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Vitamins as regulators of immune cells and immune functions

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Abstract

Vitamins are essential micronutrients that human organism cannot synthesize or does not synthesize in sufficient quantities. Consequently, vitamins need to be supplied by the diet or commensal bacteria. They have diverse functions in many biological events, including regulation of immune responses, leading to increased susceptibility to immune dysregulation and immune-related diseases, when lacking. Clinical evidence points to a continuous rise in autoimmune/inflammatory diseases and cancer throughout westernized societies over the past decade, suggesting to a stronger influence of environmental factors as opposed to genetic factors. Cancer is the second most common cause of death worldwide. Hence, it is urgent to understand the tumour microenvironment, the interactions between tumour and immune cells, and how cancers manage to escape the immune surveillance and progress. Immunotherapies are one of the most innovative treatments in cancer, enhancing immune system to fight against tumour cells. When immunotherapy works, the result can be life-changing, unfortunately, only a minority of patients benefit from it. Vitamins influence immune cells responses and immune cells are key players in the initiation and development of cancer. Hence, can dietary components, such as vitamins, impact the cancer growth by their action on immune cells? In this review, we intend to discuss the progress regarding the immune function of vitamins A, C and D with a particular focus on how they influence key immune players in steady state, as well as their potential role as immune modulators of antitumoral lymphocytes responses and as co-adjuvant of immunotherapies, such as immune checkpoint inhibitors.

The literature research was conducted using the electronic database PubMed and were selected original articles and few reviews from 2011 up to 2021. Search keywords applied were “vitamin D”, “vitamin A”, “vitamin C”, “steady state”, “immune cell functions”, “immune system”, “cancer” and "immunotherapy”.

Keywords: “Vitamins”, “Immune cells”, “Cancer”, “Immune checkpoint inhibitors”.

Resumo

As vitaminas são micronutrientes essenciais que o ser humano não consegue sintetizar ou que não sintetiza em quantidades suficientes. Têm de ser fornecidas através da dieta, ou através da sua produção pelas bactérias comensais. As vitaminas têm um papel em diversos eventos biológicos, nomeadamente na regulação de respostas imunes, pelo que, quando se encontram em concentrações deficientes aumentam o risco de desregulação imunológica. Nos últimos anos, nas sociedades ocidentais, temos assistido a um aumento de incidência de doenças autoimunes/inflamatórias e cancro, apontando para uma maior influência de fatores ambientais em oposição aos fatores genéticos. O cancro é a segunda causa de morte no mundo. Desta forma, é importante perceber as interações entre o microambiente tumoral e o sistema imune; como é que as células tumorais conseguem escapar à vigilância imune e proliferar. Um dos tratamentos mais inovadores contra o cancro é a imunoterapia, capaz de estimular as células imunes contra as células tumorais. Quando funciona, pode ser revolucionária, no entanto, apenas uma minoria de pacientes beneficia dela. As vitaminas influenciam as respostas das células imunes e as células imunes são essenciais no desenvolvimento do cancro. Será que componentes da dieta, como as vitaminas, podem influenciar células tumorais através da sua ação nas células imunes? Nesta revisão, pretendemos abordar os principais conhecimentos existentes acerca das funções das vitaminas A, C e D nas respostas imunes, com particular foco na ação destas sobre as principais células imunes na homeostase, bem como o seu potencial modulador das respostas linfocitárias com impacto no crescimento e tratamento neoplásico. Utilizou-se o PubMed para pesquisa bibliográfica e selecionou-se artigos originais e algumas revisões de 2011 até 2021. As palavras-chave utilizadas foram “vitamina D”, “vitamina A”, “vitamina C”, “estado estacionário”, “funções células imunes”, “sistema imunológico”, “cancro” e “imunoterapia”.

Palavras-chave: “Vitaminas”, “Células imunes”, “Cancro”, “Inibidores de checkpoints imunológicos”

List of Abbreviations

1,25-(OH)2D3- Calcitriol

25(OH)D- Calcifediol

5hmC-5-hydroxymethylcytosine

5mC- 5-methylcytosine

AA- Ascorbic acid

AMPK-Activated protein kinase activated protein kinase

APL-Acute promyelocytic leukemia

ATM- Ataxiatelangiectasia mutated

ATRA- All-trans retinoic acid

BATF- Basic leucine zipper transcription factor

Bcl-6 - B-cell lymphoma 6

BEC- Blood endothelial cells

Braf- B-Raf Proto-Oncogene

CAF- Cancer associated fibroblasts

CCL- C-C Motif Chemokine Ligand

CCR- C-C Motif Chemokine Receptor

CD- Cluster of differentiation

CNS- Conserved noncoding sequences

CSCs- Cancer stem cells

CSF- Colony-stimulating factor

CTL- Cytotoxic T lymphocytes

CTLA-4- Cytotoxic T lymphocyte-associated protein 4

CXCL- C-X-C motif chemokine ligand

DBP- Vitamin D-binding protein

DCs- Dendritic cells

DHA- Dehydroascorbic acid

DNA- Deoxyribonucleic acid

ECM- Collagen-rich extracellular matrix

ERK1/2- Extracellular signal-regulated kinases 1/2

FOXO3- Forkhead Box O3

FoxP3- Forkhead box P3

GAPDH- Glyceraldehyde-3-Phosphate Dehydrogenase

GLUT-Glucose transporter

GM-CSF-Granulocyte-macrophage colony-stimulating factor

GULO- Gulono-gamma-lactone oxidase

H2O2- Hydrogen peroxide

HIF-1- Hypoxia-inducible factor

HLAs- Human leukocyte antigens

HPV- Human papillomavirus

ICB- Immune checkpoint blockade

IDO- Indoleamine 2,3-dioxygenase

IFNɣ- Interferon gamma

IL- Interleukin

IL-23R-Interleukin 23 receptor

IL-6R- Interleukin 6 receptor

ILCs- Innate lymphoid cells

irAEs- Immune-related adverse effects

IRF- Interferon regulatory factor

iTregs- Inducible T regulatory cells

JAK- Janus kinase

Kras- Kirsten rat sarcoma virus

LEC- Lymphocytic endothelial cells

LPS- Lipopolysaccharide

LTi- Lymphoid tissue inducer

MAPK-Mitogen-activated protein kinase

MDSCs- Myeloid-derived suppressor cells

MHC- Major histocompatibility complex

MICA-B- MHC class I chain related-proteins A/B

miRNA- Micro Ribonucleic acid

MLN- Mesenteric lymph nodes

mRNA-Messenger ribonucleic acid

mTOR- Mammalian target of rapamycin

NFATc1/2- Nuclear factor of activated T cells

NF-kB- Nuclear factor kappa-light-chain-enhancer of activated B cells

NK- Natural Killer cells

PD-1-Programmed cell death protein 1

PD-L1- Programmed cell death protein 1 ligand

PP- Peyer's patches

RALDH- Retinaldehyde dehydrogenase

RAR- Retinoic acid receptor

RARE- Retinoic acid response elements

RDH- Retinol dehydrogenase

RORγt-RAR-related orphan receptor gamma

ROS- Reactive oxygen species

RXR- Retinoid X receptor

Smad- Suppressor of Mothers Against Decapentaplegic

STAT- signal transducer and activator of transcription

SVCT- Sodium-dependent vitamin C transporters

TAAs- Tumour-associated antigens

TAMs- Tumour associated macrophages

TCR- T-cell receptor

TET- Ten eleven translocation dioxygenases

TGF-β- Transforming growth factor beta

Th- CD4+T helper cells

TLR- Toll like receptor

TME- Tumour microenvironment

TNF- Tumor necrosis factor

TRAIL- TNF-related apoptosis-inducing ligand

TRAIL-R1- TNF-related apoptosis-inducing ligand receptor 1

Tregs- T regulatory cells

ULBP1-6- UL16 binding protein 1-6

VA- Vitamin A

VC- Vitamin C

VD- Vitamin D

VDR- Vitamin D receptor

VDRE- Vitamin D responsive elements

VEGF- Vascular endothelial growth factor

α4β7- Integrin heterodimer α4β7

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"A vitamin is a substance that makes you ill if you don't eat it." (Albert Szent-Gyorgyi, Nobel Prize in Physiology or Medicine, 1937)

Introduction

Immune system exists since the beginning of life, from bacteria to human beings, identifying and neutralising threats. The immune system is composed by a network of specialized organs, cells and proteins that enables organisms to thrive. Since invertebrates, such as insects, the immune system was already able to set up cellular and humoral responses to invading pathogens. The immune recognition in insects occurs through germline-encoded pattern recognition receptors, such as Toll, employing multiple effector mechanisms (e.g., phagocytosis, activation of antimicrobial peptides). In contrast to vertebrates, no antigen-triggered proliferative responses have yet been demonstrated. The immune system of vertebrates exhibits unique features, such as the presence of the lymphoid lineage (B cells and T cells) with a unique antigen-specific receptor randomly generated from somatic recombination processes enabling faster and efficient immune reactivity upon repeated encounters with a certain pathogen, generating immune memory (Boehm, 2012; Carr & Maggini, 2017; Nicholson, 2016). Historically, immune functions were conceptually divided into two domains, with innate responses being present in invertebrates, and adaptive responses being characteristic of vertebrate immune systems. However, some of the attributes defining innate and adaptive immunity are shared by both and essentially these two systems have co-evolved and developed many means of communication to allow a performant response. Besides the protection afforded against invading pathogens the role of the immune system is to maintain organ integrity and performance. This function relies on its ability to sense, adapt and correct environmental changes. This is critical in fighting diseases such as cancer, where the tumour microenvironment can be seen as the creation of a "novel organ" that needs support for growth. Cancer emerges as a result of the accumulation of somatic mutations. These mutations can derive new proteins (neo-antigens) that can then be recognised by the adaptive lymphocytes as "foreign", and consequently be the base of tumour elimination (Nicholson, 2016).

