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Balancing cancer immunotherapy and immune-related adverse events: The emerging role of regulatory T cells

T. Alissafi[1], A. Hatzioannou[1], A.I. Legaki, A. Varveri, Panayotis Verginis[*]

Center of Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efessiou Street, Athens, Greece

A B S T R A C T

Advances in our understanding of tumor immunity have prompted a paradigm shift in oncology, with the emergence of immunotherapy, where therapeutic agents are used to target immune cells rather than cancer cells. A real breakthrough in the field of immunotherapy came with the use of immune checkpoint inhibitors (ICI), namely antagonistic antibodies that block key immune regulatory molecules (checkpoint molecules), such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein (PD-1) and its ligand PD-L1, that under physiologic conditions suppress T cell effector function. However, despite the enormous success, a significant proportion of patients do not respond, while responses are frequently accompanied by life-threatening autoimmune related adverse events (irAEs). A major impediment in the effectiveness of ICI immunotherapy is the tumoral resistance, which is dependent on the immunosuppressive nature of tumor microenvironment (TME). Regulatory T cells (Tregs) are among the most abundant suppressive cells in the TME and their presence has been correlated with tumor progression, invasiveness as well as metastasis. Tregs are characterized by the expression of the transcription factor Foxp3 and various mechanisms ranging from cell-to-cell contact to secretion of inhibitory molecules have been implicated in their function. Notably, Tregs amply express most of the checkpoint molecules such as CTLA4, PD1 and LAG3 and therefore represent a direct target of ICI immunotherapy. Taking into consideration the critical role of Tregs in maintenance of immune homeostasis and avoidance of autoimmunity it is plausible that targeting of Tregs by ICI immunotherapy results in the development of irAEs. Since the use of ICI becomes common, and new immune checkpoint molecules are currently under clinical trials for the treatment of cancer, the occurrence of irAEs is expected to dramatically rise. Herein we review the current literature focusing on the role of Tregs in cancer evolution, ICI response and development of irAEs. Unraveling the complex mechanisms that hinder the tumor immune surveillance and in particular how ICI immunotherapy imprint on Treg activities to promote cancer regression while avoid development of irAEs, will empower the design of novel immunotherapeutic modalities in cancer with increased efficacy and diminished adverse events.

1. Introduction

Since their first discovery in the 1960s [1], $CD4^+CD25^+Foxp3^+$ T regulatory cells (Tregs) have emerged over the last twenty years as dominant regulators of T cell-mediated responses against self and non-self antigens [2,3]. Tregs mediate their function via inactivation of auto-reactive T lymphocytes, which have escaped elimination during their development in thymus and restrain excessive immune responses, thus ensuring peripheral tolerance and overall maintenance of immune homeostasis [4,5]. Furthermore, their regulatory functions affect many cells of the immune system besides T cells, including dendritic cells (DCs), macrophages, neutrophils, γδ T cells, natural killer cells (NK) and innate lymphoid cells [2,6,7]. Thus, The maintenance of a balanced, functional population of Tregs is crucial for prevention of

Abbreviations: irAEs, autoimmune related adverse events; TME, tumor microenvironment; Tregs, Regulatory T cells; Th, $CD4^+$ T helper; nTregs, natural Tregs; iTregs, induced Tregs; DCs, dendritic cells; APCs, antigen presenting cells; TILs, tumor infiltrating lymphocytes; NK, natural killer cells; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; PD1, programmed death 1; LAG3, Lymphocyte-activation gene 3; ADCC, antibody-dependent cellular cytotoxicity; mAb, monoclonal antibody ()

[*] Corresponding author. Center of Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation, Academy of Athens, 115 27, Athens, Greece.

E-mail address: pverginis@bioacademy.gr (P. Verginis).

[1] These authors contributed equally to this work.

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autoimmune manifestations and chronic inflammatory diseases.

Treg cells comprise approximately 5–10% of the peripheral $CD4^+$ T helper (Th) cells [8,9] and express high levels of CD25, the high affinity alpha chain of the interleukin-2 receptor, on the cell surface [10–12]. Nonetheless, up to date, the most definite marker characterizing Tregs is the transcription factor forkhead box P3 (Foxp3) [13,14] although in humans it might be transiently expressed as well in other cell types [15]. Foxp3 orchestrates Treg differentiation and functions, while induced Foxp3 expression by non-Treg T cells in peripheral tissues is potent of conferring suppressive activity [2]. Importantly, in humans, loss-of-function mutations in the Foxp3 gene result in the X-linked disorder, described as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX syndrome), which is characterized by severe allergies, excessive inflammation and multi-organ autoimmunity, requiring bone marrow transplantation in early childhood [16,17]. Similarly, the mouse mutant strain scurfy, which is the result of a 2-bp frameshift insertion in the mouse Foxp3, displays a lethal phenotype characterized by hyperactivation and expansion of $CD4^+$ T cells and overproduction of pro-inflammatory cytokines [12,13]. The phenotype of these disorders is the result of failed Tregs differentiation into effective immune suppressors and outlines their importance of the Foxp3 transcription factor for the normal development and function of Tregs.

Tregs are classically divided in two major cell subtypes; the thymic or natural Tregs (nTregs) and the peripheral or induced Tregs (iTregs). The first subtype arises in thymus in early life during T cell development, comprising 5–10% of total peripheral T cells, while the second subtype is generated from conventional $CD4^+FoxP3^-$ cells later in the periphery, upon up-regulation of Foxp3 transcription factor following exposure to specific tolerogenic stimuli [5,18–20]. Although their separate functions have not been yet clearly elucidated, it is undisputable that those two distinct subtypes do exert different, yet complementary roles. More specifically, nTreg cells seem to have a prominent role in recognizing self-antigens, while iTregs are implicated in establishing tolerance to non-self antigens (e.g. gut commensal bacteria and innocuous antigens present in food) [21,22]. The mechanisms underpinning the development and antigen specificities of the two subtypes probably differ and lead to a global promotion of central and peripheral tolerance.

As is shown in Table 1 Tregs preserve the immune tolerance by a plethora of mechanisms and molecules, which include cytokine secretion (including interleukin 10 –(IL-10), interleukin 35 –(IL-35), and transforming growth factor β – (TGF-β) [23–25], metabolic disruption through CD39:CD73 adenosine production or interleukin 2 (IL-2) deprivation [26–28], direct cytolysis through granzyme B [29], and modulation of DCs development and function via lymphocyte activation gene 3 (LAG3) and cytotoxic T lymphocyte associated antigen 4 (CTLA-4) [30,31]. Collectively, through their interactions and their secreted mediators, these cells inhibit the proliferation and functions of immune effector cells, while they are also capable of killing antigen presenting cells (APCs) and T cells. However, despite excessive experimental evidence regarding the biology and function of Tregs, the mechanisms and molecules dictating their contribution in the shaping of diseases, such as autoimmune syndromes, inflammatory disorders and cancer remain undetermined.

2. Tregs in cancer

Studies in mice and humans have demonstrated the central role of Tregs during neoplastic transformation and progression, as they are abundantly recruited in the tumor mass, where they mediate the formation of a tolerogenic microenvironment, functioning as powerful inhibitors of the anti-tumor immunity [18,32]. Notably, in the context of the tumor microenvironment (TME), Tregs redirect immune homeostasis towards an immunosuppressive state, thereby promoting immune evasion of cancer cells [33,34]. Importantly, intra-tumoral Tregs impair presentation of tumor antigens, disrupting their recognition and thus the elimination of cancer cells by immune effector cells. Moreover, Tregs seem to affect almost all immune cells present in the TME, besides T cells. As an outcome, due to their enhanced presence in the TME, the expansion of the cancerous lesion is promoted [32,33,35]. Collectively, their functions significantly contribute to the progressive features of cancer cells, increasing the need for unraveling the mechanisms and the cell populations mediating their recruitment and expansion in the TME in order to combat cancer more effectively.

Table 1
Treg function and recruitment in cancer.

Treg function in cancer		
Molecule	Target	Reference
IL-10	IL-10R	Maynard et al., Nat Immunol., 2007
IL-35	IL-12Rβ2	Collison et al., Nature, 2007
TGF-β	TGF-βR	Nakamura et al., JEM, 2001
CD39:CD73	ATP	Deaglio et al., JEM, 2007
IL-2 Rα (CD25)	IL-2	Thornton et al., JEM, 1998
Granzyme B	Procaspase-8, Bid	Gondek et al., J Immunol., 2005
LAG3	MHC class II molecules	Liang et al., J Immunol., 2008
CTLA-4	CD80/CD86	Grohmann et al., Nat Immunol., 2002
Treg recruitment in cancer		
Molecule	Receptor	Reference
CCL22	CCR4	Curiel et al., Nat Med, 2004
CCL5	CCR5	Wang et al., Oncogene, 2017
CCL28	CCR3, CCR10	Facciabene et al., Nature, 2011
CCL2	CCR2, CCR4	Loyher et al., Cancer Res, 2016; Sharabi et al., Nat Rev Drug Discov, 2018
CXCL12	CXCR4	Sharabi et al., Nat Rev Drug Discov, 2018
CCL8	CCR5, CCR10	Halvorsen et al., Oncoimmunology, 2016; Sharabi et al., Nat Rev Drug Discov, 2018

The mechanisms that drive intra-tumor Treg cell aggregation have not been fully elucidated. Several evidence indicate that Tregs, through the fatty acid pathway, exploit the metabolites of tumors in order to obtain energy supply. Thus, they have augmented survival capacity, which leads to their increased accumulation in the tumor masses [36]. Aside from the metabolic causes, there are still many other triggers of the enriched Treg presence in the TME. Experimental evidence implicate also the recruitment of Tregs in tumor masses through chemokines produced by either tumor or host cells (summarized in Table 1), such as CCL22, CCL5, CCL28, CCL2, and CXCL12 CXCL12 [37–43]. In particular, pancreatic [44], colon [45], ovarian and hepatocellular carcinoma cells [46] have been found to secrete CCL22, CCL5 and CCL28 in humans and experimental animal models. Additionally, CCL22 is produced by infiltrating macrophages, DCs [37] and $CD8^+$ T cells in various tumors [47], while cancer associated fibroblasts (CAFs) produce CCL5 in experimental models of breast cancer [48]. Furthermore, Tregs of cancer patients express CCR4, CCR1, CCR5, CCR10, and CXCR4 receptors and by blockading these chemokine receptors the migration of human Tregs in vitro is halted, while the recruitment of Tregs is impaired in solid tumor models [37,38,44,48]. Finally, the local spread of nTregs and the immense resistance of Tregs to reactive oxygen species (ROS) extensively produced in the TME have a pivotal role in the recruitment of Tregs in tumor sites [42,43].

