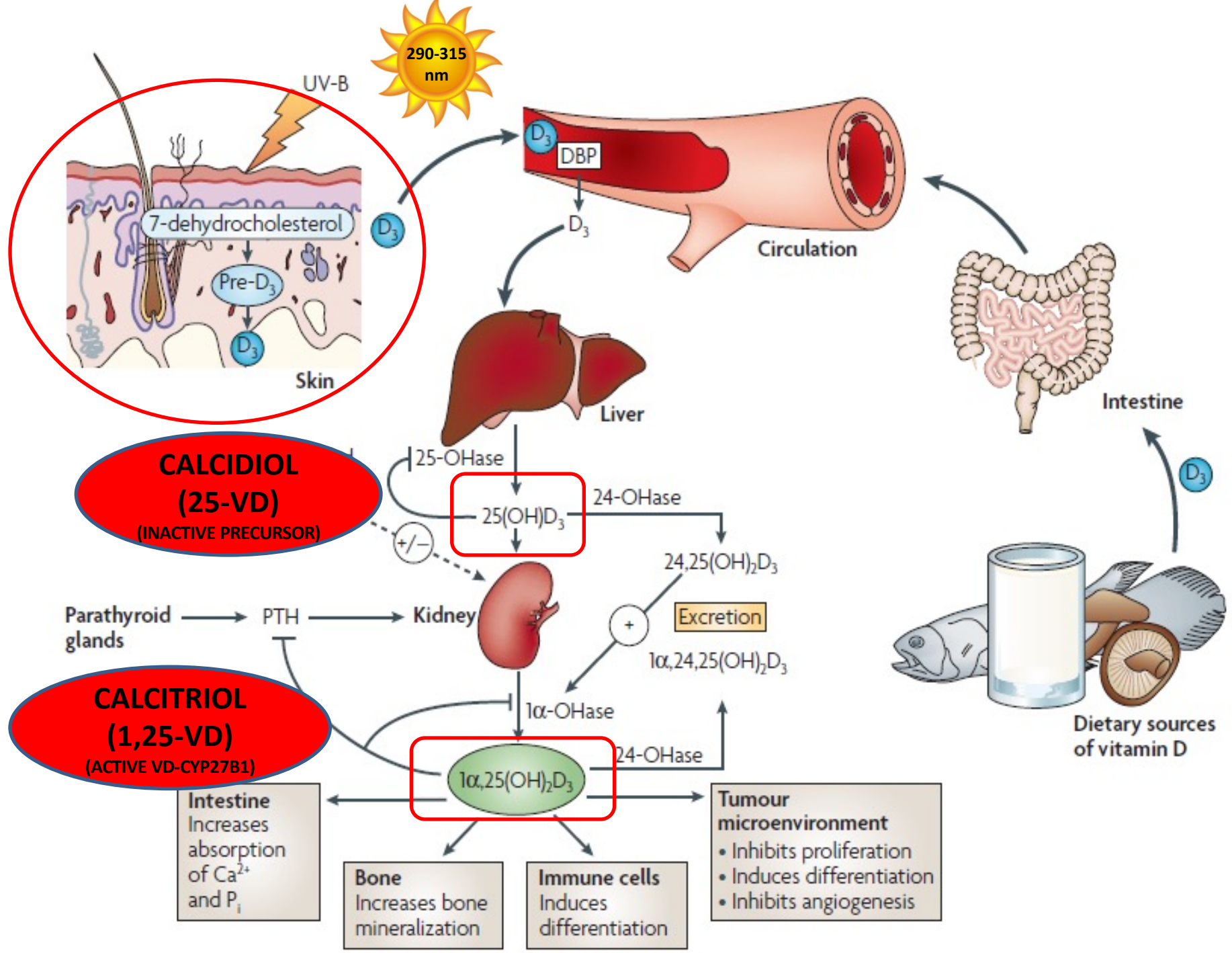


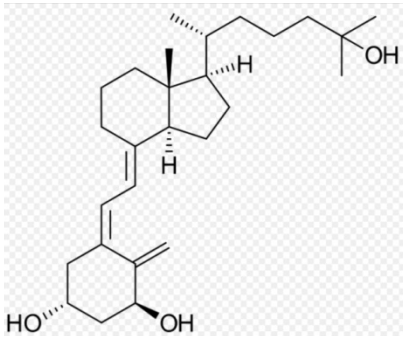
Figure 1. Vitamin D synthesis pathway (based on Bikle 2014 [14]).


UV-B
7-dehydrocholesterol
Pre-D3
D3
Skin
D3
DBP
D3
Circulation
Liver
Intestine
D3
Pi, Ca2+ and other factors
25-OHase
25(OH)D3
24-OHase
+/−
24,25(OH)2D3
Parathyroid glands
PTH
Kidney
+
Excretion
1α,24,25(OH)2D3
1α-OHase
24-OHase
1α,25(OH)2D3
Dietary sources of vitamin D
Intestine
Increases absorption of Ca2+ and Pi
Bone
Increases bone mineralization
Immune cells
Induces differentiation
Tumour microenvironment
• Inhibits proliferation
• Induces differentiation
• Inhibits angiogenesis


290-315 nm
UV-B
7-dehydrocholesterol
Pre-D3
D3
Skin
D3
DBP
D3
Circulation
Liver
Intestine
D3
CALCIDIOL
(25-VD)
(INACTIVE PRECURSOR)
25-OHase
25(OH)D3
24-OHase
+/−
24,25(OH)2D3
Parathyroid glands
PTH
Kidney
+
Excretion
1α,24,25(OH)2D3
CALCITRIOL
(1,25-VD)
(ACTIVE VD-CYP27B1)
1α-OHase
24-OHase
1α,25(OH)2D3
Dietary sources of vitamin D
Intestine
Increases absorption of Ca2+ and Pi
Bone
Increases bone mineralization
Immune cells
Induces differentiation
Tumour microenvironment
• Inhibits proliferation
• Induces differentiation
• Inhibits angiogenesis

1,25-OH_2 Vitamin D (Calcitriol)

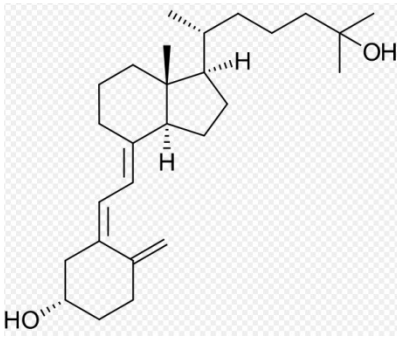
Active form. Not stored in tissues, very short blood half-life



NO MARKER!

25-OH VITAMIN D (CALCIDIOL)

PRECURSOR MAINLY STORED IN LIVER AND MUSCLES, THE MOST PRESENT IN BLOOD



MARKER!

Calcidiol: different ranges…

The ongoing D-lemma of vitamin D supplementation for nonskeletal health and bone health

Nipith Charoenngam[a,b], Arash Shirvani[a], and Michael F. Holick[a]

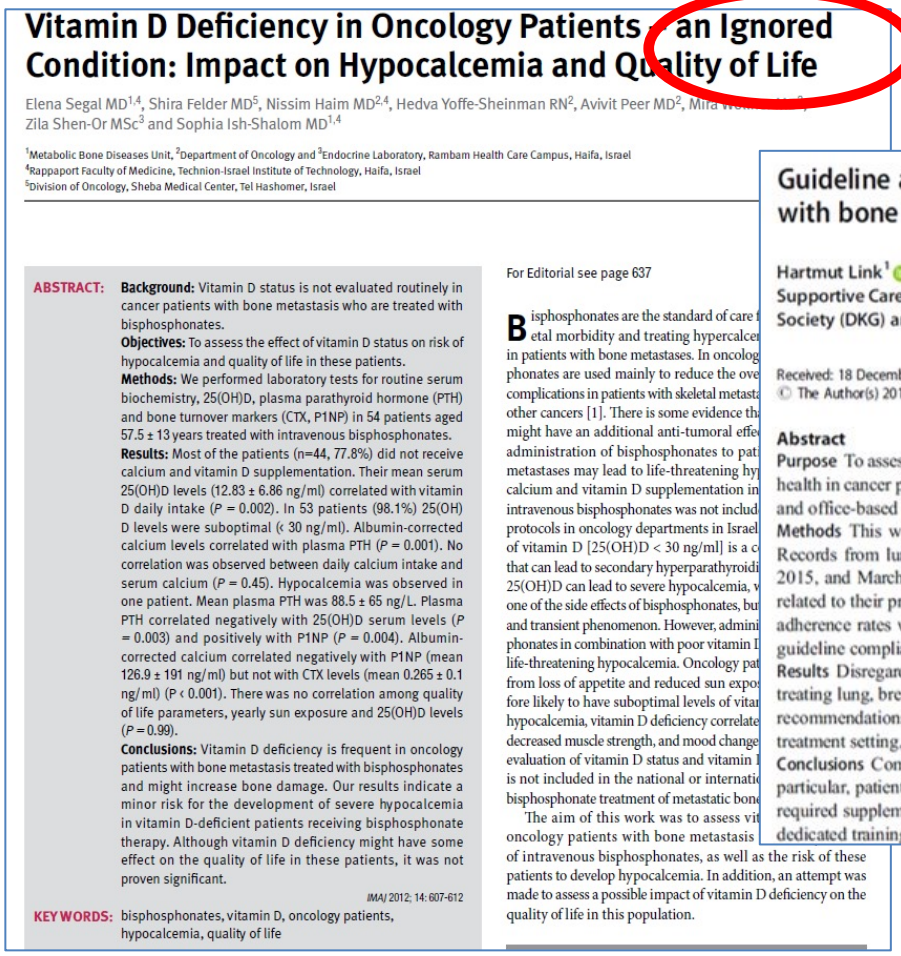
Vitamin D 25(OH)D range guidelines from various organizations:

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Testing Laboratories
Deficient	0-30 ng/ml	0-20 ng/ml	0-11 ng/ml	0-31 ng/ml
Insufficient	31-39 ng/ml	21-29 ng/ml	12-20 ng/ml	
Sufficient	40-80 ng/ml	30-100 ng/ml	>20 ng/ml	32-100 ng/ml
Toxic	>150 ng/ml			

The Vitamin D Council suggests that a level of 50 ng/ml is the ideal level to aim for. This is why the Council recommends that adults take 5,000 IU/day of vitamin D supplement in order to reach and stay at this level.

vitamindcouncil.org

- Different dosage
- Route of administration (drops, tablets, aerosol, intravenously, etc…)
- Daily? Weekly? Monthly?



Vitamin D Deficiency in Oncology Patients – an Ignored Condition: Impact on Hypocalcemia and Quality of Life

Elena Segal MD[1,4], Shira Felder MD[5], Nissim Haim MD[2,4], Hedva Yoffe-Sheinman RN[2], Avivit Peer MD[2], Mila W…, Zila Shen-Or MSc[3] and Sophia Ish-Shalom MD[1,4]

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[4]Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
[5]Division of Oncology, Sheba Medical Center, Tel Hashomer, Israel

ABSTRACT: **Background:** Vitamin D status is not evaluated routinely in cancer patients with bone metastasis who are treated with bisphosphonates.
Objectives: To assess the effect of vitamin D status on risk of hypocalcemia and quality of life in these patients.
Methods: We performed laboratory tests for routine serum biochemistry, 25(OH)D, plasma parathyroid hormone (PTH) and bone turnover markers (CTX, P1NP) in 54 patients aged 57.5 ± 13 years treated with intravenous bisphosphonates.
Results: Most of the patients (n=44, 77.8%) did not receive calcium and vitamin D supplementation. Their mean serum 25(OH)D levels (12.83 ± 6.86 ng/ml) correlated with vitamin D daily intake ($P = 0.002$). In 53 patients (98.1%) 25(OH) D levels were suboptimal (< 30 ng/ml). Albumin-corrected calcium levels correlated with plasma PTH ($P = 0.001$). No correlation was observed between daily calcium intake and serum calcium ($P = 0.45$). Hypocalcemia was observed in one patient. Mean plasma PTH was 88.5 ± 65 ng/L. Plasma PTH correlated negatively with 25(OH)D serum levels ($P = 0.003$) and positively with P1NP ($P = 0.004$). Albumin-corrected calcium correlated negatively with P1NP (mean 126.9 ± 191 ng/ml) but not with CTX levels (mean 0.265 ± 0.1 ng/ml) ($P < 0.001$). There was no correlation among quality of life parameters, yearly sun exposure and 25(OH)D levels ($P = 0.99$).
Conclusions: Vitamin D deficiency is frequent in oncology patients with bone metastasis treated with bisphosphonates and might increase bone damage. Our results indicate a minor risk for the development of severe hypocalcemia in vitamin D-deficient patients receiving bisphosphonate therapy. Although vitamin D deficiency might have some effect on the quality of life in these patients, it was not proven significant.