These past decades, we have assisted to a transformation in city lifestyle all around the world, which entails environmental changes, decreased physical activity and substantial shifts in diet, from a traditional diet, rich in fruits and plant-based fibre to a Westernized diet characterized by high-protein, fat and calorie levels (Patry & Nagler, 2021). The intestine works as the main location of nutrient absorption and comprises a unique immune system capable to differentiate between offensive pathogens and commensal microorganisms or dietary elements, balancing between pro- and anti-inflammatory responses (Arpaia et al., 2013). Westernized diet has an impact on the immune status of populations promoting a proinflammatory phenotype, with higher levels of IFN-γ, TNF and IL-6, leading to higher susceptibility to infections (Desai et al., 2016), autoimmune/inflammatory diseases (Temba et al., 2021), and cancer development (Garcia-Larsen et al., 2019; Schulz et al., 2014). Cancer is the second most common cause of death globally, being responsible for 1 in 6 deaths, making it a major public health problem affecting people of all ages (Siegel et al., 2016; "The Global Challenge of Cancer," 2020). This suggests Westernized diet may be a risk factor for cancer development and associated with worst outcomes in cancers such as colon or breast cancer (Garcia-Larsen et al., 2019; Howe et al., 2013). A sustained inflammatory environment is characterized by the presence of heterogeneous immune cells such as neutrophils, macrophages and lymphocytes. These infiltrated cells produce inflammatory cytokines, growth factors and genotoxic substances such as reactive oxygen species (ROS), inducing DNA damage and methylation. Therefore, chronic inflammation can promote carcinogenesis, tumour progression and treatment resistance (Kanda et al., 2017; Shalapour & Karin, 2015). The interplay between the immune system and the tumour microenvironment (TME) has been demonstrated to be highly relevant, crucial for cancer progression and to the response/resistance to treatment. In Westernized diet, there is low consumption of vitamins, which influence immune cells responses and immune cells are key players in the development of cancer. Hence, can dietary components, such as vitamins impact the cancer growth by their action on immune cells (Kunisawa & Kiyono, 2013)?

Therefore, the aim of this review is to question the potential of vitamins to impact cancer growth and therapy. To address this question we will present an overview of

the biological effects of vitamin A, C and D on the immune cells in homeostasis and their potential role modulating immune cells on TME and immune checkpoint inhibitors, given the importance of mortality/morbidity of cancer.

1. The role of vitamins at steady state (homeostasis) on immune cell functions

1.a. Vitamin A

- **Vitamin A biology and production**

Vitamin A (VA) is vital for the right functioning of the visual system, integrity of epithelial surfaces, maintenance of cell function and differentiation and in immune homeostasis. Since it cannot be synthesized *de novo*, diet is the only source (Guo et al., 2015). VA deficiency is associated with defects in multiple immune cell lineages and immunological functions (Hall, Grainger, et al., 2011). Lack of VA causes poor lymphoid organ development, immune dysregulation, weaker vaccination response and increased burden of infectious diseases, highlighting its importance for proper immunity (Bono et al., 2016; Oliveira et al., 2018; Spencer et al., 2014; Van De Pavert et al., 2014). Precursors of VA, such as, β-carotenes are primarily converted in retinoids in the lumen of enterocytes of the proximal portion of the gut. Afterwards, they are packed with chylomicrons and move into the general circulation, where hepatocytes capture and store them as retinol. Retinol circulates in the plasma associated to retinol-binding protein and is taken up from the blood and oxidized first to retinal by retinol dehydrogenases (RDH) and then retinal is irreversibly converted to all-trans-retinoic acid (RA), the predominant biological form of VA, by one of retinaldehyde dehydrogenase (RALDH) isoforms: RALDH1, RALDH2, or RALDH3. RA synthesis is restricted to cells that express these enzymes (Bono et al., 2016; Hall, Grainger, et al., 2011; Oliveira et al., 2018). Retinoic acid receptors (RARs) are nuclear hormone receptors expressed by lymphoid cells. They are important in the regulation of cell homing and differentiation. In response to RA, they form heterodimers with retinoid X receptors (RXRs) and bind to RA response elements (RAREs) in target genes acting as ligand-dependent transcription factors (Raverdeau & Mills, 2014). Control of the RA concentration in tissues is performed via cytochrome P450 family 26 (CYP26A1, CYP26B1, and CYP26C1), which catalyses RA in the cytosol, preventing RA

accumulation and preserving optimal physiological RA concentrations (Takeuchi et al., 2011).

- **Vitamin A and immune cells fate lineage**

Proper immune response depends on the homing ability of the effector and regulatory lymphocytes to the site of infection or injury. Even though VA has been known to be essential for a competent immune system, its underlying mechanisms are not still completely understood (Guo et al., 2015). RA has been shown to take part in directing the lineage fate of hematopoietic stem cells, dendritic cells (DCs), innate lymphoid cells (ILCs) and CD4+ T cells through activation of RAR. Even though, the factors that coordinate chromatin changes in differentiating CD4+ T helper (Th) cells are not fully defined, in addition to its classical role as a transcriptional regulator, RA-RAR has been recognized as an epigenetic regulator (Brown et al., 2015), stabilizing gene expression and promoting the maintenance of cell-fate commitment. For instance, RA synthesis is dynamically controlled at sites of T cell priming during inflammation, suggesting a potential role of RA in Th cell plasticity. Indeed, RA is essential for Th1 cell immunity (Hall, Cannons, et al., 2011; Pino-Lagos et al., 2011) and has also been implicated in Th17 cell differentiation, where its impact appears to be dose-dependent. Indeed, physiological concentrations of RA enhance Th17 cell differentiation *in vitro*, yet administration of higher concentrations of RA both *in vitro* and *in vivo* negatively regulates Th17 cell responses (Bono et al., 2016; Takahashi et al., 2012).

- **Vitamin A imprints gut homing migratory capacity in T cells by CCR9 and a4b7 expression**

RA's role in mucosal immunity was established by the discovery that both murine and human dendritic cells (DCs) harvested from the mesenteric lymph nodes (MLN) and Peyer's patches (PP) constitutively express RALDH1 and 2. These enzymes permit RA production and CD103 up-regulation (Guo et al., 2015; Rampal et al., 2016; Raverdeau & Mills, 2014). The CD103+ DCs are responsible for imprinting gut-homing specificity to T cells (both CD4+ and CD8+), via induction of chemokine receptor CCR9 and integrin heterodimer α4β7 (Bono et al., 2016). Thus, RA produced by mucosal CD103+ DC,

instructs T cells during activation, to migrate back to the site where antigen was initially encountered (Hall, Cannons, et al., 2011). RA treatment enhances the expression of both integrin α4 and CCR9 on T cells, whereas the constitutive expression of β7 can be enhanced by TGF-β. Therefore, coordination between TGF-β and RA determines T cell migratory ability (Guo et al., 2015), although RA is the main determinant of T cell migration to the gut (Hall, Cannons, et al., 2011).

Besides DCs from the gut, peripheral DCs located in the skin also express RALDH2 indicating a role for RA in immune steady state at barrier sites. This suggests that RA synthesis and signalling might be a common feature of immune response in the periphery. Elevated levels of RA in the intestine are preserved through the generation of gut-homing DC precursors in the bone marrow and additional enhancement of RA production by granulocyte-macrophage colony-stimulating factor (GM-CSF) in the intestinal DCs that then deliver RA to macrophages inducing further GM-CSF production. IL-4 has also shown to induce RALDH2 in MLN-DC (Guo et al., 2015; Zeng et al., 2013). Contrary, prostaglandin E2 blocks GM-CSF induced RALDH2 expression in both murine and human DCs, a potential suppressive mechanism of RA synthesis by DCs at a steady/inflammatory-state (Stock et al., 2011). The RA synthesis by pro-inflammatory and anti-inflammatory signals emphasizes the essential role of RA balancing between tolerance and immunity. Innate lymphoid cells (ILCs) work as primary sensors of dietary stress, sustaining barrier defence. Studies identified that mice deficient in VA, or treated with RA antagonists, have diminished ILC3s responses, associated with an impaired development of Th17 cells at the mucosal surface, enhancing susceptibility to bacterial infections such as *Citrobacter rodentium* (Bono et al., 2016; Goverse et al., 2016; Spencer et al., 2014). The absence of VA leads to expansion of IL-13-producing ILC2s which confers resistance to nematode infection (Spencer et al., 2014). Furthermore, maternal intake and consequently levels of VA regulate fetal RA signalling and the expression of RORγt, a key transcription factor for the development of lymphoid tissue inducer (LTi) cells. LTi cells, a subset of ILC3, are important in the development of secondary lymphoid organs and Peyer's patches. Hence, mice exposed to a deficient RA environment *in utero* have smaller secondary

lymphoid organs, which persist throughout adult life leading to compromised adaptive immune responses (Van De Pavert et al., 2014).

TCR-mediated signalling and CD28 costimulatory signal promotes transcription factor NFATc1 and NFATc2' nuclear translocation. NFATc2 enhances but NFATc1 inhibits RAR-RXR binding to RARE in CCR9 promoter region. Only transient T-cell activation allows CCR9 upregulation, once prolonged TCR signalling holds NFATc1 in the nucleus. This finding points to a crosstalk between RAR-RXR and TCR signalling-induced transcription factors essential to control RA-induced genes (Ohoka et al., 2011). RA also controls microRNAs (miRNA) in different T cell subsets, enabling RA to regulate gene expression in a post-transcriptional way (Jeker et al., 2012). RA and TGF-β were shown to induce expression of miRNA10a in inducible Tregs (iTreg), which inhibits expression of follicular helper T (TFh) cell master transcription factor Bcl-6 (Takahashi et al., 2012). Therefore, RA is able to regulate directly or indirectly target genes and RA-producing DCs in response to different stimuli, which coupled with other specific cytokines/chemokines can modulate peripheral T cell differentiation (Guo et al., 2015).

- **The role of VA in T cells**

The differentiation of naive T cells is shaped by many factors such as the subset of DCs and its co-stimulatory molecules and the cytokine environment surrounding T cells. RA has been shown to inhibit Th17 cell responses while promoting the generation of Tregs *in vitro*. RA enhances the TCR-mediated TGF-β-dependent conversion of naive CD4+ T cells into Tregs *in vitro* by RA-RARα axis. RA promotes RAR/RXR binding to a RARE in the enhancer 1 (CNS1) region of the *Foxp3* gene, which facilitates binding of phosphorylated Smad3 to the enhancer region. This increases the stability of Foxp3+ expression, especially when facing inflammatory cytokines (Pino-Lagos et al., 2011). However, other researchers reported that RA could induce FoxP3 expression in CD4+ T cells isolated from *Smad3* or *Smad2* knockout mice. RA synergizes with TGF-β signalling to induce Tregs via ERK1/2 pathways (L. Lu et al., 2011). These ambiguous findings may result from redundant or overlapping signalling pathways that control *Foxp3* transcription (Guo et al., 2015). iTregs generated in the presence of RA are less sensitive to pro-inflammatory cytokines as they express reduced levels of the receptor