A direct consequence of their intense accumulation and aggregation in the TME is their enhanced presence in the tumor bed. Indeed, Tregs were found significantly increased within the TME of various tumor types in humans and mice [49,50]. Higher Treg cell numbers in TME have been associated with reduced patient survival, increased likelihood for metastasis and advanced-stage disease in many types of cancer, including melanoma, pancreatic ductal adenocarcinoma and

non small-cell lung, gastric and ovarian cancer [43,51,52]. Furthermore, Treg levels have also been noted to be significantly elevated in the peripheral blood of patients of pancreatic ductal adenocarcinoma (PDAC) [53,54], gastrointestinal [55], eosophageal [56] and breast cancer [54], outlining their potential role as clinically relevant biomarkers of poor disease prognosis [53,55,56]. However, the correlation of increased Treg cell numbers both in the tumor and in the periphery with survival and disease stage remains ambiguous, as other studies have demonstrated that a stronger Treg presence is correlated with favorable prognosis [37,57]. With an absent unified system for the study and analysis of Tregs, along with the lack of Treg-specific markers, there is a difficulty in providing a definite explanation regarding the discrepancies observed in literature [3].

Besides their generation and accumulation in the tumor mass, the phenomenon of Treg induction is also quite expanded in the TME. A significant percentage of tumor-infiltrating $CD4^+$ $Foxp3^-$ T cells upregulates Foxp3, possibly due to molecules secreted in abundance by cells infiltrating the TME [3,58]; for instance, cancer cells, DCs and stromal cells such as CAFs are a major source of TGF-β, a growth factor essential for the de novo formation of Tregs in the tumor stroma, while molecules facilitating iTreg differentiation such as interleukin 10 (IL-10), indoleamine 2,3-dioxygenase (IDO), cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE2) have been found to be produced in abundance by cancer cells, immune cells (DCs and B cells) and other stromal cells in human tumor specimens. Moreover, studies in colorectal and prostate cancer have demonstrated that Tregs present in the TME proliferate robustly, in contrast to their anergic natural state [7,59]. However, the specific mechanisms responsible for the intra-tumor induction and proliferation of Treg cells remain poorly understood.

The implementation of Tregs in cancer immunology and especially in tumor immune escape mechanisms is being intensively investigated. Collective experimental and clinical evidence indicate that intra-tumoral Tregs boost tumor growth and progression by suppressing anti-tumor immune responses, promoting angiogenesis and stimulating metastasis [38,43,48]. Specifically, through Treg cell depletion in experimental murine tumor models, their role as regulators of the anti-tumor immunity has been acknowledged. Ablation of Tregs is associated with delayed tumor growth and progression [60], with direct consequences on tumor infiltrating immune cell populations: after Treg elimination, proliferation and cytokine production of T effector cells is augmented, while excessive macrophage activation is observed [61–63].

The mechanisms (see also Table 1) by which Tregs induce immune suppression in the TME have been extensively studied; thus, Tregs suppress the proliferation, activation and function of immune effector cells. First of all, they modulate the activity of APCs by engaging inhibitory co-stimulatory receptors on their surface and in that way, signaling between APCs and T cells is weakened or abolished [64,65]. On the same line, they down-regulate expression of CD80, CD86 and CD40 on DCs [66]. Secondly, Tregs, through the secretion of inhibitory cytokines (e.g. IL-10, IL-35, TGF-β), restrain the activity of immune cells [67], while they antagonize effector cells for signals or cytokines (e.g. IL-2) from APCs. Furthermore, in lymphoma and melanoma mouse models, Tregs have been demonstrated to produce perforin and granzyme B, inducing cytotoxicity and apoptosis in $CD8^+$ T cells, NK cells and DCs [68,69]. Isolated intra-tumoral Tregs are capable of inhibiting the proliferation of T cells and NK cells in vitro, by disrupting their metabolism [7,37,70]. They also inhibit the cytotoxic activities of immune effector cells, by suppressing IFN-γ production in $CD8^+$ T cells [71], down-regulating the expression of the activating receptor natural killer, group 2, member D (NKG1D) in NK cells [72] and the production of tumor necrosis factor a (TNF-α), IL-12 and CCL5 in DCs. Finally, Tregs in a sudden death state, induced by their deficient nuclear factor erythroid 2-related factor 2 (NRF2) antioxidant system and their great vulnerability to free oxygen species in the TME, produce large numbers of adenosine due to elevated release of adenosine triphosphate (ATP) and increased CD73/CD39 expression, and as a result, they engage an even more immunosuppressive profile, affecting T cell function [65,73].

Collectively, studies indicate that targeting Tregs in a clinical setting, either by depletion or functional modulation, shall prove to be an important therapeutic asset. Emerging approaches aim to define appropriate targets for selective interference with tumor-specific Tregs. Despite extensive analysis though, challenges on Tregs and their operation to cancer therapy are still undergoing.

3. Tregs and cancer immunotherapy

Immunotherapy and specifically the initiation of immune checkpoint inhibitors (ICI) generated a paradigm shift in cancer treatment. Immune checkpoints are co-inhibitory ligands that downregulate the activation and function of T cells and thus are crucial for maintaining self-tolerance and modulating the amplitude and duration of immune responses in order to avoid tissue damage. Treatment with blocking antibodies for CTLA4 (ipilimumab, tremelimumab) programmed death ligand 1 (PDL1) (durvalumab, avelumab, atezolizumab) and programmed death 1 (PD1) (nivolumab, pembrolizumab, pidilizumab, atezolizumab, nivolumab) are FDA approved and have created promising results in the therapy of melanoma, kidney cancer, colorectal cancer, head and neck malignancies and bladder cancer. New inhibitory pathways are under investigation, and drugs blocking LAG-3, T cell immunoglobulin mucin 3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT) and V-domain Ig Suppressor of T cell Activation (VISTA) are being investigated. Similar to immune checkpoint molecules, agonistic antibodies for co-stimulatory pathways such as 4-1BB and *OX40* that augment immunological responses against malignant cells are under clinical trials. Despite the promising clinical efficacy of the ICI the critical cellular targets for their function remain unknown. Tregs abundantly express both co-inhibitory (CTLA4, PD1, TIGIT, VISTA, TIM-3, LAG3) and co-stimulatory molecules (GITR, 4-1BB, ICOS, OX40) at levels that are dependent on the TME (Fig. 1), indicating that antibodies targeting these proteins could affect their function. However a definite correlation between the therapeutic efficacy and Treg numbers/function in cancer is yet to be established. In the following sections we will present data of mouse models and human studies on the effect of the most commonly used ICI on Treg survival and function.

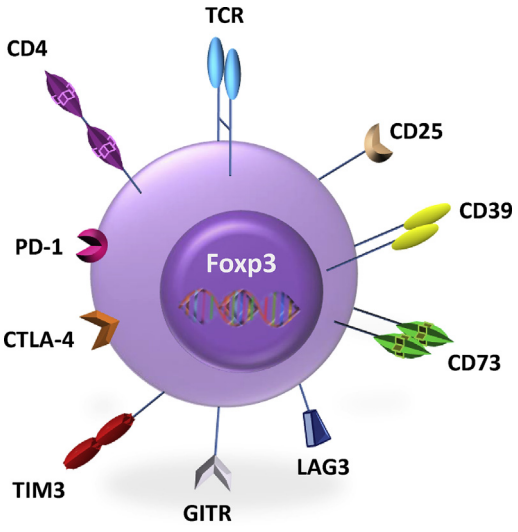

Fig. 1. Foxp3+ Tregs express various checkpoint molecules that are targets of cancer immunotherapy.

4. FDA approved immune checkpoint inhibitors

4.1. Cytotoxic T-lymphocyte–associated antigen 4 (CTLA4)

CTLA4 is constitutively expressed on Tregs [74,75] and is integral to their regulatory function at least in part by controlling CD80/86 expression [74,75] and the antigen presentation machinery in antigen presenting cells [76]. Indeed, Treg specific CTLA4 deletion is associated with a profound reduction of their suppressive capacity resulting in spontaneous development of systemic lymphoproliferation and fatal T cell mediated autoimmune disease [77]. Although is known that CTLA4 is predominantly expressed in effector Tregs in melanoma tissues compared to the other immune population infiltrating tumors [78] and anti-CTLA4 is a first line immunotherapy in melanoma, our knowledge is limited on how anti-CTLA4 affects Treg function in tumors. In preclinical cancer mouse models treatment with anti-CTLA4 results in the depletion of Tregs in the TME through antibody-dependent cellular cytotoxicity (ADCC) reliant on FCγRIV receptors thereby augmenting tumor immunity [79–83]. Nevertheless, the depletion seems to be restricted to the TME and peripheral Tregs remain intact as shown in colon cancer bearing mice [81] or even increase in anti-CTLA4 monoclonal antibody (mAb) and granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transfected tumor cell vaccine (GVAX) treated melanoma bearing mice [82]. This effect may be due to the fact that tumor-infiltrating Tregs express higher levels of CTLA4 compared to peripheral Tregs. An in vitro study revealed another aspect of anti-CTLA4 effect on Tregs through reduction of their proliferation and suppressive capacity [84]. Although Tregs are the predominant immune population expressing CTLA4 in tumors, the therapeutic effect of anti-CTLA4 is not exclusive due to Treg deletion or inhibition. When only Tregs were targeted tumor grew normally in a sophisticated set of experiments with melanoma-bearing immunodeficient $Rag^{-/-}$ (that lack T and B cells due to the deletion of recombination activating gene - Rag) mice reconstituted with different combinations of Tregs and T effectors expressing the human or the murine CTLA4 receiving GVAX and anti-murine CTLA4. Thus exclusive Treg targeting does not seem to account for the anti-tumor effect but anti-CTLA4 effect on T effector cells is also needed [84].