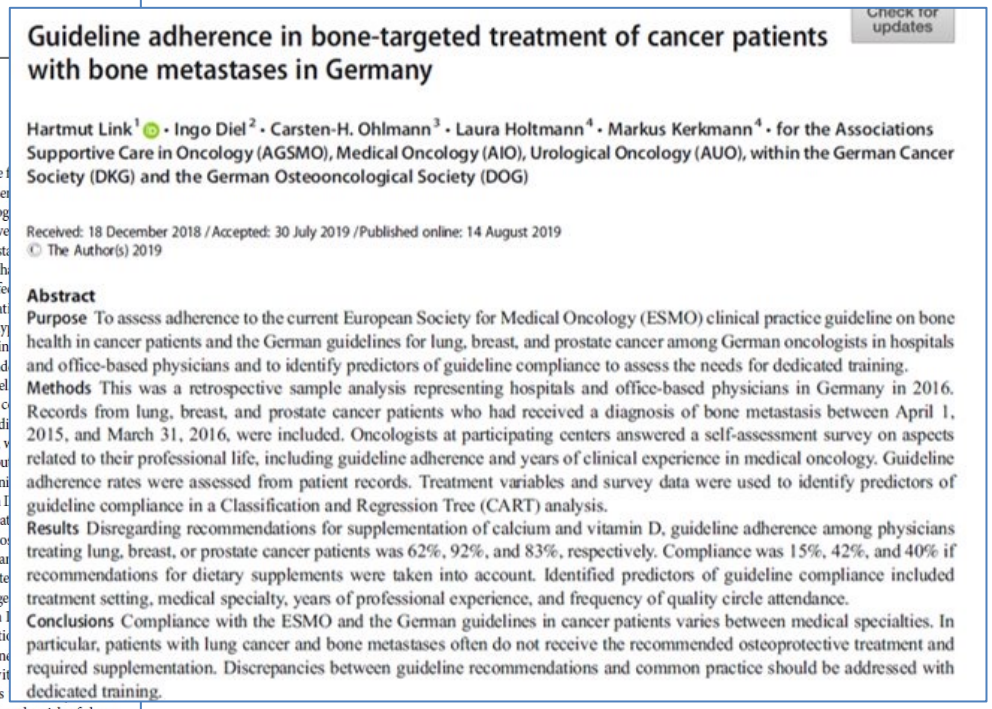
IMAJ 2012; 14: 607-612

KEY WORDS: bisphosphonates, vitamin D, oncology patients, hypocalcemia, quality of life

For Editorial see page 637

Bisphosphonates are the standard of care f… etal morbidity and treating hypercalce… in patients with bone metastases. In oncolog… phonates are used mainly to reduce the ove… complications in patients with skeletal metast… other cancers [1]. There is some evidence th… might have an additional anti-tumoral effe… administration of bisphosphonates to pat… metastases may lead to life-threatening hy… calcium and vitamin D supplementation in… intravenous bisphosphonates was not includ… protocols in oncology departments in Israel… of vitamin D [25(OH)D < 30 ng/ml] is a c… that can lead to secondary hyperparathyroidi… 25(OH)D can lead to severe hypocalcemia, … one of the side effects of bisphosphonates, bu… and transient phenomenon. However, admini… phonates in combination with poor vitamin D… life-threatening hypocalcemia. Oncology pat… from loss of appetite and reduced sun expos… fore likely to have suboptimal levels of vita… hypocalcemia, vitamin D deficiency correlate… decreased muscle strength, and mood change… evaluation of vitamin D status and vitamin D… is not included in the national or internati… bisphosphonate treatment of metastatic bone…

The aim of this work was to assess vit… oncology patients with bone metastasis … of intravenous bisphosphonates, as well as the risk of these patients to develop hypocalcemia. In addition, an attempt was made to assess a possible impact of vitamin D deficiency on the quality of life in this population.



Guideline adherence in bone-targeted treatment of cancer patients with bone metastases in Germany

Check for updates

Hartmut Link[1] · Ingo Diel[2] · Carsten-H. Ohlmann[3] · Laura Holtmann[4] · Markus Kerkmann[4] · for the Associations Supportive Care in Oncology (AGSMO), Medical Oncology (AIO), Urological Oncology (AUO), within the German Cancer Society (DKG) and the German Osteooncological Society (DOG)

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Abstract

Purpose To assess adherence to the current European Society for Medical Oncology (ESMO) clinical practice guideline on bone health in cancer patients and the German guidelines for lung, breast, and prostate cancer among German oncologists in hospitals and office-based physicians and to identify predictors of guideline compliance to assess the needs for dedicated training.

Methods This was a retrospective sample analysis representing hospitals and office-based physicians in Germany in 2016. Records from lung, breast, and prostate cancer patients who had received a diagnosis of bone metastasis between April 1, 2015, and March 31, 2016, were included. Oncologists at participating centers answered a self-assessment survey on aspects related to their professional life, including guideline adherence and years of clinical experience in medical oncology. Guideline adherence rates were assessed from patient records. Treatment variables and survey data were used to identify predictors of guideline compliance in a Classification and Regression Tree (CART) analysis.

Results Disregarding recommendations for supplementation of calcium and vitamin D, guideline adherence among physicians treating lung, breast, or prostate cancer patients was 62%, 92%, and 83%, respectively. Compliance was 15%, 42%, and 40% if recommendations for dietary supplements were taken into account. Identified predictors of guideline compliance included treatment setting, medical specialty, years of professional experience, and frequency of quality circle attendance.

Conclusions Compliance with the ESMO and the German guidelines in cancer patients varies between medical specialties. In particular, patients with lung cancer and bone metastases often do not receive the recommended osteoprotective treatment and required supplementation. Discrepancies between guideline recommendations and common practice should be addressed with dedicated training.

… Disregarding recommendations for supplementation of calcium and vitamin D, guideline adherence among physicians treating lung, breast, or prostate cancer patients was 62%, 92%, and 83%, respectively. Compliance was 15%, 42%, and 40% if recommendations for dietary supplements were taken into account. Compliance with the ESMO and the German guidelines in cancer patients varies between medical specialties. In particular, **patients with lung cancer and bone metastases often do not receive the recommended osteoprotective treatment and required supplementation**. Discrepancies between guideline recommendations and common practice should be addressed with dedicated training

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A phase I/II pharmacokinetic and pharmacogenomic study of calcitriol in combination with cisplatin and docetaxel in advanced non-small-cell lung cancer

A phase I/II clinical trial assessing the maximum tolerated dose and dose-limiting toxicities of 1,25(OH)2D3 with cisplatin/docetaxel in advanced non-small cell lung cancer patients and assessing the response rates was carried out: 60 mcg/m^2.
In addition, as a secondary outcome, they correled systemic exposure to 1,25(OH)2D3 with polymorphisms in the CYP24 enzyme.

Abstract

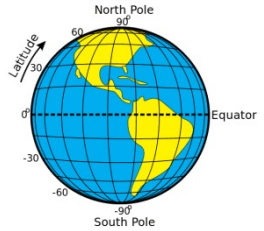
Background—Preclinical studies demonstrated antiproliferative synergy of 1,25-D_3 (calcitriol) with cisplatin. The goals of this phase I/II study were to determine the recommended phase II dose (RP2D) of 1,25-D_3 with cisplatin and docetaxel and its efficacy in metastatic non-small-cell lung cancer.

Methods—Patients were ≥18 years, PS 0–1 with normal organ function. In the phase I portion, patients received escalating doses of 1,25-D_3 intravenously every 21 days prior to docetaxel 75 mg/m^2 and cisplatin 75 mg/m^2 using standard 3 + 3 design, targeting dose-limiting toxicity (DLT) rate <33 %. Dose levels of 1,25-D_3 were 30, 45, 60, and 80 mcg/m^2. A two-stage design was employed for phase II portion. We correlated *CYP24A1* tag SNPs with clinical outcome and 1,25-D_3 pharmacokinetics (PK).

Results—34 patients were enrolled. At 80 mcg/m^2, 2/4 patients had DLTs of grade 4 neutropenia. Hypercalcemia was not observed. The RP2D of 1,25-D_3 was 60 mcg/m^2. Among 20 evaluable phase II patients, there were 2 confirmed, 4 unconfirmed partial responses (PR), and 9 stable disease (SD). Median time to progression was 5.8 months (95 % CI 3.4, 6.5), and median overall survival 8.7 months (95 % CI 7.6, 39.4). CYP24A1 SNP rs3787554 (C > T) correlated with disease progression ($P = 0.03$) and CYP24A1 SNP rs2762939 (C > G) trended toward PR/SD ($P = 0.08$). There was no association between 1,25-D_3 PK and CYP24A1 SNPs.

Conclusions—The RP2D of 1,25-D_3 with docetaxel and cisplatin was 60 mcg/m^2 every 21 days. Pre-specified endpoint of 50 % confirmed RR was not met in the phase II study. Functional SNPs in CYP24A1 may inform future studies individualizing 1,25-D_3.

Latitude, seasonality and lung cancer



- In some countries, for example the UK and in Norway, there is a significant gradient in **UVB exposure from north to south** and a **better lung cancer survival** rate in patients with **higher exposure**.
- In relation to season, in a cohort of just over **45,000 Norwegian** patients, authors found that **male lung cancer patients younger than 50 years old** had a **15% reduced** risk of dying from the disease **within 18 months when diagnosed in the summer/autumn vs. winter/spring months**.
- A meta-analysis showed that **latitude** was **positively associated** with **lung cancer incidence** rates in both men and women. There was also an independent association of **higher lung cancer incidence with lower UVB irradiance**.
- Furthermore, in a US study of 456 patients with early-stage non-small cell lung cancer, Zhou et al. found that those individuals with **higher intakes of vitamin D** and whose **surgery** occurred during the **summer** months, had **improved survival** rates and a **greater recurrence-free survival**.

