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Mini-review

Treg-mediated acquired resistance to immune checkpoint inhibitors

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ABSTRACT

T Regulatory cells (Tregs) act as a double-edged sword by regulating immune homeostasis (protective role) and inhibiting immune responses in different disease settings (pathological role). They contribute to cancer development and progression by suppressing T effector cell (Teff) functions. Decreased ratios of intratumoral $CD8^+$ T cells to Tregs have been associated with poor prognosis in most cancer types. Targeting immune checkpoints (ICs), such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death-1 (PD-1), by immune checkpoint inhibitors (ICIs) in cancer patients has been beneficial in inducing anti-tumor immune responses and improving clinical outcomes. However, response rates remain relatively low, ranging from 15 to 40% depending on cancer type. Additionally, a significant proportion of patients who initially demonstrates a clinical response can acquire resistance overtime. This acquired resistance could occur due to the emergence of compensatory mechanisms within the tumor microenvironment (TME) to evade the anti-tumor effects of ICIs. In this review, we describe the immunosuppressive role of Tregs in the TME, the effects of currently approved ICIs on Treg phenotype and function, and the mechanisms of acquired resistance to ICIs mediated by Tregs within the TME, such as the over-expression of ICs, the up-regulation of immunosuppressive molecules, and apoptotic Treg-induced immunosuppression. We also describe potential therapeutic strategies to target Tregs in combination with ICIs aiming to overcome such resistance and improve clinical outcomes. Elucidating the Treg-mediated acquired resistance mechanisms should benefit the designing of well-targeted therapeutic strategies to overcome resistance and maximize the therapeutic efficacy in cancer patients.

1. Introduction

In the light of cancer immunoediting hypothesis, cancer cells are continuously evolving to become less immunogenic, and to establish a network of immunosuppressive soluble and cellular components capable of inducing several inhibitory mechanisms to evade anti-tumor immunity, and promote tumor growth/progression [1–3]. Reduced expression of cancer antigens (i.e. neoantigens or non-self-antigens) and major histocompatibility complex class I (MHC I) is a mechanism by which cancer cells escape T effector cell (Teff) recognition, in particular cytotoxic $CD8^+$ T cells. Other mechanisms include up-regulated expression of immune checkpoints (ICs) or their ligands, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1), increased recruitment and activation of immunosuppressive cells, such as Tregs, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs; a heterogeneous population of immature granulocytes and monocytes), within the tumor microenvironment (TME) [4,5].

Tregs are immunosuppressive subset of T cells identified as $CD4^+CD25^+$ (the α-chain of IL-2 receptor), characterized by the expression of the master regulatory transcription factor, Forkhead box protein P3 (FoxP3), which is critical for the development and differentiation of Tregs [6–8]. Tregs exert pathological roles in different diseases [9]. In cancer, they promote tumorigenesis by exerting suppressive activities on effector cells, causing inactivation or apoptosis [8,10,11]. In the peripheral blood, about 20–50% of circulating $CD4^+$ T cells in melanoma, NSCLC, and gastric and ovarian cancers are Tregs; in contrast, 5–10% of circulating $CD4^+$ T cells in healthy individuals are Tregs [8,12]. In the TME, high numbers of Tregs has been positively correlated with poor prognosis in patients with melanoma, head and neck squamous cell carcinoma (HNSCC), pancreatic, colorectal, breast, ovarian and lung cancers [13,14].

Immunotherapies, such as immune checkpoint inhibitors (ICIs), have revolutionized the treatment of advanced stages of melanoma and some solid tumors [15,16]. As a monotherapy or combined therapy, the administration of monoclonal antibodies (mAbs) targeting CTLA-4 and PD-1/PD-L1 in cancer patients showed clinical efficacy in several

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cancer types, such as melanoma, non-small cell lung cancer (NSCLC), Hodgkin's lymphoma, renal cell carcinoma, and HNSCC [15,17–22]. It is important to point out that the therapeutic efficacy of ICIs and clinical response are dependent on the nature of the tumor. Tumors characterized by the absence of T cells or the lack of suitable T cell priming or activation molecules are commonly referred to as “cold tumors” or non-immunogenic, such as those from prostatic and pancreatic cancers [23–25]. These tumors demonstrate limited response rates to ICIs, predominantly, due to primary resistance and intrinsic-tumor factors [23–25]. Conversely, melanoma and lung cancer show more durable response rates to ICIs and are commonly referred to as “hot tumors” or immunogenic tumors characterized by a high number of tumor-infiltrating lymphocytes (TILs) [23,24,26]. However, a significant proportion of patients with “hot tumors” who initially respond, develop resistance to therapy overtime; hence it is called secondary or acquired resistance [22,23,27]. Tregs play a central role in the development of acquired resistance to ICIs leading to tumor relapse and poor prognosis [28–32]. Therefore, it is important to elucidate the mechanisms which trigger the Treg-mediated acquired resistance to overcome them, and maximize the clinical response and therapeutic efficacy in cancer patients.

In this review, we describe the immunosuppressive role of Tregs in the TME, the effects of currently approved ICIs on Treg phenotype and function, the mechanisms of Treg-mediated acquired resistance against ICIs, and strategies for the use of combined therapies that target Tregs in addition to ICs to overcome resistance and improve clinical outcomes.

2. Role of Tregs in cancer

Circulating naïve Tregs, defined as $CD4^+CD45RA^+CD25^-FoxP3^{low}$, possess weak immunosuppressive activity. Upon activation in the periphery (for example, tumor sites), naïve Tregs proliferate and differentiate into effector Tregs ($CD4^+CD45RA^-CD25^+FoxP3^{high}$) with a high immunosuppressive activity, and an up-regulated expression of CD25 and FoxP3 [33]. However, it remains a challenge to define Tregs based on CD25 and FoxP3 expression, due to the heterogeneity of their phenotype and activity in human [12].

Within the TME, Tregs are highly activated and immunosuppressive, characterized by an up-regulated expression of FoxP3 [11], and suppressive molecules, such as transforming growth factor-beta (TGF-β) and interleukin (IL)-10 [34], and ICs, such as CTLA-4, PD-1, T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lymphocyte activation gene-3 (LAG-3) and T-cell immunoreceptor with Ig and ITIM domains (TIGIT) [13]. As it will be discussed in the next Sections, increased numbers of intratumoral Tregs expressing ICs inhibit the proliferation and activation of cytotoxic $CD8^+$ T cells and $CD4^+$ effector T cells within the tumor, and result in the production of TGF-β and IL-10 favoring the survival and expansion of Tregs. Despite this, Tregs can augment immunosuppression by establishing a cellular network with other immunosuppressive cells within the TME, such as TAMs, MDSCs, B regulatory cells (Bregs) and cancer-associated fibroblasts (CAFs) [35]. For instance, Tregs can induce the differentiation of monocytes into TAMs (M2 macrophages with an immunosuppressive phenotype) by secreting IL-10 [36]. In addition, Tregs produce high levels of TGF-β which, in turn, triggers the conversion of resident fibroblasts into CAFs [35]. CAFs are capable of inducing $CD8^+$ T cell apoptosis, thereby promoting tumor evasion from cytotoxic T cells [37]. Treg-derived TGF-β can also act on natural killer (NK) cells to inhibit their cytotoxic activity, suggesting another mean by which Tregs contribute to the immunosuppressive network within the TME [35]. Alternatively, Tregs suppress the anti-tumor immune responses by the consumption of IL-2 via CD25 to inhibit the differentiation and proliferation of Teffs [38,39]. In addition, Tregs can release granzyme B and perforin to induce cytolysis of Teffs and reduce their numbers in the tumor [40]. Together, these data highlight the central role of Tregs within the TME in limiting the number and activity of cytotoxic T and NK cells, enhancing the infiltration and differentiation of immunosuppressive cells, for example TAMs and CAFs. This in turn creates a positive feedback loop, which continuously induces immunosuppression and promotes the survival and expansion of Tregs, thereby leading to cancer progression and tumor immune evasion.

3. Immune checkpoints and the effect of their inhibitors on Tregs

3.1. CTLA-4 and PD-1

The negative correlation between CTLA-4 and PD-1/PD-L1 expression and the overall survival rate in various cancer cases has been well-established [41–43]. CTLA-4 expression is restricted to T cells; it is constitutively expressed on Tregs, and induced on Teffs upon activation [44]. PD-1, on the other hand, is widely expressed by activated T and B lymphocytes, NK cells, mast cells and myeloid dendritic cells [22,45]. Unlike CTLA-4 ligands, which are confined to antigen-presenting cells (APCs), ligands for PD-1, namely PD-L1 and PD-L2, are widely distributed and expressed by tumor cells, immune cells, non-lymphoid and non-hematopoietic cells [46–48].

It has been proposed that CTLA-4 and PD-1 act via distinct mechanisms and during different phases of T cell activation [44,49]. CTLA-4 on Tregs binds to B7 ligands (B7-1 and B7-2; also known as CD80 and CD86) on APCs with a greater avidity compared to CD28, which is present on T cells. This in turn induces an inhibitory signal for T cell priming (priming phase) [50,51]. Alternatively, CTLA-4 down-regulates the expression of B7 ligands by a phenomenon called trans-endocytosis, where the CTLA-4-B7 complex is internalized by Tregs [52,53]. Moreover, the binding of CTLA-4 on Tregs to B7 ligands induces the expression of indoleamine 2,3-dioxygenase (IDO), tryptophan catabolic enzyme, in APCs which results in the suppression of Teff activation and proliferation, and the expansion of Tregs [54–57]. Unlike CTLA-4, PD-1 acts on Teffs to render their proliferation and activation within tumor sites (effector phase) [45]. The function of PD-1 on Tregs remains unclear; however, there is some evidence that PD-1 increases the stability of Tregs by increasing FoxP3 expression [13].

Based on the above, CTLA-4 and PD-1 inhibit Teff activation via distinct mechanisms [49], therefore, their single blockade may also alter distinct signaling pathway(s) (Fig. 1). Initially, it was thought that anti-CTLA-4 mAb acts only during the priming phase of T cell activation in the lymph node, while anti-PD-1 mAb is more important to re-activate Teffs in the TME. However, subsequent pre-clinical studies and clinical studies showed that anti-CTLA-4 mAb is also capable of inducing effects on Tregs within the TME, and that anti-PD-1 mAb could have effects on peripheral Tregs (as will be discussed in the next Section).

Up to date, there are six drugs in the form of humanized monoclonal antibodies (mAbs) against inhibitory ICs have been approved for cancer treatment: anti-CTLA-4 mAb (ipilimumab), anti-PD-1 mAbs (pembrolizumab and nivolumab), and anti-PD-L1 mAbs (atezolizumab, avelumab and durvalumab) [58]. Some of the mechanisms by which anti-CTLA-4 and anti-PD-1/PD-L1 mAbs promote anti-tumor responses have been elucidated using mouse tumor models and human ex-vivo studies (Fig. 1).

3.2. Anti-CTLA-4

Anti-CTLA-4 mAb has shown effects on T cells within the TME (Fig. 1A). For example, Simpson et al. [31] reported that the co-administration of anti-CTLA-4 mAb and GVAX (tumor cell-based vaccine that secretes GM-CSF) is selective in depleting intratumoral $CTLA\text{-}4^+$ Tregs in B16 melanoma mouse model. Authors found that anti-CTLA-4 mAb induced the depletion of intratumoral $CTLA\text{-}4^+$ Tregs in a manner that is dependent on FcγRIV receptors, which are predominantly expressed by macrophages and myeloid cells, to trigger an antibody-