In accordance to the data from mouse models ex vivo human studies showed that ipilimumab depleted the intratumoral Tregs in melanoma patients by an FCγRII-a dependent mechanism. Moreover, two small clinical studies have reported a correlation between Treg cell depletion in tumors and therapeutic response to anti-CTLA4 mAb therapy [79,85]. Nevertheless, quantitative immunohistochemistry for the evaluation of Treg densities in stage matched patients with different solid tumors treated with ipilimumab (fully human IgG1 anti-CTLA4 mAb) or tremelimumab (fully human IgG2 anti-CTLA4 mAb) did not show Treg depletion [86]. Comparable to mouse data, circulating Treg numbers are not affected by anti-CTLA4 treatment in patients with prostate, gastric and esophageal cancer [87–90]. Moreover, an international multicenter phase II trial evaluating the efficacy of tremelimunmab in patients with advanced melanoma showed no significant difference in the percentages of $CD3^+CD4^+CD25^+FOXP3^+$ in the periphery, nonetheless therapy induced a small but significant increase in their total number [91]. In accordance, patients with progressive metastatic hormone-refractory prostate cancer receiving ipilimumab [87] and melanoma patients treated with interferon a2b and tremelimumab [92] presented an expansion of $CD4^+CD25^+$ Tregs in their periphery. The majority of studies support that tumor-infiltrating Tregs are eliminated following anti-CTLA4 treatment whereas it seems that numbers of peripheral Tregs depend on the different genetic background of patients and different time point of analysis following immunotherapy [93]. Indeed, in a clinical study with bladder cancer patients treated with two doses of anti-CTLA4 before surgery the percentages of peripheral $Foxp3^+$ Tregs increased after 3 weeks while decreased after 7 weeks following treatment [93]. As already mentioned CTLA4 is an important molecule in Treg suppressive function. Thus, besides the effect of anti-CTLA4 immunotherapy on Treg numbers it may also affect Treg function. Nonetheless, results from in vitro suppression assays testing the capacity of peripheral Treg isolated from cancer patients to suppress NK and $CD8^+$ T cell killing as well as $CD4^+$ T cell proliferation are inconclusive. There are studies supporting that anti-CTLA4 did not alter the suppressive capacity of Tregs isolated from patient with renal carcinoma, progressive metastatic hormone-refractory prostate cancer and melanoma [87,90,91] while others have shown that in vitro anti-CTLA4 treatment results in diminished suppressive function and depletion of Tregs. Specifically, in vitro suppression assays with Tregs isolated from tumor specimens of patients with hepatocellular carcinoma or colorectal cancer showed that addition of anti-CTLA4 reduces the suppressive capacity [94]. Moreover, Tregs isolated from periphery of healthy donors did not suppress the proliferation of autologous $CD4^+CD25^-CD127^+$ T effector cells in the presence of tremelimumab. No deletion effect of tremelimubab on Tregs was noticed in these in vitro systems [88].

5. Programmed cell death protein 1 (PD1)

Among tumor-infiltrating lymphocytes not only activated and exhausted $CD4^+$ and $CD8^+$ T cells express PD1, but also a fraction of Tregs [95]. Nevertheless, the function of PD1 on Tregs remains unclear. Tregs suppress the function of effector CD8 T cells by direct interactions of PD1 on Tregs with PDL1 on CD8 cells. Disruption of this interaction with a blocking antibody resulted in the abolishment of Treg suppressive function in a mouse model of chronic infection [96]. On the other hand, PD1 is an inhibitor for T cell receptor (TCR) signaling. Thus PD1 blockade may result in the reinforcement of Treg activation and suppressive function. Indeed, Tregs lacking PD1 presented increase suppressive capacity and rescued mice with autoimmune pancreatitis [97]. In contrast to the role of PD1 as a co-inhibitory ligand it has been also reported as a stabilizing signal for Tregs. Specifically, in melanoma tumor models PDL1 binding to PD1-expressing Tregs maintained foxp3 expression and increased the numbers of induced Tregs Indeed, in a clinical study with bladder cancer patients treated with two doses of anti-CTLA4 before surgery the percentages of peripheral $Foxp3^+$ Tregs increased after 3 weeks while decreased after 7 weeks following treatment [98]. Moreover, in mouse tumor models only one study has reported that anti-PD1 treatment diminishes Treg numbers in the TME like it was observed with anti-CTLA4 [83].

In accordance to mouse data anti-PD1 mAb administration in melanoma patients does not affect Treg numbers in the TME [99]. Nevertheless, in a fraction of advanced gastric cancer patients treated with PD1 blockade the frequencies of proliferating effector Tregs were increased in tumors and this was correlated to rapid cancer progression (hyper-progressive disease) [100]. Although several in vitro studies have been performed for the elucidation of anti-PD1 effect on tumor infiltrating Tregs, the results remain inconclusive. On the one hand anti-PD1 mAb enhances the suppressive capacity of effector Tregs in vitro, indicating that PD1 expressed on effector Tregs is a negative regulator of Treg cell-mediated immunosuppression [100]. On the other hand anti-PD1 treatment of peripheral blood mononuclear cells from melanoma patient's downregulates Tregs suppressive function by inhibiting foxp3 expression [101,102] and inhibits their in vitro expansion [103].

6. Co-inhibitory molecules as targets in clinical trials

T cell immunoreceptor with Ig and ITIM domains (TIGIT) is a co-inhibitory receptor upregulated in T cells following activation that upon binding to its ligand (CD122 and CD155) inhibits T cells responses [104]. TIGIT expression was found upregulated in Tregs infiltrating the tumors of melanoma-bearing mice. TIGIT genetically deficient Tregs were unable to suppress the anti-tumor response because TIGIT

signaling proved to be crucial for the stability of Tregs [105].

V-domain Ig Suppressor of T cell Activation (VISTA) is a B7 family inhibitor expressed on tumor infiltrating T-lymphocyte and myeloid cells, leading to suppression of T-cell activation, proliferation and cytokine production [106]. Its extracellular domain is similar to that of PDL1. Blockage of VISTA reduces the number of Tregs and induces anti-tumor immune responses in cancer mouse models [107].

T cell immunoglobulin mucin 3 (TIM3) is a direct negative regulator of T cells mainly expressed in high levels by exhausted T cells [108]. Tim3 also marks highly active Foxp3$^+$ Tregs exhibiting enhanced suppressive function critical in maintenance of tolerance to allografts [109]. In tumor-bearing mice the majority of intra-tumoral Tregs express TIM3 in contrast to peripheral Tregs and its expression is correlated to a highly suppressive phenotype [108]. The only study that describes how TIM3 blockade affects Treg function is a transgenic HNSCC mouse model, where it has been shown that anti-TIM3 monoclonal antibody induces a reduction of CD4$^+$CD25$^+$Foxp3$^+$ Tregs accompanied by increased anti-tumor immune response [110].

Lymphocyte activation gene-3 (LAG3) is upregulated upon activation of CD4$^+$, CD8$^+$ T cells and NK cells [111]. LAG3 binds to (major histocompatibility complex) MHC class II as it structurally resembles to the CD4 co-receptor [112]. Apart from the effector cells, LAG3 is also expressed by Tregs [113]. Blockade of LAG3 on Treg cells abrogates Treg cell suppressor function in in vitro systems and in mice with lethal pneumonitis [113]. In accordance, ectopic expression of LAG3 in non-Treg CD4$^+$T cells confers suppressive activity [114]. Although the therapeutic value of anti-LAG3 is evaluated in clinical trials, data is not yet available on the expression of LAG3 on tumor-infiltrating Tregs and the effect of anti-LAG3 mAbs to Treg function in tumors.

7. Co-stimulatory molecules as targets in clinical trials

Glucocorticoid-induced TNFR-related protein (GITR) belongs to the tumor necrosis factor receptor (TNFR) family and is widely accepted as a Treg marker whose gene locus is demethylated in Tregs [115]. Results on its role on Treg function are inconclusive. For instance it has been shown that activation of GITR signaling in Tregs by agonistic GITR antibody can lead to reduced suppressor function [94,116] while another study has shown that over expression of GITR ligand (GITRL) induced an increase in Tregs in parallel with a delayed onset of experimental autoimmune encephalomyelitis [117].

GITR is highly expressed by Tregs infiltrating tumors of patients with hepatocellular carcinoma or colorectal cancer [94]. Preclinical studies in mice using agonistic antibodies targeted GITR have shown compelling antitumor activity in syngeneic mouse tumor models [118,119]. The therapeutic effect of anti-GITR antibodies has been mainly attributed to Treg depletion dependent on co-engagement of activatory FcγRs [80]. In detail anti-GITR treatment depleted Tregs at the tumor site of an anti-PD1 resistant non-small cell lung adenocarcinoma preclinical tumor model and this phenotype was correlated to the therapeutic effect of the antibody since depletion of CD4$^+$ T cells abolished anti-GITR tumor control [120].

4-1BB (CD137) is a member of the TNFR family expressed by a plethora of immune cells, such as DCs, NK cells and activated T cells. 4-1BB ligation on T cells upregulates anti-apoptotic molecules, cytokine secretion, and enhances effector function [121]. 4-1BB is also a downstream target of foxp3. It is expressed on resting Tregs and is increased upon cell activation [122]. Intra-tumoral Tregs highly express 4-1BB both in mouse models and in humans [123]. Only studies in preclinical mouse models have shown its role in promoting anti-tumor immunity [124]. Its beneficial activity has been attributed to the depletion of intra-tumoral Tregs in an activatory FcγR-dependent manner. Although other immune cells such as the CD8$^+$ T cells also expressed 4-1BB the deletion was specific for Tregs since Tregs expressed higher amounts [123]. The depleting capacity of the mAb was dependent on its isotype: IgG2a was more efficient at depleting Tregs compared to the equivalent IgG1 which presented an agonist function on CD8 T cells and not on Tregs [123].

Inducible T cell co-stimulator (ICOS) is a member of the immunoglobulin (Ig) family of co-receptor molecules presenting significant homology with the co-stimulatory molecule CD28. It is mainly expressed by activated T cells and binding to its ligand results in enhancement of their activation and proliferation [125]. In Tregs ICOS has been linked to their maintenance in non-obese diabetic (NOD) mice [126] and their proliferation and immunosuppression [127]. Human breast tumor Tregs express high levels of ICOS [128] anti-ICOS blocking antibody inhibitis Treg proliferation isolated from breast cancer patients by abolishing their interaction with ICOS lingand (ICOSL) expressed on plasmatocytoid DCs [128].

OX-40 (CD134) is a TNFR that function as a T cell co-stimulatory molecule. It is upregulated upon T cell activation and promotes T cell survival, effector function, T cell memory and enhances cell mobility [129]. OX-40 is highly expressed on tumor-infiltrating Tregs in many cancers such as melanoma, colon cancer, head and neck cancer [130]. An agonistic anti-OX40 antibody depleted the intratumoral Tregs through FcγR mediated ADCC, which correlated with tumor regression [131]. In contrast to the above preclinical studies in clinical trials the agonistic anti-OX40 antibody resulted in increased percentages of Tregs infiltrating tumors [132].