Vitamin D: Potential in the Prevention and Treatment of Lung Cancer

ROSEMARY NORTON and MARIA A O'CONNELL

Other factors relating VD and lung cancer…

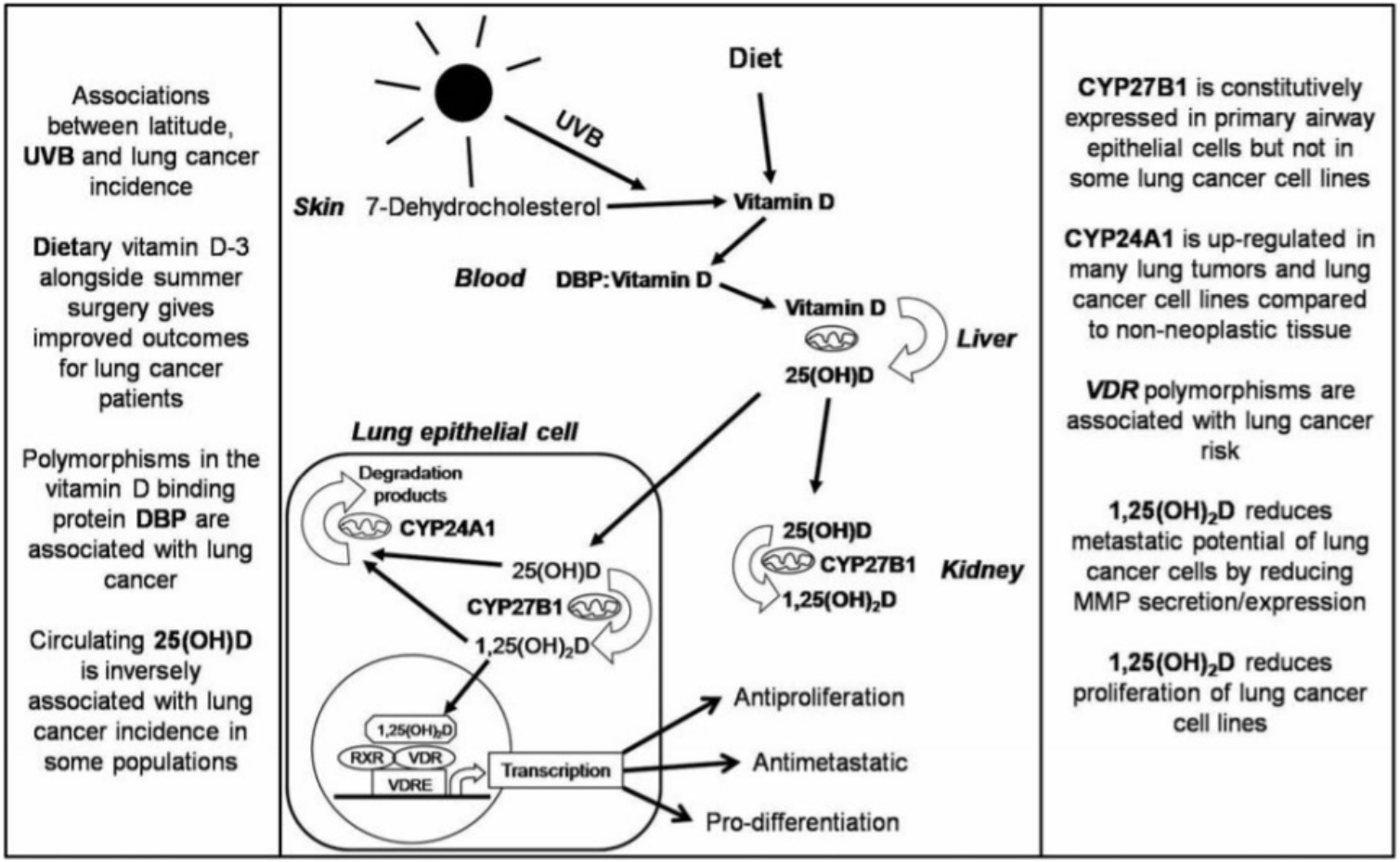
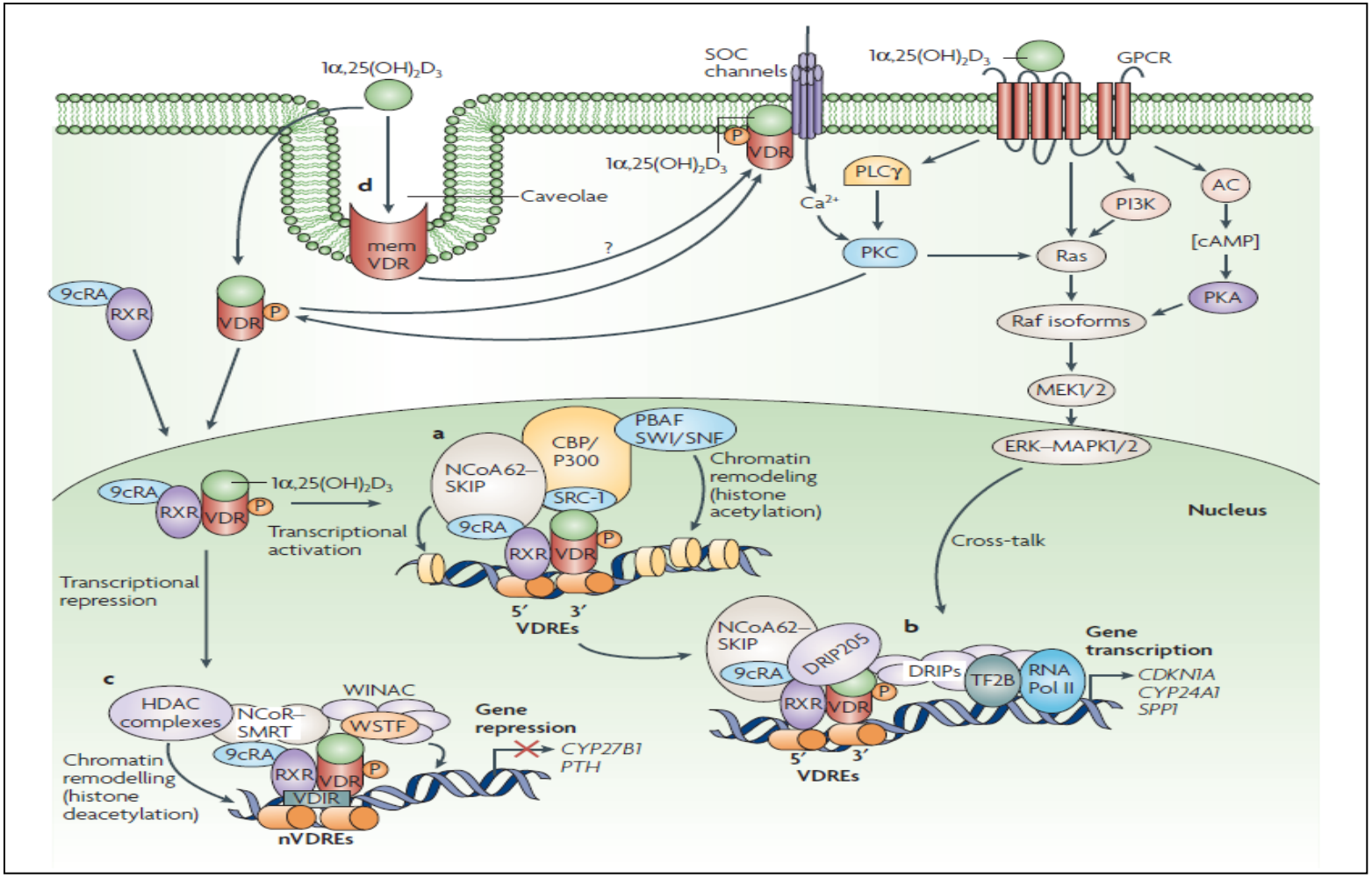


Figure 2. *Summary of the associations between lung cancer and the vitamin D pathway.*

Vitamin D: Potential in the Prevention and Treatment of Lung Cancer

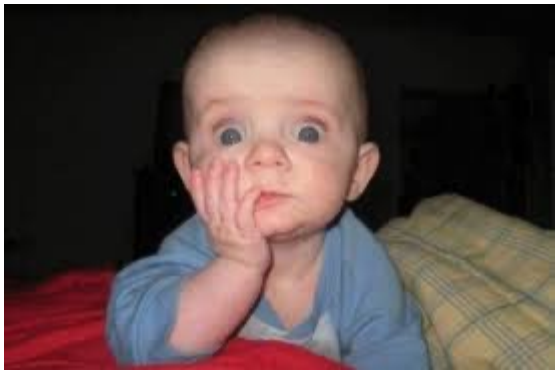
ROSEMARY NORTON and MARIA A O'CONNELL

Vitamin D activity: the vitamin D receptor (VDR)

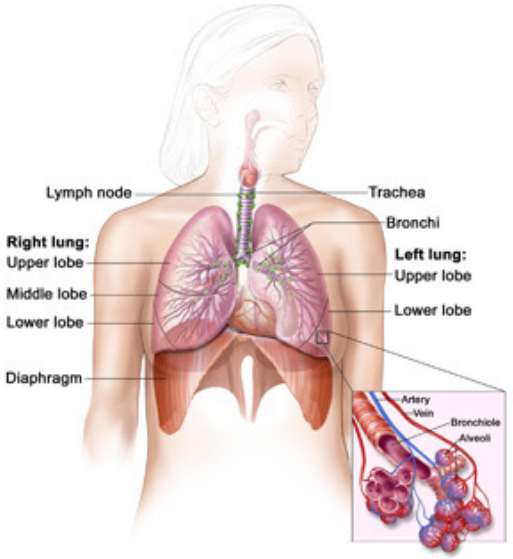


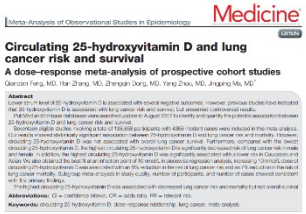
Vitamin D activities

- *Calcium and Phophorous Omeostasis*
- *Bones remodelling*
- *Muscles contraction*
- *Paracrin regulation of cellular growing (including tumors)*
- *Blood pressure regulation*
- *Insulin secretion*
- *Inflammation*
- *Neoangiogenesis*
- *Antimicrobial activity*
- *Antitumoral activity*
- *Immunomodulatory activity*
- *Gene expression regulation in DRUG ADME*
- *Antimetastatic activity*



Antitumoral activity in lung cancer




Meta-Analysis of Observational Studies in Epidemiology

Medicine

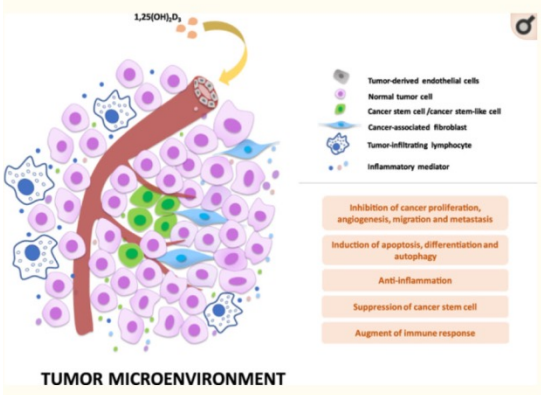
Circulating 25-hydroxyvitamin D and lung cancer risk and survival

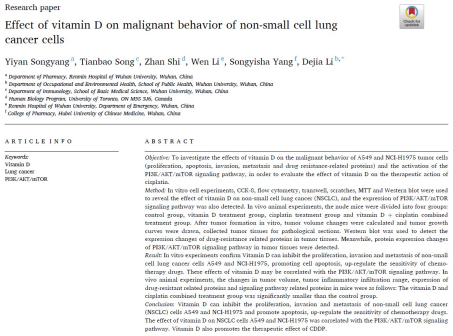
A dose-response meta-analysis of prospective cohort studies

Qianqian Feng, MD, Hai Zhang, MD, Zhengqin Dong, MD, Yang Zhou, MD, Jingqing Ma, MD

The immunomodulatory function of vitamin D in lung cancer

- 25-VD can inhibit the activity of mammalian target of **rapamycin** in lung cancer cells and **raise** its level of expression, which can **induce** the **autophagy** of tumor cells.
- 25-VD can **induce** the expression of major antioxidant protein–superoxide dismutase **SOD1** and **SOD2**, thereby **inhibiting** the formation of **lung cancer** to some extent.
- VD can inhibit cellular **proliferation** and **angiogenesis**, while **improving** cellular **differentiation** and **apoptosis**.
- **24,25(OH)2D3** is biologically active in lung cancer and prolonged the **survival** rate of LLC mice, exhibiting both **antimetastatic** and **analgesic** effects.
- **1,25(OH)2D3** reduces **tumor metastasis** and recurrence and increases **tumor immunity** in the LLC model.




Research paper

Effect of vitamin D on malignant behavior of non-small cell lung cancer cells

Yiyan Songyang, Tianbao Song, Zhan Shi, Wen Li, Songyisha Yang, Dejia Li

ARTICLE INFO

ABSTRACT

Metastatic growth of lung cancer cells is extremely reduced in Vitamin D receptor knockout mice☆

Kimie Nakagawa [a,*], Akihiko Kawaura [a], Shigeaki Kato [b], Eiji Takeda [c], Toshio Okano [a]

[a] Department of Hygienic Sciences, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan
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Abstract

Lung metastatic neoplasms are the major cause of cancer mortality. Despite the progress of diagnostic techniques and improvements in surgical procedures, the prognosis of patients with lung cancer is generally poor, even in the early stages of cancer [Cancer: Principles and Practice of Oncology, vol. 1, fifth ed., Lippincott-Raven, New York, 1997, p. 849]. Epidemiological studies indicate a positive correlation with the prevalence of cancers and low serum levels of Vitamin D metabolites [Am. J. Clin. Nutr. 54 (1991) 193s; Cancer Epidemiol. Biomark. Prev. 9 (2000) 1059]. 1α,25-Dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] is a potent inhibitor of cancer cell proliferation in vitro [Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 4990; Endocrinol. 139 (1998) 1046; Mol. Endocr. 15 (2001) 1127]. There is, however, no report demonstrating that 1α,25$(OH)_2D_3$ is operative in vivo to inhibit metastatic growth of cancer cells. To verify this possibility, we generated a stable transfectant of the Lewis lung carcinoma (LLC) cell expressing green fluorescent protein (GFP) and examined its metastatic activity in wild-type mice and Vitamin D receptor (VDR) knockout mice that exhibit no Vitamin D-dependent calcemic activity and extremely high serum levels of 1α,25$(OH)_2D_3$ due to the overexpression of the 1α-hydroxylase gene [Nat. Genet. 16 (1997) 391; Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 9831]. Here, we show that 1α,25$(OH)_2D_3$ inhibits metastatic growth of lung cancer cells in the defined animal model and may work as an intrinsic factor for prevention of metastasis in intact animals. These findings establish a critical role for 1α,25$(OH)_2D_3$ in lung metastatic neoplasms and provide a new model for metastasis of malignant cells.

Keywords: VDR knockout mice; Vitamin D; Lung cancer; Tumor; Metastasis

22-Oxa-1α,25-dihydroxyvitamin D_3 inhibits metastasis and angiogenesis in lung cancer

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1α,25-Dihydroxyvitamin D_3 (1α,25-D_3) has potent antiproliferative and anti-invasive properties *in vitro* in cancer cells. However, its calcemic effect *in vivo* limits its therapeutic applications. Here, we report the efficacy of 22-oxa-1α,25-dihydroxyvitamin D_3 (22-oxa-1α,25-D_3), a low calcemic analog of vitamin D_3 against the development of metastatic lung carcinoma after an intravenous injection of green fluorescent protein-transfected Lewis lung carcinoma (LLC-GFP) cells in C57BL/6 mice. The mice injected with tumor cells were implanted simultaneously with osmotic minipumps containing either 1α,25-D_3, 22-oxa-1α,25-D_3 or vehicle. The 1α,25-D_3 treatment group had been hypercalcemic, but the 22-oxa-1α,25-D_3 and vehicle treatment groups remained normocalcemic for the duration of the experiment. The total number of lung metastases, lung weight and the expression of GFP mRNA in the lung were markedly decreased in 1α,25-D_3 and 22-oxa-1α,25-D_3-treated mice. In the *in vitro* experiment, 1α,25-D_3 and 22-oxa-1α,25-D_3 reduced the expression of matrix metalloproteinase (MMP)-2, MMP-9, vascular endothelial growth factor and parathyroid hormone-related protein in LLC-GFP cells. Furthermore, in the angiogenesis assay, the number of tumor cells or basic fibroblast growth factor-induced angiogenesis was reduced in 1α,25-D_3 and 22-oxa-1α,25-D_3-treated mice. Moreover, using a new experimental model of vitamin D receptor (VDR) null mutant ($VDR^{-/-}$) mice with corrected hypocalcemia and hypervitaminosis D, we examine the anti-cancer effect of 22-oxa-1α,25-D_3 without other functions induced by 22-oxa-1α,25-D_3 in the host. In the $VDR^{-/-}$ mice, 22-oxa-1α,25-D_3 directly inhibited the metastatic activity of LLC-GFP cells in a dose-dependent manner without exerting a direct influence on the calcemic activity or other actions regulated by 22-oxa-1α,25-D_3 in the host. These results indicate that the inhibition of metastasis and angiogenesis-inducing activity in cancer cells seemed to be a major mechanism responsible for the anti-cancer effects of 22-oxa-1α,25-D_3. Our findings show that 22-oxa-1α,25-D_3 is beneficial for the prevention of metastasis in lung carcinoma.