for IL-6, a Th17 instructing cytokine. Th17 cells differentiate from naive T cells stimulated by TCR, CD28, TGF-β and IL-6, *in vitro,* activating STAT3 which promotes the expression of *RORɤt* leading to the lineage specification producing proinflammatory cytokines like IL-17, IL-21, and IL-22. RA opposes STAT3 activation in part by downmodulating IL-6R, interferon (IFN) regulatory factor 4 (IRF4) and IL-23R. Thus, RA inhibits Th17 cell differentiation (Basu et al., 2015; Collins et al., 2011; Huber et al., 2011). Overall, the role of RA in generating Tregs seems counterintuitive given the epidemiological data supporting a role for RA against infectious disease. Consistent with the latter, there are also studies indicating a proinflammatory facet of RA, demonstrating that RA helps to maintain Th1 lineage and is essential to produce IFN-ɤ and IL-17 from Th1 and Th17 cells, respectively (Rampal et al., 2016). Emerging data scrutinizing the effects of RA on effector lymphocytes *in vivo* have helped to understand this paradox. RA signalling is critical for Th1 mediated immunity during inflammatory responses. *Rarα*-/- T cells do not differentiate into Th1 or Th17 even if cultured *in vitro* under Th1 or Th17 polarizing conditions (Hall, Cannons, et al., 2011), supporting a role of RA in their differentiation. RA-RARα sustained the expression of Th1 cell-associated genes during naive T-cell differentiation while repressing the signature of Th17 cell-associated genes, such as *Runx1* and *Il6rα*. In addition, BATF-IRF4 (key transcription factors for the differentiation towards Th17 phenotype) target genes were expressed in the absence of RA signalling, modulating chromatin accessibility to facilitate binding of STAT3 and RORɤt. IRF8 an identified member of the Th1 cell transcriptional network, whose expression depends on RA signalling, limits plasticity of Th1 cells by binding to BATF and repressing Th17 differentiation. For this reason, removal of RA signalling in Th1-differentiating cells enhances deviation towards a Th17 cell phenotype, making RA essential for limiting Th1 cell conversion into Th17 effectors, preventing pathogenic Th17 responses *in vivo* (Brown et al., 2015; Hall, Cannons, et al., 2011). In the presence of IL-15, RA acts through DCs decreasing naive T cell conversion into Tregs and enhancing Th1 cell polarization (Depaolo et al., 2011). To evaluate whether RA could restore protective immunity *in vivo*, mice with VA insufficiency, which were treated with RA for 5 days and then inoculated orally with *Toxoplasma gondii*, were found to express higher IFNɤ than controls. In addition, in intestinal mucosa, RA was essential to generate Th1 and Th17 cell responses (Hall,

Cannons, et al., 2011). Other studies, however, suggest that RA may have a dose-dependent effect over T cell differentiation. At pharmacological or high doses (≥10 nM), RA has been shown to impair the differentiation of human Th17 and Th1 cells while inducing the generation of Tregs *in vitro* (Brown & Noelle, 2015). At physiological or lower concentrations (1nM), RA favours Th1 and Th17 cell differentiation *in vitro*. Taken together these observations suggest that RA may have a dual effect favouring Tregs induction and suppression of Th1 and Th17 responses in the steady state, while inducing Th1 and limiting Th17 cell mediated immunity during inflammation (Bono et al., 2016; Raverdeau & Mills, 2014). Finally, VA may also participate in the induction of Th2 responses. *In vitro* studies with naive T cells cultured in the presence of VA, or an RXR agonist, induce IL-4 by T cells, pointing to a Th2 differentiation effect via stimulation of RXR pathway (Bono et al., 2016). However, studies trying to clarify the role of RA in the regulation of Th2 cells are conflicting, given that RA was also found to decrease *Gata-3* mRNA, reducing Th2 differentiation (Wu et al., 2013). In figures 1 and 2 a summary of the effect of VA is presented.

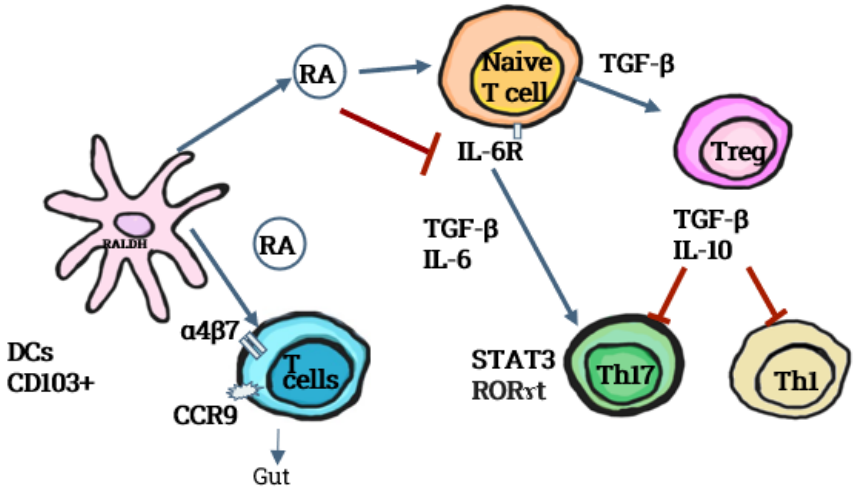

Figure 1*- Summary of the effect of retinoic acid action in homeostasis*

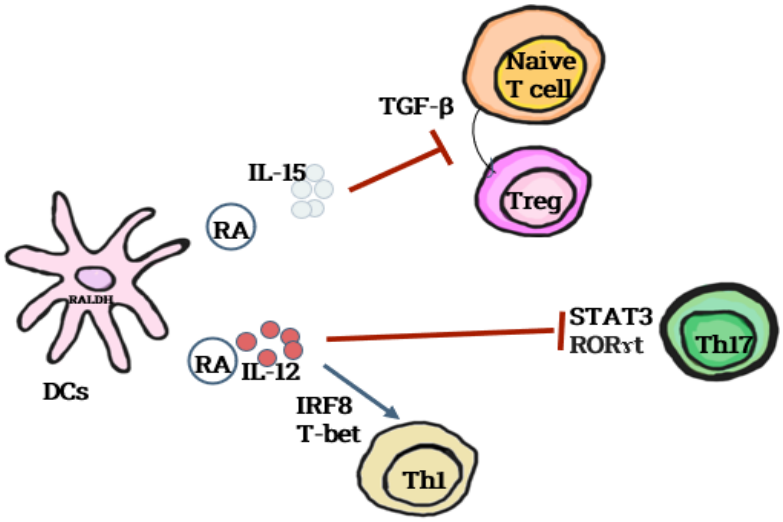

Figure 2- *Action of retinoic acid in inflammatory processes*

Adding to the requirement of RA for the development of Th cells, RA is also essential for the survival of effector CD8+ T cells during the expansion phase. Selective disruption of RA signalling in CD8+ T cells disables their capability to undergo effective clonal expansion *in vivo* interfering with their functional responses (Guo et al., 2012, 2014).

The use of antagonists and agonists of RA signalling as well as genetically engineered mouse models has expanded our knowledge of how RA controls immune responses apart from its task in controlling gut homing. Its deficiency leads to a wide range of immunological dysfunctions (Guo et al., 2015). Moreover, therapeutic translation is hindered by some drawbacks from high doses of retinoid therapy that encompasses teratogenicity, especially in women. Retinoids in current clinical use are pan-RAR agonists. Selective agonists for RARα, the one implicated in effector T cell responses, may lead to lesser side effects. For that reason, RA could be joined to protocols for Tregs expansion, while also enhancing gut homing capacity which could be valuable in inflammatory intestinal diseases (Bono et al., 2016). It remains some questions to be answered, like what signalling pathways are induced by physiological and pharmacological RA concentrations; what other transcription factors cooperate with RARs defining phenotype of immune cells and what genes in T cells are controlled by

RA. Further studies are necessary to establish the role of RA in Th cell generation across different *in vivo* settings.

1.b. Vitamin C

- **Vitamin C biology and production**

Vitamin C (VC), a powerful antioxidant, and a cofactor for multiple gene regulatory enzymes, protects important biomolecules (eg., proteins, lipids, nucleic acids) from damage generated by cellular metabolism oxidants or toxins. Human beings do not carry a functional copy of the gulono-gamma-lactone oxidase *(GULO)*-gene, responsible to synthesize ascorbic acid (AA) in the liver. Consequently, humans depend on dietary sources of AA (Van Gorkom et al., 2018). VC absorption is tightly controlled. There are two forms of VC, the reduced form, AA, and the oxidized form, dehydroascorbic acid (DHA). DHA is transported into cells via glucose transporters (GLUT) 1, 3, and 4 passively along the concentration gradient. AA is actively transported via sodium-dependent vitamin C transporters (SVCT) 1 and 2 (Hong et al., 2016). Hypovitaminosis C is common in Western populations mostly due to limited body stores and lifestyles that either limit the intake or increase the VC requirements (e.g., pollution, smoking, alcohol, drug abuse, infections and inflammatory diseases). VC acts as a co-factor for the prolyl and lysyl hydroxylase enzymes that stabilize the tertiary structure of collagen and promote oxidant scavenging activity protecting against ROS-induced damage (Lauer et al., 2013). VC deficiency results in a potentially fatal disease, scurvy, denoted by the weakening of collagenous structures, characterized by poor wound healing, bleeding gums and bruising. Furthermore, individuals with scurvy also have impaired immunity, being highly susceptible to potentially fatal infections. Infection-induced inflammation increases the metabolic demand of VC. Hence, it is necessary to ensure adequate intakes, especially in high-risk deficiency VC groups such as the elderly (Carr & Maggini, 2017).

- **Neutrophils and macrophages**

VC accumulates in immune cells including DCs, neutrophils, monocytes, macrophages and T cells acting as an antioxidant, protecting these cells from ROS produced during

immune responses in inflammatory situations and oxidative burst (Demaret et al., 2015). The expression of SVCT-2 on immune cells (Carr & Maggini, 2017), enables leukocytes, such as neutrophils and monocytes to actively accumulate VC. Neutrophils in an initial inflammatory stage can enhance various functions including chemotaxis, phagocytosis, generation of ROS and ultimately microbial killing. VC helps to enhance apoptosis and clearance of neutrophils from inflammatory sites, by macrophages. This decreases necrosis/NETosis and potential tissue damage (Carr & Maggini, 2017). Supplementation of healthy humans with 1 g/day (10 times more than the daily recommendation) of VC was shown to modulate mononuclear cell-derived response enhancing the production of IL-10 (anti-inflammatory cytokine) and decreasing the expression of TNF-α following an inflammatory stimulus (eg., lipopolysaccharide (LPS)) (Canali et al., 2014).

- **NK cells and T cells**

AA improves, *in vitro*, the proliferation and activity of NK cells, cytotoxic innate lymphocytes, especially relevant against viruses and tumour surveillance. NK cells isolated from *Gulo*−/− mice showed a significant decrease in killing capacity *in vitro*, with reduced expression of perforin, granzyme B and activating receptors CD69 and NKG2D, contrary to AA-supplemented or wild-type mice (J. E. Kim et al., 2012).