Overall, inhibitory antibodies against co-inhibitory molecules and agonistic antibodies against co-stimulatory molecules seem to exert their anti-tumor effect in part by deleting the suppressive arm of the TME, the Tregs and/or by abolishing their suppressive function. Nevertheless, more studies are needed to clarify the effect of cancer therapeutic monoclonal antibodies on Tregs. It seems that their effect on Tregs depends on the genetic background of the patients, the isotype of the antibody and the time point of examination. Nevertheless, none of the already conducted studies have elucidated the exact mechanism and pathway in Tregs altered by this type of treatment.

8. Implication of tregs in irAEs development following ICI therapy

By unleashing the breaks of the immune system, ICI give rise to a wide spectrum of severe and occasionally life-threatening autoimmune manifestations, described as irAEs [133–136]. As the use of ICI becomes more common, especially as first- and second-line treatments, and new immunotherapies are currently under clinical trials, the occurrence of irAEs is expected to rise.

irAEs occur in up to 70% of patients treated with anti-CTLA4 and in 50% of those receiving anti-PD1/PDL1 antibodies [137–139]. IrAEs usually develop within the first few weeks to months after treatment initiation. However, irAEs can present at any time, including after cessation of immune checkpoint blockade therapy, and may wax and wane over time. A review of recent literature indicates that different grades of adverse events affecting nearly every organ system have been reported in association with cancer immunotherapy. irAEs can generally involve the gastrointestinal, liver, skin, nervous and endocrine systems. Management of irAEs includes interruption/discontinuation of therapy, the use of immunosuppressant such as systemic high-dose corticosteroids and antibodies against tumor-necrosis factor (TNFα) [136,140]. The CTLA4 and PD1/PDL1 inhibitors utilize distinct mechanisms to mediate their effects on T cell response, resulting in distinct toxicity patterns and toxicity kinetics. To this end, colitis is the most prevalent adverse event following anti-CTLA4 monotherapy [141], whereas thyroiditis and pneumonitis [142] are more common in anti-PD1/PDL1 treated patients [143]. Grade 1 (characterized as asymptomatic, minimally symptomatic, or radiographic or laboratory change) and 2 events (characterized by mild-to-moderate or persistent symptoms) are most common in the skin and the bowel, whereas grade 3 (moderate-to-severe symptoms) and 4 toxicities (life-threatening symptoms) are prevalent in the digestive tract (14% for anti-PD1 and 25% for anti-CTLA4) and warrant attention [144,145], as in extreme

Table 2
Summary of irAEs following ICI therapy.

Target	Drug	Trial	Phase	Type of cancer	irAEs
CTLA4	Ipilimumab			Melanoma	intestinal (colitis); liver (hepatitis); skin; nerve; and hormone gland (especially the pituitary, adrenal, and thyroid glands)
PD1	Nivolumab			Melanoma, non–small-cell lung cancer, renal-cell carcinoma, hepatocellular carcinoma, classic Hodgkin's lymphoma, squamous-cell carcinoma of the head and neck, urothelial carcinoma, colorectal cancer	Lung (pneumonitis); intestinal (colitis)); liver (hepatitis); hormone gland; kidney (neuphritis); skin; brain (encephalitis)
PD1	Pembrolizumab			Melanoma, non–small-cell lung cancer, classic Hodgkin's lymphoma, squamous- cell carcinoma of the head and neck, urothelial carcinoma, gastric cancer, solid tumors	Lung (pneumonitis); intestinal (colitis)); liver (hepatitis); hormone gland; kidney (neuphritis); skin; brain (encephalitis)
PDL1	Atezolizumab			Non–small-cell lung cancer, urothelial carcinoma	Pneumonitis; hepatitis; colitis; endocrinopathies
PDL1	Avelumab			Merkel-cell carcinoma, urothelial carcinoma	Pneumonitis; hepatitis; colitis; endocrinopathies
PDL1	Durvalumab			Urothelial carcinoma	Pneumonitis; hepatitis; colitis; endocrinopathies
TIM3	MBG453	NCT02608268	I/II	Advanced malignancies	
	MEDI9447	NCT02503774	I	Solid tumors	
LAG3	IMP321	NCT00732082	I	Pancreatic cancer	
		NCT00349934	I	Breast cancer	
		NCT02614833	II	Breast cancer	
	BMS-986016	NCT01968109	I	Melanoma	
	LAG525	NCT02460224	I/II	Solid malignancies	
TIGIT	OMP-31M32	NCT03119428	I	Solid tumors	
4-1BB	Utomilumab	NCT02179918	I	Solid tumors	No dose-limiting toxicities, most were grade 1–2 AEs
		NCT01307267	I	Renal cell carcinoma, melanoma, Non–small-cell lung cancer, Hodgkin's lymphoma, squamous-cell carcinoma of the head and neck	
	Urelumab	NCT02253992	I/II	Solid tumors and non Hodgkin's lymphoma	Elevated transaminases and grade3-4 hepatitis
OX40	9B12	NCT01644968	I	Solid tumors	Grade 3 or more lymphopenia
	MOXR 0916	NCT02410512	I	Solid tumors	No dose limiting toxicities
GITR	TRX-518	NCT01239134	I	Solid tumors	No dose-limiting toxicities or grade 3–5 AEs
	BMS-986156	NCT02598960	I	Solid tumors	grade 4 creatine phosphokinase elevation
	AMG 228	NCT02437916	I	refractory colorectal cancer, head and neck squamous cell carcinoma, urothelial carcinoma, and melanoma	hypophosphatemia, anemia, and fever

cases these can be severe and life threatening. Importantly, almost all patients (93%) develop irAEs following concurrent anti-PD1 and anti-CTLA4 combinatorial therapy with an increase in grade 3 or 4 irAEs (50%) (summarized in Table 2). Since there are no standardized diagnostic criteria for irAEs, there is a concern that irAEs are under-reported; therefore, their true burden in clinical practice may be more extensive than has been so far reported [146]. Moreover, organ involvement in an autoimmune response is unpredictable from patient to patient, maybe due to variations in genetics, epigenetics, or microbiota environment, and/or because of polymorphisms in the checkpoint molecules [147]. While, there is literature that correlates responsiveness to checkpoint blockade with increased risk of developing severe autoimmune adverse events [148,149], however, other studies have failed to identify such correlation [150], thus no safe conclusion about the efficacy-toxicity relationship can be yet drawn.

Although the specific pathogenic processes of irAEs, remain largely unknown, several mechanisms have been proposed to be implicated in irAEs development such as genetic predisposition, gut microbiome, epitope spreading and cross-presentation of neoantigens (reviewed elsewhere: [151,152]). Here we will focus on the possible role of Tregs in the pathogenesis of irAEs.

As autoimmunity and cancer represent the two sides of the same coin perhaps it is not surprising that as we manipulate the immune system to treat cancer through the use of checkpoint therapy, we inevitably unbalance the vital mechanisms that regulate self tolerance, inducing an array of irAEs. This is in part related to the loss of Treg homeostasis which is essential for maintaining immune tolerance (Fig. 2). In support, depletion of Tregs in tumor models has been studied in Foxp3$^{DTR-GFP}$ mice, (that express a diphtheria toxin receptor (DTR) under the Foxp3 promoter allowing depletion of Tregs following DT administration), where the majority of mice clear the tumor but subsequently succumb to multi-organ autoimmunity [6], through expansion of effector T cells in affected organs and increase in IFNγ and TNF in the serum [153]. Currently used ICI can also target Tregs, as several checkpoint molecules, including CTLA4 and PD1 are highly expressed on their surface, thus it is possible that development of irAEs can be partly attributed to Treg de-stabilization. In this line, Alissafi et al., recently demonstrated that anti-CTLA4 disrupts the crosstalk between Foxp3$^+$ Tregs and antigen-presenting cells to promote autoimmunity [76]. The classical hypothesis of checkpoint inhibition was formulated based on the notion that anti-CTLA4 mAbs mediate their cancer immunity by inactivating CTLA-4:B7 interactions through *trans*-endocytosis of B7.1/B7.2 ligands and subsequent down-modulation of their co-stimulatory interaction with CD28 on T effector cells [154]. Since, human and mouse CTLA4 insufficiency is characterized by a complex immune dysregulation syndrome that leads to fatal autoimmunity [147,155–157], it can be assumed that irAEs will be the price to pay for efficient ICI therapy. On the contrary, it has been implied that instead of blocking B7-CTLA4 interaction, the therapeutic effects of anti-CTLA4 can be due to ADCC mediated depletion of Tregs by Fcγ receptor expressing macrophages present in the TME [79,81,82]. This systemic depletion of Tregs might result in loss of peripheral tolerance and development of irAEs.

PD1 inhibitors demonstrate lower prevalence of irAEs compared to anti-CTLA4 that can be attributed to distinct modes of action. Whereas CTLA4 is constitutively expressed on Tregs, PD1 expression, in contrast, is limited to a smaller sub-population of Tregs, thus restricting the adverse effects of its blockers. Studies on knockout mice have reported a significant role for PD1 in the autoimmune response. *Pdcd1*-knockout mice develop lupus-like glomerulonephritis, arthritis, autoimmune dilated cardiomyopathy, gastritis, sub-acute type 1 diabetes mellitus and lethal myocarditis depending on their genetic background [158–162]. Furthermore, Francisco et al. have demonstrated that PDL1$^{-/-}$ antigen-presenting cells cannot convert naive CD4 T cells to iTreg cells and a rapid onset of a fatal inflammatory phenotype develops in PDL1$^{-/-}$PDL2$^{-/-}$ Rag$^{-/-}$ recipients of naive CD4 T cells [163].