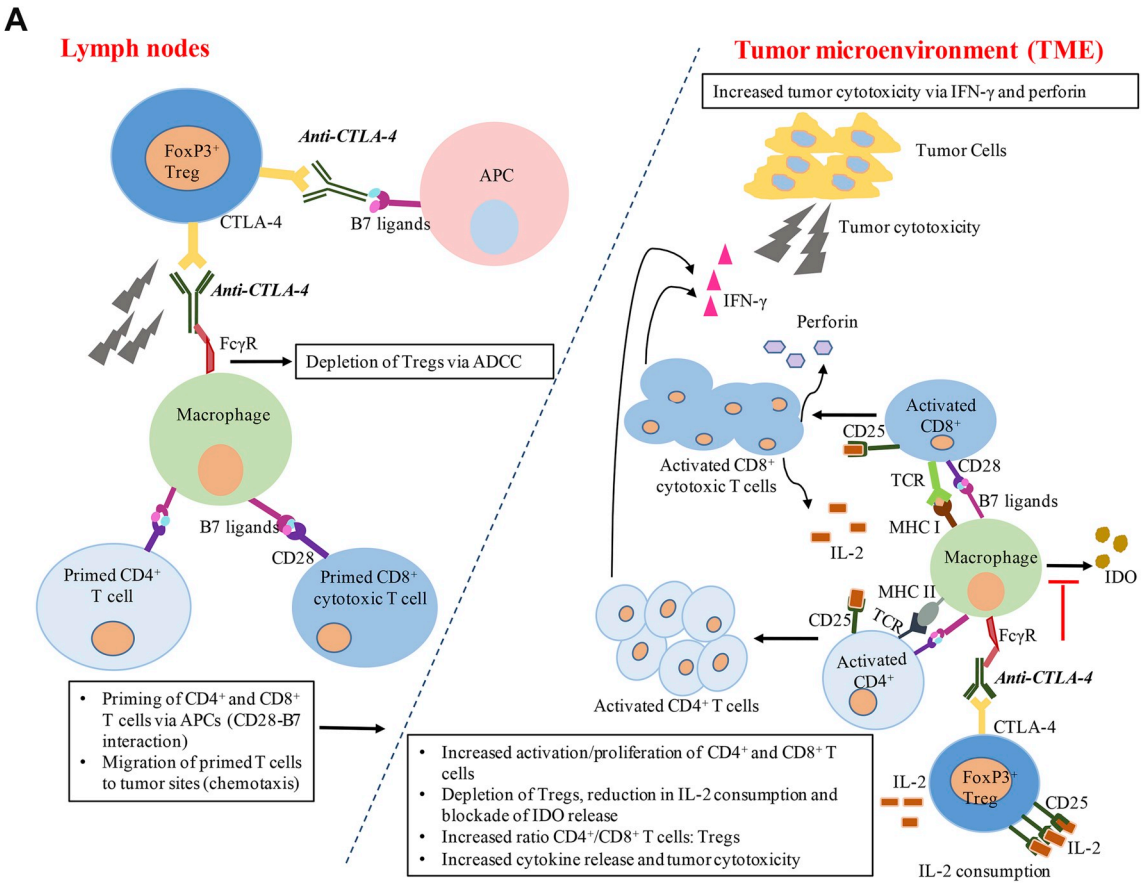

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Fig. 1. A proposed model of the therapeutic effect of blocking immune checkpoints. (A) In lymph nodes, anti-CTLA-4 mAb induces Treg depletion by binding to Fcγ receptors, which are predominantly expressed by macrophages, to trigger an antibody dependent cellular cytotoxicity (ADCC). Blocking of CTLA-4 allows the binding of B7 ligands (on APCs or macrophages) with CD28 on T cells, hence, it leads to enhanced priming of $CD4^+$ and $CD8^+$ T cells (priming phase), which subsequently migrate into the TME, via chemotaxis, where they become activated. Anti-CTLA-4 mAb also depletes intratumoral Tregs, reduces IL-2 consumption and allows Teff activation/proliferation in tumor sites, thereby increasing the ratio of Teffs:Tregs, production of IL-2, IFN-γ and perforin by $CD8^+$ T cytotoxic cells, and causing tumor cytotoxicity mediated by IFN-γ and perforin. Anti-CTLA-4 mAb blocks the release of immunosuppressive indoleamine 2,3-dioxygenase (IDO), tryptophan catabolic enzyme, by APCs, thereby promoting Teff activation and proliferation. **(B)** In the TME, anti-PD-1 or anti-PD-L1 mAb reduces the expression of FoxP3 in Tregs, thereby reducing Tregs stability/survival. Anti-PD-1 or anti-PD-L1 mAb blocks the inhibitory signal of $PD\text{-}L1^+$ tumor cells and re-activates $CD4^+$ and $CD8^+$ Teffs, and their subsequent proliferation and production of IFN-γ and perforin (effector phase). Antigen-presenting cell (APC); T cell receptor (TCR); Major histocompatibility complex class I (MHC I) or II (MHC II); Forkhead box protein P3 (FoxP3).

mediated dependent cellular cytotoxicity (ADCC) [31]. Similarly, ex-vivo studies by Romano et al. [59] demonstrated that anti-CTLA-4 mAb (ipilimumab) in melanoma patients depletes intratumoral Tregs in a manner that is dependent on FcγRIIIA receptor, which is expressed by $CD16^+$ monocytes, allowing for Tregs cytotoxicity and killing via ADCC. This was also confirmed in other studies demonstrating that the anti-tumor effects induced by anti-CTLA-4 mAb are completely dependent on the Fc domain, and that blocking the B7 and CTLA-4 interaction alone may not have a therapeutic effect [60,61]. Using a mouse model of melanoma and live imaging technology, Ingram and colleagues reported that expression of CTLA-4 is confined to the TME [62], suggesting that anti-CTLA-4 mAb could be used as a targeted therapy for the tumor tissue without affecting other tissue sites. Unlike its effects on intratumoral Tregs, Simpson et al. [31] found that the co-treatment with anti-CTLA-4 mAb/GVAX, in a mouse model of melanoma, increases the number of Tregs and $CD4^+$ Teffs in the lymph nodes. Consistent with this, Selby et al. [63] found that targeting CTLA-4 in CT26 and MC38 tumor-bearing mice (colon cancer models) also depletes intratumoral Tregs in a selective manner, without affecting Treg numbers in the peripheral blood and secondary lymphoid organs. The sensitivity of intratumoral Tregs to anti-CTLA-4 mAb in B16 melanoma and colon cancer models [31,63] could be attributed to the differential expression of CTLA-4 on intratumoral Tregs versus circulating Tregs or those present in lymphoid tissues. In other words, anti-CTLA-4 mAb selectively depletes Tregs in the tumors, but not in the blood, spleen and lymphoid organs, because Tregs in the TME express much higher levels of surface CTLA-4 than those present in the spleen/lymphoid organs or circulating in the blood. Another study by Kavanagh and colleagues demonstrated that anti-CTLA-4 mAb does not affect the number of circulating Tregs in prostate cancer patients, but rather increases the number of activated Teffs [64]. Collectively, these findings further highlight the advantage of using anti-CTLA-4 mAb as a targeted therapy for Treg depletion within the TME without altering their frequency in the circulation, spleen or lymphoid organs. This may also reduce the risk of unfavorable adverse events related to the lack of Tregs.

In both mice [63,65] and humans [66–69], anti-CTLA-4 mAb has been effective in increasing intratumoral Teff:Treg ratio and improving clinical outcomes. Other studies showed that the clinical outcome in melanoma patients treated with anti-CTLA-4 mAb is associated with an increase in the T cell receptor (TCR) repertoire diversity, primarily, in the memory T cells pool, which in turn increases the reactivity of T cells to tumor antigens [70–72].

3.3. Anti-PD-1

Like anti-CTLA-4 mAb, anti-tumor immune responses mediated by anti-PD-1 mAb are also associated with an increase in intratumoral Teff:Treg ratio and enhanced Teff functions, such as the secretion of IFN-γ and IL-2 [27] (Fig. 1). However, the signaling mechanisms by which anti-PD-1 mAb induces such effects are different to those mediated by anti-CTLA-4 mAb [44]. It is believed that upon the binding of PD-1 to PD-L1, inhibitory signals are generated to inhibit the TCR activating signal, thereby suppressing Teff proliferation and activation (as reviewed in Refs. [44,73]). The importance of PD-1 in peripheral Treg differentiation remains controversial [74,75]. Wang et al. [76] showed that blockade of PD-1 by pembrolizumab in peripheral blood mononuclear cells (PBMCs) from melanoma patients increases the proliferation and activation of cytotoxic T cells and down-regulation of FoxP3 in Tregs, implicating that PD-1 could have an effect on Tregs. However, in vitro studies using PBMCs from healthy donors and breast cancer patients showed that anti-PD-1 mAb has no effect on the level of $FoxP3^+$ Tregs, and that the effect of PD-1 blockade was more prominent in $CD4^+CD25^-$ T cell subset, which is comprised of non-Tregs and/or non-activated T cells [77]. Ribas et al. [78] demonstrated that anti-PD-1 mAb in melanoma patients does not affect Treg numbers within the TME, but rather increases $CD8^+$ cytotoxic T cells reactivity and enhances their accumulation in the tumor. Consistent with this, anti-PD-1 mAb in MC38 colon cancer model did not affect Treg numbers within the TME, but increased $CD4^+$ and $CD8^+$ T cell numbers and IFN-γ levels [79]. Furthermore, inhibition of PD-1 showed therapeutic efficacy in patients with melanoma [76] and metastatic renal cell carcinoma [80] associated with increased levels of IFN-γ, and enhanced $CD8^+$ T cell accumulation in the tumor, suggesting that anti-PD-1 mAb can induce Teff activation.

3.4. Anti-CTLA-4 and anti-PD-1/PD-L1

In some patients, the combined blockade of CTLA-4 and PD-1/PD-L1 axis has maximized the therapeutic efficacy compared to their single blockade [15,81,82]. This could be attributed to the different mechanisms that each of CTLA-4 and PD-1 regulates (as discussed above) [22]. In addition, blocking PD-1/PD-L1 may induce other T cell-independent anti-tumor immune responses, since the blockade of these molecules will target other cell types (such as B lymphocytes, NK cells, myeloid cells and some DCs) [27,83]. For example, it has been shown that targeting PD-1/PD-L1 axis promotes the proliferation and activation of B lymphocytes, and their production of cytokines [84], which in turn positively modulate the anti-tumor immune responses [85]. Other studies highlighted the efficacy of targeting PD-1/PD-L1 in reducing the immunosuppressive activity of Bregs in cancer [86,87]. Additionally, Ray et al. [88] demonstrated that blocking PD-1/PD-L1 axis may have a therapeutic efficacy in multiple myeloma patients by preventing the interaction between $PD\text{-}L1^+$ DCs and $PD\text{-}1^+$ NK cells, which in turn restores the cytotoxicity of NK cells.

4. Resistance to ICIs

Limited response rates and resistance to the currently approved ICIs have been observed in cancer patients [22,23,27]. Acquired resistance against combined ICIs remains to be a challenge in cancer treatment due to the emergence of compensatory pathways, within the TME, to evade the anti-tumor response induced by ICIs. This can occur as a result of tumor intrinsic factors, such as altered expression of immune regulatory molecules on tumor cells [89–95], or extrinsic factors such as cytokine/growth factor/chemokine milieu within TME, expression of molecules on tumor-infiltrating immune cells, and the composition of tumor-infiltrating lymphocytes [23,24].

Tumor extrinsic-mediated mechanisms are associated with the activation of signaling pathways, which lead to a decrease in $CD8^+$ T

cell:Treg ratio, the induction of MDSCs, and production of immunosuppressive mediators and pro-tumorigenic factors (e.g. VEGF) [24,96–98]. For example, a positive correlation between the number of MDSCs and poor disease prognosis has been reported in melanoma [99] and lung cancer patients [100]. Furthermore, MDSCs act as immunosuppressive cells capable of promoting resistance to anti-CTLA-4 and/or anti-PD-1 mAbs by secreting soluble factors that impair the activation of cytotoxic T cells [99,101]. Tregs represent another cellular mechanism by which tumor resistance to ICIs could be acquired. In this review, we focus on the Treg-mediated resistance mechanisms.

4.1. Treg-mediated resistance mechanisms and therapeutic strategies to overcome resistance

The mechanisms by which Tregs induce tumor resistance against ICIs have not fully been elucidated. However, a number of molecules and signaling pathways have been associated with the resistance of Tregs against approved therapies targeting CTLA-4 and PD-1/PD-L1 molecules as it will be discussed below. Therapeutic strategies to overcome the Treg-mediated resistance will be also addressed.

4.1.1. Up-regulation of other immune checkpoints

Apart from CTLA-4 and PD-1, Tregs within the TME express up-regulated levels of other immune checkpoints/co-inhibitory receptors, namely TIM-3, LAG-3, TIGIT [13] and V-domain containing Ig suppressor of T cell activation (VISTA) [102] and possibly others. As depicted in Fig. 2, up-regulated levels of these molecules on Tregs block Teff activation, enhance the accumulation of Tregs and promote tumorigenesis.

4.1.1.1. TIM-3. It was initially thought to be selectively expressed by $CD4^+$IFN-γ^- Th1 cells [103], and its role was confined to regulating Teff function and cytokine production [104]; for instance, ex-vivo studies showed that the expression of TIM-3 on intratumoral $CD8^+$ T cells results in reduced levels of IFN-γ, and therefore reduced the anti-tumor immune response in lung cancer [105]. However, its importance in regulating intratumoral Tregs has become evident. High numbers of $TIM\text{-}3^+$ Tregs were detected in tumor tissues from patients with lung, cervical, ovarian, hepatocellular and colon cancers [105,106], and mouse tumor tissues [107]. Furthermore, $TIM\text{-}3^+$ Tregs show enhanced suppressive activity compared to $TIM\text{-}3^-$ Tregs as measured by the level of IL-10, granzymes and perforin released in tumor tissues from melanoma and colon cancer mouse models [107]. In vitro studies showed that inhibition of TIM-3 is beneficial in restoring the activity of antigen-specific proliferation and cytokine production in exhausted T cells, providing a rationale for using anti-TIM-3 mAb [104]. Acquired resistance to anti-PD-1 mAb has been associated with the up-regulation of TIM-3 on $CD4^+$ and $CD8^+$ T cells, as well as increased levels of its ligand, galectin-9, in tumor samples from lung cancer patients and a mouse lung cancer model [108]. Tumor-infiltrating lymphocytes from patients with HNSCC treated with anti-PD-1 mAb also showed up-regulated levels of TIM-3 [109]. In line with this, Oweida et al. [110] reported that response to radiotherapy (RT) and anti-PD-L1 combined therapy was compensated by the up-regulation of TIM-3 on $CD8^+$ T cells and Tregs causing tumor relapse and limited survival rate in head and neck tumor mouse model [110]. Concurrent treatment with anti-TIM-3 and anti-PD-L1 mAbs combined with RT resulted in a durable response associated with an increase in $CD8^+$ T cell:Treg ratio and a delay in tumor growth. Together, these findings indicate that the re-emergence of $TIM\text{-}3^+$ Tregs contribute to tumor relapse and progression, thereby providing resistance against anti-PD-1/PD-L1 mAb [110].