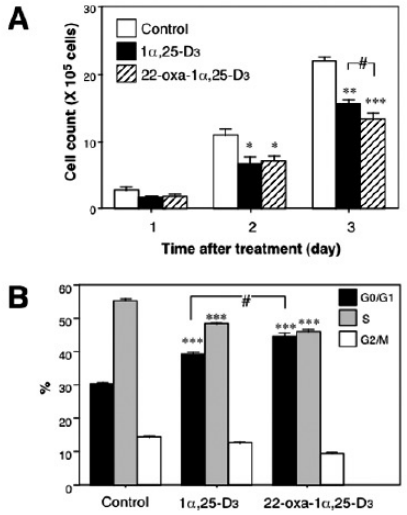
Introduction

Lung cancer is the most common cause of cancer death in the world. Lung cancer frequently metastasizes to the systemic lymph nodes and distant organs, including the liver, lung, kidney and bone, and >90% of deaths from lung cancer can be attributed to metastases (1). In Japan, an estimated 90 000 new cases of lung cancer were diagnosed in 2003, and lung cancer will remain the leading cause of cancer death. Therefore, metastasis to multiple organs is a critical problem for patients with lung cancer. The prevention and treatment of the cancer metastases are clinically important.

The active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-D_3), is a major regulator of calcium homeostasis, and is critically important for the normal mineralization of bone. In addition to the small intestine, bone and kidney, a multitude of other sites of action for this steroid hormone have been discovered. This insight resulted mainly from the observation that the vitamin D receptor (VDR), which mediates the hormone's genomic activity, is expressed in almost all tissues of the human body. Previous studies have shown that 1α,25-D_3 and its analogs are able to reduce the invasiveness of metastatic cancer cells *in vitro* (2,3). Using different animal models, it has now been confirmed that 1α,25-D_3 and its analogs suppressed invasion and metastasis and exerted an anti-angiogenic activity *in vivo* as well. In melanoma, lung, prostate, colon and breast cancer models, a reduction in the number and size of metastatic nodules has been observed in animals treated with 1α,25-D_3 and its analogs compared with untreated animals (4–7). However, the hypercalcemic activity of 1α,25-D_3 has precluded its application as a pharmacological agent. For this reason, various synthetic vitamin D_3 compounds with reduced calcemic activity have been developed. 22-Oxa-1α,25-dihydroxyvitamin D_3 (22-oxa-1α,25-D_3) is an analog of vitamin D that has reduced calcemic effects but demonstrates a strong action on cell differentiation (8,9). The weaker calcemic effect of 22-oxa-1α,25-D_3 has been mainly attributed to its short half-life in the blood stream (10). Previous studies have shown that 22-oxa-1α,25-D_3 reduced tumor size and tumor weight significantly, without increasing the serum calcium concentration in a number of different breast cancer cell *in vivo* models, including nude mice implanted with human breast cancer cells and rats carrying DMBA-induced breast tumors (9,11). However, despite the promising effects of 22-oxa-1α,25-D_3 in animal models of cancer, the molecular mechanisms behind these anti-cancer effects have not been clarified.

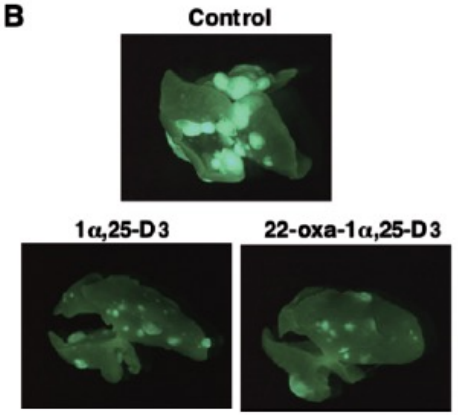
In the present study, we examined the effects of 22-oxa-1α,25-D_3 on the metastasis of lung cancer in mice. We have

Abbreviations: 1α,25-D_3, 1α,25-dihydroxyvitamin D_3; GFP, green fluorescent protein; HHM, hormonal hypercalcemia of malignancy; LLC, Lewis lung carcinoma; MMP, matrix metalloproteinase; 22-oxa-1α,25-D_3, 22-oxa-1α,25-dihydroxyvitamin D_3; PKA, protein kinase A; PTHrP, parathyroid hormone-related peptide; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor.



Nakagawa and co-workers generated a fluorescent stable transfectant of the LLC to view metastasis and found that **1,25(OH)2D3** and its analog **22-oxa-1α 25D3** significantly **reduced growth and metastasis**

VD and some analogs have antiproliferative properties in several lung cancer epithelial cell lines expressing VDR. This antiproliferative effects seem to be mediated in part by stalling the cell cycle at the G1/S checkpoint by increasing inhibitors and reducing activators of the cyclin-dependent kinase complexes which prevent DNA synthesis and cell growth.



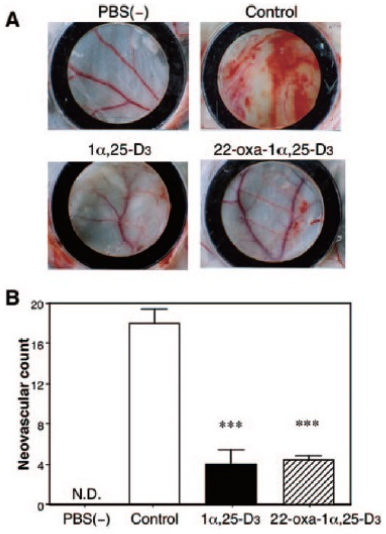


Fig. 5. Effects of 1α,25-D_3 and 22-oxa-1α,25-D_3 on LLC-GFP cell-induced angiogenesis in the mouse dorsal air sac model. (**A**) The chamber containing LLC-GFP cells or PBS(−), which was the negative control, was implanted into a subcutaneous dorsal air sac. At the same time, a osmotic minipump with control (vehicle), 1α,25-D_3 or 22-oxa-1α,25-D_3, was implanted on the opposite side of the chamber ring in the mice. Ten days after implantation, the mice were killed and the chambers were removed from the fascia. The area that had been in contact with the chamber was photographed. (**B**) Measurement of neovascular counts in the skin of mice bearing Millipore chambers containing LLC-GFP cells. ND, the neovascular vessels were not detected. Each bar represents the mean ± SE. $^{***}P < 0.001$ versus vehicle-treated group ($n = 10$).

Effects of continuous treatment with 22-oxa-1α,25-D_3 on the development of lung metastases in the LLC-GFP cell injected $VDR^{+/+}$ mice and $VDR^{-/-}$ mice fed a high calcium and vitamin D-deficient diet

$VDR^{-/-}$ mice exhibit hypocalcemia and extremely high serum levels of 1α,25-D_3. However, we reported previously that feeding these animals a high calcium and vitamin D-deficient diet resulted in the complete elimination of 1α,25-D_3 and the correction of calcium levels in the serum of both $VDR^{+/+}$ and

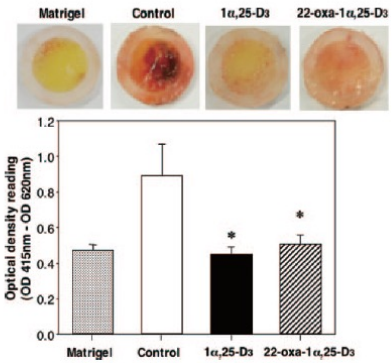


Fig. 6. Effects of 1α,25-D_3 and 22-oxa-1α,25-D_3 on bFGF-induced angiogenesis in the *in vitro* chamber angiogenesis assay. At the time of the implantation of the chamber ring to store the Matrigel-containing bFGF, a osmotic minipump with 1α,25-D_3 or 22-oxa-1α,25-D_3, was implanted on the other side of the chamber ring in the mice. Ten days after implantation, the mice were killed and the chambers were removed from the fascia. The angiogenic factor bFGF induced angiogenesis on day 10 post-implantation as determined from OD readings at 415 nm. Each bar represents the mean ± SE. $^{*}P < 0.05$ versus vehicle-treated group ($n = 10$).

Authors also found that both compounds reduced angiogenesis and invasiveness by inhibiting matrix metalloproteinase 9 (**MMP9**) and **MMP2** expression and **VEGF (vascular endothelial grow factor)**.

Establishment of a regression model of bone metabolism markers for the diagnosis of bone metastases in lung cancer

Zhongliang Zhu[†], Guangyu Yang[†], Zhenzhen Pang, Jiawei Liang, Weizhong Wang[*†] and Yonglie Zhou[*†]

Abstract

Background: The aim of this study was to establish a regression equation model of serum bone metabolism markers. We analyzed the diagnostic value of bone metastases in lung cancer and provided laboratory evidence for the early clinical treatment of bone metastases in lung cancer.

Methods: A total of 339 patients with non-metastatic lung cancer, patients with lung cancer with bone metastasis, and patients with benign lung disease who were treated in our hospital from July 2012 to October 2015 were included. A total of 103 patients with lung cancer in the non-metastatic group, 128 patients with lung cancer combined with bone metastasis group, and 108 patients with benign lung diseases who had nontumor and nonbone metabolism-related diseases were selected as the control group. Detection and analysis of type I collagen carboxyl terminal peptide β-special sequence (β-CTX), total type I procollagen amino terminal propeptide (TPINP), N-terminal-mid fragment of osteocalcin (N-MID), parathyroid hormone (PTH), vitamin D (VitD3), alkaline phosphatase (ALP), calcium (CA), phosphorus (P), cytokeratin 19 fragment (F211), and other indicators were performed. Four multiple regression models were established to determine the best diagnostic model for lung cancer with bone metastasis.

Results: Analysis of single indicators of bone metabolism markers in lung cancer was performed, among which F211, β-CTX, TPINP, and ALP were significantly different ($P < 0.05$). The ROC curve of each indicator was less than 0.712. Based on the multiple regression models, the fourth model was the best and was much better than a single indicator with an AUC of 0.856, a sensitivity of 70.0%, a specificity of 91.0%, a positive predictive value of 82.5%, and a negative predictive value of 72.0%.

Conclusion: Multiple regression models of bone metabolism markers were established. These models can be used to evaluate the progression of lung cancer and provide a basis for the early treatment of bone metastases.

Keywords: Bone metabolism markers, Lung cancer, Bone metastases

No effect of VD levels was suggested among the different groups

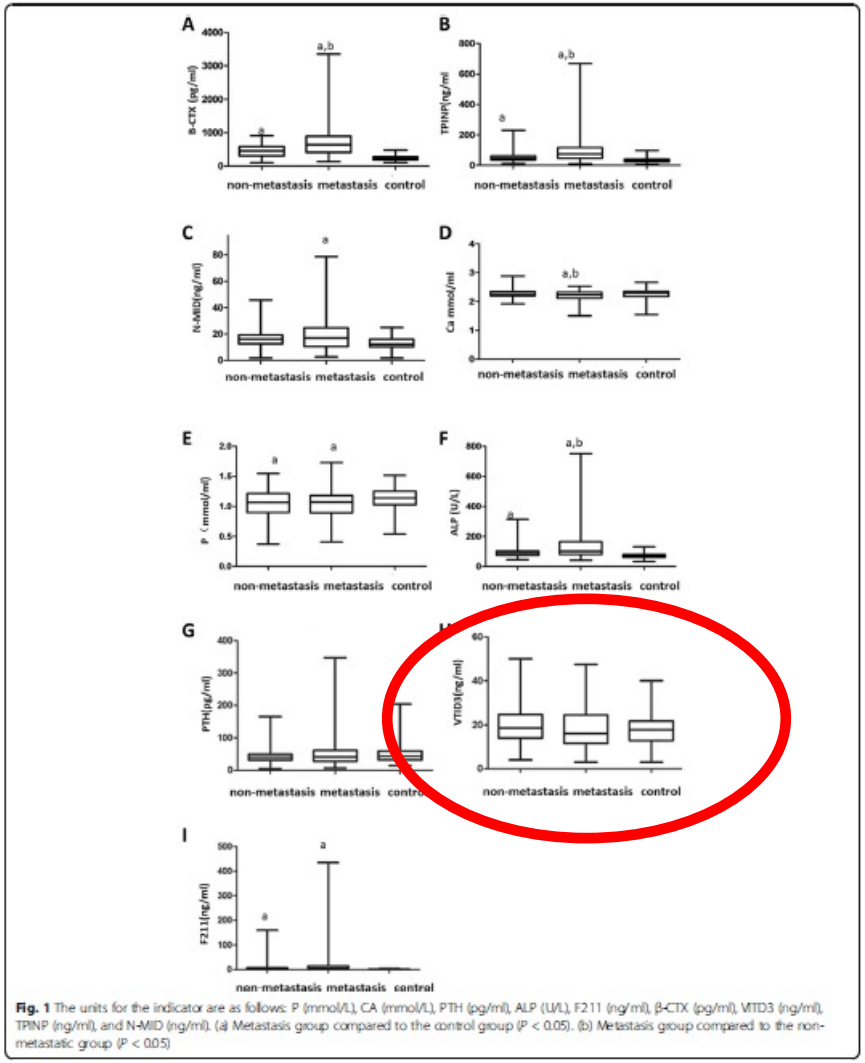
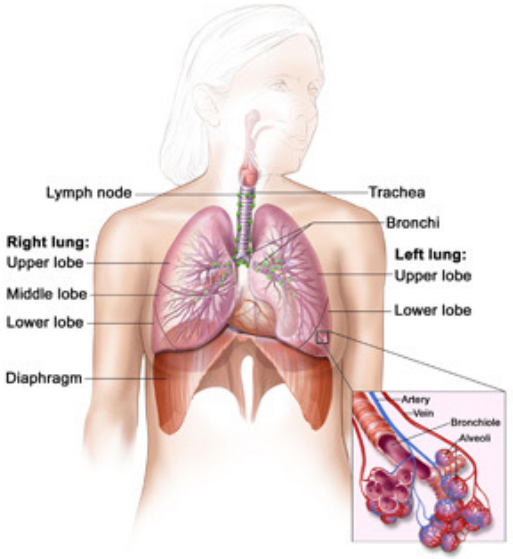