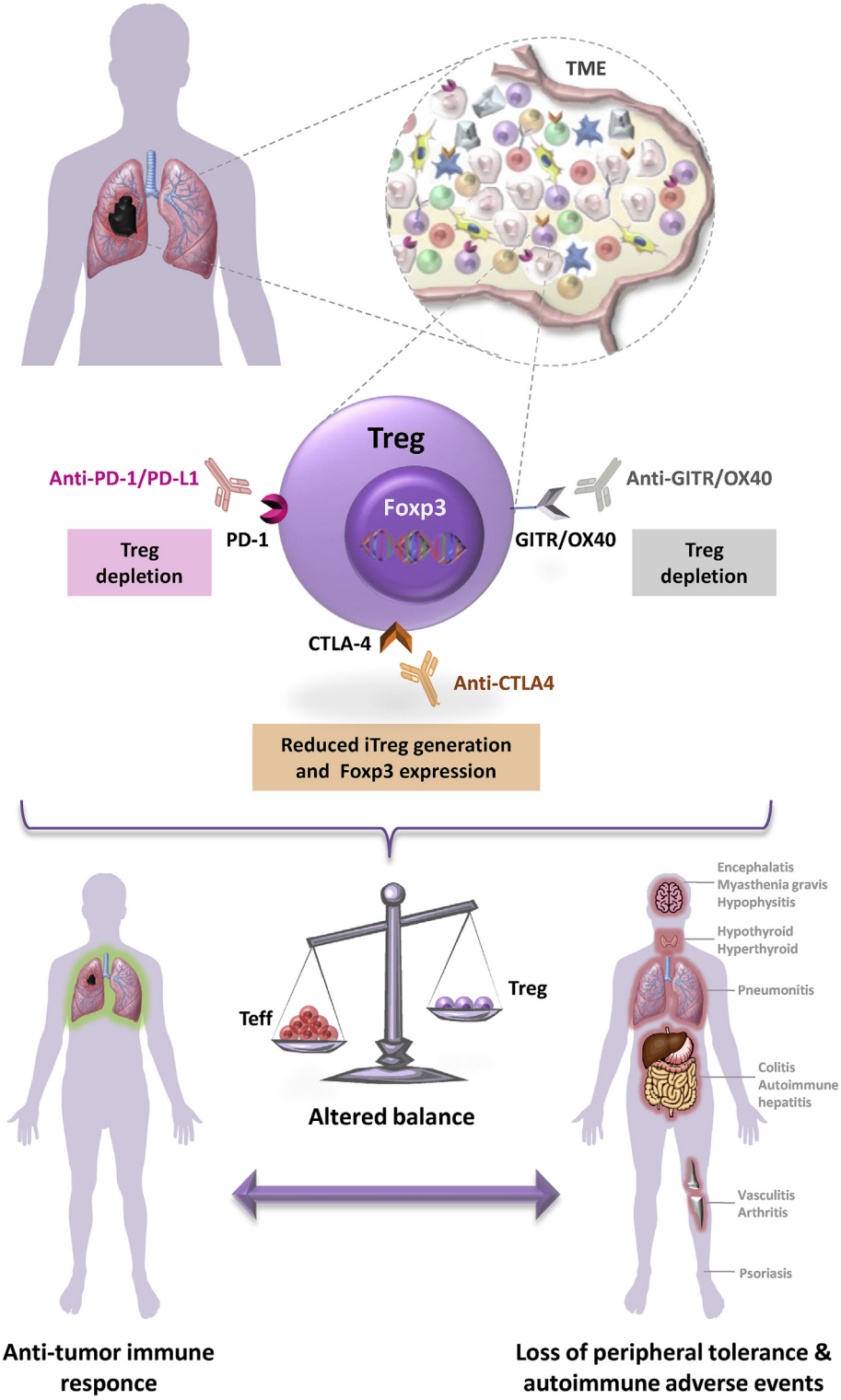

Fig. 2. Treg cell targeting by ICI immunotherapy may shift the balance towards irAEs development. ICI immunotherapy targets Treg cells via multiple mechanisms. This interaction could induce a re-programming of Tregs leading to irAEs development.

Moreover, Zhang et al. highlighted the significance of PD1 expression in Tregs to maintain their suppressive function, by crossing a down-modulated FoxP3 knock-in mouse (that did not show any marks of autoimmunity) with a PD1–deficient one. The generated mouse developed severe pancreatitis and died soon after birth due to accelerated generation of exFoxp3 Tregs [97].

Blockade of co-inhibitory receptors like TIM3, LAG3 and TIGIT whose expression is inducible and mostly restricted on intra-tumoral Tregs might result in reduced autoimmune adverse events as implied by the use of mice deficient for these co-inhibitory receptors that do not develop spontaneous autoimmunity [164–166]. Nonetheless, their efficacy and safety are yet to be determined in larger clinical trials.

Systematic delivery of 4-1BB, OX40, and GITR agonists promote anti-tumor responses through potential activation of peripheral lymphocytes by FcγR-mediated depletion of Tregs, a process inevitably bound to exacerbate autoimmune side effects. Indeed, phase I clinical

trials of mAbs verified the development of irAEs in patients treated with these agonists. In this line, 9B12 is a murine IgG monoclonal agonistic antibody against OX40 that was studied in a phase I clinical trial in 30 patients with metastatic solid malignancies [132] demonstrated transient grade 3 or higher lymphopenia in 7 patients [132]. In addition, up to 90% of patients (27/30) experienced electrolyte imbalances, anemia, and fever in a first clinical trial with AMG 228, an agonistic IgG1 monoclonal antibody of GITR, in 30 patients with refractory colorectal cancer, head and neck squamous cell carcinoma, urothelial carcinoma, and melanoma. Finally, the 4-1BB preliminary results from clinical trial showed that at least 10% of patients developed grade 3–4 transaminase elevation, and 7% of the 123 enrolled patients developed serious adverse events grade 4 severe hepatitis leading to discontinuation of 4-1BB in 5% of study patients [167].

Overall, it is becoming apparent that further research is needed in order to fully comprehend the mechanisms underlying the pathophysiology of irAEs to draw definite conclusions on how Tregs are related to autoimmune unwanted side effects arising from checkpoint blockade. Moreover, additional clinical trials using larger cohorts of patients are required to understand the potential benefits and undesirable side effects associated with the combination of anti-CTLA4/anti-PD1/anti-PDL1 with new generation ICI for cancer immunotherapy. Finally, developing improved relevant preclinical humanized animal models to mimic irAEs following ICI are urgently needed to study the possible consequences of ICI on immune homeostasis and identify the mechanisms that can be targeted to fight tumorogenesis while minimizing autoimmune adverse events. A very promising candidate for treating tumors without developing irAEs would be the specific targeting of intra-tumoral Tregs without compromising peripheral Treg homeostasis. However, such a daunting task requires a thorough knowledge of the origin, development, phenotype, and homeostasis of the tumor-specific Tregs.

9. Conclusions

Despite the emerging success of cancer immunotherapy and the advances in our understanding of tumor tolerance mechanisms, still cancer remains one of the leading causes of death globally with an estimation of 21 million cases around the world by 2030. Molecular diagnostics and personalized therapy as well as identification of novel immunotherapeutic regimens that will induce a robust clinical response remain unmet needs in oncology. The fact that most patients remain not responding to ICI, suggests that unappreciated mechanisms of resistance exist. Tregs not only possess a central role in tumor immune evasion but also constitute a fundamental obstacle for the success of cancer immunotherapy. With the abundant expression of various checkpoint molecules, Tregs are a direct target of ICI immunotherapy, however the mechanisms that ICI re-program Tregs remain elusive. In addition, the importance of Tregs in ICI resistance, tumor recurrence and irAEs development remain largely unknown. Specifically, the transcriptional and functional features of Tregs that infiltrate tumors and how these signatures altered upon ICI immunotherapy towards development of irAEs are solely unexplored. Finally, whether peripheral Tregs in cancer patients share similar signatures with tumor-infiltrating Tregs and could predict response to immunotherapy remains elusive. Delineating the characteristics and function of Tregs in cancer and in particular in ICI-mediated irAEs evolvement, may lead in the development of targeted effective therapeutic regimens and diminished autoimmune unwanted manifestations.

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References

[1] Y. Nishizuka, T. Sakakura, Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice, Science 166 (1969) 753–755.
[2] S. Sakaguchi, T. Yamaguchi, T. Nomura, M. Ono, Regulatory T cells and immune tolerance, Cell 133 (2008) 775–787.
[3] A. Hatziioannou, T. Alissafi, P. Verginis, Myeloid-derived suppressor cells and T regulatory cells in tumors: unraveling the dark side of the force, J. Leukoc. Biol. 102 (2017) 407–421.
[4] K. Wing, S. Sakaguchi, Regulatory T cells exert checks and balances on self tolerance and autoimmunity, Nat. Immunol. 11 (2010) 7–13.
[5] C.S. Hsieh, H.M. Lee, C.W. Lio, Selection of regulatory T cells in the thymus, Nat. Rev. Immunol. 12 (2012) 157–167.
[6] A. Tanaka, S. Sakaguchi, Regulatory T cells in cancer immunotherapy, Cell Res. 27 (2017) 109–118.
[7] I. Pedroza-Pacheco, A. Madrigal, A. Saudemont, Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy, Cell. Mol. Immunol. 10 (2013) 222–229.
[8] S.E. Weber, J. Harbertson, E. Godebu, G.A. Mros, R.C. Padrick, B.D. Carson, et al., Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells in vivo, J. Immunol. 176 (2006) 4730–4739.
[9] A. Sharma, D. Rudra, Regulatory T cells as therapeutic targets and mediators, Int. Rev. Immunol. (2019) 1–21.
[10] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, M. Toda, Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases, J. Immunol. 155 (1995) 1151–1164.
[11] A.R. Almeida, N. Legrand, M. Papiernik, A.A. Freitas, Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers, J. Immunol. 169 (2002) 4850–4860.
[12] L. Lu, J. Barbi, F. Pan, The regulation of immune tolerance by FOXP3, Nat. Rev. Immunol. 17 (2017) 703–717.
[13] J.D. Fontenot, M.A. Gavin, A.Y. Rudensky, Foxp3 programs the development and function of CD4+CD25+ regulatory T cells, Nat. Immunol. 4 (2003) 330–336.
[14] S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3, Science 299 (2003) 1057–1061.
[15] M.A. Gavin, T.R. Torgerson, E. Houston, P. DeRoos, W.Y. Ho, A. Stray-Pedersen, et al., Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 6659–6664.
[16] C.L. Bennett, J. Christie, F. Ramsdell, M.E. Brunkow, P.J. Ferguson, L. Whitesell, et al., The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3, Nat. Genet. 27 (2001) 20–21.
[17] S. Sakaguchi, K. Fukuma, K. Kuribayashi, T. Masuda, Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease, J. Exp. Med. 161 (1985) 72–87.
[18] W. Zou, Regulatory T cells, tumour immunity and immunotherapy, Nat. Rev. Immunol. 6 (2006) 295–307.
[19] T. Alissafi, A. Hatzioannou, M. Ioannou, T. Sparwasser, J.R. Grun, A. Grutzkau, et al., De novo-induced self-antigen-specific Foxp3+ regulatory T cells impair the accumulation of inflammatory dendritic cells in draining lymph nodes, J. Immunol. 194 (2015) 5812–5824.
[20] P. Verginis, K.A. McLaughlin, K.W. Wucherpfennig, H. von Boehmer, I. Apostolou, Induction of antigen-specific regulatory T cells in wild-type mice: visualization and targets of suppression, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3479–3484.
[21] E.V. Russler-Germain, S. Rengarajan, C.S. Hsieh, Antigen-specific regulatory T-cell responses to intestinal microbiota, Mucosal Immunol. 10 (2017) 1375–1386.
[22] K.S. Kim, S.W. Hong, D. Han, J. Yi, J. Jung, B.G. Yang, et al., Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine, Science 351 (2016) 858–863.
[23] C.L. Maynard, L.E. Harrington, K.M. Janowski, J.R. Oliver, C.L. Zindl, A.Y. Rudensky, et al., Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10, Nat. Immunol. 8 (2007) 931–941.
[24] L.W. Collison, C.J. Workman, T.T. Kuo, K. Boyd, Y. Wang, K.M. Vignali, et al., The inhibitory cytokine IL-35 contributes to regulatory T-cell function, Nature 450 (2007) 566–569.
[25] K. Nakamura, A. Kitani, W. Strober, Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta, J. Exp. Med. 194 (2001) 629–644.
[26] S. Deaglio, K.M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, et al., Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression, J. Exp. Med. 204 (2007) 1257–1265.
[27] A.M. Thornton, E.M. Shevach, CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production, J. Exp. Med. 188 (1998) 287–296.
[28] P. Pandiyan, L. Zheng, S. Ishihara, J. Reed, M.J. Lenardo, CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells, Nat. Immunol. 8 (2007) 1353–1362.
[29] D.C. Gondek, L.F. Lu, S.A. Quezada, S. Sakaguchi, R.J. Noelle, Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism, J. Immunol. 174 (2005) 1783–1786.