4.1.1.2. LAG-3. It is expressed on activated Tregs, Teffs and B lymphocytes, as well as NK cells and dendritic cells. It binds to MHC II on APCs with a higher avidity than CD4 [111,112], causing the down-regulation of antigen-specific $CD4^+$ Teff responses and cytokine production [113–115]. Another report demonstrated that LAG-3 has another ligand, galectin-3 expressed by tumor cells [116]; upon receptor-ligand binding, anti-tumor responses are abolished within the TME as a result of suppressed $CD8^+$ T cell cytotoxic functions [114,117]. Patients with NSCLC and HNSSC show high numbers of $LAG\text{-}3^+$ Tregs within the TME [118,119]. Based on ex vivo studies, intratumoral $LAG3^+$ Tregs from melanoma and colorectal cancer patients are highly proliferative and suppressive [113]. In mouse tumor models, the absence or blockade of LAG-3 in Tregs modestly

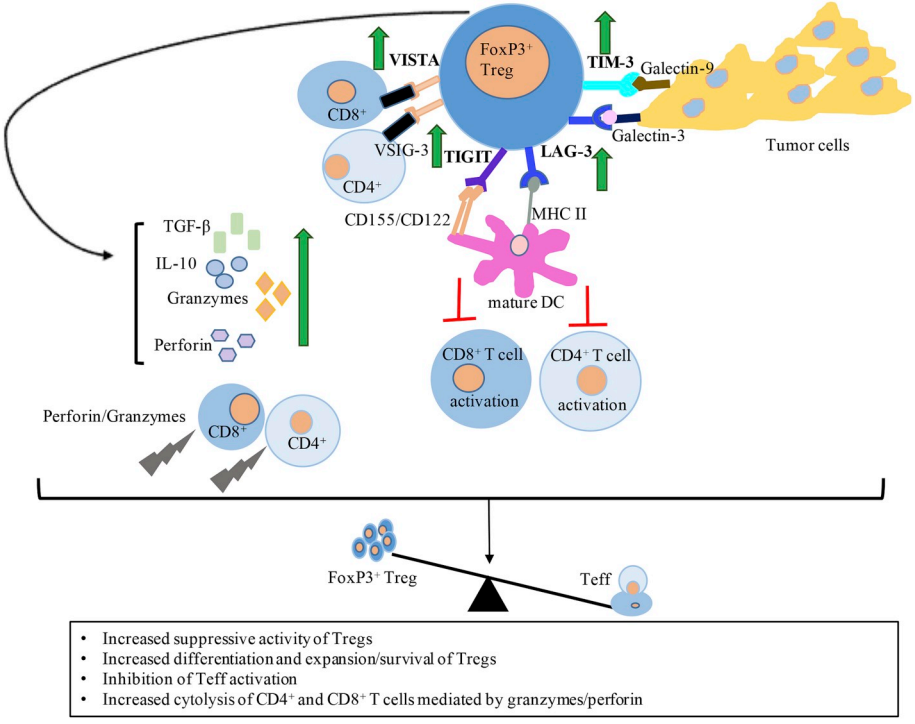

Fig. 2. Up-regulated expression of immune checkpoints on Tregs within the TME. TIM-3 binds to its ligand galectin-9 on tumor cells, LAG-3 binds to its ligand galectin-3 on tumor cells or MHC II on DCs, TIGIT binds to CD155/CD122 on DCs, and VISTA binds to VSIG-3 on CD4 and CD8 T cells resulting in the blockade of Teff activation. Up-regulated expression (as indicated by green arrows) of TIM-3, LAG-3, TIGIT and VISTA on Tregs also enhance their suppressive activity as measured by increased secretion of TGF-β/IL-10, and granzymes/perforin. In turn, TGF-β and IL-10 will increase the immunosuppressive activity of Tregs and promote their differentiation and survival, while granzymes/perforin will induce Teff cytolysis. T effector cell (Teff); immunoglobulin and mucin domain-containing protein 3 (TIM-3); lymphocyte activation gene-3 (LAG-3); T-cell immunoreceptor with Ig and ITIM domains (TIGIT); V-domain containing Ig suppressor of T cell activation (VISTA); V-Set and immunoglobulin domain containing 3 (VSIG-3); transforming growth factor-beta (TGF-β); interleukin-10 (IL-10). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

reduces the suppressive activity of Tregs, particularly the secretion of immunosuppressive molecules (IL-10 and TGF-β), and increases cytokine production by Teffs [120,121]. Huang et al. [122] showed that anti-CTLA-4 or anti-PD-1 mAb in mouse ovarian cancer model resulted in the up-regulation of LAG-3 on $CD8^+$ T cells, suggesting a potential resistance mechanism. The co-blockade of CTLA-4 and LAG-3 or PD-1 and LAG-3 showed better therapeutic efficacy, associated with increased numbers of $CD8^+$ T cells and/or reduced numbers of Tregs in the tumor, compared to targeting CTLA-4 or PD-1 alone [122].

4.1.1.3. TIGIT. It is another IC, expressed by activated $CD4^+$ T cells, $CD8^+$ T cells, Tregs and NK cells [123,124]. It competes with CD226 (on T cells) and binds to CD112 and CD155 ligands (on dendritic cells) causing the inhibition of Teff activation [124,125]. In B16 melanoma mouse model, intratumoral $CD8^+TIGIT^+$ T cells were characterized by reduced levels of IL-2 and TNF production, while intratumoral $TIGIT^+$ Tregs exhibited increased suppressive activity as measured by IL-10 levels [126]. Using the same model, adoptive transfer experiments showed that TIGIT promotes tumorigenesis by enhancing the suppressive activity of Tregs [126]. Therefore, it was rationalized that targeting TIGIT could be an alternative therapeutic strategy for suppressing Treg function and mediating anti-tumor immune responses. Blocking TIGIT in glioma mouse model showed beneficial outcomes associated with increased Teff:Treg ratio, however, the co-blockade of PD-1 and TIGIT was more sustained in increasing the survival rate in tumor-bearing mice compared to those treated with anti-TIGIT mAb alone [127]. Recent data by Zhang et al. [128] demonstrated that TIGIT blockade improved the response of tumor-bearing mice treated with anti-PD-1 mAb, suggesting that TIGIT up-regulation can provide resistance to immunotherapy.

4.1.1.4. VISTA. It is another inhibitory IC, belongs to CD28-B7 family, and expressed by Tregs, Teffs, NK cells, macrophages and dendritic cells [129]. VISTA binds to its ligand, V-Set and immunoglobulin domain containing 3 (VSIG-3), to block Teff proliferation and reduce their cytokine release [130], and acts on naïve T cells to induce their differentiation into Tregs [102]. VISTA expression is highly up-regulated on Tregs within the TME compared with its level on peripheral Tregs, suggesting its potential importance in intratumoral Treg function [131]. The blockade of VISTA enhances Teff accumulation and activation in mouse tumors, reduces Treg numbers, and selectively decreases the number of MDSCs, thereby inducing anti-tumor immune responses [131]. Furthermore, the co-blockade of VISTA and PD-L1 in B16 melanoma and CT26 colon cancer mouse models showed better efficacy in reducing tumor growth and increasing survival rates than targeting each molecule alone, suggesting a synergy between anti-VISTA and anti-PD-L1 mAbs in inducing Teff activation [132]. On the other hand, Kondo et al. [133] showed that combined inhibition of CTLA-4 and VISTA is more effective in tumor regression and increasing Teff:Treg ratio than the co-blockade of PD-1 and VISTA in HNSCC mouse model. In human, a positive correlation between VISTA expression and poor survival rates in patients with oral squamous cell carcinoma was reported [134]. Patients with prostate cancer treated with ipilimumab showed up-regulated levels of $VISTA^+$ tumor-infiltrating lymphocytes and $VISTA^+$ macrophages with M2 phenotype, indicating that VISTA up-regulation could be a compensatory resistance mechanism [135].

Collectively, these data suggest that up-regulated levels of TIM-3, LAG-3, TIGIT and VISTA on intratumoral T cells, including Tregs, act as compensatory pathways to allow immune response evasion and promote tumor growth/progression (as shown in Fig. 2). Therefore, the combined inhibition of TIM-3, LAG-3 or TIGIT, VISTA with anti-CTLA-4 mAb or anti-PD-1/PD-L1 mAbs could provide better therapeutic means for targeting Treg function and overcome resistance, thereby maximizing the efficacy of current cancer therapies. Clinical trials to examine the therapeutic efficacy of mAbs against these molecules are currently ongoing in a range of cancer patients (as reviewed in Refs. [13,102]).

4.1.2. Survival of Tregs within TME

There is some evidence suggesting that targeting CTLA-4 and/or PD-1/PD-L1 is not sufficient for the full elimination of Tregs. Preliminary data, as reviewed by Whiteside [73], suggested that in vitro inhibition of CTLA-4 in Tregs, isolated from healthy donors, does not fully deplete Tregs and the remaining survived Tregs are able to proliferate, expand and induce immunosuppressive activity. In line with this, other studies showed that anti-CTLA-4 mAb does not affect the number of circulating Tregs in patients with prostate cancer [64], and gastric and esophageal adenocarcinomas [136,137], but rather increases the number of activated Teffs. In head and neck mouse model, Oweida et al. [110] found that a residue of intratumoral Tregs, which are highly proliferative, remained within the TME following the co-inhibition of PD-1 and TIM-3 and resulted in tumor relapse. The addition of anti-CD25 following the dual inhibition of PD-L1 and TIM-3 combined with RT, on the other hand, resulted in a more sustained survival response [110]. Consistent with this, the efficacy of targeting Tregs, using anti-CD25 antibody, has been shown in tumor mouse models characterized by an increase in tumor-infiltrating $CD8^+$ and $CD4^+$ T cells and the production of inflammatory cytokines such as IFN-γ [10]. Another study by Yu et al. [32] showed that intratumoral administration of anti-CD4 antibody in mice with late stage tumor depletes Tregs and leads to tumor rejection, and the up-regulation of IFN-γ and down-regulation of IL-10 in tumor tissues. Together, these findings further emphasize the importance of Tregs in tumor progression and resistance, and indicate that ICIs can partially deplete intratumoral Tregs. Furthermore, the use of methylation agents targeting Treg-specific demethylated region (TSDR), which is important for a stable FoxP3 expression and the maintenance of intratumoral Tregs [138,139] could have promising therapeutic effects associated with reduced numbers of intratumoral $FoxP3^+$ Treg [140]. Alternatively, the use of neutralizing antibodies or antagonists against chemokines or chemokine receptors associated with Treg chemotaxis can be used to target Tregs in tumor sites [141,142]. It was shown by Sugiyama et al. [142] that CCR4 is predominately expressed by immunosuppressive Tregs in melanoma. The blockade of CCR4, using anti-CCR4 mAb, in PBMCs isolated from melanoma patients induced the generation of $CD4^+$ and $CD8^+$ T cells specific for cancer-testis antigens [142]. In vivo, anti-CCR4 mAb (mogamulizmab) resulted in the depletion of melanoma-infiltrating Tregs, and the increase of $CD4^+$ and $CD8^+$ T cell-mediated anti-tumor response in adult T-cell leukemia/lymphoma (ATL) patients [142]. They also showed that blocking CCR4 is more selective in depleting immunosuppressive Tregs ($CD4^+FoxP3^+$) than targeting CD25, which was associated with depleting a fraction of non-Tregs defined as $CD4^+FoxP3^-$ [142]. Mogamulizmab has been approved in Japan to treat ATL, a condition that is highly associated with CCR4-expressing Tregs [142]. The therapeutic efficacy of mogamulizmab, as a monotherapy, is under investigation in ongoing early phase clinical trials (NCT01929486) [143], or in combination with either anti-CTLA-4 mAb (NCT02301130), anti-PD-1 mAb (NCT02476123 and NCT02705105) or anti-PD-L1 mAb (NCT02444793) in advanced solid tumors.

