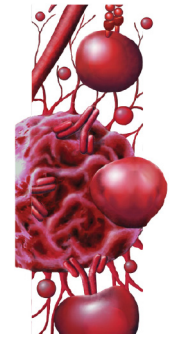
Editorial

Immunotherapy



The link between calcitriol and anticancer immunotherapy: vitamin D as the possible balance between inflammation and autoimmunity in the immune-checkpoint blockade

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"We are thus inclined to hypothesize a proportionally better prognostic role respectively for normal basal vitamin D levels and for recovering levels after proper repletion in advanced cancer patients, compared with the worse prognosis of vitamin D deficient patients who are unable to raise their levels. Similarly, the possible predictive role of vitamin D status for response to CKI might follow this proportional strength"

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The advent of the new immunotherapy with immune-checkpoint inhibitors (CKI) is currently revolutionizing the world of oncology, both in the advanced and early cancer setting.

After the approval of the anti-CTLA-4 antibody ipilimumab for the systemic treatment of metastatic melanoma, a cascade of subsequent therapeutic indications of further CKI took place for several advanced cancers during the last decade [1,2]. Likewise, anti-CTLA-4, anti-PD-1 and anti-PD-L1 antibodies are currently in study for the adjuvant setting in several cancer types after the first positive data still coming from melanoma [2,3].

The great challenge of immune-checkpoint blockade is still represented by the lack of consistent predictive factors. Despite the evidence of conditions favoring response to CKI, for instance mismatch repair deficiency [4], PD-L1 expression, high mutational burden, loss of function of genes involved in antigen presentation and IFN-γ signaling among many others [5,6], each of these represents an imperfect marker, whose negativity does not exclude a good response to immunotherapy. Several studies are still investigating potentially predictive biomarkers.

The role of vitamin D blood levels (25[OH]D, calcidiol) has been largely explored in the field of cancer, ranging from the assessment of hypovitaminosis D in cancer patient populations to the investigation of the possible prophylactic role of vitamin D supplementation to prevent cancer, basing on the preclinical evidence of an antiproliferative effect of calcitriol [7,8]. On the other hand, it is well known that the role of the active form of vitamin D ($1,25[OH]_2D$, calcitriol) and of vitamin D receptor (VDR) is crucial for the proper activation of the immune system, particularly for T-cell differentiation and their effector function [9]. This evidence, in our opinion, constitutes the rationale for a possible pivotal link between vitamin D and the new anticancer immunotherapy with CKI, whose main goal is T-cell activation (Figure 1).

Interestingly, most of immune cells express VDR and hold enzyme CYP27B1, used for the internal conversion of circulating 25(OH)D to the active VDR-ligand calcitriol. In fact, circulating levels of vitamin D are too low to affect immune responses *in vivo* and immune upregulation by calcitriol is dose dependent [9,10]. Not only antigen presenting cells such as monocytes, macrophages and dendritic cells, but also activated T cells are capable to produce $1,25(OH)_2D$ in sufficiently high concentrations to affect vitamin D responsive genes, by binding vitamin D responsive elements. These signals upregulate the enzyme phosphoinositide phospholipase C-γ1 (PLC-γ1), a pivotal molecule for the classical T-cell receptor (TCR) signaling pathway (Figure 1) [6,8]. In turn, antigen-specific