[30] B. Liang, C. Workman, J. Lee, C. Chew, B.M. Dale, L. Colonna, et al., Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II, J. Immunol. 180 (2008) 5916–5926.

[31] U. Grohmann, C. Orabona, F. Fallarino, C. Vacca, F. Calcinaro, A. Falorni, et al., CTLA-4-Ig regulates tryptophan catabolism in vivo, Nat. Immunol. 3 (2002) 1097–1101.

[32] E.M. Shevach, Mechanisms of foxp3+ T regulatory cell-mediated suppression, Immunity 30 (2009) 636–645.

[33] M. Najafi, B. Farhood, K. Mortezaee, Contribution of regulatory T cells to cancer: a review, J. Cell. Physiol. 234 (2019) 7983–7993.

[34] W.L. Byrne, K.H. Mills, J.A. Lederer, G.C. O'Sullivan, Targeting regulatory T cells in cancer, Cancer Res. 71 (2011) 6915–6920.

[35] T.B. Shabaneh, A.K. Molodtsov, S.M. Steinberg, P. Zhang, G.M. Torres, G.A. Mohamed, et al., Oncogenic BRAF(V600E) governs regulatory T-cell recruitment during melanoma tumorigenesis, Cancer Res. 78 (2018) 5038–5049.

[36] J.W. Locasale, Serine, glycine and one-carbon units: cancer metabolism in full circle, Nat. Rev. Cancer 13 (2013) 572–583.

[37] T.J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, et al., Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival, Nat. Med. 10 (2004) 942–949.

[38] A. Facciabene, X. Peng, I.S. Hagemann, K. Balint, A. Barchetti, L.P. Wang, et al., Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells, Nature 475 (2011) 226–230.

[39] E.C. Halvorsen, M.J. Hamilton, A. Young, B.J. Wadsworth, N.E. LePard, H.N. Lee, et al., Maraviroc decreases CCL8-mediated migration of CCR5(+) regulatory T cells and reduces metastatic tumor growth in the lungs, OncoImmunology 5 (2016) e1150398.

[40] X. Wang, M. Lang, T. Zhao, X. Feng, C. Zheng, C. Huang, et al., Cancer-FOXP3 directly activated CCL5 to recruit FOXP3(+)Treg cells in pancreatic ductal adenocarcinoma, Oncogene 36 (2017) 3048–3058.

[41] P.L. Loyher, J. Rochefort, C. Baudesson de Chanville, P. Hamon, G. Lescaille, C. Bertolus, et al., CCR2 influences T regulatory cell migration to tumors and serves as a biomarker of cyclophosphamide sensitivity, Cancer Res. 76 (2016) 6483–6494.

[42] T. Koga, M. Mizui, N. Yoshida, K. Otomo, L.A. Lieberman, J.C. Crispin, et al., KN-93, an inhibitor of calcium/calmodulin-dependent protein kinase IV, promotes generation and function of Foxp3(+) regulatory T cells in MRL/lpr mice, Autoimmunity 47 (2014) 445–450.

[43] A. Sharabi, M.G. Tsokos, Y. Ding, T.R. Malek, D. Klatzmann, G.C. Tsokos, Regulatory T cells in the treatment of disease, Nat. Rev. Drug Discov. 17 (2018) 823–844.

[44] M.C. Tan, P.S. Goedegebuure, B.A. Belt, B. Flaherty, N. Sankpal, W.E. Gillanders, et al., Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer, J. Immunol. 182 (2009) 1746–1755.

[45] L.Y. Chang, Y.C. Lin, J. Mahalingam, C.T. Huang, T.W. Chen, C.W. Kang, et al., Tumor-derived chemokine CCL5 enhances TGF-beta-mediated killing of CD8(+) T cells in colon cancer by T-regulatory cells, Cancer Res. 72 (2012) 1092–1102.

[46] L. Ren, Y. Yu, L. Wang, Z. Zhu, R. Lu, Z. Yao, Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer, Oncotarget 7 (2016) 75763–75773.

[47] S. Spranger, R.M. Spaapen, Y. Zha, J. Williams, Y. Meng, T.T. Ha, et al., Up-regulation of PD-L1, Ido, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells, Sci. Transl. Med. 5 (2013) 200ra116.

[48] W. Tan, W. Zhang, A. Strasner, S. Grivennikov, J.Q. Cheng, R.M. Hoffman, et al., Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling, Nature 470 (2011) 548–553.

[49] Y. Mizukami, K. Kono, Y. Kawaguchi, H. Akaike, K. Kamimura, H. Sugai, et al., CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of Foxp3+ regulatory T cells in gastric cancer, Int. J. Cancer 122 (2008) 2286–2293.

[50] Z. Ao, S.H. Shah, L.M. Machlin, R. Parajuli, P.C. Miller, S. Rawal, et al., Identification of cancer-associated fibroblasts in circulating blood from patients with metastatic breast cancer, Cancer Res. 75 (2015) 4681–4687.

[51] A. Facciabene, G.T. Motz, G. Coukos, T-regulatory cells: key players in tumor immune escape and angiogenesis, Cancer Res. 72 (2012) 2162–2171.

[52] Y. Togashi, K. Shitara, H. Nishikawa, Regulatory T cells in cancer immunosuppression - implications for anticancer therapy, Nat. Rev. Clin. Oncol. 16 (2019) 356–371.

[53] N. Hiraoka, K. Onozato, T. Kosuge, S. Hirohashi, Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions, Clin. Cancer Res. 12 (2006) 5423–5434.

[54] U.K. Liyanage, T.T. Moore, H.G. Joo, Y. Tanaka, V. Herrmann, G. Doherty, et al., Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma, J. Immunol. 169 (2002) 2756–2761.

[55] T. Sasada, M. Kimura, Y. Yoshida, M. Kanai, A. Takabayashi, CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression, Cancer 98 (2003) 1089–1099.

[56] F. Ichihara, K. Kono, A. Takahashi, H. Kawaida, H. Sugai, H. Fujii, Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers, Clin. Cancer Res. 9 (2003) 4404–4408.

[57] W.H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, Nat. Rev. Cancer 12 (2012) 298–306.

[58] A.M. Bilate, J.J. Lafaille, Induced CD4+Foxp3+ regulatory T cells in immune tolerance, Annu. Rev. Immunol. 30 (2012) 733–758.

[59] A.L. Chang, J. Miska, D.A. Wainwright, M. Dey, C.V. Rivetta, D. Yu, et al., CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory T cells and myeloid-derived suppressor cells, Cancer Res. 76 (2016) 5671–5682.

[60] E.L. Kachikwu, K.S. Iwamoto, Y.P. Liao, J.J. DeMarco, N. Agazaryan, J.S. Economou, et al., Radiation enhances regulatory T cell representation, Int. J. Radiat. Oncol. Biol. Phys. 81 (2011) 1128–1135.

[61] K.G. Elpek, C. Lacelle, N.P. Singh, E.S. Yolcu, H. Shirwan, CD4+CD25+ T regulatory cells dominate multiple immune evasion mechanisms in early but not late phases of tumor development in a B cell lymphoma model, J. Immunol. 178 (2007) 6840–6848.

[62] M. Onda, K. Kobayashi, I. Pastan, Depletion of regulatory T cells in tumors with an anti-CD25 immunotoxin induces CD8 T cell-mediated systemic antitumor immunity, Proc. Natl. Acad. Sci. U. S. A. 116 (10) (2019) 4575–4582.

[63] C. Asseman, S. Mauze, M.W. Leach, R.L. Coffman, F. Powrie, An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation, J. Exp. Med. 190 (1999) 995–1004.

[64] M. Caridade, L. Graca, R.M. Ribeiro, Mechanisms underlying CD4+ Treg immune regulation in the adult: from experiments to models, Front. Immunol. 4 (2013) 378.

[65] T. Maj, W. Wang, J. Crespo, H. Zhang, S. Wei, L. Zhao, et al., Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor, Nat. Immunol. 18 (2017) 1332–1341.

[66] N. Larmonier, M. Marron, Y. Zeng, J. Cantrell, A. Romanoski, M. Sepassi, et al., Tumor-derived CD4(+)CD25(+) regulatory T cell suppression of dendritic cell function involves TGF-beta and IL-10, Cancer Immunol. Immunother. 56 (2007) 48–59.

[67] C.A. Bauer, E.Y. Kim, F. Marangoni, E. Carrizosa, N.M. Claudio, T.R. Mempel, Dynamic Treg interactions with intratumoral APCs promote local CTL dysfunction, J. Clin. Investig. 124 (2014) 2425–2440.

[68] X. Cao, S.F. Cai, T.A. Fehniger, J. Song, L.I. Collins, D.R. Piwnica-Worms, et al., Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance, Immunity 27 (2007) 635–646.

[69] A. Boissonnas, A. Scholer-Dahirel, V. Simon-Blancal, L. Pace, F. Valet, A. Kissenpfennig, et al., Foxp3+ T cells induce perforin-dependent dendritic cell death in tumor-draining lymph nodes, Immunity 32 (2010) 266–278.