4.1.3. Treg-induced TGF-β activation and production

Treg-induced TGF-β activation (membrane-bound form) and the release of soluble forms could be another mechanism by which Tregs promote cancer growth/progression, and suppress anti-tumor immune responses (as shown in Fig. 3). Immunosuppressive intratumoral Tregs are characterized by secreting high levels of TGF-β, within the TME [144–146]. TGF-β, in turn, is a crucial mediator for FoxP3 induction, Treg differentiation and maintenance, and Treg suppressive activity [146,147]. In B cell non-Hodgkin lymphoma, both membrane-bound and soluble forms of TGF-β can alter the function and composition of tumor-infiltrating lymphocytes favoring Treg accumulation and their

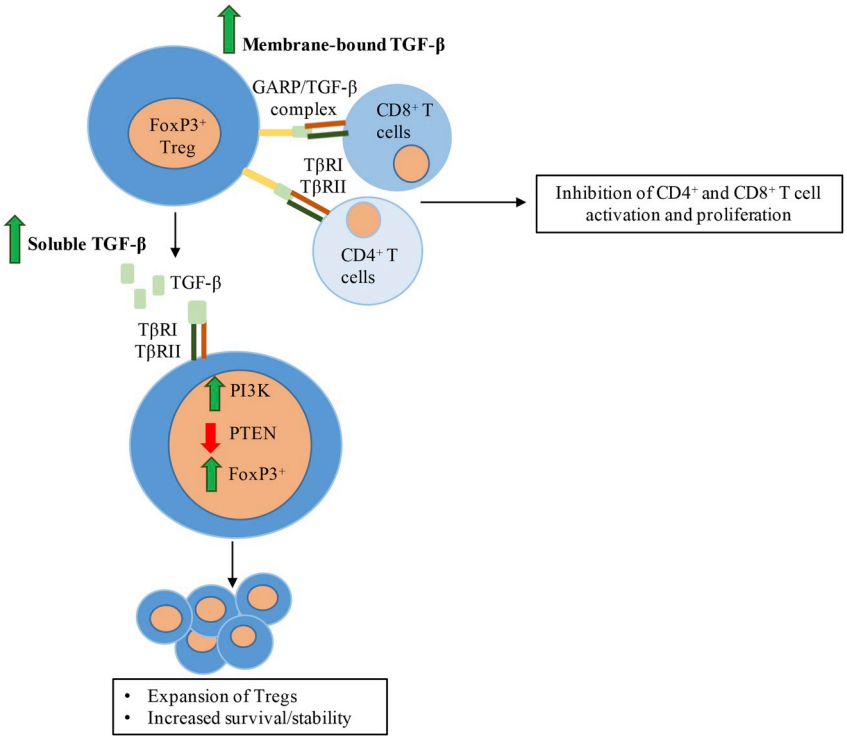

Fig. 3. Increased production of TGF-β (membrane-bound or soluble form) by Tregs within the TME. Up-regulated levels of soluble and increased activation of membrane-bound forms of TGF-β (as indicated by green arrows) promote the expansion of Tregs and block the activation of Teffs. Both forms of TGF-β are capable of promoting the expansion/stability of Tregs. GARP/TGF-β complex (membrane-bound form) binds to TGF-β receptors (TβRI and TβRII) on Teffs ($CD4^+$ and $CD8^+$ T cells) to render their activation and inhibit their proliferation. Upon ligand-receptors interaction, soluble form of TGF-β activates PI3Kinase (PI3K) signaling pathway in Tregs which up-regulates FoxP3 expression and induces the loss of PTEN expression leading to the expansion of Tregs. Phosphatidylinositol 3-kinase (PI3K); glycoprotein-A repetitions predominant (GARP); Phosphatase and tensin homolog (PTEN). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

suppressive activity [148]. In vitro studies showed that stimulated Tregs produce active TGF-β1 in a manner that is dependent on glycoprotein-A repetitions predominant (GARP), a transmembrane protein [149]; however, this active form of TGF-β1 is a membrane-bound form, and it exerts its immunosuppressive action on Teffs through cell-contact [150]. In vivo, anti-GARP mAb inhibits the immunosuppressive activity of Tregs in a xenograft-versus-host-disease model induced by the transfer of human PBMC into immune-deficient mice [151]. Together, these data indicate the importance of TGF-β1 in Tregs and its immunosuppressive function and suggest that GARP could be a good therapeutic target for Treg-induced TGF-β1 activation and Treg suppressive function in cancer to enhance anti-tumor immune responses [152].

Targeting TGF-β in Tregs could be another therapeutic approach to induce anti-tumor responses and activate Teffs. For instance, Ravi and colleagues showed that simultaneous inhibition of CTLA-4 or PD-L1 with TGF-β signaling significantly reduced tumor-infiltrating Tregs, promoting anti-tumor cytotoxic effects in a melanoma mouse model [153]. An engineered antibody targeting either CTLA-4 or PD-L1 fused to a TGFβRII ectodomain sequence (anti-CTLA-4-TGFβRII or anti-PD-L1-TGFβRII) was used to examine the in vivo effect of inhibiting the autocrine/paracrine TGF-β signaling within a TGF-β-enriched TME on tumor progression in human melanoma (A375) tumor bearing mice [153]. Unlike anti-CTLA-4, treating A375-tumor bearing mice with anti-CTLA-4-TGFβRII fused antibody significantly reduced tumor-infiltrating Treg numbers, and increased the proportion of $CD8^+$ T cells expressing IFN-γ. Similar in vivo effects were also obtained for anti-PD-L1-TGFβRII fused antibody, in comparison to anti-PD-L1 mAb treatment using the same mouse model. In vitro, stimulated human PBMC with IL-2, TGF-β and anti-CD3/anti-CD28-coated beads treated with anti-CTLA-4-TGFβRII fused antibody abrogated the TGF-β-mediated FoxP3 expression, unlike stimulated human PBMC treated with anti-CTLA-4 mAb alone [153]. Together, these data indicate that simultaneous targeting ICs and TGF-β may provide an immunotherapeutic strategy targeting Tregs with a better efficacy compared to targeting ICs alone. Hence, this combined therapy could be beneficial against cancers which exhibit a limited response to current ICIs. Despite this, other in vitro findings by Mariathasan and colleagues showed that TGF-β is associated with the development of resistance against anti-PD-L1 mAb (atezolizumab) by acting on non-immune cells in patients with metastatic urothelial cancer [154]. Compared to the single blockade, targeting TGF-β in combination with anti-PD-L1 mAb in EMT6 (mouse mammary carcinoma) and MC38 models showed therapeutic efficacy, associated with an accumulation of cytotoxic $CD8^+$ T cells in the center of the tumor and reduced levels of TGF-β within the stromal cells [154]. No effects on Tregs were seen in tumor-bearing mice co-treated with anti-TGF-β and anti-PD-L1 mAbs. These data indicate that the inhibition of TGF-β signaling pathway is not only limited to immune cells, implicating that TGF-β could promote cancer progression independently of Tregs [154].

4.1.4. Increased activation of PI3K signaling pathway in Tregs

Phosphatidylinositol 3-kinase (PI3K) signaling pathway controls the survival and function of Tregs [94]. In vivo, the inhibition of PI3K signaling results in the loss of phosphatase and tensin homolog (PTEN), a tumor suppressor gene, in intratumoral Tregs which subsequently causes the reduction of Treg proliferation, increases $CD8^+$ T cell activation, and results in tumor regression [94,155]. Consistent with this, the administration of PI3K inhibitor in B16 melanoma and CT26 mouse models was shown to be effective in enhancing the anti-tumor response associated with reduced number of Tregs and increased number of $CD8^+$ T cells in the TME [156]. It was also demonstrated that in vitro inhibition of PI3K selectively reduces the proliferation of human Tregs ($CD4^+CD25^+$) with little effects on $CD4^+CD25^-$ non-Tregs [156]. Furthermore, the use of PI3K inhibitor in combination with anti-CTLA-4 and/or anti-PD-1 mAbs increased the efficacy of these mAbs and maximized the response in murine models of melanoma [94] and HNSCC [109]. Collectively, these data suggest that targeting PI3K, using small molecule inhibitors, in addition to the blockade of ICs could selectively target Tregs, and potentially alleviate the Treg-mediated resistance.

The link between PI3K signaling pathway and TGF-β has been established in cancer metastasis by promoting epithelial to mesenchymal cell transition (EMT) [147,157]. As discussed above, both molecules

have been implicated in tumor immunity, at least in part, by regulating Treg stability and suppressive activity. Therefore, we could propose that increased activation of membrane-bound TGF-β and production of soluble TGF-β by Tregs, within the TME, also up-regulates the activation of PI3K in Tregs which subsequently leads to increased expression of FoxP3 causing the expansion/stability of Tregs, and increasing their suppressive function (Fig. 3).

4.1.5. Hypoxic TME induces highly suppressive apoptotic Tregs

The TME is a hypoxic environment enriched with reactive oxygen species (ROS), oxidative agents, which trigger cell damage and apoptosis [158]. FoxP3$^+$ Tregs can induce the adenosine-mediated immunosuppression pathway, which involves the ectoenzymes CD39 and CD73 facilitating the conversion of extracellular adenosine triphosphate (ATP), an inflammatory mediator, to extracellular adenosine (immunosuppressive mediator) [29,38]. CD73 expression has been positively correlated with poor prognosis in melanoma patients [159]. Up-regulated expression of *Nt5e* (gene encodes CD73), a 5′ ectonucleotidase, in Tregs has been associated with increased inhibition of Teff activation/proliferation and cytokines release [29,38]. Furthermore, melanoma patients with acquired resistance against anti-PD-1 mAb showed an up-regulation in CD73 expression [160]. Based on this knowledge, Maj et al. [29] proposed a mechanism by which intratumoral Tregs become more immunosuppressive and undergo apoptosis, as a result of the hypoxic TME (as summarized in Fig. 4), to provide a resistance against anti-PD-L1 mAb treatment. It was demonstrated that FoxP3$^+$ Tregs are more susceptible to ROS-induced cell damage/apoptosis, while CD4$^+$FoxP3$^-$ T cells exhibit an adequate antioxidant response countering ROS [29]. As a result, ATP is released by the apoptotic Tregs, and de-phosphorylated by CD39 and CD73 (which are present on Tregs) into adenosine (Fig. 4). Subsequently, extracellular adenosine binds to its receptor, adenosine 2A (A2A), on Teffs causing the accumulation of intracellular 3′-5′-cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA) signaling pathway, which in turn inhibits the function of Teffs, suppresses their proliferation [161–163], inhibits the expression of cytokines in CD4$^+$ and CD8$^+$ T cells [29], and may inhibit the TCR-mediated signaling in Teffs [164] (Fig. 4). On the contrary, the inhibition of A2A receptor in mouse models of melanoma elevated the number of tumor-infiltrating CD8$^+$ T cells and promoted tumor regression [165]. Taken together, these findings highlight the immunosuppressive role of CD73 in the Treg-mediated acquired resistance, and A2A receptor signaling pathway in regulating anti-tumor immune responses and cancer progression. Therefore, the use of metabolic inhibitors to target this signaling pathway could offer a new therapeutic strategy to inhibit Treg immunosuppressive function and overcome resistance to ICIs. The blockade of ICs in combination with CD73 inhibition is ongoing in a clinical trial (NCT02503774) [166].

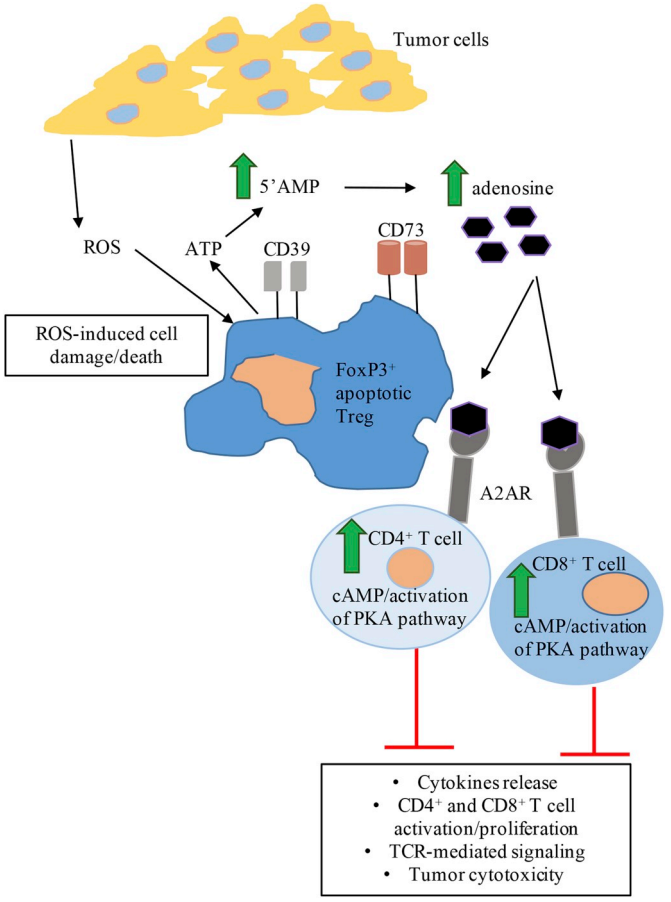

Fig. 4. Hypoxia and apoptotic Tregs. The TME is a hypoxic environment enriched with ROS; these oxidative molecules, as indicated by green arrows, trigger Treg apoptosis resulting in increased secretion of ATP (inflammatory molecule) from apoptotic Tregs and the de-phosphorylation of ATP to become adenosine (immunosuppressive molecule) by CD39 and CD73 ectoenzymes expressed on Tregs. Extracellular adenosine binds to its receptor, A2AR on Teffs, causes accumulation of intracellular cAMP and the activation of PKA signaling pathway in Teffs, which in turn inhibits their functions, suppresses their proliferation, inhibits their expression of cytokines, and may inhibit the TCR-mediated signaling. Adenosine triphosphate (ATP); 3′-5′-cyclic adenosine monophosphate adenosine monophosphate (cAMP); Adenosine A2A receptor (A2AR); protein kinase A (PKA); T cell receptor (TCR). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusions, challenges and future directions