The role of VC in lymphocytes is not yet totally clear. Activated lymphocytes acquire SVCT-2, achieving intracellular AA concentrations 10–100 fold higher than plasma levels, which may suggest an essential role in these cells (Hong et al., 2016). AA is able to regulate gene expression through direct action on transcription factors. VC also acts as a cofactor in enzymatic reactions through iron-, copper- and 2-oxoglutarate-dependent dioxygenases, including ten eleven translocation (TET) family dioxygenases and histone demethylases such as Jumonji C family. These are responsible for DNA and histone demethylation reactions, thus modulating gene expression (Agathocleous et al., 2017; Minor et al., 2013). As a result, one can hypothesize that the enhancement of the activity of these enzymes could be a mechanism by which AA promotes T cell development in thymus and later differentiation. *In vitro* studies revealed that AA is a critical component for the transition of double-negative (DN, CD4- CD8-) precursors to

double-positive (DP, CD4+ CD8+), a stage where TCRαβ selection occurs and promotes proliferation and improved T-lineage development (Huijskens et al., 2014). The action of AA was not due to its antioxidant properties. Manning et al. findings suggest an AA-dependent removal of repressive histone modifications in early development of T cell induction and maintenance of *Cd8a* gene expression. Thus, the role of AA in this process is at least partially due to regulation of *Cd8a* gene expression. This model of AA-dependent epigenetic gene regulation proposes a system that integrates environmental stimulus (dietary nutrients) with a transcriptional switch through epigenetic alterations (Manning et al., 2013; Van Gorkom et al., 2018).

It has been suggested that AA can stimulate a shift of immune response from Th2 to Th1 cells (Van Gorkom et al., 2018). For example, murine bone marrow-derived naive DCs pre-treated with AA, before being activated with LPS, secreted less IL-5 and more IL-12, IFN-γ and IL-15 due to higher phosphorylation of p38 and ERK1/2 and increased activation of NF-kB leading T cells towards Th1 phenotype (Jeong et al., 2011). Under Th17 polarizing conditions, VC treated cells, but not control cells, differentiated into Foxp3 expressing Tregs. VC has been reported to induce demethylation of CpG motifs in the Foxp3 intronic element (CNS2), which is indispensable for stable expression of Foxp3 in Tregs (Sasidharan Nair et al., 2016). Therefore, VC may stabilize the expression of Foxp3 even in the presence of IL-6. Only one study found AA, through its epigenetic actions, may increase IL-17 expression, promoting Th17 polarization of murine naive CD4+ T cells (M. H. Song et al., 2017).

Concluding, animal studies show that AA promotes Th1 differentiation at the expense of Th2 polarization. Stable expression of Foxp3 in iTreg cells is dependent on DNA demethylation of the intronic element CNS2 by TET2, which uses AA as co-factor, with better suppressive capacity *in vivo* in murine model (Sasidharan Nair et al., 2016; Yue et al., 2016). There are limited records showing that Th17 polarization is promoted by AA acting as an epigenetic regulator. Therefore, VC, a low-price micronutrient, appears to modulate the immune system to set up and maintain a suitable response, which could be crucial in contexts of inflammatory diseases or cancer. However, most of these conclusions are based on *in vitro* studies with different conditions (concentration, incubation time) making it hard to appraisal.

1.c. Vitamin D

- **Generalities and its metabolism**

Besides its impact in calcium and phosphate homeostasis (Charoenngam & Holick, 2020), vitamin D (VD) has also a noteworthy role in immune function. In the immune system it acts as a regulator with preponderance towards tolerance induction. This raised the question, could VD be a potential immune modulator in the treatment or prevention of immune-mediated illnesses (Martens et al., 2020)? Indeed, there is epidemiological data connecting low levels of serum 25-hydroxyvitamin D3 (25-(OH)D3) and the increased risk of developing or exacerbating infectious and inflammatory/autoimmune diseases, such as multiple sclerosis (Duan et al., 2014; Soilu-Hänninen et al., 2012), type 1 diabetes (Cooper et al., 2011) and inflammatory bowel disease (Martens et al., 2020). Differing from the previous vitamins, humans do not depend exclusively on VD acquired from diet or supplements, as it can also be obtained through endogenous production from ultraviolet B irradiation of the skin. VD requires both 25- and 1α-hydroxylation for its activation. The 25-hydroxylation occurs in the liver by multiple enzymes (eg., CYP2DII, CYP2D25, CYP3A4, CYP2R1, and CYP27A1), that convert VD to 25-(OH)D3. Then, 1α-hydroxylase (CYP27B1), expressed by different cell types (eg., kidneys, skin, immune cells, bone cells, placenta) converts 25-(OH)D3 into calcitriol (1,25-(OH)2D3), the active metabolite of VD. VD and its metabolites travel bounded to the vitamin D-binding protein (DBP). Like VA, VD receptor (VDR) belongs to the nuclear receptor superfamily, which after ligand binding leads to heterodimerization with RXR and act on the VD responsive elements (VDRE) in the promoter region of target genes in the nucleus (Charoenngam & Holick, 2020; Martens et al., 2020)

There is a lot of debate about the best serum level of VD and how much and in what ways it should be given, if necessary, for immune and overall health benefits. The world population has a significant VD insufficiency likely due to better solar protection, decreased sun exposure as people are becoming more obese, barely participating in open-air activities (Ganji et al., 2012). For instance, VD values less than 20 ng/mL were associated with superior all-cause mortality (Dudenkov et al., 2018). Regarding the best replacement regimen of VD, randomized controlled trials showed that an intake

of 1040 IU per day is vital to correct deficiency, while an intake of 400 IU per day is required to rectify insufficiency and achieve (in both cases) a concentration >20 ng/mL (Cashman et al., 2017).

- **Vitamin D and immune cells**

The influence of VD on immune cells is not straightforward as the expression of VDR, 25- and 1α-hydroxylases in immune cells, such as monocytes, macrophages, DCs and lymphocytes, depend on their activation status (Charoenngam & Holick, 2020). Higher calcitriol concentrations in DCs from skin-draining lymph nodes leads to epidermal tropism in T cells through expression of CCR10, while suppressing gut-homing properties (CCR9), typically induced by RA. This suggests VDR and RARα may have opposing signalling, possibly due to competition for their shared heterodimeric partner, RXR. This competition has been recognized in T cells: RA induces integrin α4β7 and CCR9 intestinal T-cell homing, antagonized by 1,25-OH-VD3 *in vitro* (Bscheider & Butcher, 2016).

- **Macrophages**

The level of 1α-hydroxylase in macrophages and monocytes is upregulated upon activation by immune stimuli, such as IFN-γ or toll like receptors (TLR) ligands. In turn, rising levels of 1,25(OH)2D3 stimulates VDR-RXR signalling, increasing the production of antimicrobial cathelicidin LL-37 that fights against pathogens (Barlow et al., 2011; Tripathi et al., 2013). Increased levels of VD on macrophages also increases the production of IL-10 and decreases inflammatory stimuli (eg., IL-1β, IL-2, IL-6, IL-17, TNF-α and cyclo-oxygenase-2(COX-2))(Martens et al., 2020). The downregulation of inflammatory cytokines happens through the upregulation of MAPK phosphatase-1 by calcitriol and subsequent inhibition of LPS-induced p38 activation (Y. Zhang et al., 2012). Therefore, VD supplementation could be a complement in inflammatory disease (Wang et al., 2014). The 1,25-(OH)2D3-VDR signalling on macrophages also attenuates TLR-mediated inflammation by downregulating miRNA-155 leaving uninhibited the negative feedback regulation of inflammation done by suppressor of cytokine signalling 1 (SOCS-1). Besides, miR-155 has also been detected in immune cells such as dendritic cells, B and T cells, so it is probable that vitamin D may regulate immune

activities of these immune cells also through miRNA-155 (Y. Chen et al., 2013). Calcitriol also function as an antioxidant on monocytes, upregulating glutathione reductase and glutamate-cysteine ligase which reduces the formation of ROS (Jain & Micinski, 2013).

- **Neutrophils and NK cells**

Both NK cells and neutrophils express VDR. On neutrophils 1,25-(OH)2D3 boosts their destructive ability against pathogens through improved expression of cathelicidin, α and β-defensins, while lowering the production of proinflammatory cytokines IL-6, IL-8, and IL-12, and inducing the anti-inflammatory cytokine, IL-4, in neutrophils infected with Pneumococci (Subramanian et al., 2017). In NK cells it results in a decreased development from hematopoietic stem cells, and in the successful NK cells, with an impaired functional status identified by a reduction of IFN-γ and granzymes A and B (Weeres et al., 2014).

- **DCs and T cells**

Antigen-presenting DCs are an important source of 1α-hydroxylase, specially upon contact with LPS or during T cell priming. DCs produce and release calcitriol, subsequently affecting T cell responses (Jeffery et al., 2012). In addition, calcitriol modulates DCs to be less mature and have a tolerogenic phenotype, downregulating major histocompatibility complex (MHC), cluster of differentiation (CD) 80, CD40, CD86 (co-stimulatory molecules) and CD54 (adhesion molecule) causing a decrease in antigen presentation. It culminates in a diminished expression of IL-17, IFNγ, IL-21, IL-6 and IL-12, increased expression of IL-10, TGF-β, switching the activation of effector T cell response towards a regulatory phenotype (Ferreira et al., 2011). Therefore, DCs could be an important tool to stimulate or restore antigen-specific tolerance in autoimmune diseases (Bscheider & Butcher, 2016). DCs production of calcitriol is affected by the calcifediol bioavailability and by DBP concentration. DBP can sequester calcifediol and inhibits the production of calcitriol in T cells influencing the capacity of immune cells to uptake calcifediol (Kongsbak et al., 2014).

Direct effect of VD in T cells depends on their activation status as they gain higher VDR concentration upon activation. Although calcitriol can directly affect activated T cells, it is doubtful that its circulating levels are enough to influence their phenotype. As T cells have limited 1α-hydroxylase expression, DCs could fulfil this function, producing calcitriol locally influencing T-cell phenotype. The presence of calcitriol leads to a more tolerogenic phenotype of T lymphocytes, promoting a shift from Th1 and Th17 to Th2 (IL-4, IL-5, IL-9, IL-13) by suppressing the expression of Th1 (IL-2, IFN-Y, TNF-α) and Th17 (IL-17, IL-21) cytokines (Charoenngam & Holick, 2020). Treatment of naive human T cells with 1,25(OH)2D3 enhances the expression of FoxP3, by VDR binding to *Foxp3* gene, inducing CD4+CD25+Tregs (Kang et al., 2012). VD can also boost the absolute number of Tregs without interfering with their suppressive functionality, which can be important to inflammatory diseases (Prietl et al., 2014). Like Th cells, cytotoxic T lymphocytes (CTL) express both 1α-hydroxylase and VDR when activated. VDR expression is important for homeostatic CD8+ T proliferation as *VDR* knockout CD8+ T cells following activation proliferated faster and increased the production of IL-2 *in vitro* and *in vivo* leading to a dysregulated CD8+ T cells response. For that reason, VDR is necessary, participating as a negative co-regulator of naive CD8+T cell proliferation and IL-2 production by memory CD8+ T cells. Thus, VDR is critical for controlling the rate of proliferation of naive CD8+ T cells (J. Chen et al., 2014). In figure 3, a summary of the effect of VD is presented.