Fig. 1 The units for the indicator are as follows: P (mmol/L), CA (mmol/L), PTH (pg/ml), ALP (U/L), F211 (ng/ml), β-CTX (pg/ml), VITD3 (ng/ml), TPINP (ng/ml), and N-MID (ng/ml). (a) Metastasis group compared to the control group ($P < 0.05$). (b) Metastasis group compared to the non-metastatic group ($P < 0.05$)

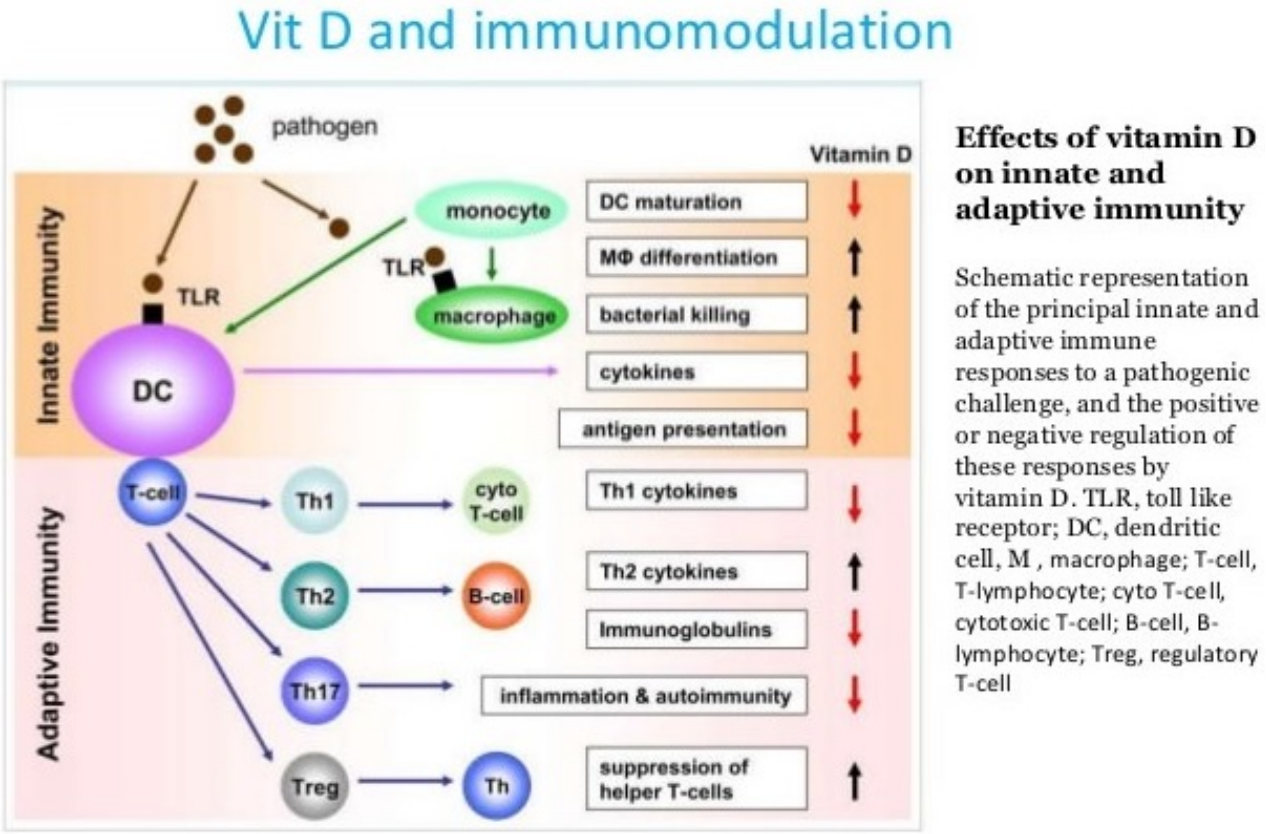
Tumor Resistance to Vitamin D

- In the lung, **normal** airway epithelial cells and alveolar macrophages **express CYP27B1**, converting 25(OH)D to its more active form. Furthermore, dendritic cells and lymphocytes also express this enzyme. In contrast to normal airway epithelial cells, some **small cell and non-small cell cancer cell lines** have been shown to express very **low CYP27B1** or none at all. This suggests that lung cancer cells may inhibit CYP27B1 expression, and hence formation of active 1,25(OH)2D3, in order **to prevent its antiproliferative effects**.
- In contrast, **increased CYP27B1 expression** has been reported in alveolar macrophages from patients with lung cancer, with **highest** expression being found in more **advanced stages of lung cancer**. This may be a means by which the **tumor evades the immune system** by modulating cytokine production and suppressing immune cell function by increasing vitamin D, or it may be the body's response to increase 1,25(OH)2D3 in order to activate its antiproliferative and anticancer properties.

Immunomodulatory activity in lung cancer



Immune system regulation role



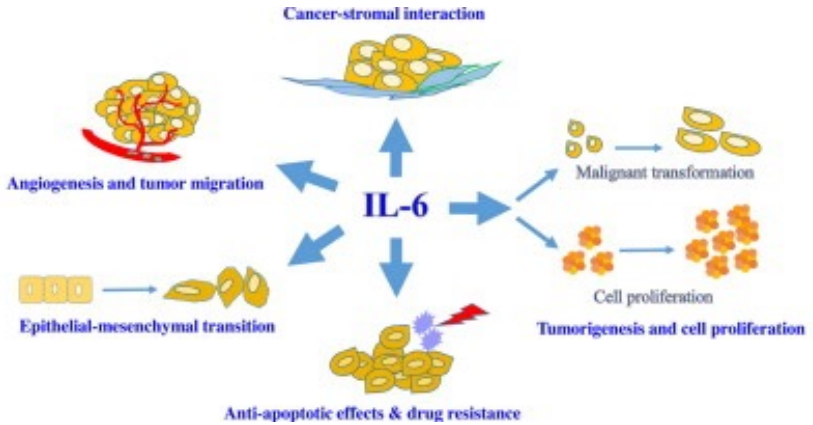
Effects of vitamin D on innate and adaptive immunity

Schematic representation of the principal innate and adaptive immune responses to a pathogenic challenge, and the positive or negative regulation of these responses by vitamin D. TLR, toll like receptor; DC, dendritic cell, M , macrophage; T-cell, T-lymphocyte; cyto T-cell, cytotoxic T-cell; B-cell, B-lymphocyte; Treg, regulatory T-cell

Endocrinol Metab Clin North Am. 2010 Jun;39(2):365-79, Vitamin D and the immune system: new perspectives on an old theme. Hewison M

The immunomodulatory function of vitamin D in lung cancer

- VD immunomodulatory properties are the **inhibition of prostaglandins**, **proteases** and **pro-inflammatory** cytokines **through** modulation of signaling pathways that include p38 mitogen activated protein kinase (**MAPK**) and nuclear factor kappa-light-chain-enhancer of activated B cells (**NFκB**).
- Particularly, 1,25(OH)2D3 **reduces** interleukin-6 (**IL-6**) production in primary airway epithelial cells, but not in the lung cancer cell lines NCI-H292 and A549. IL-6 is a key cytokine involved in the initiation and extension of the immune response and elevated levels have been implicated in lung cancer.



Vitamin D: Potential in the Prevention and Treatment of Lung Cancer

ROSEMARY NORTON and MARIA A O'CONNELL

RESEARCH ARTICLE

Aerosol 1,25-dihydroxyvitamin D3 supplementation: A strategy to boost anti-tumor innate immune activity

Francesca Bianchi[1,2], Michele Sommariva[1], Valentino Le Noci[1], Simone Camelliti[1], Nicoletta Gagliano[1], Marta Giussani[3], Andrea Balsari[1,2], Elda Tagliabue[2], Lucia Sfondrini[1]*

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* lucia.sfondrini@unimi.it

Aerosol delivery could represent a feasible approach to supplement 1,25(OH)2D3 directly to the lungs improving the activation of local immunity against cancer

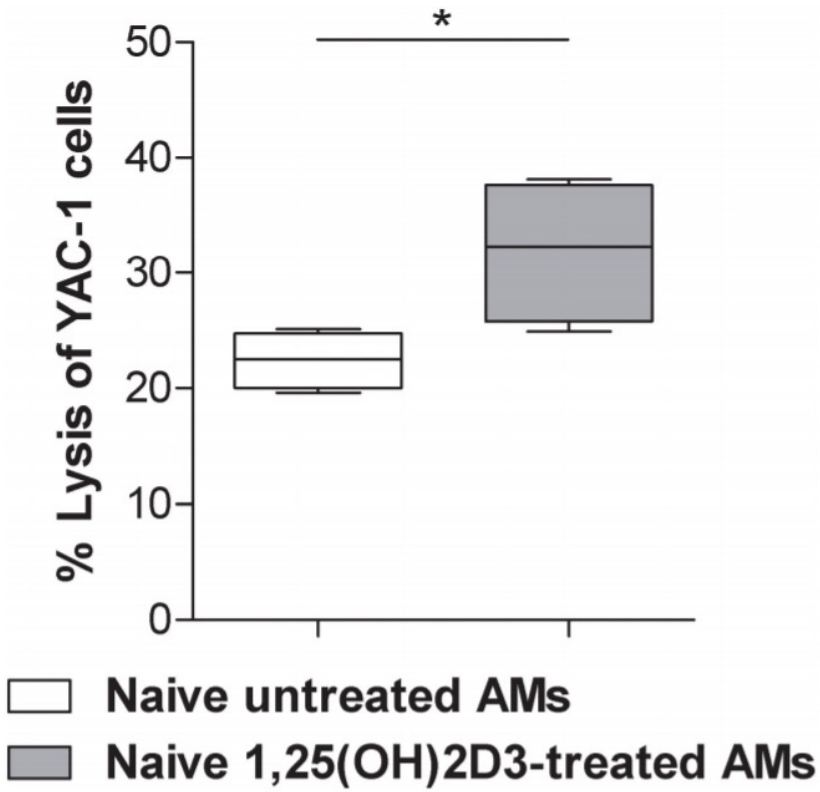
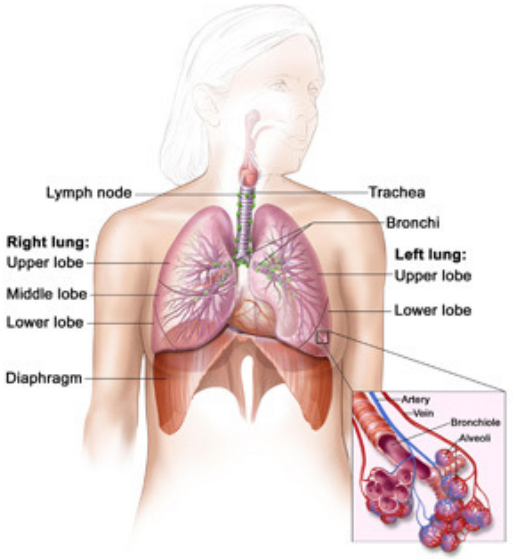


Fig 1. 1,25(OH)2D3 increased the ability of murine alveolar macrophages to stimulate in vitro NK cell cytotoxicity. NK cells, from spleen of C57BL/6 healthy mice, co-cultured with 1,25(OH)2D3-pretreated lung macrophages significantly increased the percentage of lysis of YAC-1 cells, as compared to NK cells co-cultured with untreated lung macrophages. Unpaired t test; *$p<0.05$.