The response to ICIs could be dependent on the type of molecular and cellular networks found in the TME. For example, tumor tissues comprising of a high tumor burden, and large numbers of highly immunosuppressive immune cells, such as Tregs expressing high levels of CTLA-4 and PD-1, are more susceptible to regression upon the single or combined inhibition of CTLA-4 and PD-1/PD-L1 pathway(s) [23]. Overtime, and as result of pressure selection, some tumors become less immunogenic by producing more self-antigens to enhance Treg function, and acquiring compensatory pathways to evade the anti-tumor immune responses. This reflects what occurs in human when patients who initially respond to therapy acquire resistance to ICIs, which could be triggered by Tregs [24]. To overcome this resistance and increase anti-tumor immune responses, several clinical trials are underway to examine the therapeutic efficacy of combining different ICIs together [13], or with small molecule inhibitors, such as metabolic inhibitors, epigenetic modifiers, and immune stimulating agents [98,167]. Furthermore, it is crucial to determine the levels of Tregs in the peripheral blood and tumor tissues and to characterize their phenotype, during and after the inhibition of ICs for the identification of predictive biomarkers associated with Treg-mediated resistance and response to ICIs. This in turn will help in identifying the most suitable treatment for cancer patients and maximizing the therapeutic efficacy. Further insights into the mechanisms and molecular pathways by which ICs regulate T cell function, including Tregs, are needed to design better immunotherapeutic strategies which could require the inhibition of multiple ICs at different time-points based on disease progression and cancer stage.

Conflicts of interest

The authors declare no conflicts of interest.

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References

[1] M.W. Teng, J. Galon, W.H. Fridman, M.J. Smyth, From mice to humans: developments in cancer immunoediting, J. Clin. Investig. 125 (2015) 3338–3346.
[2] G.P. Dunn, A.T. Bruce, H. Ikeda, L.J. Old, R.D. Schreiber, Cancer immunoediting: from immunosurveillance to tumor escape, Nat. Immunol. 3 (2002) 991–998.
[3] R.D. Schreiber, L.J. Old, M.J. Smyth, Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion, Science 331 (2011) 1565–1570.
[4] W. Zou, L. Chen, Inhibitory B7-family molecules in the tumour microenvironment, Nat. Rev. Immunol. 8 (2008) 467–477.
[5] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Canc. 12 (2012) 252–264.
[6] S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3, Science 299 (2003) 1057–1061.
[7] R. Khattri, T. Cox, S.A. Yasayko, F. Ramsdell, An essential role for Scurfin in CD4 + CD25 + T regulatory cells, Nat. Immunol. 4 (2003) 337–342.
[8] Y. Takeuchi, H. Nishikawa, Roles of regulatory T cells in cancer immunity, Int. Immunol. 28 (2016) 401–409.
[9] N. Cools, P. Ponsaerts, V.F. Van Tendeloo, Z.N. Berneman, Regulatory T cells and human disease, Clin. Dev. Immunol. 2007 (2007) 89195–89205.
[10] J. Shimizu, S. Yamazaki, S. Sakaguchi, Induction of tumor immunity by removing CD25 + CD4 + T cells: a common basis between tumor immunity and autoimmunity, J. Immunol. 163 (1999) 5211–5218.
[11] K. Shitara, H. Nishikawa, Regulatory T cells: a potential target in cancer immunotherapy, Ann. N. Y. Acad. Sci. 1417 (2018) 104–115.
[12] S. Sakaguchi, M. Miyara, C.M. Costantino, D.A. Hafler, FOXP3 + regulatory T cells in the human immune system, Nat. Rev. Immunol. 10 (2010) 490–500.
[13] V. Sasidharan Nair, E. Elkord, Immune checkpoint inhibitors in cancer therapy: a focus on T-regulatory cells, Immunol. Cell Biol. 96 (2018) 21–33.
[14] B. Chaudhary, E. Elkord, Regulatory T Cells in the Tumor Microenvironment and Cancer Progression: Role and Therapeutic Targeting, Vaccines, Basel), 2016, p. 4.
[15] J. Larkin, F.S. Hodi, J.D. Wolchok, Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, N. Engl. J. Med. 373 (2015) 1270–1271.
[16] H. Tang, J. Qiao, Y.X. Fu, Immunotherapy and tumor microenvironment, Cancer Lett. 370 (2016) 85–90.
[17] K. Bardhan, T. Anagnostou, V.A. Boussiotis, The PD1:PD-L1/2 pathway from discovery to clinical implementation, Front. Immunol. 7 (2016) 550.
[18] H. Borghaei, L. Paz-Ares, L. Horn, D.R. Spigel, M. Steins, N.E. Ready, L.Q. Chow, E.E. Vokes, E. Felip, E. Holgado, F. Barlesi, M. Kohlhaufl, O. Arrieta, M.A. Burgio, J. Fayette, H. Lena, E. Poddubskaya, D.E. Gerber, S.N. Gettinger, C.M. Rudin, N. Rizvi, L. Crino, G.R. Blumenschein Jr., S.J. Antonia, C. Dorange, C.T. Harbison, F. Graf Finckenstein, J.R. Brahmer, Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer, N. Engl. J. Med. 373 (2015) 1627–1639.
[19] R.L. Ferris, G. Blumenschein Jr., J. Fayette, J. Guigay, A.D. Colevas, L. Licitra, K.J. Harrington, S. Kasper, E.E. Vokes, C. Even, F. Worden, N.F. Saba, L.C.I. Docampo, R. Haddad, T. Rordorf, N. Kiyota, M. Tahara, M. Lynch, V. Jayaprakash, L. Li, M.L. Gillison, Nivolumab vs investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression, Oral Oncol. 81 (2018) 45–51.
[20] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbe, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, W.J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma, N. Engl. J. Med. 363 (2010) 711–723.
[21] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P.D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L. McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kollia, A. Gupta, J.M. Wigginton, M. Sznol, Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (2012) 2443–2454.
[22] J.A. Seidel, A. Otsuka, K. Kabashima, Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations, Front Oncol 8 (2018) 86–99.
[23] P. Sharma, S. Hu-Lieskovan, J.A. Wargo, A. Ribas, Primary, adaptive, and acquired resistance to cancer immunotherapy, Cell 168 (2017) 707–723.
[24] J.M. Pitt, M. Vetizou, R. Daillere, M.P. Roberti, T. Yamazaki, B. Routy, P. Lepage, I.G. Boneca, M. Chamaillard, G. Kroemer, L. Zitvogel, Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors, Immunity 44 (2016) 1255–1269.
[25] D. Kabacaoglu, K.J. Ciecielski, D.A. Ruess, H. Algul, Immune checkpoint inhibition for pancreatic ductal adenocarcinoma: current limitations and future options, Front. Immunol. 9 (2018) 1878–1901.
[26] D. Liu, R.W. Jenkins, R.J. Sullivan, Mechanisms of resistance to immune checkpoint blockade, Am. J. Clin. Dermatol. 20 (2019) 41–54.
[27] J.S. O'Donnell, G.V. Long, R.A. Scolyer, M.W. Teng, M.J. Smyth, Resistance to PD1/PDL1 checkpoint inhibition, Cancer Treat Rev. 52 (2017) 71–81.
[28] A. Facciabene, G.T. Motz, G. Coukos, T-regulatory cells: key players in tumor immune escape and angiogenesis, Cancer Res. 72 (2012) 2162–2171.
[29] T. Maj, W. Wang, J. Crespo, H. Zhang, W. Wang, S. Wei, L. Zhao, L. Vatan, I. Shao, W. Szeliga, C. Lyssiotis, J.R. Liu, I. Kryczek, W. Zou, Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor, Nat. Immunol. 18 (2017) 1332–1341.
[30] R.P. Petersen, M.J. Campa, J. Sperlazza, D. Conlon, M.B. Joshi, D.H. Harpole Jr., E.F. Patz Jr., Tumor infiltrating Foxp3 + regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients, Cancer 107 (2006) 2866–2872.
[31] T.R. Simpson, F. Li, W. Montalvo-Ortiz, M.A. Sepulveda, K. Bergerhoff, F. Arce, C. Roddie, J.Y. Henry, H. Yagita, J.D. Wolchok, K.S. Peggs, J.V. Ravetch, J.P. Allison, S.A. Quezada, Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma, J. Exp. Med. 210 (2013) 1695–1710.
[32] P. Yu, Y. Lee, W. Liu, T. Krausz, A. Chong, H. Schreiber, Y.X. Fu, Intratumor depletion of CD4 + cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors, J. Exp. Med. 201 (2005) 779–791.
[33] M. Miyara, Y. Yoshioka, A. Kitoh, T. Shima, K. Wing, A. Niwa, C. Parizot, C. Taflin, T. Heike, D. Valeyre, A. Mathian, T. Nakahata, T. Yamaguchi, T. Nomura, M. Ono, Z. Amoura, G. Gorochov, S. Sakaguchi, Functional delineation and differentiation dynamics of human CD4 + T cells expressing the FoxP3 transcription factor, Immunity 30 (2009) 899–911.
[34] A. Taylor, J. Verhagen, K. Blaser, M. Akdis, C.A. Akdis, Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells, Immunology 117 (2006) 433–442.
[35] M. Najafi, B. Farhood, K. Mortezaee, Contribution of regulatory T cells to cancer: a review, J. Cell. Physiol. 234 (2019) 7983–7993.
[36] M.M. Tiemessen, A.L. Jagger, H.G. Evans, M.J. van Herwijnen, S. John, L.S. Taams, CD4 + CD25 + Foxp3 + regulatory T cells induce alternative activation of human monocytes/macrophages, Proc Natl Acad Sci U S A, vol. 104, 2007, pp. 19446–19451.
[37] M.A. Lakins, E. Ghorani, H. Munir, C.P. Martins, J.D. Shields, Cancer-associated fibroblasts induce antigen-specific deletion of CD8 (+) T Cells to protect tumour cells, Nat. Commun. 9 (2018) 948.
[38] A.M. Thornton, J. Lu, P.E. Korty, Y.C. Kim, C. Martens, P.D. Sun, E.M. Shevach, Helios(+) and Helios(-) Treg subpopulations are phenotypically and functionally distinct and express dissimilar TCR repertoires, Eur. J. Immunol. 49 (3) (2019) 398–412.
[39] T. Chinen, A.K. Kannan, A.G. Levine, X. Fan, U. Klein, Y. Zheng, G. Gasteiger, Y. Feng, J.D. Fontenot, A.Y. Rudensky, An essential role for the IL-2 receptor in Treg cell function, Nat. Immunol. 17 (2016) 1322–1333.
[40] X. Cao, S.F. Cai, T.A. Fehniger, J. Song, L.I. Collins, D.R. Piwnica-Worms, T.J. Ley, Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance, Immunity 27 (2007) 635–646.
[41] P. Hu, Q. Liu, G. Deng, J. Zhang, N. Liang, J. Xie, J. Zhang, The prognostic value of cytotoxic T-lymphocyte antigen 4 in cancers: a systematic review and meta-analysis, Sci. Rep. 7 (2017) 42913.
[42] C. Le Goux, D. Damotte, S. Vacher, M. Sibony, N.B. Delongchamps, A. Schnitzler, B. Terris, M. Zerbib, I. Bieche, G. Pignot, Correlation between messenger RNA expression and protein expression of immune checkpoint-associated molecules in bladder urothelial carcinoma: a retrospective study, Urol. Oncol. 35 (2017) 257–263.
[43] G. Santoni, C. Amantini, M.B. Morelli, D. Tomassoni, M. Santoni, O. Marinelli, M. Nabissi, C. Cardinali, V. Paolucci, M. Torniai, S. Rinaldi, F. Morgese, G. Bernardini, R. Berardi, High CTLA-4 expression correlates with poor prognosis in thymoma patients, Oncotarget 9 (2018) 16665–16677.
[44] E.I. Buchbinder, A. Desai, CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition, Am. J. Clin. Oncol. 39 (2016) 98–106.
[45] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, Annu. Rev. Immunol. 26 (2008) 677–704.
[46] Y.E. Latchman, S.C. Liang, Y. Wu, T. Chernova, R.A. Sobel, M. Klemm, V.K. Kuchroo, G.J. Freeman, A.H. Sharpe, PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells, Proc Natl Acad Sci U S A, vol. 101, 2004, pp. 10691–10696.
[47] G.J. Freeman, A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, L. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, T. Honjo, Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation, J. Exp. Med. 192 (2000) 1027–1034.
[48] E.N. Rozali, S.V. Hato, B.W. Robinson, R.A. Lake, W.J. Lesterhuis, Programmed death ligand 2 in cancer-induced immune suppression, Clin. Dev. Immunol. 2012 (2012) 656340.
[49] R.V. Parry, J.M. Chemnitz, K.A. Frauwirth, A.R. Lanfranco, I. Braunstein, S.V. Kobayashi, P.S. Linsley, C.B. Thompson, J.L. Riley, CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms, Mol. Cell. Biol. 25 (2005) 9543–9553.
[50] A.V. Collins, D.W. Brodie, R.J. Gilbert, A. Iaboni, R. Manso-Sancho, B. Walse, D.I. Stuart, P.A. van der Merwe, S.J. Davis, The interaction properties of costimulatory molecules revisited, Immunity 17 (2002) 201–210.
[51] C.J. Wang, R. Kenefeck, L. Wardzinski, K. Attridge, C. Manzotti, E.M. Schmidt, O.S. Qureshi, D.M. Sansom, L.S. Walker, Cutting edge: cell-extrinsic immune regulation by CTLA-4 expressed on conventional T cells, J. Immunol. 189 (2012) 1118–1122.
[52] F.A. Schildberg, S.R. Klein, G.J. Freeman, A.H. Sharpe, Coinhibitory pathways in