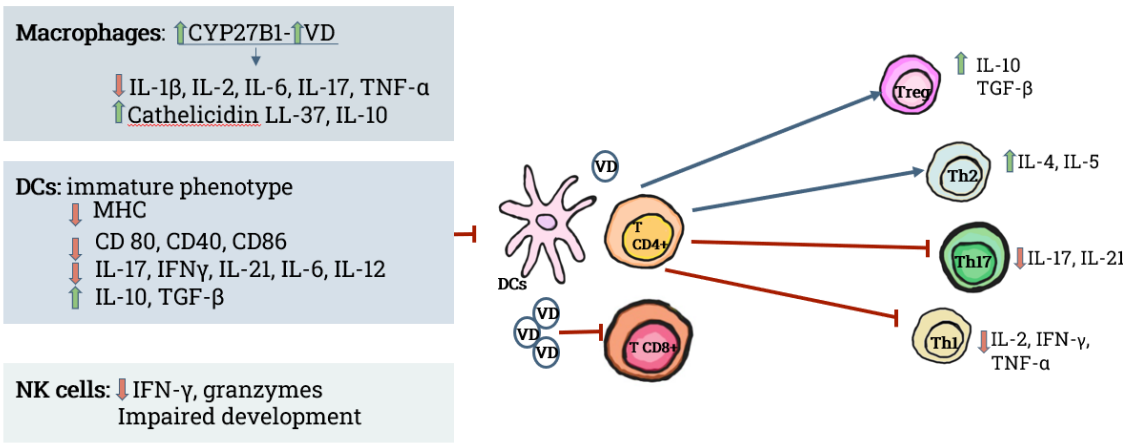

Figure 3- *Summary of the effect of vitamin D action in homeostasis*

Therefore, VD looks like an essential regulator of the immune system maintaining tolerance, making it appealing in the treatment of immune-mediated diseases. However, the translation of these findings into solid results in clinical trials has been unsuccessful. Aspects regarding the start of intervention, dosing regimens and who would benefit the most from VD are critical factors that need to be kept in mind when designing controlled trials to investigate whether supplementation with VD can prevent or modify the course of inflammatory/autoimmune diseases. For now, preventing VD deficiency seems to stimulate immune tolerance and lessen susceptibility to autoimmune diseases.

Summing up, in steady state, VA and VD act on immune cells by binding to nuclear receptors. They have a critical role in gut homing migration of T cells, subsequently regulating systemic immune responses (Cantorna et al., 2019). VD appears to have an important immune-regulatory preponderance towards tolerance induction, as low VD seems to increase the risk and the activity of immune-related diseases. Additionally, VC protects the body against oxidative challenges, acts as a cofactor for multiple biosynthetic and gene regulatory enzymes with immune-modulating effects (Carr & Maggini, 2017).

2. The role of immune cells in the tumour microenvironment (TME)

- **Tumour growth control: evasion of immune system surveillance**

Cancer develops as a result of accumulation of genetic somatic mutations accompanied by multifaceted interactions between the tumour, the immune system and the local host tissue (Y. L. Chen et al., 2012). Neoplastic transformation is characterised by substantial changes in the surrounding connective tissue and stroma, resulting in the emergence of a pathological TME. The TME is a complex milieu of non-malignant cells that includes elements such as cancer associated fibroblasts (CAF), which produce a collagen-rich extracellular matrix (ECM), blood endothelial cells (BEC), pericytes, lymphocytic endothelial cells (LEC), accountable for tumour vasculature, and resident/infiltrating lymphocytes or leukocytes such as macrophages (Vatner & Formenti, 2015). The dynamic and mutualistic connection between the various cell

types of the TME evolves throughout the history of the tumour progression inducing substantial molecular, metabolic, cellular, and physical alterations (Speiser et al., 2016; Turley et al., 2015). These interactions supply growth factors and structural support (cytokines, chemokines, inhibitory factors, angiogenesis), promoting protumour immune infiltrates. As a result, these interactions support solid cancer growth (Turley et al., 2015).

Cancer formation and evolution is related with accumulation of genetic mutations and/or disruption of normal regulatory systems, through an evolutionary process subsequent to cell division. This may lead to the expression of tumour-associated antigens (TAAs), which can be recognized by T cells and activate antitumor immune responses. This results in destruction and/or selection of tumour escape clones, promoting an inflammatory state that, if chronic, can become an immunosuppressive state (McGranahan & Swanton, 2015; Teng et al., 2015). Neoantigens can arise as a result of somatic mutation, anticancer treatments (e.g., chemotherapy or radiation therapy), or by targeting epigenetic regulatory systems or medications that interfere with DNA repair pathways. Non-small-cell lung cancers from heavy cigarette smokers, for example, had much more mutations than non-smokers (Govindan et al., 2012; Huang et al., 2011). While the majority of these mutations are likely to be passenger events with no selection benefit to the cancer cell, a small percentage will be cancer driver events with a selective advantage. Chronic inflammation, a hallmark of cancer, is associated with tissue remodelling and metabolic changes, angiogenesis, lymphangiogenesis, as well as chronic activation of inflammatory immune cells, which promote proliferation of stromal cells, all of which support neoplastic cell survival and tumour progression (Palucka & Coussens, 2016; Turley et al., 2015). Thus, the host immune system plays an important role in cancer growth.

- **Cancer immunoediting**

One crucial feature of cancer is its ability to evade detection and prevent eradication by the immune system. How do cancer cells accomplish this? The classic notion of immune cells as facilitators of tumour rejection has given way to a more sophisticated perspective of leukocytes. Leukocytes have both tumour-inhibitory and tumour-

promoting effects, as they also promote chronic inflammation, shape tumour immunogenicity, and suppress antitumor immunity (Coussens et al., 2013). Tumour-promoting chronic inflammation encourages neoplastic cell survival, with myeloid cells secreting factors such as IL-6 and GM-CSF, which stimulates the recruitment and proliferation of immature and immunosuppressive myeloid cells. Chronic inflammation is also associated with Th2 responses and suppressed activation of CD8+ T cell proliferation promoting tumour development. On the other hand, tumour-limiting acute inflammation causes neoplastic cell death associated with Th1 responses (Palucka & Coussens, 2016). This is why individuals who have chronic inflammatory diseases (e.g. chronic infections such as HPV responsible for cervical cancer or *Helicobacter pylori* elevating the risk for gastric cancer) or situations of low-grade chronic inflammation (eg. cigarette smokers or obesity) are at bigger risk for developing cancer (Hirsch et al., 2021). Cancer-associated inflammation contributes to genomic instability, epigenetic changes, angiogenesis and, ultimately, cancer cell proliferation (Hanahan & Weinberg, 2011).

The process during which the cancer evolves so that the immune system changes from preventing to supporting tumour growth is called: cancer immunoediting. It consists of three stages: elimination, equilibrium, and escape (the 3E's) (Teng et al., 2015). In the **elimination** phase, in the early stages of tumour development, immune cells collaborate to kill cancer cells, even before they become clinically visible. Although the elimination phase has not been directly observed *in vivo*, studies have shown that immunodeficient mice developed earlier onset of cancer compared with *wild-type* mice (Diamond et al., 2011).

Macrophages, the innate immune cells present in all tissue, may be the first cell type in contact with transformed cells. They have been shown capable to recognize cancer cells and attacking them via cellular engulfment by phagocytosis, bypassing the induction of cell death (Chao et al., 2012; Jaiswal et al., 2010). At an early stage the first sign of inflammation can stimulate the recruitment of DCs to the TME (Böttcher et al., 2018). DCs by MHC class I and class II molecules allows the TAAs presentation priming naive CD8+ and CD4+ T lymphocytes (Turley et al., 2015). Naive CD8+ T cells differentiate into CTLs, the main anti-cancer effector cells. In this phase, CD4+ Th1 cells

are also an important immune weapon against cancer (Palucka & Coussens, 2016). Th1 anti-tumoral response, through the discharged of proinflammatory cytokines such as IL-2, TNF-α, and the most potent anti-tumour cytokine IFN-γ, promotes the activity of CTLs, proinflammatory M1 macrophages, NK cells and an overall increase in TAAs presentation (Hanahan & Coussens, 2012). In most cancers, the presence of tumour-infiltrating CD8+ T cells and Th1 cytokines is linked with a better prognosis in terms of overall survival and disease-free survival (Gonzalez et al., 2018). Less immunogenic tumour cells that were not eliminated during this phase persist in the **equilibrium** phase. In this next phase, the immune system and developing tumour enter into a temporary state of balance that controls tumour progression by a selective pressure applied by adaptive immunity, notably CD4+ Th1 response, CD8+ T cells, IFN-γ and opposing cytokines, IL-12 and IL-23 (Braumüller et al., 2013; Teng et al., 2012). Although this persistent anti-tumour immune pressure inhibits tumour progression, ultimately, it results in the selection of less immunogenic cancer cells capable of resisting, evading, or suppressing the antitumor immune response (Teng et al., 2015). Tumour cells suffer editing during equilibrium as a result of genetic (DNA mutations) and epigenetic changes (altered gene expression). This genome instability contributes to intratumour heterogeneity (Burrell et al., 2013a), a hallmark allowing clonal survival and therapy resistance, which is influenced by immune responses (McGranahan & Swanton, 2015). This culminates in the **escape** phase and in the emergence of clinically visible tumours (Teng et al., 2015). In the escape phase tumour becomes infiltrated by pro-tumourigenic leukocytes, like anti-inflammatory-macrophages (M2), driven by IL-4 or IL-13 (Jiang et al., 2018), myeloid-derived suppressor cells (MDSCs) and immune-suppressive T cells (T. Lu et al., 2011), such as Th2 responses and induction of Tregs associated with decreased CTL response (Teng et al., 2015). These immunosuppressive cells are attracted by tumour-derived chemokines such as CCL2, CSF1, CCL5, CCL22, CXCL5, CXCL8, and CXCL12 (DeNardo et al., 2011; Halama et al., 2016; Nakatsumi et al., 2017; Qian et al., 2011). Tumour cell escape methods can be divided into three categories:

- Reduced immune recognition due to downregulation/loss of TAAs, less antigen-presenting machinery or lack of costimulatory molecules avoiding the recognition by

cytotoxic T cells. DCs are both friend and foe to the antitumour immune response. Initially they are vital for cytotoxic antitumor immunity. But in established tumours, DCs take on an immunosuppressive phenotype aiding immune evasion (Schouppe et al., 2013; Tran Janco et al., 2015). They do not receive maturation signs as they are exposed to high levels of IL-10 (Ruffell et al., 2014) and TGF-β (Speiser et al., 2016) in the TME, expressing low quantities of costimulatory molecules (eg. CD80 and CD86) and express regulatory and immunosuppressive molecules such as the programmed cell death protein 1 ligand (PD-L1) (Harimoto et al., 2013; Krempski et al., 2011) resulting in T-cell anergy and tolerance. By downregulating MHC molecules, certain cancer cells become less immunogenic, consequently insensitive to CD8+ T cells (Montesion et al., 2021). For example, some non-small cell lung cancer have a loss of heterozygosity in human leukocyte antigens (HLAs), resulting in immune evasion by presenting fewer antigens (McGranahan et al., 2017). Notably, HLA loss has been associated with a poor outcome response to checkpoint blockade immunotherapy in melanoma and lung cancer patients (Chowell et al., 2018). On the other hand, this loss of MHC molecules expression, makes tumour cells a target to NK cells. MHC class I molecules are ligands for inhibitory receptors on NK cell surface, contributing to self-tolerance (Paul & Lal, 2017). However, the loss of MHC I by tumour cells results in a reduced inhibitory signal in NK cells, activating them. Furthermore, signalling via the activating receptor, NKG2D, on NK cells enhances IFN-γ release (Paul et al., 2016), which induces tumour killing together with the released cytotoxic membrane-disturbing proteins, perforin and granzyme, without prior sensitization or identification of a specific antigen (Liu et al., 2012). However, in most cases, tumour-infiltrating NK cells are unable to eradicate MHC I-deficient tumour cells, as tumour cells promote shedding of NKG2D ligand (eg. MICA-B and ULBP1-6) as a mechanism of immune escape, significantly reducing NK activation (Iannello et al., 2016).

- Increased expression of prosurvival or growth factor genes (e.g., Bcl-2, Her2/neu) (Das et al., 2015) or upregulation of CTL resistance pathways, such as inhibitory checkpoints that control T-cell differentiation (e.g., CTLA-4) and function (e.g.PD-1) on immune effector cells (Teng et al., 2015). IFN-γ, produced during effector immune anti-tumour activity upregulates the expression of PD-L1 on tumour cells and on immune

cells of TME, attenuating T cell activity by its binding to PD-1 present on activated T cells (Krempski et al., 2011; Teng et al., 2015).

- Establishment of an immunosuppressive TME via production of cytokines, such as TGF-β, IL-4, IL-6, IL-13, IL-8, IL-10, and metabolic factors (e.g., adenosine, prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO)) (Spranger et al., 2013), mainly produced by myeloid cells from the TME (eg. tumour-associated macrophages (TAMs), myeloid derived suppressor cells (MDSCs)). FOXO3 is overexpressed in murine prostate cancer DCs. It causes an increase in the expression of IDO, arginase, and TGF-β while decreasing the expression of costimulatory molecules (Watkins et al., 2011). They promote a change in tumour stroma and an immunosuppressive environment, disrupting the development of anti-tumour immune actions, promoting Th2 CD4+ T cells, Tregs, and M2-polarized macrophages (Tran Janco et al., 2015).

The role of the immune system in the setting of cancer is not straightforward. Cancer immunoediting illustrates how the immune system can limit and/or boost tumour growth. Indeed, cancer is visible to the immune system during early neoplasia identifying and rejecting it**.** This opens up immunotherapeutic possibilities to restore anticancer immunity in the TME (Speiser et al., 2016).

2.a. Vitamins A, C and D as immune modulators of anti-tumoral response

- **Vitamin A**

Retinoids have become viewed as a potentially effective chemotherapeutic and chemopreventive agents. Retinoids target neoplastic cells directly by inducing differentiation and limiting cell proliferation by halting the cell cycle at G1 phase. ATRA has been used successfully in the treatment of acute promyelocytic leukemia (APL) (Y. S. Kim et al., 2015). ATRA has the potential to induce differentiation of HL-60 cells, leukaemia cell line, to macrophages. ATRA also causes post-maturation apoptosis in APL-blasts, sensitizing human cancer cells to TRAIL-induced apoptosis by up-regulating transcription of TRAIL-R1. The ability of ATRA to stimulate TRAIL-R1 expression was decreased by RAR antagonists but was enhanced by various RAR agonists (Dhandapani et al., 2011). Beside its effect on APL, retinoids' chemopreventive and therapeutic

benefits in solid tumours remain debatable (Connolly et al., 2013). RA has been shown to suppress mammary carcinogenesis in mice and reduce human breast cancer proliferation (Ribeiro et al., 2014). However, resistance mechanisms have been proposed. RA action in cancer cells is influenced by decreased retinoid uptake, enhanced retinoid degradation by cytochrome P450 (Su et al., 2015), active drug efflux and downregulation of RAR expression (Y. S. Kim et al., 2015). The methylation rate of the *RARβ* gene is higher in solid tumours, such as breast cancer, than in normal tissues, resulting in diminished or lost expression (Sun et al., 2011). Altogether this suggests that the use of RARα agonists would be preferable to the use of RAR pan-agonists, such as RA, which also activates the RARγ and promotes tumour growth, as opposed to the others, which mediate antiproliferative effects (Ribeiro et al., 2014).

- **Macrophages**

Retinoids are also important in the anti-tumour process because they help the regulation of differentiation, recruitment and polarization of macrophages. This is important once macrophages play a critical role in cancer development (Jiang et al., 2018). Fenretinide, a synthetic derivative of ATRA, is an effective cancer treatment, owing to its anti-angiogenic and pro-apoptotic properties (Mittal et al., 2014). It accumulates specially in breast tissue and has minimal toxicity, triggering apoptosis by producing ROS. In colon cancer, fenretinide showed to block M2 macrophage polarization by inhibiting the phosphorylation of STAT6 (Dong et al., 2017). The suppression of M2 macrophages could be one of the mechanisms that lie beneath the impact of retinoids (Dobrotkova et al., 2018). RA decreases TAMs production of VEGF and IL-8 leading to a slower growth and reduction in vascularity (Jiang et al., 2018). RA also inhibits the recruitment and activation of monocytes involved in tumour angiogenesis, by directly limiting migratory chemotactic factors such as CCL2 and TGF-β1. Although, until this point, RA has been thought to be an anti-cancer agent, Devalaraja et al. showed its tumorigenic capability. Blocking RA synthesis or signalling in TME increases the number of immunostimulatory DCs by promoting the expression of its transcription factor Irf4, improving T cell-dependent anti-tumour immunity, which might have an synergistic effect with immune checkpoint blockade (Devalaraja et al., 2020).

- **T cells**

RA signalling-deficient CD8+ T cells were unable to expand, accumulate and produce IFN-γ, resulting in increased tumour expansion (Guo et al., 2012). One can wonder if RA in TME, rich in suppressive cytokines (e.g. TGF-β), particularly at late stages of tumour progression, could stimulate Tregs. However, when RA signalling was inhibited in T cells, no significant alterations were seen in Tregs in TME (Guo et al., 2012). Extra effort should be put into studying vitamin A signalling-deficient Tregs in TME to see if Treg functioning in TME is dependent on vitamin A signalling. In figure 4 a summary of the effect of VA in TME is presented.

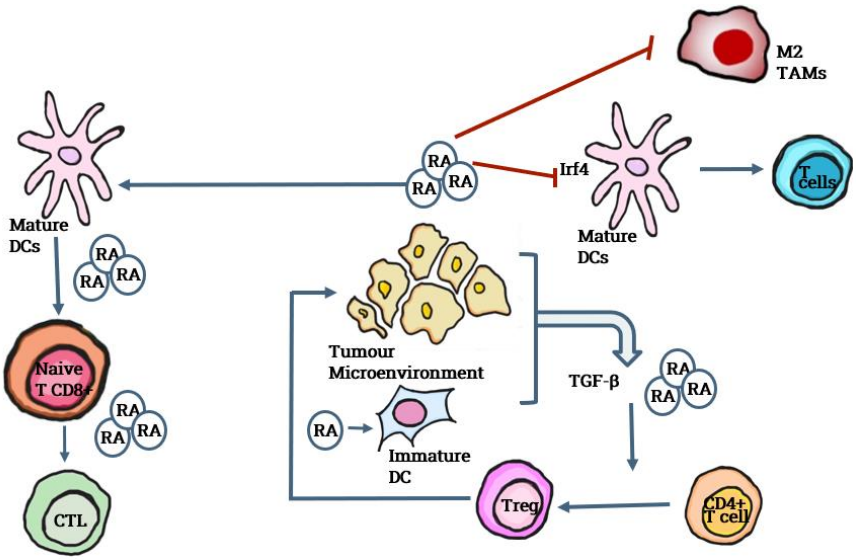

Figure 4- *Summary of vitamin A action on immune cells in cancer environment*

- **Vitamin C**

Cancer patients have lower plasma VC levels than healthy people (Huijskens et al., 2016). Despite recent indications suggesting an anti-tumour effect for high-dose ascorbate, its use in cancer remains controversial, since potential mechanisms of action are still unknown and lack convincing data (Campbell et al., 2016). Tissue features such as high cell density, poor perfusion (Kuiper et al., 2014), and reduced expression/activity of transporters are all limiting factors for ascorbate uptake in malignancies (Shenoy et al., 2018). The mode of VC administration can result in

substantial varied plasma concentrations. In humans oral absorption of ascorbate is closely restricted and bioavailability of VC raises when taken intravenously, which is well tolerated (Stephenson et al., 2013). VC revealed a double-edged sword effect on melanoma, depending on its concentration. Unlike intravenous VC, VC oral administration accelerated melanoma tumour growth in mice. Oral VC reaches lower plasma concentrations, which stimulated Braf and Akt pathways, contributing to its pro-melanoma impact. Yet, it is early to infer that eating fruits and vegetables on a daily basis increases the risk of melanoma, patients should be cautious using oral VC supplements (Yang et al., 2017).