Role of VD in drug ADME



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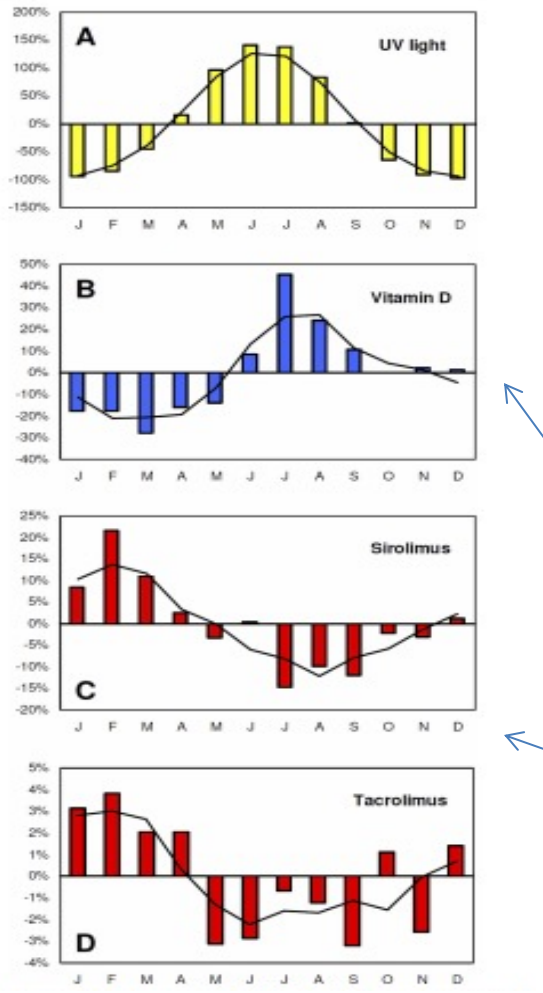
Accelerated Communication

Seasonal Variation in Blood Drug Concentrations and a Potential Relationship to Vitamin D

Jonatan D. Lindh, Marine L. Andersson, Erik Eliasson, and Linda Björkhem-Bergman

Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital Huddinge, Stockholm, Sweden

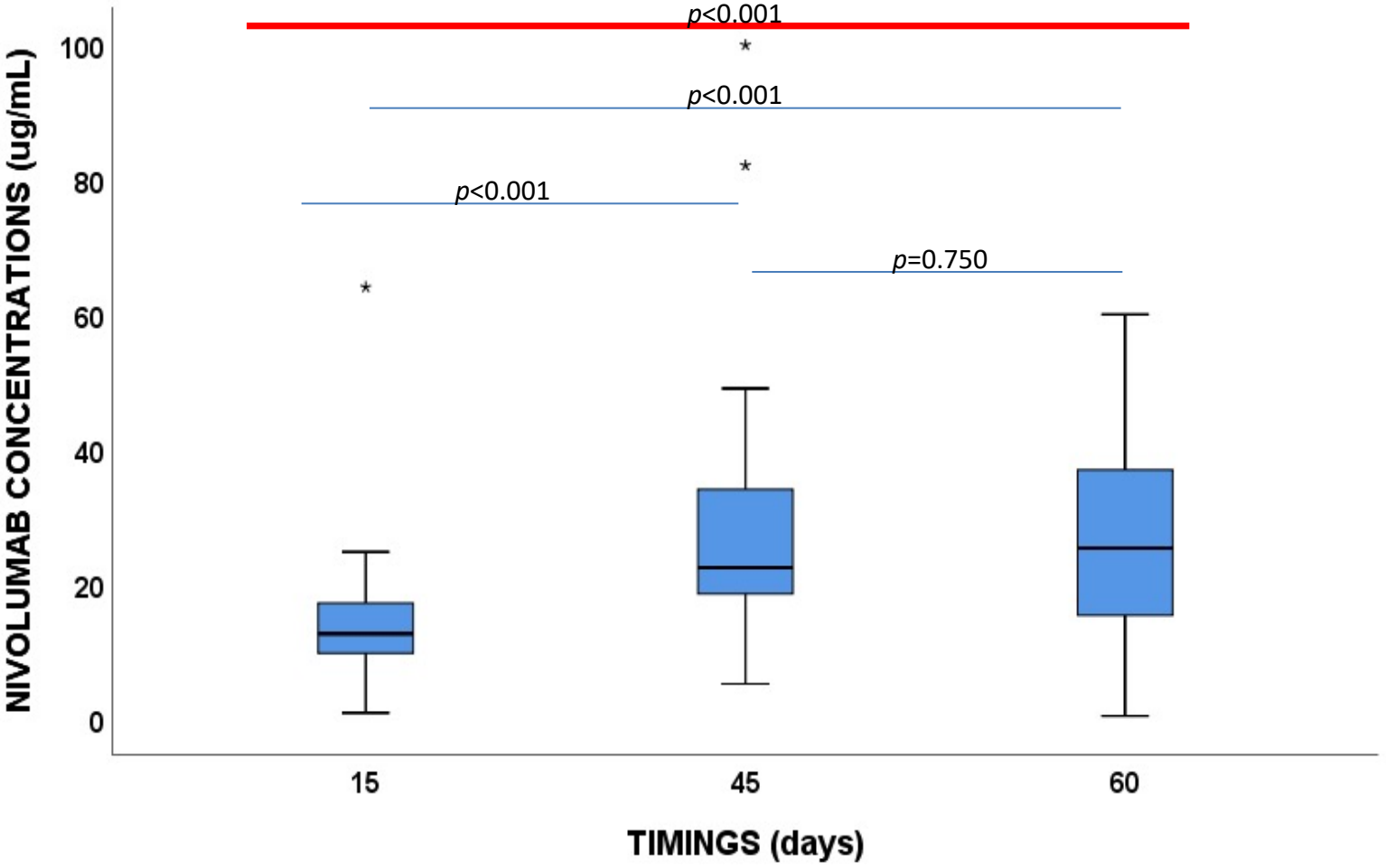
Received January 11, 2011; accepted February 24, 2011



VD is able to induce drug-related cytochromes and transporters, thus higher VD levels are associated to reduced drug concetrations

OPPOSITE TREND: Vitamin D increase is related to reduction in tacrolimus and sirolimus concentrations

NIVOLUMAB RESULTS: PLASMA LEVELS



Median nivolumab concentrations:

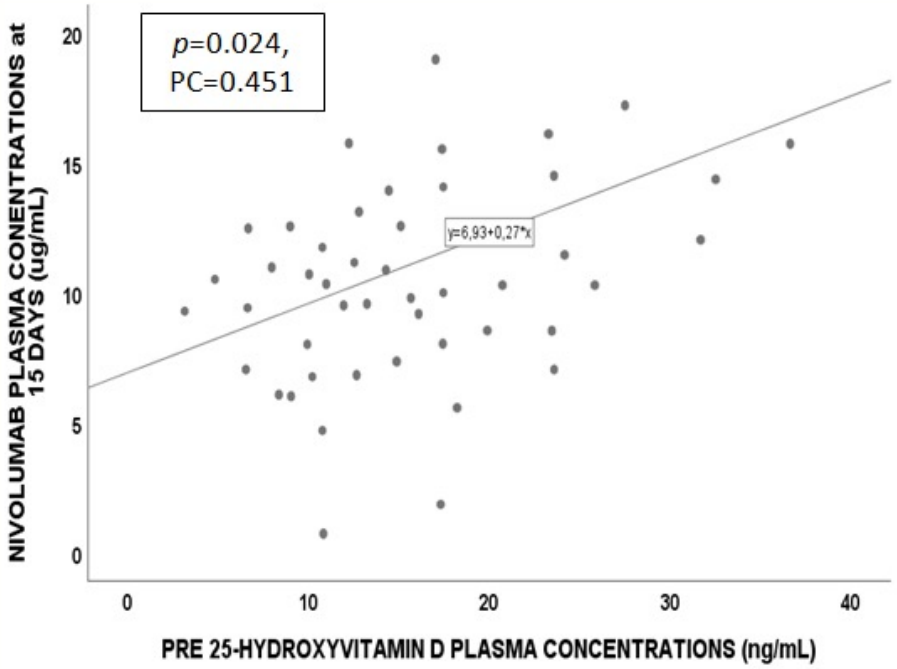
-12.48 µg/mL (IQR:9.54-17.13) at 15 days

- 22.31 µg/mL (IQR:18.30-34.88) at 45 days

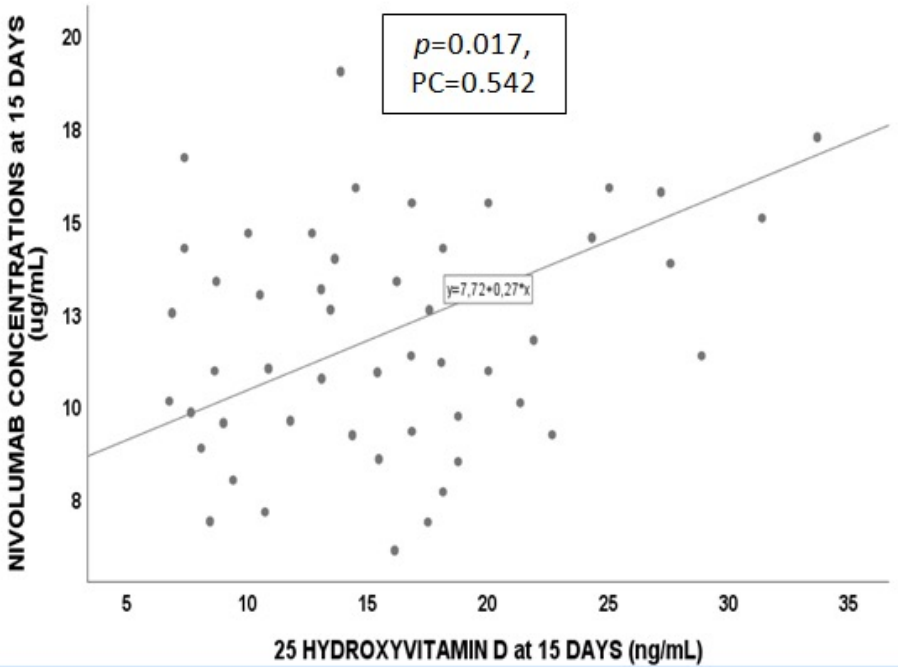
-25.19 µg/mL (IQR:14.34-38.93) at 60 days

No anti-nivolumab antibodies were found

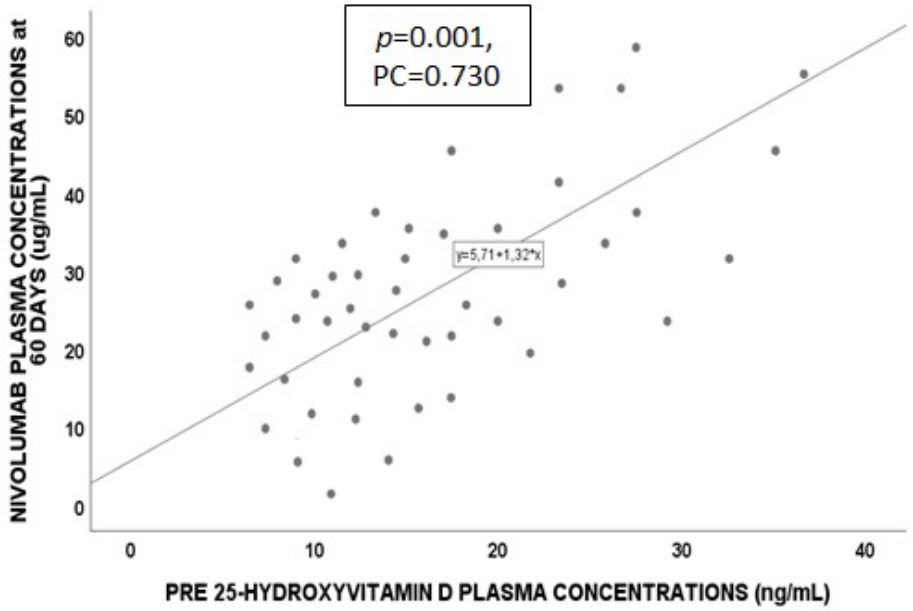
NIVOLUMAB RESULTS: correlations at 15 DAYS