Future Medicine

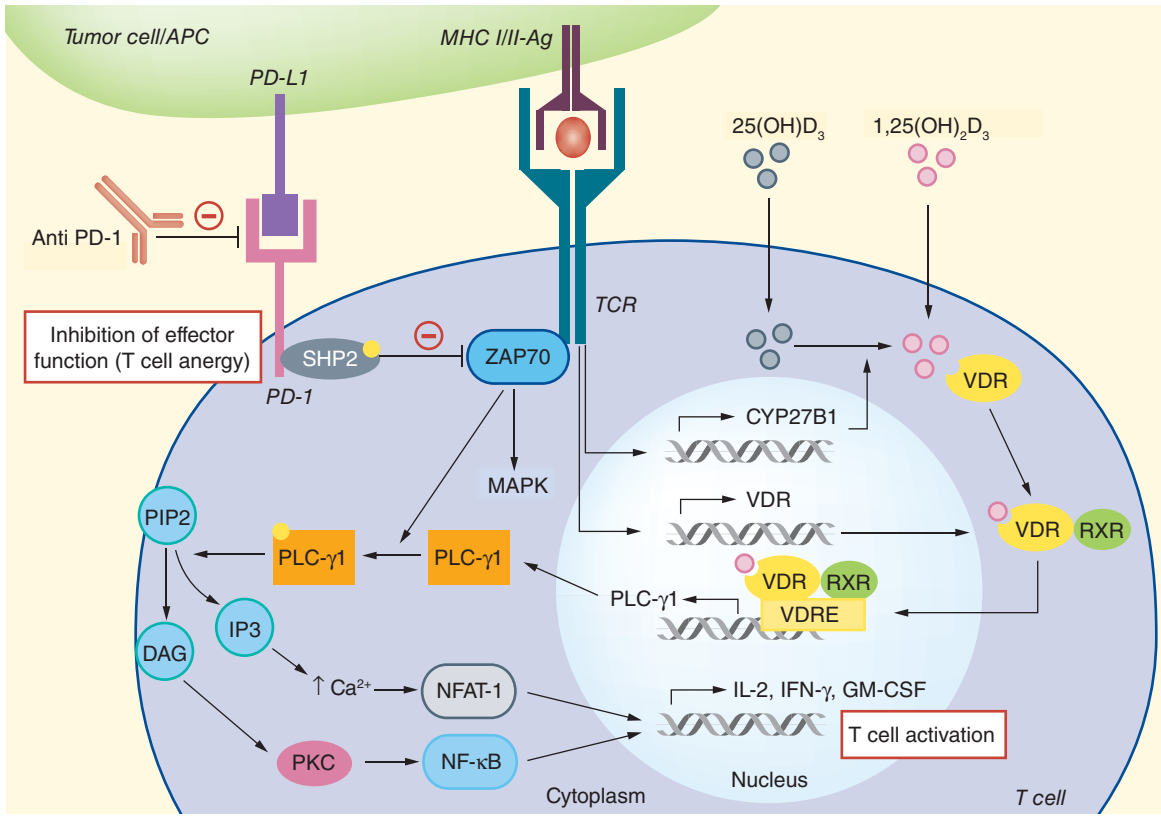

Figure 1. The role of vitamin D and vitamin D receptor signaling in T-cell activation.
Circulating 25(OH)D_3 is absorbed by T cell and converted into the active form (1,25[OH]$_2D_3$) through CYP27B1. VDR binds to 1,25(OH)$_2D_3$ and dimerizes with RXR. VDR/RXR complex translocates into the nucleus and induces the transcription of PLC-γ1 through interaction with VDRE on specific genes. TCR signaling also induces the transcription of CYP27B1 and of VDR. After TCR engagement, ZAP 70 can phosphorylate and activate PLC-γ1. Once activated, PLC-γ1 can catalyze the hydrolysis of PIP2 into IP3 and DAG. IP3 increases the cytoplasmic levels of calcium, resulting in nuclear import of NFAT-1. DAG activates PKC, which is responsible of NF-κB induction. The transcription factors NFAT-1 and NF-κB translocate into the nucleus and mediate the transcription of cytokines involved in T-cell response, such as IL-2, IFN-γ and GM-CSF. ZAP70 is also responsible of the MAPK cascade activation. The interaction between PD-1 and PD-L1 (which can be expressed on the surface of tumor cell or antigen-presenting cell) downmodulate T-cell response through inhibition of ZAP70 by SHP2. Anti-PD-1 monoclonal antibodies, targeting PD-1 receptor on T-cell surface and preventing the binding with its ligand PD-L1, block this inhibitory signal and enhance the immune response.
Ag: Antigen; DAG: Diacylglycerol; PLC-γ1: Phosphoinositide Phospholipase C-γ1; PKC: Protein kinase C; RXR: Retinoid X receptor; TCR: T-cell receptor; VDR: Vitamin D receptor; VDRE: Vitamin D responsive element.

triggering of TCR expressed on the surface of naive T cells have the intracellular effect to upregulate VDR, thus enhancing vitamin D signaling and establishing a virtuous circle of T-lymphocyte activation [9]. Moreover, a negative feedback mechanism also dependent from VDR has been demonstrated to control potential over-reactions of immune system, for example, through the VDR-mediated inhibition of IL-2 gene transcription (IL-2 is secreted by T cells in response to TCR activation and is needed for their proliferation) [9].

These mechanisms explain how the balance resulting from calcitriol–VDR interaction finally results in a controlled activation of T-cell differentiation and effector function, allowing proper response against infections or cancer after antigen presentation, meanwhile avoiding excessive autoimmune activation [11]. This is also indirectly proven by the association found between low serum levels of vitamin D and development of autoimmune diseases, indeed known to be latitude dependent (due to the low exposure to sunlight at higher degrees of latitude) [12,13], and by the link between low serum levels of 25(OH)D and higher susceptibility to various infections [14–16].

With the aim of predicting the possible T-cell response to immune-checkpoint blockade in cancer patients, we think that we cannot ignore the significant role of vitamin D in balancing inflammation and autoimmunity. Obviously, we do not expect everything to be linear, and further unpredictable variables need careful consideration

besides the mere 25(OH)D serum levels. Among these, the allelic variations of VDR (inclusive of nonfunctional variants) and the VDR modulation by several extracellular and intracellular signals should be considered [9]. Moreover, the vitamin D-binding protein may also play an important role, inhibiting 25(OH)D-induced T-cell response by sequestering calcidiol and blocking its conversion to calcitriol in T cells [11]. Interestingly, vitamin D-binding protein decrease was strongly associated with longer overall survival in advanced renal cancer patients treated with atezolizumab (anti-PD-L1 CKI), from the exploratory biomarkers' analysis of an important Phase Ia trial by McDermott *et al.* (Hazard ratio [HR]: 3.38; 95% CI: 1.45–7.89), confirming our previous assertion about this further key issue [17].