Anti-cancer effect of VC is believed to be achieved by two mechanisms: H2O2-induced oxidative stress and DNA demethylation mediated by TET enzymes activation. VC works as an antioxidant at physiological concentrations. However, at high/pharmacological concentrations (> 100 µM) achievable intravenously, it functions as a pro-oxidant, promoting the creation of significant levels of ROS, such as H2O2. ROS are cytotoxic to tumour cells *in vitro*, causing DNA damage and ATP depletion. This leads to ATM/AMPK activation and mTOR inhibition (Gillberg et al., 2018; Ma et al., 2014). While normal cells have enough catalase to impair H2O2, cancer cells, which absorb VC preferentially, have decreased catalase activity. As a result, they store up more H2O2 than normal cells (Lee et al., 2019). High amounts DHA, absorbed via enhanced GLUT1 expression, cause an energy crisis due to ROS accumulation, which disables the glycolytic enzyme GAPDH in human colorectal cancer cells with KRAS or BRAF mutations, resulting in cell death (Yun et al., 2015). However, there is little evidence of its activity in tumours *in vivo*. Nevertheless, intravenous VC inhibited tumour growth and eradicated liver CSCs, due to their high SVCT-2 expression. It resulted in cell cycle arrest and apoptosis, associated with better disease-free survival (Cho et al., 2018; Lv et al., 2018)**.**

VC is also a cofactor for hydroxylases responsible to disable the expression of the transcription factor hypoxia-inducible factor (HIF-1). As solid tumours grow, areas of inadequate vascularization emerge, which leads to hypoxia, activating hypoxia-inducible factor 1 (HIF-1) (Ratcliffe, 2013). HIF-1 regulates proteins that promote angiogenesis, such as VEGF, which promotes cancer cell resistance to chemo- and

radiation treatment. Tumours with increased ascorbate levels, on the other hand, had less HIF-1 protein and decreased expression of downstream gene products (Kuiper et al., 2014; Kuiper & Vissers, 2014). Therefore, increased VC availability enhances HIF hydroxylase activity, which leads to decreased HIF-1 levels. Consequently, it diminished VEGF levels and angiogenic activity, resulting in slower tumour growth (Campbell et al., 2015, 2016; Dachs et al., 2016). Currently, there are a number of human clinical trials aiming to investigate the clinical anti-tumour value of VC (Jacobs et al., 2015; Ma et al., 2014; Stephenson et al., 2013; J. L. Welsh et al., 2013). It was demonstrated that intravenous ascorbic acid enhances the anticancer activity of gemcitabine against human and murine pancreatic cancer cells. Besides, it is safe and well tolerated by cancer patients. This advocates the use of VC in conjunction with cytotoxic chemotherapy (Espey et al., 2011; Monti et al., 2012; J. L. Welsh et al., 2013). It is uncertain how the growth delay is affected. The appropriate dose or schedule is also unknown. This mechanistic knowledge should lead to more rigorous clinical trial design (Campbell et al., 2016)**.**

The discovery of VC as a co-factor of epigenetic regulators, such as TET and Jumonji dioxygenases led to a novel perspective on the possible VC activity in cancer (Gillberg et al., 2018). During the development of many solid cancers, normal epigenetic regulation is impaired, and DNA methylation is one of the earliest and prevalent occurrences. The 5-hydroxymethylcytosine (5hmC) is the first oxidative result in the active demethylation of 5-methylcytosine (5mC) by TET enzymes, which levels are much decreased in diverse malignancies, indicating a reduction/loss of TET activity (Haffner et al., 2011).This could be due to inactivating mutations, decreased TET gene expression, or a lack of TET cofactors. Furthermore, in cancer TME the release of free radicals consumes VC and enough VC may be critical to maintain normal 5hmC levels and improve response of epigenetic therapy (Gillberg et al., 2018). Xu et al., showed that TET2 drives the IFN-γ/JAK/STAT/TET signalling pathway, which controls chemokine, PD-L1 expression, lymphocyte invasion and consequently cancer immunity. IFN-γ stimulation activates JAK, resulting in phosphorylation and nuclear translocation of the STAT1 transcription factor, promoting TET2 recruitment, binding to STAT1. The STAT1-TET2 interaction activates STAT1 target genes. When TET2

expression is reduced or deleted, the expression of PD-L1, Th1-type chemokine genes, CXCL9, CXCL10, and CXCL11 are reduced. Both PD-L1 and chemokine genes are repressed by DNA methylation in noninflamed cells and are activated by TET2-mediated demethylation after inflammation and IFN-γ stimulation. Lower/deletion of TET action was linked to lower levels of Th1-type chemokines and tumour-infiltrating lymphocytes, allowing tumours to escape antitumor immunity and resist anti-PD-L1 therapy, promoting its growth. TET activity is enhanced by VC, raising chemokines and tumour-infiltrating lymphocytes, resulting in enhanced antitumor immunity and anti-PD-L1 therapy efficacy, as well as extended lifetime of tumour-bearing animals (Xu et al., 2019). The loss of TET activity or mutations in JAK2 and JAK1 inactivate the IFN-γ/ JAK/STAT/TET pathway which confers resistance to anti–PD-1/PD-L1 therapy. Nevertheless, only a small percentage of anti–PD-1 resistant tumours were discovered to have loss-of-function mutations in this IFN-γ/JAK/STAT/TET genes (Shin et al., 2017; Xu et al., 2019; Zaretsky et al., 2016). The VC as non-toxic epigenetic therapy for cancer prevention and treatment has increased the importance of this vital vitamin in human biology (Cimmino et al., 2018). Although, there is no robust scientific evidence showing the effectiveness of vitamins on cancer, VC showed cytotoxic activity against some cancer cell types, including breast cancer, melanoma, and colon cancer, but not to normal tissues (Cha et al., 2013; Yang et al., 2017; Yun et al., 2015). Despite breakthroughs in early identification and cancer treatment (surgery, cytotoxic, hormonal, biological drugs, etc.), still many patients develop recurring disease after curative surgery. *In vitro*, combining high-dose of VC with standard anti-cancer medicines, showed therapeutic benefits against cancer cells (Lee et al., 2019). Intravenous VC has been shown to inhibit ovarian cancer in animal models acting synergistically combined with carboplatin and paclitaxel, since the concentration of chemotherapy that produces cell killing could be decreased and increased cancer-free survival time. It also diminished chemotherapy-related neurologic, dermatologic, and bone marrow damage in ovarian cancer patients (Ma et al., 2014).

- **DC and T cells**

DCs treated with AA generated higher Th1 cytokine and IFN-γ, together with less Th2-cytokines, boosting tumour effect of CD4+ and CD8+ T cells (Jeong et al., 2011, 2014).

Adoptive transfer of CD8+ T cells was only able to impair tumour growth when lymphocytes were obtained from high-dose VC-treated mice. This implies that the impact of VC in tumour growth relies on T cells, especially CD8+ T cells, although in this VC-treated model, the presence of CD4+ T cells also plays important role to trigger the cytotoxic potential of CD8+ T cells (Magrì et al., 2020). Therefore, high-dose VC modulates infiltration of the TME by cells of the immune system and delays cancer growth in a T cell–dependent manner. A fully competent immune system is required to maximize the anti-proliferative effect of VC in tumour growth (Magrì et al., 2020).

- **NK cells**

NK cells isolated from ovarian cancer-bearing and AA-depleted mice revealed a significant drop in killing power *in vitro,* with less IFN-γ and lower synthesis of the cytolytic proteins perforin and granzyme B, when compared to AA-supplemented mice. Accordingly, NK cells from AA-depleted mice had lower levels of CD69 and NKG2D activating receptors (J. E. Kim et al., 2012). Thus, AA seems to be important for normal NK cell function. This might be significant for cancer treatment, especially after chemotherapy, where NK cells could provide some protection while T cells have not yet recovered from the treatment (Van Gorkom et al., 2018)

- **Macrophages**

VC is a cofactor of hydroxylases which lessens the expression of the transcription factor HIF-1. This is important since HIF-1 activation is required for monocyte function, leading to M2 macrophages formation in TME (Colegio et al., 2014). When expressing HIF cancer cells gain properties to evade immunological attack by suppressing macrophage phagocytosis (Fu et al., 2020)**.**

Further research into the specific action mechanism of VC is required. The evidence for AA's anticancer effects in human cancer was restricted to case reports and observational and uncontrolled research. Despite this encouraging information, there is no high-quality evidence that VC supplementation improves the effect of chemotherapy or lessens its toxicity in cancer patients. Therefore, high-quality double-

blind placebo-controlled *in vivo* trials are required before VC can be advised (Jacobs et al., 2015). In figure 5 a summary of the effect of VC in the TME is presented.

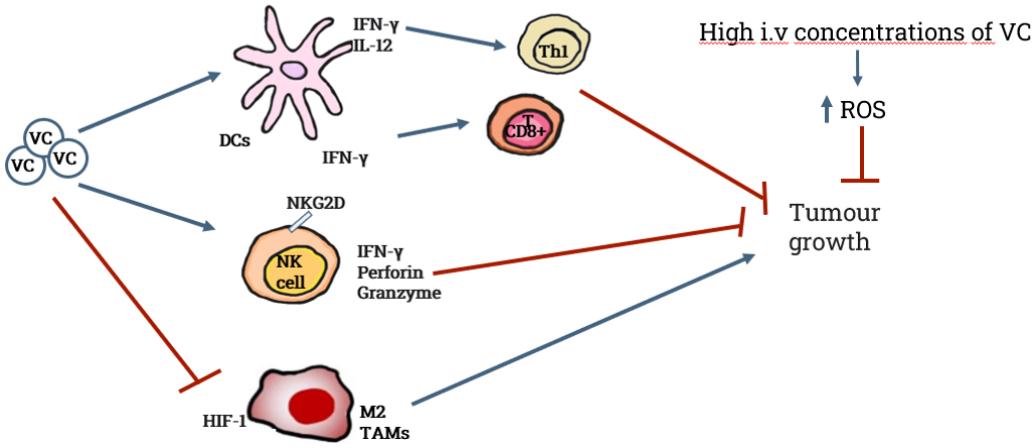

Figure 5- *Summary of VC action on immune cells in cancer environment*

- **Vitamin D**

Preclinical data from breast, prostate, and colon cancer cells strongly suggest that VD deficiency increases the risk of developing cancer, and that avoiding deficiency or supplementing with vitamin D may be an inexpensive and safe way to reduce cancer incidence and improve cancer prognosis and outcome. Several cancer cells have abnormally high basal expression levels of 24-hydroxylase, rendering them resistant to calcitriol activity (Feldman et al., 2014). Increased VDR expression, required for VD activity, in breast and prostate tumours is related with a better prognosis and lower risk of dying (Ditsch et al., 2012; Hendrickson et al., 2011).