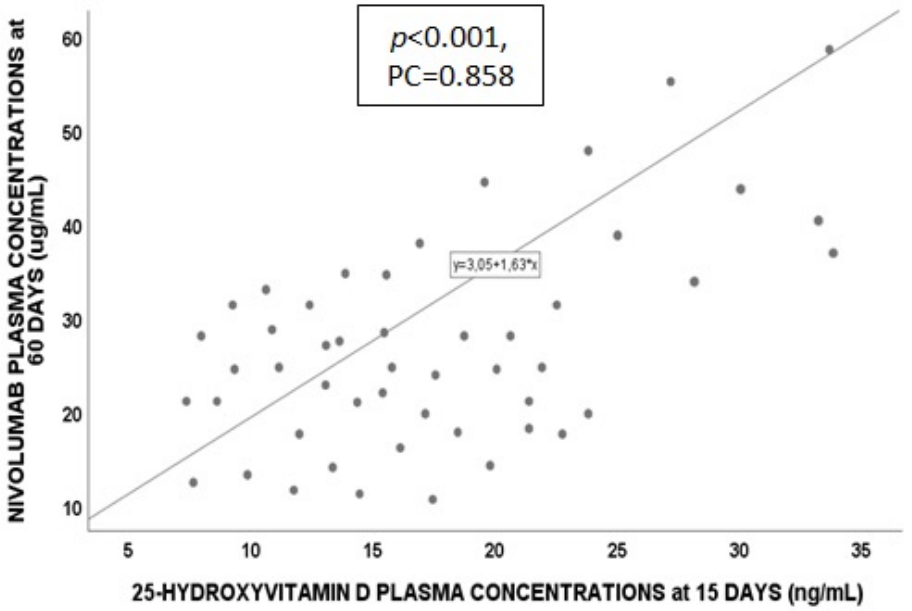


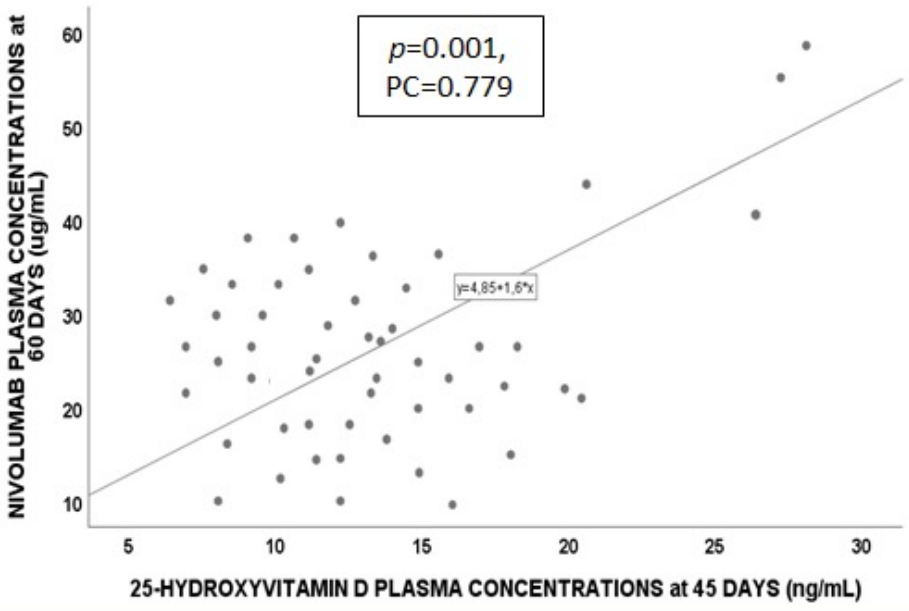
Direct correlation with 25-hydroxyvitamin D3, but not with 1,25- dihydroxyvitamin D3…

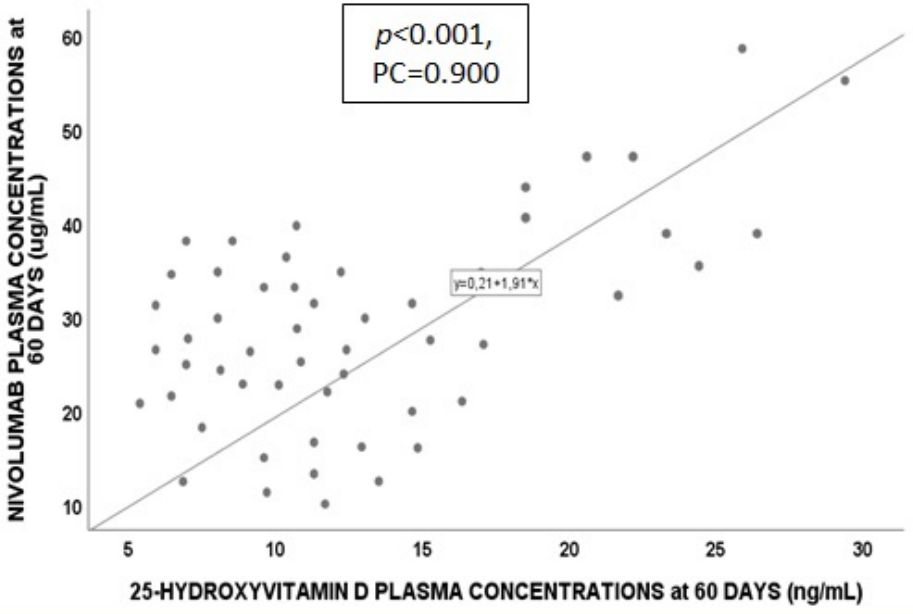


NIVOLUMAB RESULTS: correlations at 60 DAYS

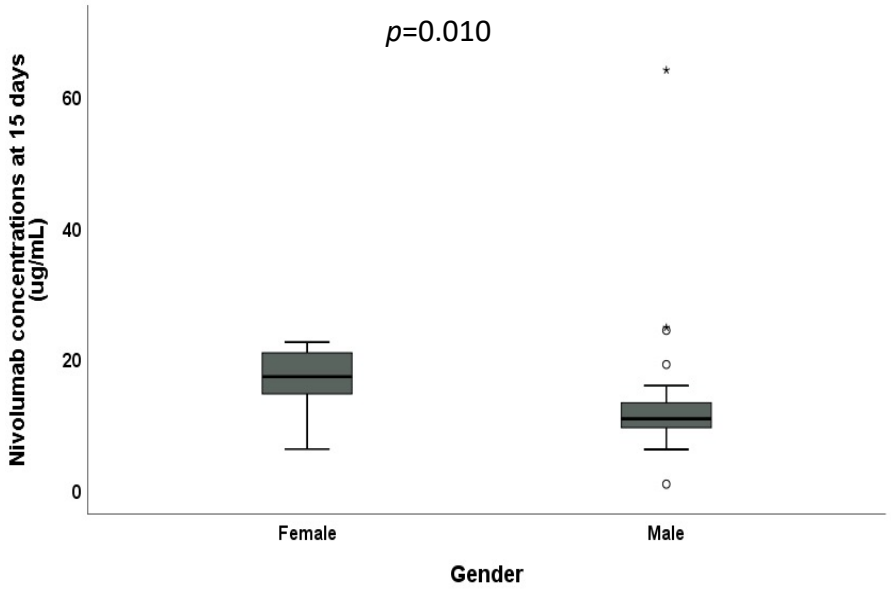


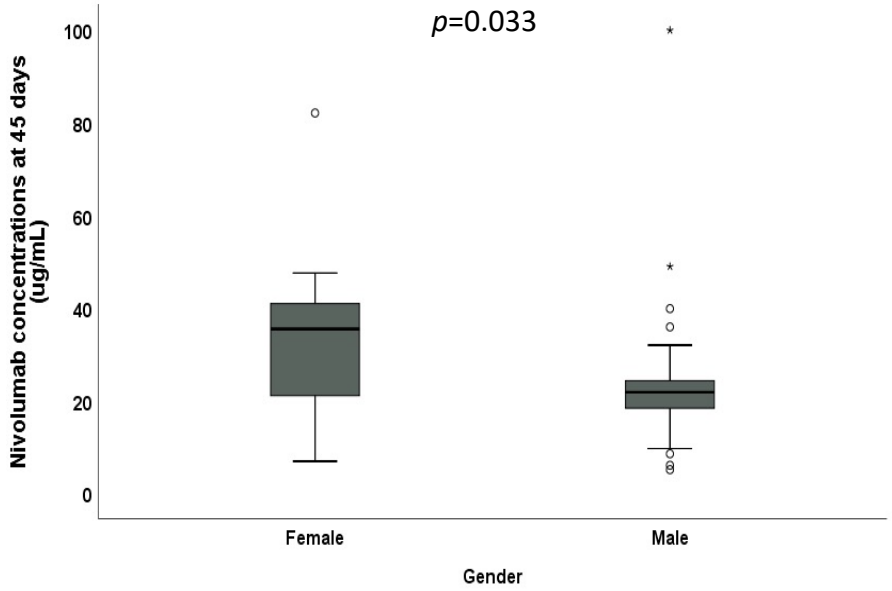


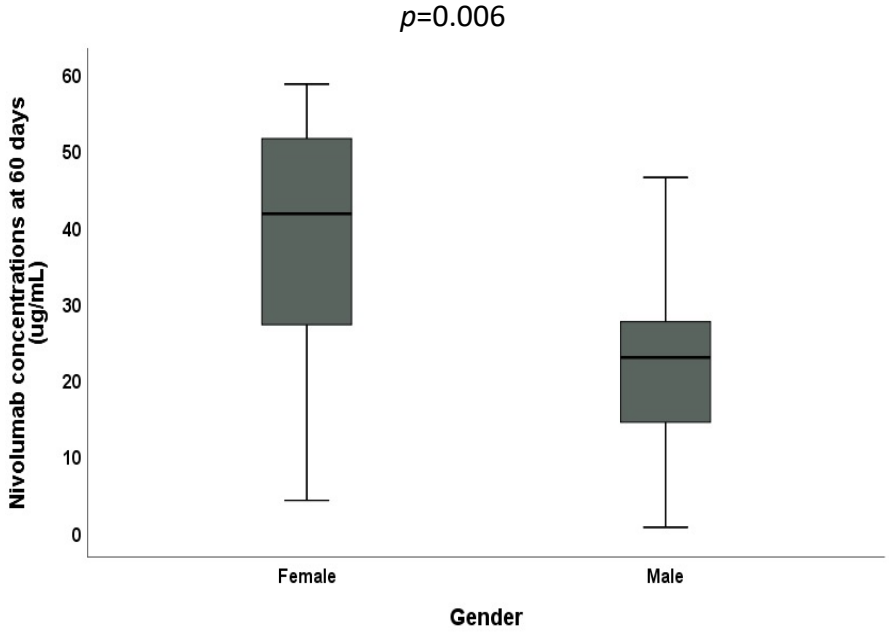




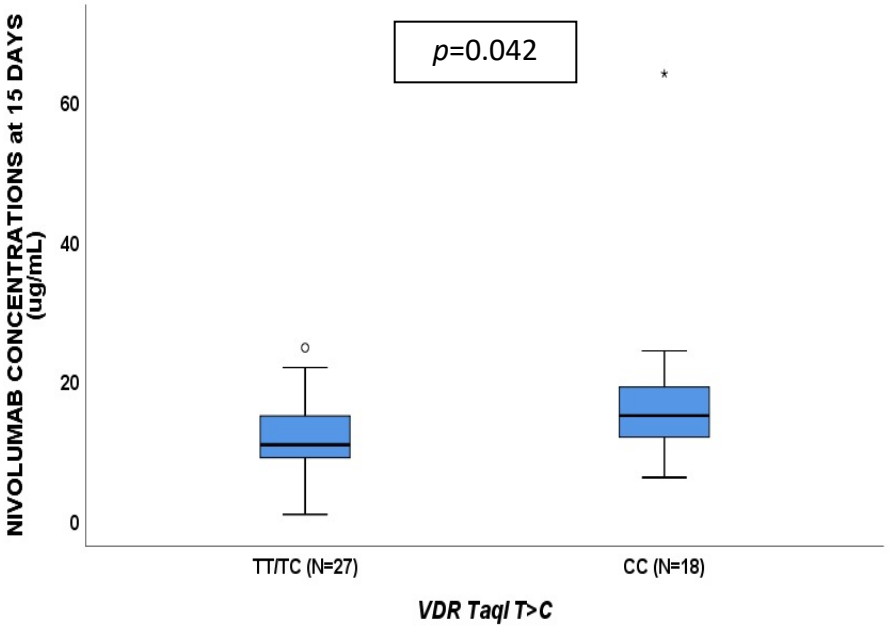
NIVOLUMAB RESULTS: CONCENTRATIONS and GENDER