[70] X.L. Yuan, L. Chen, M.X. Li, P. Dong, J. Xue, J. Wang, et al., Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner, Clin. Immunol. 134 (2010) 277–288.

[71] M. Bu, Y. Shen, W.L. Seeger, S. An, R. Qi, J.A. Sanderson, et al., Ovarian carcinoma-infiltrating regulatory T cells were more potent suppressors of CD8(+) T cell inflammation than their peripheral counterparts, a function dependent on TIM3 expression, Tumour Biol 37 (2016) 3949–3956.

[72] F. Ghiringhelli, C. Menard, M. Terme, C. Flament, J. Taieb, N. Chaput, et al., CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner, J. Exp. Med. 202 (2005) 1075–1085.

[73] A. Sharma, D. Rudra, Emerging functions of regulatory T cells in tissue homeostasis, Front. Immunol. 9 (2018) 883.

[74] T. Takahashi, T. Tagami, S. Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, et al., Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4, J. Exp. Med. 192 (2000) 303–310.

[75] B. Birebent, R. Lorho, H. Lechartier, S. de Guibert, M. Alizadeh, N. Vu, et al., Suppressive properties of human CD4+CD25+ regulatory T cells are dependent on CTLA-4 expression, Eur. J. Immunol. 34 (2004) 3485–3496.

[76] T. Alissafi, A. Banos, L. Boon, T. Sparwasser, A. Ghigo, K. Wing, et al., Tregs restrain dendritic cell autophagy to ameliorate autoimmunity, J. Clin. Investig. 127 (2017) 2789–2804.

[77] K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, et al., CTLA-4 control over Foxp3+ regulatory T cell function, Science 322 (2008) 271–275.

[78] D. Ha, A. Tanaka, T. Kibayashi, A. Tanemura, D. Sugiyama, J.B. Wing, et al., Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 609–618.

[79] F. Arce Vargas, A.J.S. Furness, K. Litchfield, K. Joshi, R. Rosenthal, E. Ghorani, et al., Fc effector function contributes to the activity of human anti-CTLA-4 antibodies, Cancer Cell 33 (2018) 649–663 e4.

[80] Y. Bulliard, R. Jolicoeur, M. Windman, S.M. Rue, S. Ettenberg, D.A. Knee, et al., Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies, J. Exp. Med. 210 (2013) 1685–1693.

[81] M.J. Selby, J.J. Engelhardt, M. Quigley, K.A. Henning, T. Chen, M. Srinivasan, et al., Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells, Cancer Immunol Res 1 (2013) 32–42.

[82] T.R. Simpson, F. Li, W. Montalvo-Ortiz, M.A. Sepulveda, K. Bergerhoff, F. Arce, et al., 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.

[83] S.C. Wei, J.H. Levine, A.P. Cogdill, Y. Zhao, N.A.S. Anang, M.C. Andrews, et al., Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade, Cell 170 (2017) 1120–1133 e17.

[84] K.S. Peggs, S.A. Quezada, C.A. Chambers, A.J. Korman, J.P. Allison, Blockade of

CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies, J. Exp. Med. 206 (2009) 1717–1725.
[85] E. Romano, M. Kusio-Kobialka, P.G. Foukas, P. Baumgaertner, C. Meyer, P. Ballabeni, et al., Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 6140–6145.
[86] A. Sharma, S.K. Subudhi, J. Blando, J. Scutti, L. Vence, J. Wargo, et al., Anti-CTLA-4 immunotherapy does not deplete FOXP3(+) regulatory T cells (Tregs) in human cancers, Clin. Cancer Res. 25 (2019) 1233–1238.
[87] B. Kavanagh, S. O'Brien, D. Lee, Y. Hou, V. Weinberg, B. Rini, et al., CTLA4 blockade expands FoxP3+ regulatory and activated effector CD4+ T cells in a dose-dependent fashion, Blood 112 (2008) 1175–1183.
[88] 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.
[89] C. Ralph, E. Elkord, D.J. Burt, J.F. O'Dwyer, E.B. Austin, P.L. Stern, et al., 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.
[90] A.V. Maker, P. Attia, S.A. Rosenberg, Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade, J. Immunol. 175 (2005) 7746–7754.
[91] C. Menard, F. Ghiringhelli, S. Roux, N. Chaput, C. Mateus, U. Grohmann, et al., 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.
[92] A.A. Tarhini, L.H. Butterfield, Y. Shuai, W.E. Gooding, P. Kalinski, J.M. Kirkwood, Differing patterns of circulating regulatory T cells and myeloid-derived suppressor cells in metastatic melanoma patients receiving anti-CTLA4 antibody and interferon-alpha or TLR-9 agonist and GM-CSF with peptide vaccination, J. Immunother. 35 (2012) 702–710.
[93] C.I. Liakou, A. Kamat, D.N. Tang, H. Chen, J. Sun, P. Troncoso, et al., CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14987–14992.
[94] A. Pedroza-Gonzalez, G. Zhou, S.P. Singh, P.P. Boor, Q. Pan, D. Grunhagen, et al., GITR engagement in combination with CTLA-4 blockade completely abrogates immunosuppression mediated by human liver tumor-derived regulatory T cells ex vivo, OncoImmunology 4 (2015) e1051297.
[95] R. Montler, R.B. Bell, C. Thalhofer, R. Leidner, Z. Feng, B.A. Fox, et al., OX40, PD-1 and CTLA-4 are selectively expressed on tumor-infiltrating T cells in head and neck cancer, Clin Transl Immunology 5 (2016) e70.
[96] H.J. Park, J.S. Park, Y.H. Jeong, J. Son, Y.H. Ban, B.H. Lee, et al., PD-1 upregulated on regulatory T cells during chronic virus infection enhances the suppression of CD8+ T cell immune response via the interaction with PD-L1 expressed on CD8+ T cells, J. Immunol. 194 (2015) 5801–5811.
[97] B. Zhang, S. Chikuma, S. Hori, S. Fagarasan, T. Honjo, Nonoverlapping roles of PD-1 and FoxP3 in maintaining immune tolerance in a novel autoimmune pancreatitis mouse model, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 8490–8495.
[98] C. Stathopoulou, A. Gangaplara, G. Mallett, F.A. Flomerfelt, L.P. Liniany, D. Knight, et al., PD-1 inhibitory receptor downregulates asparaginyl endopeptidase and maintains Foxp3 transcription factor stability in induced regulatory T cells, Immunity 49 (2018) 247–263 e7.
[99] A. Ribas, D.S. Shin, J. Zaretsky, J. Frederiksen, A. Cornish, E. Avramis, et al., PD-1 blockade expands intratumoral memory T cells, Cancer Immunol Res 4 (2016) 194–203.
[100] T. Kamada, Y. Togashi, C. Tay, D. Ha, A. Sasaki, Y. Nakamura, et al., PD-1(+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 9999–10008.
[101] 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.
[102] 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.
[103] J. Rosenblatt, B. Glotzbecker, H. Mills, B. Vasir, D. Tzachanis, J.D. Levine, et al., PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine, J. Immunother. 34 (2011) 409–418.
[104] X. Yu, K. Harden, L.C. Gonzalez, M. Francesco, E. Chiang, B. Irving, et al., The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells, Nat. Immunol. 10 (2009) 48–57.
[105] S. Kurtulus, K. Sakuishi, S.F. Ngiow, N. Joller, D.J. Tan, M.W. Teng, et al., TIGIT predominantly regulates the immune response via regulatory T cells, J. Clin. Investig. 125 (2015) 4053–4062.
[106] J.L. Lines, L.F. Sempere, T. Broughton, L. Wang, R. Noelle, VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy, Cancer Immunol Res 2 (2014) 510–517.
[107] I. Le Mercier, W. Chen, J.L. Lines, M. Day, J. Li, P. Sergent, et al., VISTA regulates the development of protective antitumor immunity, Cancer Res. 74 (2014) 1933–1944.
[108] K. Sakuishi, S.F. Ngiow, J.M. Sullivan, M.W. Teng, V.K. Kuchroo, M.J. Smyth, et al., TIM3(+)FOXP3(+) regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer, OncoImmunology 2 (2013) e23849.
[109] S. Gupta, T.B. Thornley, W. Gao, R. Larocca, L.A. Turka, V.K. Kuchroo, et al., Allograft rejection is restrained by short-lived TIM-3+PD-1+Foxp3+ Tregs, J. Clin. Investig. 122 (2012) 2395–2404.
[110] J.F. Liu, L. Wu, L.L. Yang, W.W. Deng, L. Mao, H. Wu, et al., Blockade of TIM3 relieves immunosuppression through reducing regulatory T cells in head and neck cancer, J. Exp. Clin. Cancer Res. 37 (2018) 44.
[111] F. Triebel, S. Jitsukawa, E. Baixeras, S. Roman-Roman, C. Genevee, E. Viegas-Pequignot, et al., LAG-3, a novel lymphocyte activation gene closely related to CD4, J. Exp. Med. 171 (1990) 1393–1405.
[112] 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.
[113] C.T. Huang, C.J. Workman, D. Flies, X. Pan, A.L. Marson, G. Zhou, et al., Role of LAG-3 in regulatory T cells, Immunity 21 (2004) 503–513.
[114] C.J. Workman, D.A. Vignali, Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223), J. Immunol. 174 (2005) 688–695.
[115] N. Ohkura, M. Hamaguchi, H. Morikawa, K. Sugimura, A. Tanaka, Y. Ito, et al., T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development, Immunity 37 (2012) 785–799.
[116] J. Shimizu, S. Yamazaki, T. Takahashi, Y. Ishida, S. Sakaguchi, Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance, Nat. Immunol. 3 (2002) 135–142.
[117] R.W. van Olffen, N. Koning, K.P. van Gisbergen, F.M. Wensveen, R.M. Hoek, L. Boon, et al., GITR triggering induces expansion of both effector and regulatory CD4+ T cells in vivo, J. Immunol. 182 (2009) 7490–7500.
[118] M.J. Turk, J.A. Guevara-Patino, G.A. Rizzuto, M.E. Engelhorn, S. Sakaguchi, A.N. Houghton, Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells, J. Exp. Med. 200 (2004) 771–782.
[119] K. Ko, S. Yamazaki, K. Nakamura, T. Nishioka, K. Hirota, T. Yamaguchi, et al., Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells, J. Exp. Med. 202 (2005) 885–891.
[120] J.E. Schoenhals, T.R. Cushman, H.B. Barsoumian, A. Li, A.P. Cadena, S. Niknam, et al., Anti-glucocorticoid-induced tumor necrosis factor-related protein (GITR) therapy overcomes radiation-induced Treg immunosuppression and drives abscopal effects, Front. Immunol. 9 (2018) 2170.
[121] C. Chester, M.F. Sanmamed, J. Wang, I. Melero, Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies, Blood 131 (2018) 49–57.
[122] A. Marson, K. Kretschmer, G.M. Frampton, E.S. Jacobsen, J.K. Polansky, K.D. MacIsaac, et al., Foxp3 occupancy and regulation of key target genes during T-cell stimulation, Nature 445 (2007) 931–935.
[123] S.L. Buchan, L. Dou, M. Remer, S.G. Booth, S.N. Dunn, C. Lai, et al., Antibodies to costimulatory receptor 4-1BB enhance anti-tumor immunity via T regulatory cell depletion and promotion of CD8 T cell effector function, Immunity 49 (2018) 958–970 e7.
[124] I. Melero, D. Hirschhorn-Cymerman, A. Morales-Kastresana, M.F. Sanmamed, J.D. Wolchok, Agonist antibodies to TNFR molecules that costimulate T and NK cells, Clin. Cancer Res. 19 (2013) 1044–1053.
[125] A. Hutloff, A.M. Dittrich, K.C. Beier, B. Eljaschewitsch, R. Kraft, I. Anagnostopoulos, et al., ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28, Nature 397 (1999) 263–266.
[126] M. Kornete, E. Sgouroudis, C.A. Piccirillo, ICOS-dependent homeostasis and function of Foxp3+ regulatory T cells in islets of nonobese diabetic mice, J. Immunol. 188 (2012) 1064–1074.
[127] Y. Chen, S. Shen, B.K. Gorentla, J. Gao, X.P. Zhong, Murine regulatory T cells contain hyperproliferative and death-prone subsets with differential ICOS expression, J. Immunol. 188 (2012) 1698–1707.
[128] J. Faget, N. Bendriss-Vermare, M. Gobert, I. Durand, D. Olive, C. Biota, et al., ICOS-ligand expression on plasmacytoid dendritic cells supports breast cancer progression by promoting the accumulation of immunosuppressive CD4+ T cells, Cancer Res. 72 (2012) 6130–6141.
[129] G.J. Webb, G.M. Hirschfield, P.J. Lane, OX40, OX40L and autoimmunity: a comprehensive review, Clin. Rev. Allergy Immunol. 50 (2016) 312–332.
[130] S. Aspeslagh, S. Postel-Vinay, S. Rusakiewicz, J.C. Soria, L. Zitvogel, A. Marabelle, Rationale for anti-OX40 cancer immunotherapy, Eur. J. Cancer 52 (2016) 50–66.
[131] Y. Bulliard, R. Jolicoeur, J. Zhang, G. Dranoff, N.S. Wilson, J.L. Brogdon, OX40 engagement depletes intratumoral Tregs via activating FcgammaRs, leading to antitumor efficacy, Immunol. Cell Biol. 92 (2014) 475–480.
[132] B.D. Curti, M. Kovacsovics-Bankowski, N. Morris, E. Walker, L. Chisholm, K. Floyd, et al., OX40 is a potent immune-stimulating target in late-stage cancer patients, Cancer Res. 73 (2013) 7189–7198.
[133] M. Freeman-Keller, Y. Kim, H. Cronin, A. Richards, G. Gibney, J.S. Weber, Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes, Clin. Cancer Res. 22 (2016) 886–894.
[134] S.M. Amos, C.P. Duong, J.A. Westwood, D.S. Ritchie, R.P. Junghans, P.K. Darcy, et al., Autoimmunity associated with immunotherapy of cancer, Blood 118 (2011) 499–509.
[135] J.M. Michot, C. Bigenwald, S. Champiat, M. Collins, F. Carbonnel, S. Postel-Vinay, et al., Immune-related adverse events with immune checkpoint blockade: a comprehensive review, Eur. J. Cancer 54 (2016) 139–148.
[136] J.S. Weber, J.C. Yang, M.B. Atkins, M.L. Disis, Toxicities of immunotherapy for the practitioner, J. Clin. Oncol. 33 (2015) 2092–2099.
[137] J.R. Brahmer, S.S. Tykodi, L.Q. Chow, W.J. Hwu, S.L. Topalian, P. Hwu, et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer, N.