the B7-CD28 ligand-receptor family, Immunity 44 (2016) 955–972.
[53] O.S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E.M. Schmidt, J. Baker, L.E. Jeffery, S. Kaur, Z. Briggs, T.Z. Hou, C.E. Futter, G. Anderson, L.S. Walker, D.M. Sansom, Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4, Science 332 (2011) 600–603.
[54] A. Curti, S. Trabanelli, V. Salvestrini, M. Baccarani, R.M. Lemoli, The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology, Blood 113 (2009) 2394–2401.
[55] U. Grohmann, R. Bianchi, C. Orabona, F. Fallarino, C. Vacca, A. Micheletti, M.C. Fioretti, P. Puccetti, Functional plasticity of dendritic cell subsets as mediated by CD40 versus B7 activation, J. Immunol. 171 (2003) 2581–2587.
[56] U. Grohmann, C. Orabona, F. Fallarino, C. Vacca, F. Calcinaro, A. Falorni, P. Candeloro, M.L. Belladonna, R. Bianchi, M.C. Fioretti, P. Puccetti, CTLA-4-Ig regulates tryptophan catabolism in vivo, Nat. Immunol. 3 (2002) 1097–1101.
[57] D.H. Munn, M.D. Sharma, A.L. Mellor, Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells, J. Immunol. 172 (2004) 4100–4110.
[58] M. Davies, E.A. Duffield, Safety of checkpoint inhibitors for cancer treatment: strategies for patient monitoring and management of immune-mediated adverse events, ImmunoTargets Ther. 6 (2017) 51–71.
[59] E. Romano, M. Kusio-Kobialka, P.G. Foukas, H. Bichat, P. Baumgaertner, C. Meyer, P. Ballabeni, O. Michielin, B. Weide, P. Romero, D.E. Speiser, FcgRIIIA (CD16)-expressing monocytes mediate the depletion of tumor-infiltrating Tregs via Ipilimumab-dependent ADCC in melanoma patients, J. Immunother. Cancer 2 (2014) O14-O14.
[60] X. Du, F. Tang, M. Liu, J. Su, Y. Zhang, W. Wu, M. Devenport, C.A. Lazarski, P. Zhang, X. Wang, P. Ye, C. Wang, E. Hwang, T. Zhu, T. Xu, P. Zheng, Y. Liu, A reappraisal of CTLA-4 checkpoint blockade in cancer immunotherapy, Cell Res. 28 (2018) 416–432 .
[61] F. Tang, X. Du, M. Liu, P. Zheng, Y. Liu, Anti-CTLA-4 antibodies in cancer immunotherapy: selective depletion of intratumoral regulatory T cells or checkpoint blockade? Cell Biosci. 8 (2018) 30–32.
[62] J.R. Ingram, O.S. Blomberg, M. Rashidian, L. Ali, S. Garforth, E. Fedorov, A.A. Fedorov, J.B. Bonanno, C. Le Gall, S. Crowley, C. Espinosa, T. Biary, E.J. Keliher, R. Weissleder, S.C. Almo, S.K. Dougan, H.L. Ploegh, M. Dougan, Anti-CTLA-4 therapy requires an Fc domain for efficacy, Proc Natl Acad Sci U S A, vol. 115, 2018, pp. 3912–3917.
[63] M.J. Selby, J.J. Engelhardt, M. Quigley, K.A. Henning, T. Chen, M. Srinivasan, A.J. Korman, Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells, Cancer Immunol. Res. 1 (2013) 32–42.
[64] B. Kavanagh, S. O'Brien, D. Lee, Y. Hou, V. Weinberg, B. Rini, J.P. Allison, E.J. Small, L. Fong, CTLA4 blockade expands FoxP3+ regulatory and activated effector CD4+ T cells in a dose-dependent fashion, Blood 112 (2008) 1175–1183.
[65] J.F. Grosso, M.N. Jure-Kunkel, CTLA-4 blockade in tumor models: an overview of preclinical and translational research, Cancer Immun. 13 (2013) 5–18.
[66] C. Menard, F. Ghiringhelli, S. Roux, N. Chaput, C. Mateus, U. Grohmann, S. Caillat-Zucman, L. Zitvogel, C. Robert, Ctla-4 blockade confers lymphocyte resistance to regulatory T-cells in advanced melanoma: surrogate marker of efficacy of tremelimumab? Clin. Cancer Res. 14 (2008) 5242–5249.
[67] S. Nizar, J. Copier, B. Meyer, M. Bodman-Smith, C. Galustian, D. Kumar, A. Dalgleish, T-regulatory cell modulation: the future of cancer immunotherapy? Br. J. Canc. 100 (2009) 1697–1703.
[68] A.A. Tarhini, H. Edington, L.H. Butterfield, Y. Lin, Y. Shuai, H. Tawbi, C. Sander, Y. Yin, M. Holtzman, J. Johnson, U.N. Rao, J.M. Kirkwood, Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab, PLoS One 9 (2014) e87705.
[69] R.R. Huang, J. Jalil, J.S. Economou, B. Chmielowski, R.C. Koya, S. Mok, H. Sazegar, E. Seja, A. Villanueva, J. Gomez-Navarro, J.A. Glaspy, A.J. Cochran, A. Ribas, CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in humans, Clin. Cancer Res. 17 (2011) 4101–4109.
[70] M.A. Postow, M. Manuel, P. Wong, J. Yuan, Z. Dong, C. Liu, S. Perez, I. Tanneau, M. Noel, A. Courtier, N. Pasqual, J.D. Wolchok, Peripheral T cell receptor diversity is associated with clinical outcomes following ipilimumab treatment in metastatic melanoma, J. Immunother. Cancer 3 (2015) 23.
[71] E. Cha, M. Klinger, Y. Hou, C. Cummings, A. Ribas, M. Faham, L. Fong, Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients, Sci. Transl. Med. 6 (2014) 238ra270.
[72] N. van Rooij, M.M. van Buuren, D. Philips, A. Velds, M. Toebes, B. Heemskerk, L.J. van Dijk, S. Behjati, H. Hilkmann, D. El Atmioui, M. Nieuwland, M.R. Stratton, R.M. Kerkhoven, C. Kesmir, J.B. Haanen, P. Kvistborg, T.N. Schumacher, Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma, J. Clin. Oncol. 31 (2013) e439–442.
[73] T.L. Whiteside, FOXP3+ Treg as a therapeutic target for promoting anti-tumor immunity, Expert Opin. Ther. Targets 22 (2018) 353–363.
[74] X. Chen, D. Fosco, D.E. Kline, L. Meng, S. Nishi, P.A. Savage, J. Kline, PD-1 regulates extrathymic regulatory T-cell differentiation, Eur. J. Immunol. 44 (2014) 2603–2616.
[75] K.K. Ellestad, G. Thangavelu, C.L. Ewen, L. Boon, C.C. Anderson, PD-1 is not required for natural or peripherally induced regulatory T cells: severe autoimmunity despite normal production of regulatory T cells, Eur. J. Immunol. 44 (2014) 3560–3572.
[76] W. Wang, R. Lau, D. Yu, W. Zhu, A. Korman, J. Weber, PD1 blockade reverses the suppression of melanoma antigen-specific CTL by CD4+ CD25(Hi) regulatory T cells, Int. Immunol. 21 (2009) 1065–1077.
[77] S.M. Toor, A.S. Syed Khaja, I. Alkurd, E. Elkord, In-vitro effect of pembrolizumab on different T regulatory cell subsets, Clin. Exp. Immunol. 191 (2018) 189–197.
[78] A. Ribas, D.S. Shin, J. Zaretsky, J. Frederiksen, A. Cornish, E. Avramis, E. Seja, C. Kivork, J. Siebert, P. Kaplan-Lefko, X. Wang, B. Chmielowski, J.A. Glaspy, P.C. Tumeh, T. Chodon, D. Pe'er, B. Comin-Anduix, PD-1 blockade expands intratumoral memory T cells, Cancer Immunol. Res. 4 (2016) 194–203.
[79] M.J. Selby, J.J. Engelhardt, R.J. Johnston, L.S. Lu, M. Han, K. Thudium, D. Yao, M. Quigley, J. Valle, C. Wang, B. Chen, P.M. Cardarelli, D. Blanset, A.J. Korman, Preclinical development of ipilimumab and nivolumab combination immunotherapy: mouse tumor models, in vitro functional studies, and cynomolgus macaque toxicology, PLoS One 11 (2016) e0161779.
[80] T.K. Choueiri, M.N. Fishman, B. Escudier, D.F. McDermott, C.G. Drake, H. Kluger, W.M. Stadler, J.L. Perez-Gracia, D.G. McNeel, B. Curti, M.R. Harrison, E.R. Plimack, L. Appleman, L. Fong, L. Albiges, L. Cohen, T.C. Young, S.D. Chasalow, P. Ross-Macdonald, S. Srivastava, M. Jure-Kunkel, J.F. Kurland, J.S. Simon, M. Sznol, Immunomodulatory activity of nivolumab in metastatic renal cell carcinoma, Clin. Cancer Res. 22 (2016) 5461–5471.
[81] M.A. Postow, J. Chesney, A.C. Pavlick, C. Robert, K. Grossmann, D. McDermott, G.P. Linette, N. Meyer, J.K. Giguere, S.S. Agarwala, M. Shaheen, M.S. Ernstoff, D. Minor, A.K. Salama, M. Taylor, P.A. Ott, L.M. Rollin, C. Horak, P. Gagnier, J.D. Wolchok, F.S. Hodi, Nivolumab and ipilimumab versus ipilimumab in untreated melanoma, N. Engl. J. Med. 372 (2015) 2006–2017.
[82] M.K. Callahan, C.E. Horak, M.A. Curran, T. Hollman, D.A. Schaer, J. Yuan, A.M. Lesokhin, S. Kitano, Q. Hong, C.E. Ariyan, K.J. Busam, W. Feely, M. Jure-Kunkel, J. Grosso, J.S. Simon, A.J. Korman, J.M. Wigginton, A.K. Gupta, M. Sznol, J.D. Wolchok, Peripheral and tumor immune correlates in patients with advanced melanoma treated with combination nivolumab (anti-PD-1, BMS-936558, ONO-4538) and ipilimumab, J. Clin. Oncol. 31 (2013) 3003-3003.
[83] M. Versteven, J.M.J. Van den Bergh, E. Marcq, E.L.J. Smits, V.F.I. Van Tendeloo, W. Hobo, E. Lion, Dendritic cells and programmed death-1 blockade: a joint venture to combat cancer, Front. Immunol. 9 (2018) 394.
[84] M.L. Thibult, E. Mamessier, J. Gertner-Dardenne, S. Pastor, S. Just-Landi, L. Xerri, B. Chetaille, D. Olive, PD-1 is a novel regulator of human B-cell activation, Int. Immunol. 25 (2013) 129–137.
[85] G.J. Yuen, E. Demissie, S. Pillai, B lymphocytes and cancer: a love-hate relationship, Trends Cancer 2 (2016) 747–757.
[86] H. Guan, Y. Wan, J. Lan, Q. Wang, Z. Wang, Y. Li, J. Zheng, X. Zhang, Z. Wang, Y. Shen, F. Xie, PD-L1 is a critical mediator of regulatory B cells and T cells in invasive breast cancer, Sci. Rep. 6 (2016) 35651.
[87] Z. Ren, H. Peng, Y.X. Fu, PD-1 shapes B cells as evildoers in the tumor microenvironment, Cancer Discov. 6 (2016) 477–478.
[88] A. Ray, D.S. Das, Y. Song, P. Richardson, N.C. Munshi, D. Chauhan, K.C. Anderson, Targeting PD1-PDL1 immune checkpoint in plasmacytoid dendritic cell interactions with T cells, natural killer cells and multiple myeloma cells, Leukemia 29 (2015) 1441–1444.
[89] M. Sade-Feldman, Y.J. Jiao, J.H. Chen, M.S. Rooney, M. Barzily-Rokni, J.P. Eliane, S.L. Bjorgaard, M.R. Hammond, H. Vitzthum, S.M. Blackmon, D.T. Frederick, M. Hazar-Rethinam, B.A. Nadres, E.E. Van Seventer, S.A. Shukla, K. Yizhak, J.P. Ray, D. Rosebrock, D. Livitz, V. Adalsteinsson, G. Getz, L.M. Duncan, B. Li, R.B. Corcoran, D.P. Lawrence, A. Stemmer-Rachamimov, G.M. Boland, D.A. Landau, K.T. Flaherty, R.J. Sullivan, N. Hacohen, Resistance to checkpoint blockade therapy through inactivation of antigen presentation, Nat. Commun. 8 (2017) 1136.
[90] J.M. Zaretsky, A. Garcia-Diaz, D.S. Shin, H. Escuin-Ordinas, W. Hugo, S. Hu-Lieskovan, D.Y. Torrejon, G. Abril-Rodriguez, S. Sandoval, L. Barthly, J. Saco, B. Homet Moreno, R. Mezzadra, B. Chmielowski, K. Ruchalski, I.P. Shintaku, P.J. Sanchez, C. Puig-Saus, G. Cherry, E. Seja, X. Kong, J. Pang, B. Berent-Maoz, B. Comin-Anduix, T.G. Graeber, P.C. Tumeh, T.N. Schumacher, R.S. Lo, A. Ribas, Mutations associated with acquired resistance to PD-1 blockade in melanoma, N. Engl. J. Med. 375 (2016) 819–829.
[91] N. McGranahan, R. Rosenthal, C.T. Hiley, A.J. Rowan, T.B.K. Watkins, G.A. Wilson, N.J. Birkbak, S. Veeriah, P. Van Loo, J. Herrero, C. Swanton, T.R. Consortium, Allele-specific HLA loss and immune escape in lung cancer evolution, Cell 171 (2017) 1259–1271 e1211.
[92] M.S. Rooney, S.A. Shukla, C.J. Wu, G. Getz, N. Hacohen, Molecular and genetic properties of tumors associated with local immune cytolytic activity, Cell 160 (2015) 48–61.
[93] S. George, D. Miao, G.D. Demetri, D. Adeegbe, S.J. Rodig, S. Shukla, M. Lipschitz, A. Amin-Mansour, C.P. Raut, S.L. Carter, P. Hammerman, G.J. Freeman, C.J. Wu, P.A. Ott, K.K. Wong, E.M. Van Allen, Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma, Immunity 46 (2017) 197–204.
[94] W. Peng, J.Q. Chen, C. Liu, S. Malu, C. Creasy, M.T. Tetzlaff, C. Xu, J.A. McKenzie, C. Zhang, X. Liang, L.J. Williams, W. Deng, G. Chen, R. Mbofung, A.J. Lazar, C.A. Torres-Cabala, Z.A. Cooper, P.L. Chen, T.N. Tieu, S. Spranger, X. Yu, C. Bernatchez, M.A. Forget, C. Haymaker, R. Amaria, J.L. McQuade, I.C. Glitza, T. Cascone, H.S. Li, L.N. Kwong, T.P. Heffernan, J. Hu, R.L. Bassett Jr., M.W. Bosenberg, S.E. Woodman, W.W. Overwijk, G. Lizee, J. Roszik, T.F. Gajewski, J.A. Wargo, J.E. Gershenwald, L. Radvanyi, M.A. Davies, P. Hwu, Loss of PTEN promotes resistance to T cell-mediated immunotherapy, Cancer Discov. 6 (2016) 202–216.
[95] S. Spranger, R. Bao, T.F. Gajewski, Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity, Nature 523 (2015) 231–235.
[96] T. Voron, E. Marcheteau, S. Pernot, O. Colussi, E. Tartour, J. Taieb, M. Terme, Control of the immune response by pro-angiogenic factors, Front Oncol 4 (2014) 70.