Nevertheless, considering the extremely high prevalence of hypovitaminosis D in cancer patients [7,18], some initial consideration about the possible utility of supplementation could be done for candidates to immune-checkpoint blockade. Recognizing low 25(OH)D levels as a consequence of chronic inflammation rather than the cause, as well as dropping the rationale for the losing strategy of a prophylactic supplementation for cancer prevention in healthy subjects [8], led to the explanation of such a high incidence of vitamin deficiency among advanced cancer patients.

As instance, we assessed basal vitamin D levels of metastatic renal cancer patients undergoing therapy with CKI [19]. Of the first ten consecutive patients, all pretreated with tyrosine kinase inhibitors, 25(OH)D levels assessed before the first treatment with nivolumab (anti-PD1 CKI) revealed deficiency in 80% of cases (eight patients). Hypovitaminosis was severe (<20 ng/ml) in seven cases and moderate (<30 ng/ml) in one. The only two patients without deficiency had vitamin D values near to the lower limit of normality. Oral vitamin D supplementation was given when necessary, likely confounding the possible influence of vitamin D deficiency on the outcome of CKI. Vitamin D normal values after 2 months of therapy were recovered in most of cases. Interestingly, the only patient who achieved a good objective response to treatment had normal values of vitamin D before therapy.

The prophylactic or adjuvant vitamin D supplementation for anticancer purposes may be useless due to the weak basis of a possible antiproliferative effect. Conversely, hypothesizing an immunological rationale, the effectiveness of vitamin D as a kind of 'anticancer agent' may be closely linked to the presence of antigens.

Indeed, not surprisingly, a significant association of vitamin D deficiency with worse outcomes (HR: 2.06; 95% CI: 1.10–3.87) was demonstrated in patients with stage IV metastatic melanoma, but not in patients with nonmetastatic melanoma [20], reinforcing the hypothesis of an antigen-dependent immunological effect of vitamin D against cancer. Moreover, from the same study, patients with metastatic melanoma who were vitamin D deficient at the first measurements had significantly worse outcomes (HR: 2.26; 95% CI: 1.23–4.17) compared with patients properly vitamin D replete. A strong rationale for supplementation was provided by the evidence that patients initially vitamin D deficient (≤20 ng/ml) and with subsequent insufficient increase or a decrease in their 25(OH)D levels had significantly worse outcomes (HR: 4.68; 95% CI: 1.05–20.88) compared with vitamin D replete patients with an increase of ≥20 ng/ml on a subsequent measurement. In conclusion, both initial vitamin D deficiency and inadequate repletion portend a worse prognosis in patients with metastatic melanoma, with more prognostic significance than LDH (already well known as a strong prognostic factor in this disease) [20].

We are thus inclined to hypothesize a proportionally better prognostic role respectively for normal basal vitamin D levels and for recovering levels after proper repletion in advanced cancer patients, compared with the worse prognosis of vitamin D deficient patients who are unable to raise their levels. Similarly, the possible predictive role of vitamin D status for response to CKI might follow this proportional strength.

Based on such hypothesis, we are conducting a multicenter, perspective, exploratory trial (the PROVIDENCE study) to investigate the potential role of hypovitaminosis D and of cholecalciferol supplementation on treatment outcome in advanced cancer patients undergoing immunotherapy with CKI (any type – anti-PD-1, anti-PD-L1 or anti-CTLA4 – and any treatment line) for all primary cancer types. At least 250 consecutive patients with metastatic disease candidate to receive immunotherapy will be enrolled at five Italian institutions (2 years enrollment, 1 year follow-up). Serum levels of 25(OH)D will be measured before CKI starting and then every 3 months, prescribing three different supplementation schedules basing on the entity of deficiency (cut-off 30, 20 and 10 ng/ml). We planned a stratification based on primary tumor, ECOG performance status and treatment line. Time to treatment failure will represent the primary end point. Prevalence of hypovitaminosis D among subgroups, effectiveness of supplementation, overall survival, progression-free survival and response rate according to immune-related radiologic response criteria will be evaluated as secondary end points. Outcomes of patients with normal basal 25(OH)D levels will be compared with those with basal hypovitaminosis D. A further group will be enrolled while

ongoing immunotherapy, allowing an exploratory comparison of the primary study group with a cohort of patients with a misdiagnosed vitamin D deficiency.

In conclusion, hypovitaminosis D has a relevant prevalence in advanced cancer patients. Considering the role of the vitamin D endocrine system in T-cell activation, the assessment of 25(OH)D levels and the initiation of a proper supplementation before immunotherapy with CKI should be considered also with the aim to enhance responsiveness to immune-checkpoint blockade. Hypothesis-generating data about this issue may be provided by the PROVIDENCE study.

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