Engl. J. Med. 366 (2012) 2455–2465.
[138] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (2012) 2443–2454.
[139] C. Devaud, L.B. John, J.A. Westwood, P.K. Darcy, M.H. Kershaw, Immune modulation of the tumor microenvironment for enhancing cancer immunotherapy, OncoImmunology 2 (2013) e25961.
[140] J.S. Weber, K.C. Kahler, A. Hauschild, Management of immune-related adverse events and kinetics of response with ipilimumab, J. Clin. Oncol. 30 (2012) 2691–2697.
[141] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, et al., Improved survival with ipilimumab in patients with metastatic melanoma, N. Engl. J. Med. 363 (2010) 711–723.
[142] J. Naidoo, X. Wang, K.M. Woo, T. Iyriboz, D. Halpenny, J. Cunningham, et al., Pneumonitis in patients treated with anti-programmed death-1/programmed death ligand 1 therapy, J. Clin. Oncol. 35 (2017) 709–717.
[143] O. Abdel-Rahman, H. ElHalawani, M. Fouad, Risk of endocrine complications in cancer patients treated with immune check point inhibitors: a meta-analysis, Future Oncol. 12 (2016) 413–425.
[144] S.L. Topalian, M. Sznol, D.F. McDermott, H.M. Kluger, R.D. Carvajal, W.H. Sharfman, et al., Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab, J. Clin. Oncol. 32 (2014) 1020–1030.
[145] J.S. Weber, R. Dummer, V. de Pril, C. Lebbe, F.S. Hodi, Patterns of onset and resolution of immune-related adverse events of special interest with ipilimumab: detailed safety analysis from a phase 3 trial in patients with advanced melanoma, Cancer 119 (2013) 1675–1682.
[146] T.W. Chen, A.R. Razak, P.L. Bedard, L.L. Siu, A.R. Hansen, A systematic review of immune-related adverse event reporting in clinical trials of immune checkpoint inhibitors, Ann. Oncol. 26 (2015) 1824–1829.
[147] K. Klocke, S. Sakaguchi, R. Holmdahl, K. Wing, Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E2383–E2392.
[148] M. Vetizou, J.M. Pitt, R. Daillere, P. Lepage, N. Waldschmitt, C. Flament, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, Science 350 (2015) 1079–1084.
[149] A. Snyder, V. Makarov, T. Merghoub, J. Yuan, J.M. Zaretsky, A. Desrichard, et al., Genetic basis for clinical response to CTLA-4 blockade in melanoma, N. Engl. J. Med. 371 (2014) 2189–2199.
[150] T.Z. Horvat, N.G. Adel, T.O. Dang, P. Momtaz, M.A. Postow, M.K. Callahan, et al., Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at memorial sloan kettering cancer center, J. Clin. Oncol. 33 (2015) 3193–3198.
[151] M.A. Postow, R. Sidlow, M.D. Hellmann, Immune-related adverse events associated with immune checkpoint blockade, N. Engl. J. Med. 378 (2018) 158–168.
[152] C.H. June, J.T. Warshauer, J.A. Bluestone, Is autoimmunity the Achilles' heel of cancer immunotherapy? Nat. Med. 23 (2017) 540–547.
[153] J. Liu, S.J. Blake, H. Harjunpaa, K.A. Fairfax, M.C. Yong, S. Allen, et al., Assessing immune-related adverse events of efficacious combination immunotherapies in preclinical models of cancer, Cancer Res. 76 (2016) 5288–5301.
[154] O.S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E.M. Schmidt, et al., Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4, Science 332 (2011) 600–603.
[155] N. Mitsuiki, C. Schwab, B. Grimbacher, What did we learn from CTLA-4 insufficiency on the human immune system? Immunol. Rev. 287 (2019) 33–49.
[156] H.S. Kuehn, W. Ouyang, B. Lo, E.K. Deenick, J.E. Niemela, D.T. Avery, et al., Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4, Science 345 (2014) 1623–1627.
[157] E.A. Tivol, F. Borriello, A.N. Schweitzer, W.P. Lynch, J.A. Bluestone, A.H. Sharpe, Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4, Immunity 3 (1995) 541–547.
[158] H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor, Immunity 11 (1999) 141–151.
[159] H. Nishimura, T. Okazaki, Y. Tanaka, K. Nakatani, M. Hara, A. Matsumori, et al., Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice, Science 291 (2001) 319–322.
[160] T. Okazaki, Y. Otaka, J. Wang, H. Hiai, T. Takai, J.V. Ravetch, et al., Hydronephrosis associated with antiurothelial and antinuclear autoantibodies in BALB/c-Fcgr2b-/-Pdcd1-/- mice, J. Exp. Med. 202 (2005) 1643–1648.
[161] J. Wang, I.M. Okazaki, T. Yoshida, S. Chikuma, Y. Kato, F. Nakaki, et al., PD-1 deficiency results in the development of fatal myocarditis in MRL mice, Int. Immunol. 22 (2010) 443–452.
[162] J. Wang, T. Yoshida, F. Nakaki, H. Hiai, T. Okazaki, T. Honjo, Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11823–11828.
[163] L.M. Francisco, V.H. Salinas, K.E. Brown, V.K. Vanguri, G.J. Freeman, V.K. Kuchroo, et al., PD-L1 regulates the development, maintenance, and function of induced regulatory T cells, J. Exp. Med. 206 (2009) 3015–3029.
[164] C.A. Sabatos, S. Chakravarti, E. Cha, A. Schubart, A. Sanchez-Fueyo, X.X. Zheng, et al., Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance, Nat. Immunol. 4 (2003) 1102–1110.
[165] T. Miyazaki, A. Dierich, C. Benoist, D. Mathis, Independent modes of natural killing distinguished in mice lacking Lag3, Science 272 (1996) 405–408.
[166] N. Joller, J.P. Hafler, B. Brynedal, N. Kassam, S. Spoerl, S.D. Levin, et al., Cutting edge: TIGIT has T cell-intrinsic inhibitory functions, J. Immunol. 186 (2011) 1338–1342.
[167] J.A. Marin-Acevedo, B. Dholaria, A.E. Soyano, K.L. Knutson, S. Chumsri, Y. Lou, Next generation of immune checkpoint therapy in cancer: new developments and challenges, J. Hematol. Oncol. 11 (2018) 39.