[97] X.D. Liu, A. Hoang, L. Zhou, S. Kalra, A. Yetil, M. Sun, Z. Ding, X. Zhang, S. Bai, P. German, P. Tamboli, P. Rao, J.A. Karam, C. Wood, S. Matin, A. Zurita, A. Bex, A.W. Griffioen, J. Gao, P. Sharma, N. Tannir, K. Sircar, E. Jonasch, Resistance to antiangiogenic therapy is associated with an immunosuppressive tumor microenvironment in metastatic renal cell carcinoma, Cancer Immunol. Res. 3 (2015) 1017–1029.
[98] R.W. Jenkins, D.A. Barbie, K.T. Flaherty, Mechanisms of resistance to immune checkpoint inhibitors, Br. J. Canc. 118 (2018) 9–16.
[99] C. Gebhardt, A. Sevko, H. Jiang, R. Lichtenberger, M. Reith, K. Tarnanidis, T. Holland-Letz, L. Umansky, P. Beckhove, A. Sucker, D. Schadendorf, J. Utikal, V. Umansky, Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab, Clin. Cancer Res. 21 (2015) 5453–5459.
[100] J. Ma, H. Xu, S. Wang, Immunosuppressive role of myeloid-derived suppressor cells and therapeutic targeting in lung cancer, J. Immunol. Res. 2018 (2018) 6319649.
[101] C. Krieg, M. Nowicka, S. Guglietta, S. Schindler, F.J. Hartmann, L.M. Weber, R. Dummer, M.D. Robinson, M.P. Levesque, B. Becher, High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy, Nat. Med. 24 (2018) 144–153.
[102] S. Burugu, A.R. Dancsok, T.O. Nielsen, Emerging targets in cancer immunotherapy, Semin. Canc. Biol. 52 (2018) 39–52.
[103] L. Monney, C.A. Sabatos, J.L. Gaglia, A. Ryu, H. Waldner, T. Chernova, S. Manning, E.A. Greenfield, A.J. Coyle, R.A. Sobel, G.J. Freeman, V.K. Kuchroo, Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease, Nature 415 (2002) 536–541.
[104] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nat. Immunol. 6 (2005) 1245–1252.
[105] X. Gao, Y. Zhu, G. Li, H. Huang, G. Zhang, F. Wang, J. Sun, Q. Yang, X. Zhang, B. Lu, TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression, PLoS One 7 (2012) e30676.
[106] J. Yan, Y. Zhang, J.P. Zhang, J. Liang, L. Li, L. Zheng, Tim-3 expression defines regulatory T cells in human tumors, PLoS One 8 (2013) e58006.
[107] K. Sakuishi, S.F. Ngiow, J.M. Sullivan, M.W. Teng, V.K. Kuchroo, M.J. Smyth, A.C. Anderson, TIM3(+)FOXP3(+) regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer, OncoImmunology 2 (2013) e23849.
[108] S. Koyama, E.A. Akbay, Y.Y. Li, G.S. Herter-Sprie, K.A. Buczkowski, W.G. Richards, L. Gandhi, A.J. Redig, S.J. Rodig, H. Asahina, R.E. Jones, M.M. Kulkarni, M. Kuraguchi, S. Palakurthi, P.E. Fecci, B.E. Johnson, P.A. Janne, J.A. Engelman, S.P. Gangadharan, D.B. Costa, G.J. Freeman, R. Bueno, F.S. Hodi, G. Dranoff, K.K. Wong, P.S. Hammerman, Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints, Nat. Commun. 7 (2016) 10501.
[109] G. Shayan, R. Srivastava, J. Li, N. Schmitt, L.P. Kane, R.L. Ferris, Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer, OncoImmunology 6 (2017) e1261779.
[110] A. Oweida, M.K. Hararah, A. Phan, D. Binder, S. Bhatia, S. Lennon, S. Bukkapatnam, B. Van Court, N. Uyanga, L. Darragh, H.M. Kim, D. Raben, A.C. Tan, L. Heasley, E. Clambey, R. Nemenoff, S.D. Karam, Resistance to radiotherapy and PD-L1 blockade is mediated by TIM-3 upregulation and regulatory T-cell infiltration, Clin. Cancer Res. 24 (2018) 5368–5380.
[111] B. Huard, P. Prigent, M. Tournier, D. Bruniquel, F. Triebel, CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins, Eur. J. Immunol. 25 (1995) 2718–2721.
[112] F. Triebel, S. Jitsukawa, E. Baixeras, S. Roman-Roman, C. Genevee, E. Viegas-Pequignot, T. Hercend, LAG-3, a novel lymphocyte activation gene closely related to CD4, J. Exp. Med. 171 (1990) 1393–1405.
[113] C. Camisaschi, C. Casati, F. Rini, M. Perego, A. De Filippo, F. Triebel, G. Parmiani, F. Belli, L. Rivoltini, C. Castelli, LAG-3 expression defines a subset of CD4(+)CD25(high)Foxp3(+) regulatory T cells that are expanded at tumor sites, J. Immunol. 184 (2010) 6545–6551.
[114] T. Kouo, L. Huang, A.B. Pucsek, M. Cao, S. Solt, T. Armstrong, E. Jaffee, Galectin-3 shapes antitumor immune responses by suppressing CD8+ T cells via LAG-3 and inhibiting expansion of plasmacytoid dendritic cells, Cancer Immunol. Res. 3 (2015) 412–423.
[115] C.J. Workman, D.A. Vignali, The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells, Eur. J. Immunol. 33 (2003) 970–979.
[116] H. Ahmed, D.M. AlSadek, Galectin-3 as a potential target to prevent cancer metastasis, Clin. Med. Insights Oncol. 9 (2015) 113–121.
[117] E. Baixeras, B. Huard, C. Miossec, S. Jitsukawa, M. Martin, T. Hercend, C. Auffray, F. Triebel, D. Piatier-Tonneau, Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens, J. Exp. Med. 176 (1992) 327–337.
[118] T. Wei, J. Zhang, Y. Qin, Y. Wu, L. Zhu, L. Lu, G. Tang, Q. Shen, Increased expression of immunosuppressive molecules on intratumoral and circulating regulatory T cells in non-small-cell lung cancer patients, Am. J. Cancer Res. 5 (2015) 2190–2201.
[119] W.W. Deng, L. Mao, G.T. Yu, L.L. Bu, S.R. Ma, B. Liu, J.S. Gutkind, A.B. Kulkarni, W.F. Zhang, Z.J. Sun, LAG-3 confers poor prognosis and its blockade reshapes antitumor response in head and neck squamous cell carcinoma, OncoImmunology 5 (2016) e1239005.
[120] C.T. Huang, C.J. Workman, D. Flies, X. Pan, A.L. Marson, G. Zhou, E.L. Hipkiss, S. Ravi, J. Kowalski, H.I. Levitsky, J.D. Powell, D.M. Pardoll, C.G. Drake, D.A. Vignali, Role of LAG-3 in regulatory T cells, Immunity 21 (2004) 503–513.
[121] T. Okamura, K. Fujio, S. Sumitomo, K. Yamamoto, Roles of LAG3 and EGR2 in regulatory T cells, Ann. Rheum. Dis. 71 (Suppl 2) (2012) i96–100.
[122] R.Y. Huang, A. Francois, A.R. McGray, A. Miliotto, K. Odunsi, Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer, OncoImmunology 6 (2017) e1249561.
[123] R.J. Johnston, L. Comps-Agrar, J. Hackney, X. Yu, M. Huseni, Y. Yang, S. Park, V. Javinal, H. Chiu, B. Irving, D.L. Eaton, J.L. Grogan, The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function, Cancer Cell 26 (2014) 923–937.
[124] X. Yu, K. Harden, L.C. Gonzalez, M. Francesco, E. Chiang, B. Irving, I. Tom, S. Ivelja, C.J. Refino, H. Clark, D. Eaton, J.L. Grogan, The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells, Nat. Immunol. 10 (2009) 48–57.
[125] S. Tahara-Hanaoka, K. Shibuya, Y. Onoda, H. Zhang, S. Yamazaki, A. Miyamoto, S. Honda, L.L. Lanier, A. Shibuya, Functional characterization of DNAM-1 (CD226) interaction with its ligands PVR (CD155) and nectin-2 (PRR-2/CD112), Int. Immunol. 16 (2004) 533–538.
[126] S. Kurtulus, K. Sakuishi, S.F. Ngiow, N. Joller, D.J. Tan, M.W. Teng, M.J. Smyth, V.K. Kuchroo, A.C. Anderson, TIGIT predominantly regulates the immune response via regulatory T cells, J. Clin. Investig. 125 (2015) 4053–4062.
[127] A.L. Hung, R. Maxwell, D. Theodros, Z. Belcaid, D. Mathios, A.S. Luksik, E. Kim, A. Wu, Y. Xia, T. Garzon-Muvdi, C. Jackson, X. Ye, B. Tyler, M. Selby, A. Korman, B. Barnhart, S.M. Park, J.I. Youn, T. Chowdhury, C.K. Park, H. Brem, D.M. Pardoll, M. Lim, TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM, OncoImmunology 7 (2018) e1466769.
[128] Q. Zhang, J. Bi, X. Zheng, Y. Chen, H. Wang, W. Wu, Z. Wang, Q. Wu, H. Peng, H. Wei, R. Sun, Z. Tian, Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity, Nat. Immunol. 19 (2018) 723–732.
[129] L. Wang, R. Rubinstein, J.L. Lines, A. Wasiuk, C. Ahonen, Y. Guo, L.F. Lu, D. Gondek, Y. Wang, R.A. Fava, A. Fiser, S. Almo, R.J. Noelle, VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses, J. Exp. Med. 208 (2011) 577–592.
[130] J. Wang, G. Wu, B. Manick, V. Hernandez, M. Renelt, C. Erickson, J. Guan, R. Singh, S. Rollins, A. Solorz, M. Bi, J. Li, D. Grabowski, J. Dirkx, C. Tracy, T. Stuart, C. Ellinghuysen, D. Desmond, C. Foster, V. Kalabokis, VSIG-3 as a ligand of VISTA inhibits human T-cell function, Immunology 156 (2019) 74–85.
[131] I. Le Mercier, W. Chen, J.L. Lines, M. Day, J. Li, P. Sergent, R.J. Noelle, L. Wang, VISTA regulates the development of protective antitumor immunity, Cancer Res. 74 (2014) 1933–1944.
[132] J. Liu, Y. Yuan, W. Chen, J. Putra, A.A. Suriawinata, A.D. Schenk, H.E. Miller, I. Guleria, R.J. Barth, Y.H. Huang, L. Wang, Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses, Proc Natl Acad Sci U S A, vol. 112, 2015, pp. 6682–6687.
[133] Y. Kondo, T. Ohno, N. Nishii, K. Harada, H. Yagita, M. Azuma, Differential contribution of three immune checkpoint (VISTA, CTLA-4, PD-1) pathways to antitumor responses against squamous cell carcinoma, Oral Oncol. 57 (2016) 54–60.
[134] L. Wu, W.W. Deng, C.F. Huang, L.L. Bu, G.T. Yu, L. Mao, W.F. Zhang, B. Liu, Z.J. Sun, Expression of VISTA correlated with immunosuppression and synergized with CD8 to predict survival in human oral squamous cell carcinoma, Cancer Immunol. Immunother. 66 (2017) 627–636.
[135] J. Gao, J.F. Ward, C.A. Pettaway, L.Z. Shi, S.K. Subudhi, L.M. Vence, H. Zhao, J. Chen, H. Chen, E. Efstathiou, P. Troncoso, J.P. Allison, C.J. Logothetis, Wistuba II, M.A. Sepulveda, J. Sun, J. Wargo, J. Blando, P. Sharma, VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer, Nat. Med. 23 (2017) 551–555.
[136] S. Khan, D.J. Burt, C. Ralph, F.C. Thistlethwaite, R.E. Hawkins, E. Elkord, Tremelimumab (anti-CTLA4) mediates immune responses mainly by direct activation of T effector cells rather than by affecting T regulatory cells, Clin. Immunol. 138 (2011) 85–96.
[137] C. Ralph, E. Elkord, D.J. Burt, J.F. O'Dwyer, E.B. Austin, P.L. Stern, R.E. Hawkins, F.C. Thistlethwaite, Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma, Clin. Cancer Res. 16 (2010) 1662–1672.
[138] Y. Zheng, S. Josefowicz, A. Chaudhry, X.P. Peng, K. Forbush, A.Y. Rudensky, Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate, Nature 463 (2010) 808–812.
[139] H. Ma, W. Gao, X. Sun, W. Wang, STAT5 and TET2 cooperate to regulate FOXP3-TSDR demethylation in CD4(+) T cells of patients with colorectal cancer, J. Immunol. Res. 2018 (2018) 6985031.
[140] C. Zhuo, Y. Xu, M. Ying, Q. Li, L. Huang, D. Li, S. Cai, B. Li, FOXP3+ Tregs: heterogeneous phenotypes and conflicting impacts on survival outcomes in patients with colorectal cancer, Immunol. Res. 61 (2015) 338–347.
[141] S. Spranger, H.K. Koblish, B. Horton, P.A. Scherle, R. Newton, T.F. Gajewski, Mechanism of tumor rejection with doublets of CTLA-4, PD-1/PD-L1, or Ido blockade involves restored IL-2 production and proliferation of CD8(+) T cells directly within the tumor microenvironment, J. Immunother. Cancer 2 (2014) 3.
[142] D. Sugiyama, H. Nishikawa, Y. Maeda, M. Nishioka, A. Tanemura, I. Katayama, S. Ezoe, Y. Kanakura, E. Sato, Y. Fukumori, J. Karbach, E. Jager, S. Sakaguchi, Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking antitumor immune responses in humans, Proc Natl Acad Sci U S A, vol. 110, 2013, pp. 17945–17950.
[143] K. Kurose, Y. Ohue, H. Wada, S. Iida, T. Ishida, T. Kojima, T. Doi, S. Suzuki, M. Isobe, T. Funakoshi, K. Kakimi, H. Nishikawa, H. Udono, M. Oka, R. Ueda, E. Nakayama, Phase ia study of FoxP3+ CD4 Treg depletion by infusion of a