  - **T cells**

Epidemiological studies concerning VD and cancer revealed that areas of the world distant from the equator, with less sunlight, had an increased incidence of colon and prostate cancers, implying that VD insufficiency is a risk factor for cancer (Feldman et al., 2014). In agreement, animal studies revealed that severe VD deficiency, or deletion of the VDR gene (Zheng et al., 2012), rises cancer risk, increasing tumour cells proliferation and reduced apoptosis (Kovalenko et al., 2011). Immune cells in TME can convert 25(OH)D to bioactive calcitriol. In tumours with strong immune infiltration,

high plasma 25(OH)D levels associate with lower risk of colorectal cancer. This suggests a role of VD in cancer immunoprevention through tumour–host interaction (M. Song et al., 2016). However, a completed multicentric, randomized, placebo-controlled study showed that VD supplement did not lower incidence of invasive cancer compared to placebo (Manson et al., 2019). Nevertheless, Karkeni et al. demonstrated *in vivo* modulation of breast cancer tumour development and inflammation, by injecting a mouse model with VD. The VD-treated group displayed increased tumour-infiltrating CD8+ T cells, with greater granzyme B and PD-1 expression levels, revealing a more active profile than the control group. Interestingly, in high-fat diets, VD had the reverse effect, with a decrease in CD8+ T cell infiltration. This discloses the significance of nutrition in tumour formation. Thus, depending on the diet, VD supplementation has contrasting effects on CD8+T cells and consequently on tumour progression (Karkeni et al., 2019). This could be justified by a change in VD metabolism in adipocytes, namely 25-hydroxylase in overweight mice. This increases 25-hydroxylation, increasing local synthesis of 25(OH)D in adipose tissue (J. M. Park et al., 2015). It causes a drop in plasma 25(OH)D, which may decrease CD8+ T cell infiltration. In situations where VD favours access for cytolytic T cells to the tumour site, VD supplementation could be used with immune checkpoint immunotherapy (T-cell activation inducer) in the treatment of breast cancer, only if these findings are confirmed in human randomized clinical trials (Karkeni et al., 2019).

Chronic inflammation in the gut has showed to be a risk factor for colon cancer in humans. Animal studies have connected anti-cancer properties of VD, by modulating inflammation and immunological responses in the gut. According to clinical data, VDR is expressed in early stages of colon cancer but decreases during colon cancer growth. Its downregulation has been connected with the upregulation of transcriptional repressors such SNAIL, which blocks the VDR promoter directly (J. Welsh, 2012). In addition, mice fed with a western-style diet (poor in VD) develop benign and malignant colonic tumours. The western-style diet boosts wnt signalling, critical to maintain adequate stem cell development along the crypt-villus axis. *In vitro*, VD inhibits the release of IL-1b by TAMs, which stimulates wnt signalling in colon cancer cells; however, this has not been studied *in vivo* (J. Welsh, 2012).

2.b. Immunotherapy

Immunotherapy has gained global attention and came out as the “new hope” for cancer treatment, once immune evasion is an important characteristic of cancer. Oncoimmunology is an evolving field, as indicated by the success and development of immunotherapies, using the immune system to combat cancer. As each tumour produces its unique immunosuppressive TME, cancer immunotherapy must be tailored to each patient (Whiteside et al., 2016). T cells face several inhibitory stimuli within tumours, limiting their activation, proliferation, and effector actions. Immune checkpoints, such as the programmed cell death protein 1 (PD-1) or CTLA-4) are co-inhibitory receptors on T lymphocytes. For that reason, this inhibitory immune checkpoints are possible targets for immune checkpoint blockade (ICB), enabling inhibited T cell activity to be reactivated. ICB, one of many immunotherapeutics, has shown exceptional efficacy in a variety of cancers (Hamid et al., 2013; Topalian et al., 2012). ICB has received a lot of attention once some cancer cells use physiological pro-tolerogenic pathways, such as PD-L1/PD-1, to avoid immune detection. PD-L1 is commonly overexpressed on cancer cells, endothelial cells and myeloid cells impairing T cell activity, due to its binding of PD-1 on activated T cells. Expression of PD-L1 in the TME is associated with a worst prognosis, cancer proliferation, despite adequate tumour-infiltrating lymphocytes (Topalian et al., 2012).

ICB has good results in tumours with high mutational load associated to environmental exposure, such as melanoma (Snyder et al., 2014), bladder, and lung cancers and/or with deactivation of DNA mismatch repair (Le et al., 2017), increasing tumour neoantigens. This may trigger immune surveillance, which can be exacerbated by ICB therapy (Germano et al., 2017). Surprisingly, several individuals with high mutation burden do not respond to ICBs (Y. J. Park et al., 2018). Other variables and patient characteristics may also modulate immunotherapy response. Overall, *in situ* immune infiltration is crucial for tumour elimination. Still, not all immune infiltrates are equal, the quality of the immune response is a vital for treatment effectiveness.

2.c. Vitamins A, C and D as complementary/supportive therapy in cancer checkpoint immunotherapy

Given their potency, the efficacy of ICB can be limited by immune-related adverse effects (irAEs). The presence of irAEs indicates that ICB has activated the patient's immune system, but it is unclear if it matches increased anticancer immunity. Almost any organ can be affected irAE, usually involving the gastrointestinal tract, endocrine glands, skin, and liver. Less frequently can lead to type 1 diabetes and myocarditis (Johnson et al., 2016). Hence, the aim is to develop safe combinations to improve the efficacy of ICB and limit irAE.

- **Vitamin D**

Given that VD has been shown to reduce T cell-mediated immune responses and once irAE are likely due to enhancement of the immune system, there is growing interest in combining VD with ICB to alleviate them. VD could be an interesting substitute for the main treatment for irAEs, that is corticosteroids. In addition, VD through VDR promotes transcription of *PD-L1* gene, increasing its expression in human epithelial and myeloid cells, blocking anti-tumour activity of T cells (Dankers et al., 2017; Dimitrov et al., 2017). Thus, it is plausible that concomitant VD and ICB administration may help to enhance the efficacy of ICB and simultaneously attenuate irAEs. VD leads to a more tolerogenic phenotype of DCs, with increased expression of IL-10, TGF-β, inhibiting the activation of effector T cells response and enhancing Tregs towards a regulatory phenotype (Ferreira et al., 2011; Kang et al., 2012). However in a breast tumour model study VD increased the tumour-infiltrating active CD8+ T cells (Karkeni et al., 2019). This shows that future research is needed to examine the direct possible beneficial potential of VD for cancer patients on irAE-induced by cancer immunotherapy.

- **Vitamin C**

Research has shown that VC may boost the immune response of anti-PD-L1 therapy (Fu et al., 2020). IL-6 maintains PD-L1 stability (Chan et al., 2019). IL-6 is suppressed by VC-activated TET 2 decreasing inflammation (Q. Zhang et al., 2015), which can boost

anti-PD1 ICB action. Interestingly, combination of VC and anti-PD1 treatments enhances intratumoral cytotoxic T cells and NK cells actions pointing to a strong immunological activity (Q. Zhang et al., 2015). Thus, VC might be a potential adjuvant therapy enhancing anti-PD-1/PD-L1 immunotherapy sensitivity (Magrì et al., 2020). In light of this, a possible decrease of the doses of ICB in combination with VC may be investigated, in order to reduce toxicity or adverse effects caused by ICB.

No evidence of vitamin A possibly influencing ICB was found.

Conclusion

Optimal levels of vitamins A, C and D are required to ensure suitable immune functions. Vitamin A is important for lymphoid organ development, inducing gut homing receptors on T cells unlike vitamin D that suppresses immune responses. Vitamin A showed to primarily promote the generation of Tregs, while inhibiting Th17 cells and was essential for the survival of effector CD8+T cells. Vitamin D appears to have a preponderance towards tolerance induction, controlling CTL proliferation as low vitamin D seems to be associated with inflammation and dysregulated immune function. Conversely, vitamin C besides its antioxidant effect, also acts as a cofactor for multiple gene regulatory enzymes with immune-modulating effects, increasing effector actions of NK cells, Th1 and CTL. Therefore, inadequate vitamin intake has been shown to possibly disrupt host immunity and predisposing humans to inflammatory diseases and cancer. Chronic inflammation is a hallmark of cancer, that promotes an immunosuppressive environment, allowing cancer cells to evade immune destruction. Although VC may appear the most promising in cancer, caution needs to be taken as by epigenetic mechanisms it is also capable of inducing Tregs. Despite breakthroughs in early identification and cancer treatment, there are still many patients that develop recurring disease. Therefore, it would be beneficial to include in the staging of cancer, the kind of immune cells infiltrated promoting inflammation guiding other potential therapies such as immunotherapy.

To answer our initial question, although there is evidence pointing to the potential of vitamins as adjuvant therapies in cancer, there is a lack of randomized, double-blind human clinical trials supporting this information, and consequently, supporting their

use in clinical practice. Ultimately, the complex interactions and mechanism of action of vitamins in the regulation of the immune system in steady state or in cancer are worthy of continued investigation, to understand the underlying mechanisms and develop therapeutic combinations to improve treatments in immune-influenced diseases, such as cancer.

Annexes

Target/ Drug Name	Company	Identifier	Tumour-type	Therapy partner	Phase
			Vitamin A		
Tretinoin	Ohio State University Comprehensive Cancer Center	NCT04919369	Non-Small Cell Lung Carcinoma	Atezolizumab	1
Vesanoid	University of Colorado, Denver	NCT02403778	Melanoma	Ipilimumab	2
			Vitamin C		
Vitamin C	Fudan University	NCT04516681	Colorectal Cancer	FOLFOXIRI	3
Ascorbic acid	National Cancer Institute (NCI)	NCT02516670	Prostate Cancer	Docetaxel	2
Vitamin C	Clifford Hospital	NCT03799094	Non-Small-Cell Lung carcinoma	Tyrosine kinase inhibitor	1, 2
Ascorbic acid	National Cancer Institute (NCI), National Institutes of Health (NIH), Holden Comprehensive Cancer Center, McGuff Pharmaceuticals, Inc	NCT02420314	Non-Small-Cell Lung carcinoma	Paclitaxel Carboplatin	2

Ascorbic acid	National Institutes of Health (NIH), National Cancer Institute (NCI), Holden Comprehensive Cancer Center, McGuff Pharmaceuticals, Inc	NCT02905578	Pancreas Cancer	Gemcitabine Paclitaxel	2
Vitamin D					
Cholecalciferol	National Cancer Institute (NCI)	NCT04677816	Triple Negative Breast Cancer	Doxorubicin Cyclophosphamide paclitaxel	2
Cholecalciferol	National Cancer Institute (NCI)	NCT04094688	Colorectal Adenocarcinoma	Bevacizumab Oxaliplatin Leucovorin Fluorouracil Irinotecan Hydrochloride	3
Paricalcitol	Stand Up To Cancer, Lustgarten Foundation, American Association for Cancer Research	NCT03520790	Pancreatic Cancer	Gemcitabine Paclitaxel	1,2

Table: Examples of clinical trials assessing the potential role of vitamins in cancer. This list only includes active and/or recruiting, interventional trials from http://clinicaltrials.gov/ until 01 May 2022, on solid tumours (Melanoma, Lung, Colon, Breast, and Pancreas). Of note, trials prioritising combinatorial

therapies were selected over the individual therapy, and approaches were not repeated once cited from a given study, even when promoted from different pharmaceutical companies.

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