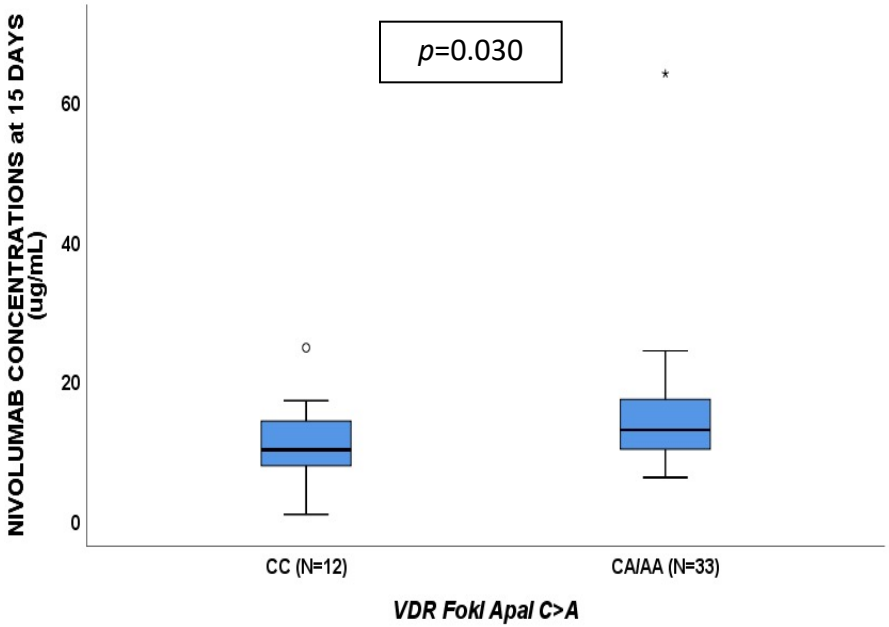


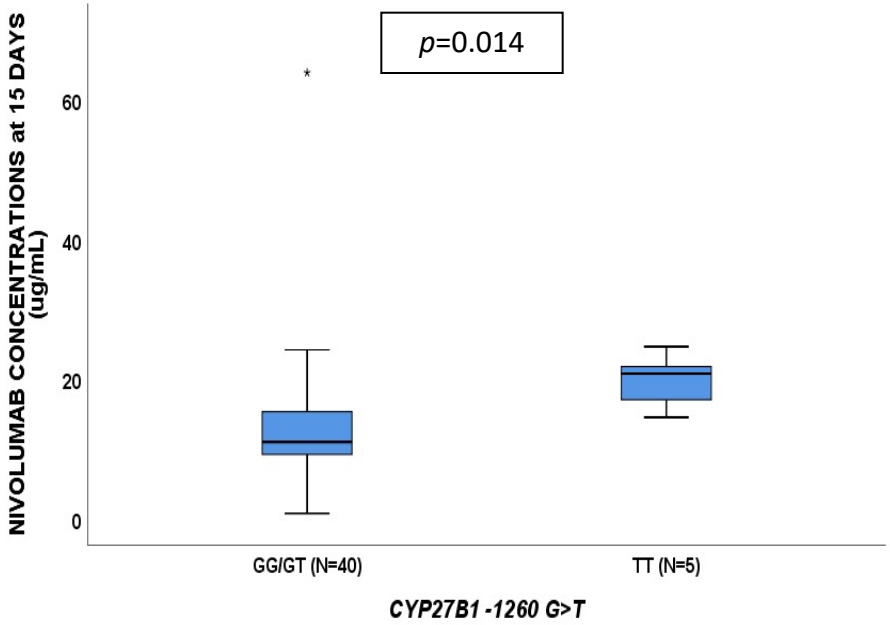




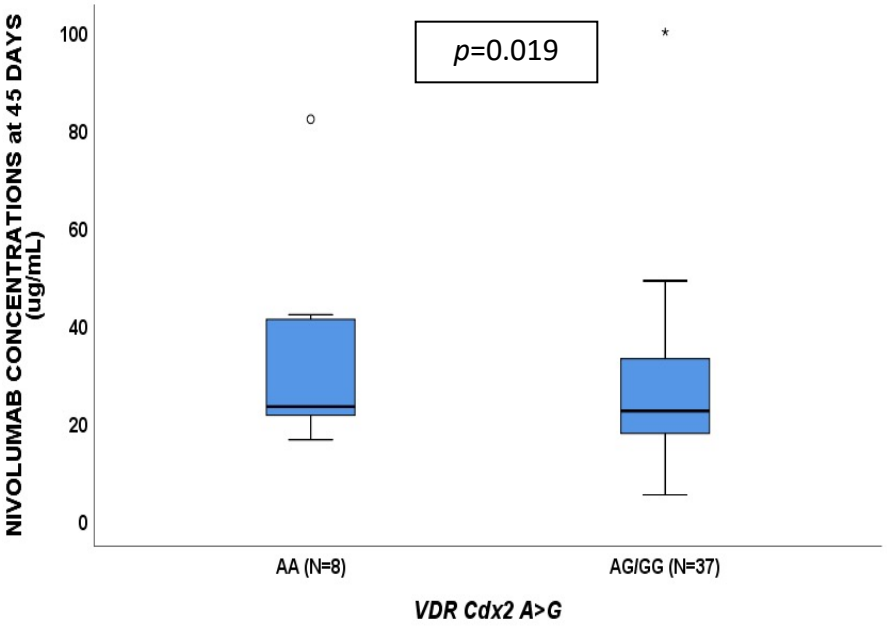
NIVOLUMAB RESULTS: genetics 15 DAYS

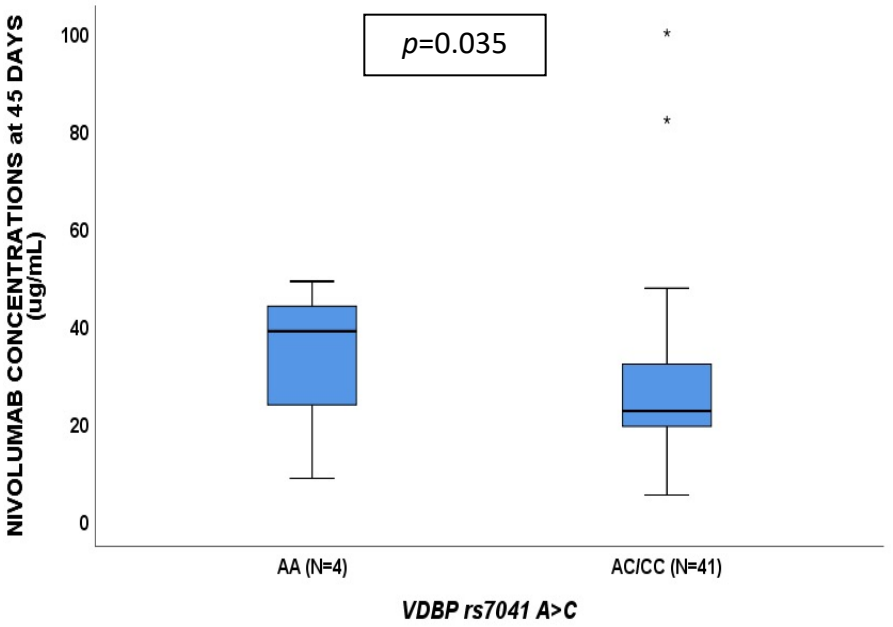


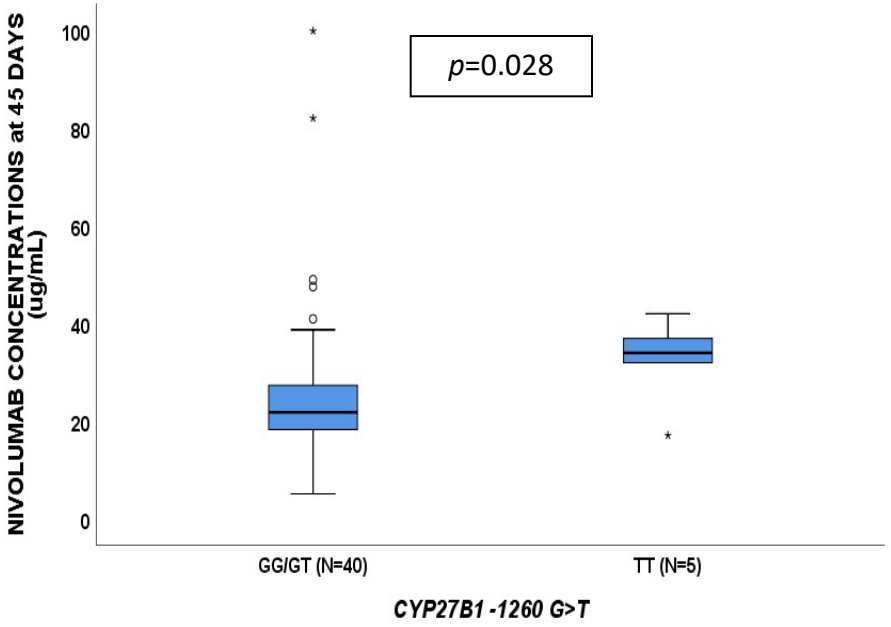




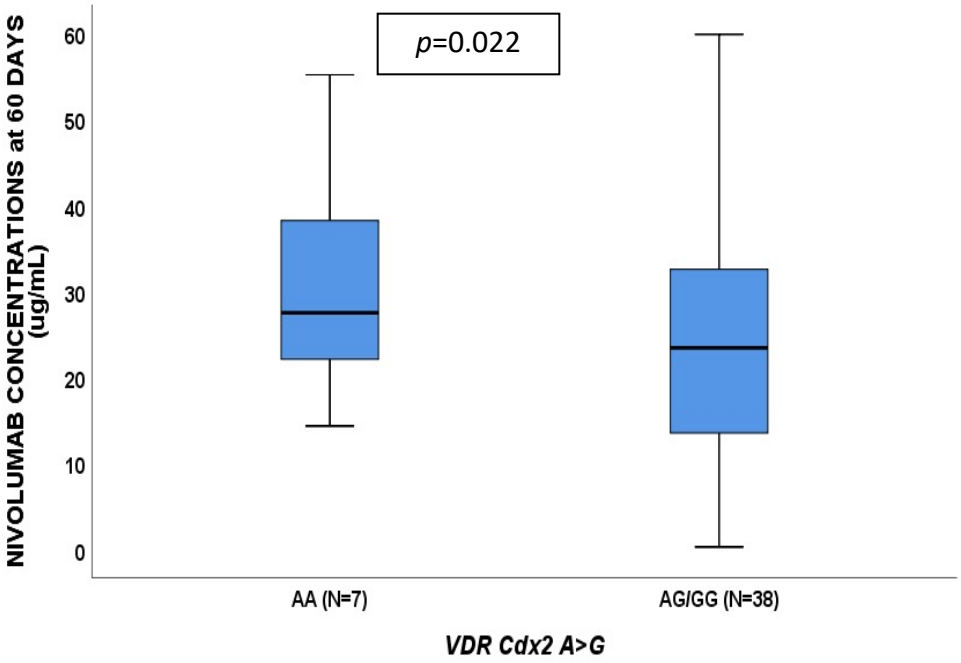
NIVOLUMAB RESULTS: genetics 45 DAYS

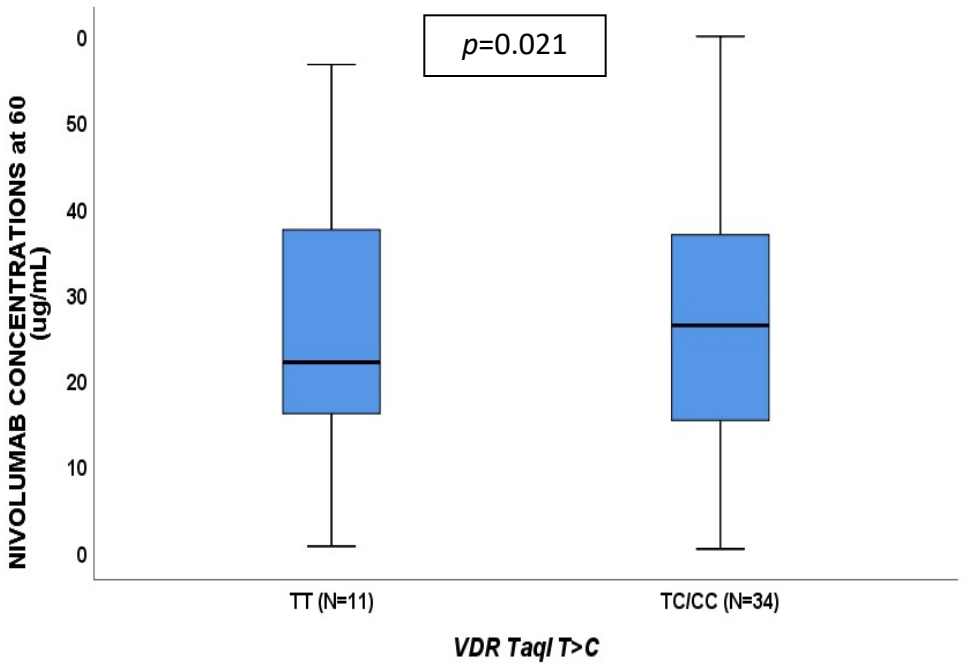






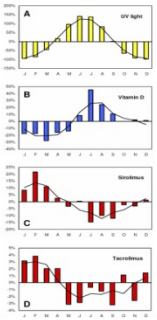
NIVOLUMAB RESULTS: genetics 60 DAYS





NIVOLUMAB CONCLUSIONS

RESUMING…



- NIVOLUMAB CONCENTRATIONS at 15d and 60d are directly correlated with 25-VD.
- Women have higher drug levels.
- 15d exposure is higher in *VDR* TaqI CC, ApaI CA/AA and CYP27B1-1260 TT genotype patients.
- 45d exposure is higher in *VDR* Cdx2 AA, *GC* (VDBP) AA and *CYP27B1-1260* TT genotype patients.
- 60d exposure is higher in *VDR* Cdx2 AA and TaqI TC/CC genotype patients.
- 18.7 µg/mL at 15d are predicted by baseline 25-VD at < 10 ng/mL (all) and *GC* AC/CC genotype.
- Progression is predicted by baseline 25-VD at < 10 ng/mL, BMI > 25 Kg/m2 and *CYP24A1 8620* AG/GG genotype.
- Overall survivall is predicted by baseline 25-VD at < 10 ng/mL (all died) and *VDR* Cdx2 AG/GG and ApaI CA/AA (all died) genotypes.

- ***Nivolumab exposure is directly correlated with VD concentrations (in contrast for what observed for other drugs (e.g. tacrolimus)), but in accordance with studies reporting low VD levels associated with low intrinsec IgG concentrations.***
- ***Nivolumab concentrations are increased in females: estrogen could affect protein turnover; in addition, progression is more present in individuals with BMI > 25 Kg/m^2 (tipically men).***
- ***25-hydroxyvitamin D levels at baseline < 10 ng/mL was able to predict nivolumab concentrations < 18.7 µg/mL, progression, survival and toxicity; for this reason, it is important to monitor vitamin D levels during life, also considering genetics.***

Conclusions…

- VD **role** in inhibiting angiogenesis and proliferation, improving immunity activation, apoptosis and cellular differentiations in lung cancer has been demonstrated, altough some conflicting data.
- VD is also able to affect **drug concentrations**
- Further studies are needed to clarify these aspects: **collaborations have to be improved!**

Thanks for your attention!