humanized anti-CCR4 antibody, KW-0761, in cancer patients, Clin. Cancer Res. 21 (2015) 4327–4336.
[144] J. Seoane, R.R. Gomis, TGF-beta family signaling in tumor suppression and cancer progression, Cold Spring Harb. Perspect. Biol. 9 (2017).
[145] L. Strauss, C. Bergmann, M. Szczepanski, W. Gooding, J.T. Johnson, T.L. Whiteside, A unique subset of CD4+CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment, Clin. Cancer Res. 13 (2007) 4345–4354.
[146] R. Bommireddy, T. Doetschman, TGFbeta1 and Treg cells: alliance for tolerance, Trends Mol. Med. 13 (2007) 492–501.
[147] M.O. Li, R.A. Flavell, TGF-beta: a master of all T cell trades, Cell 134 (2008) 392–404.
[148] Z.Z. Yang, D.M. Grote, S.C. Ziesmer, B. Xiu, N.R. Yates, F.J. Secreto, L.S. Hodge, T.E. Witzig, A.J. Novak, S.M. Ansell, Soluble and membrane-bound TGF-beta-mediated regulation of intratumoral T cell differentiation and function in B-cell non-Hodgkin lymphoma, PLoS One 8 (2013) e59456.
[149] O. Dedobbeleer, J. Stockis, B. van der Woning, P.G. Coulie, S. Lucas, Cutting edge: active TGF-beta1 released from GARP/TGF-beta1 complexes on the surface of stimulated human B lymphocytes increases class-switch recombination and production of IgA, J. Immunol. 199 (2017) 391–396.
[150] J. Stockis, W. Fink, V. Francois, T. Connerotte, C. de Smet, L. Knoops, P. van der Bruggen, T. Boon, P.G. Coulie, S. Lucas, Comparison of stable human Treg and Th clones by transcriptional profiling, Eur. J. Immunol. 39 (2009) 869–882.
[151] J. Cuende, S. Lienart, O. Dedobbeleer, B. van der Woning, G. De Boeck, J. Stockis, C. Huygens, D. Colau, J. Somja, P. Delvenne, M. Hannon, F. Baron, L. Dumoutier, J.C. Renauld, H. De Haard, M. Saunders, P.G. Coulie, S. Lucas, Monoclonal antibodies against GARP/TGF-beta1 complexes inhibit the immunosuppressive activity of human regulatory T cells in vivo, Sci. Transl. Med. 7 (2015) 284ra256.
[152] E.M. Shevach, Garp as a therapeutic target for modulation of T regulatory cell function, Expert Opin. Ther. Targets 21 (2017) 191–200.
[153] R. Ravi, K.A. Noonan, V. Pham, R. Bedi, A. Zhavoronkov, I.V. Ozerov, E. Makarev, V.A.A., P.T. Wysocki, R. Mehra, S. Nimmagadda, L. Marchionni, D. Sidransky, I.M. Borrello, E. Izumchenko, A. Bedi, Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFbeta enhance the efficacy of cancer immunotherapy, Nat. Commun. 9 (2018) 741–754.
[154] S. Mariathasan, S.J. Turley, D. Nickles, A. Castiglioni, K. Yuen, Y. Wang, E.E. Kadel III, H. Koeppen, J.L. Astarita, R. Cubas, S. Jhunjhunwala, R. Banchereau, Y. Yang, Y. Guan, C. Chalouni, J. Ziai, Y. Senbabaoglu, S. Santoro, D. Sheinson, J. Hung, J.M. Giltnane, A.A. Pierce, K. Mesh, S. Lianoglou, J. Riegler, R.A.D. Carano, P. Eriksson, M. Hoglund, L. Somarriba, D.L. Halligan, M.S. van der Heijden, Y. Loriot, J.E. Rosenberg, L. Fong, I. Mellman, D.S. Chen, M. Green, C. Derleth, G.D. Fine, P.S. Hegde, R. Bourgon, T. Powles, TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells, Nature 554 (2018) 544–548.
[155] K. Ali, D.R. Soond, R. Pineiro, T. Hagemann, W. Pearce, E.L. Lim, H. Bouabe, C.L. Scudamore, T. Hancox, H. Maecker, L. Friedman, M. Turner, K. Okkenhaug, B. Vanhaesebroeck, Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer, Nature 510 (2014) 407–411.
[156] R. Abu-Eid, R.N. Samara, L. Ozbun, M.Y. Abdalla, J.A. Berzofsky, K.M. Friedman, M. Mkrtichyan, S.N. Khleif, Selective inhibition of regulatory T cells by targeting the PI3K-Akt pathway, Cancer Immunol. Res. 2 (2014) 1080–1089.
[157] J.J. Lebrun, The dual role of TGFbeta in human cancer: from tumor suppression to cancer metastasis, ISRN Mol. Biol. 2012 (2012) 381428.
[158] V. Petrova, M. Annicchiarico-Petruzzelli, G. Melino, I. Amelio, The hypoxic tumour microenvironment, Oncogenesis 7 (2018) 10.
[159] A. Young, S.F. Ngiow, J. Madore, J. Reinhardt, J. Landsberg, A. Chitsazan, J. Rautela, T. Bald, D.S. Barkauskas, E. Ahern, N.D. Huntington, D. Schadendorf, G.V. Long, G.M. Boyle, M. Holzel, R.A. Scolyer, M.J. Smyth, Targeting adenosine in BRAF-mutant melanoma reduces tumor growth and metastasis, Cancer Res. 77 (2017) 4684–4696.
[160] J. Reinhardt, J. Landsberg, J.L. Schmid-Burgk, B.B. Ramis, T. Bald, N. Glodde, D. Lopez-Ramos, A. Young, S.F. Ngiow, D. Nettersheim, H. Schorle, T. Quast, W. Kolanus, D. Schadendorf, G.V. Long, J. Madore, R.A. Scolyer, A. Ribas, M.J. Smyth, P.C. Tumeh, T. Tuting, M. Holzel, MAPK signaling and inflammation link melanoma phenotype switching to induction of CD73 during immunotherapy, Cancer Res. 77 (2017) 4697–4709.
[161] M. Mandapathil, B. Hilldorfer, M.J. Szczepanski, M. Czystowska, M. Szajnik, J. Ren, S. Lang, E.K. Jackson, E. Gorelik, T.L. Whiteside, Generation and accumulation of immunosuppressive adenosine by human CD4+CD25highFOXP3+ regulatory T cells, J. Biol. Chem. 285 (2010) 7176–7186.
[162] M. Sitkovsky, D. Lukashev, S. Deaglio, K. Dwyer, S.C. Robson, A. Ohta, Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells, Br. J. Pharmacol. 153 (Suppl 1) (2008) S457–S464.
[163] G. Borsellino, M. Kleinewietfeld, D. Di Mitri, A. Sternjak, A. Diamantini, R. Giometto, S. Hopner, D. Centonze, G. Bernardi, M.L. Dell'Acqua, P.M. Rossini, L. Battistini, O. Rotzschke, K. Falk, Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression, Blood 110 (2007) 1225–1232.
[164] V.L. Wehbi, K. Tasken, Molecular mechanisms for cAMP-mediated immunoregulation in T cells - role of anchored protein kinase a signaling units, Front. Immunol. 7 (2016) 222.
[165] V. Umansky, I. Shevchenko, A.V. Bazhin, J. Utikal, Extracellular adenosine metabolism in immune cells in melanoma, Cancer Immunol. Immunother. 63 (2014) 1073–1080.
[166] C.M. Hay, E. Sult, Q. Huang, K. Mulgrew, S.R. Fuhrmann, K.A. McGlinchey, S.A. Hammond, R. Rothstein, J. Rios-Doria, E. Poon, N. Holoweckyj, N.M. Durham, C.C. Leow, G. Diedrich, M. Damschroder, R. Herbst, R.E. Hollingsworth, K.F. Sachsenmeier, Targeting CD73 in the tumor microenvironment with MEDI9447, Oncoimmunology 5 (2016) e1208875.
[167] K.M. Mahoney, P.D. Rennert, G.J. Freeman, Combination cancer immunotherapy and new immunomodulatory targets, Nat. Rev. Drug Discov. 14 (2015) 561–584.