The Role Of PD-1/PD-L1 Axis In Treg Development And Function: Implications For Cancer Immunotherapy

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Abstract: During the past decade, immunotherapy targeting immune checkpoints has become an important component of the treatment paradigm for numerous malignancies, especially PD-1/PD-L1 blockade which was demonstrated to rejuvenate disabled T cells in cancer patients to achieve long-term remissions. However, the clinical outcome of PD-1/PD-L1 targeted monotherapy against solid malignancies is not satisfactory which may be related with the intricate tumor microenvironment. As a vital suppressive immunocyte in tumor microenvironment, Tregs are characterized by PD-1 and PD-L1 and demonstrated to contribute to the tumor progression. The latest studies have suggested that Tregs might be involved in the treatment of PD-1/PD-L1 blockade and PD-1/PD-L1 axis could influence Treg differentiation and function. However, the complicated relationship between PD-1/PD-L1 pathway and Tregs has not been fully clarified. Here, we explored the role of PD-1/PD-L1 axis in Treg development and function, as well as the potential mechanisms of PD-1/PD-L1 blockade resistance related with Tregs. Meanwhile, we discussed the combination therapy aimed at targeting PD-1/PD-L1 axis and Tregs, hoping to provide novel insights for the future cancer treatment.

Keywords: PD-1, PD-L1, tregs, CTLA-4, combination therapy

Introduction

Over the years, immunotherapy targeting immune checkpoints has shed a light on cancer treatment. Especially last year, James P. Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine for the discovery of cancer treatment by inhibiting the immune checkpoint programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Differed from chemotherapies and targeted therapies, checkpoint blockade reprograms immune response to tumors and appears to have longer-term benefit for cancer patients after the whole treatment course.¹ To date, there are several FDA-approved PD-1/PD-L1 inhibitors used in cancer treatment: pembrolizumab, nivolumab, atezolizumab, avelumab and durvalumab. Even though their side effects are considered manageable and well tolerated when compared with chemoradiotherapy or other immunotherapy drugs,² the clinical outcome of PD-1/PD-L1 blockade against solid malignancies is not satisfactory and the response rate is only 20%–30% when employed as monotherapy.³ In addition to the gene mutations among different cancers, the low response and resistance to PD-1/PD-L1 blockade may be related with the complicated tumor microenvironment (TME).

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As an important suppressive immunocyte in TME, regulatory T cells (Tregs) are demonstrated to contribute to cancer development and progression, and their absence will lead to tumor eradication.⁴ To date, the role of PD-1/PD-L1 pathway in the regulation of Tregs differentiation and function has not been fully clarified. Woods DM et al have evaluated the predictive significance of Tregs in melanoma patients in response to nivolumab and reported that Tregs exhibited a decreased inhibitory activity in responding patients,⁵ suggesting Tregs might be involved in the treatment of PD-1/PD-L1 inhibitors, and their proportion and function would influence the effect of PD-1/PD-L1 blockade.

In this review, we summarized the immunoregulation mechanisms of PD-1 pathway and Tregs at first. Then, we reviewed the research advances on the role of PD-1/PD-L1 pathway in Treg development and function, as well as the potential mechanisms of PD-1/PD-L1 blockade resistance related with Tregs. Finally, we discussed the current researches about the combination therapy aimed at targeting PD-1/PD-L1 pathway and Tregs which could improve the therapeutic effect of immunotherapy.

PD-1/PD-L1 Axis

PD-1 is a transmembrane molecular belonging to the immunoglobulin CD28 family, encoded by *pdc1* gene and composed of 288 amino acid residues. PD-1 is expressed on multiple immunocytes, including activated T cells, B cells, NK cells, monocytes and DCs.⁶ PD-L1 (CD274 or B7-H1) is the ligand of PD-1 belonging to the B7 family. In addition to T cells, B cells, Tregs, macrophages and DCs, PD-L1 is widely expressed on non-blood cells such as vascular endothelial cells, mesenchymal stem cells, reticular fibroblasts, islet cells and so on. More importantly, PD-L1 is highly expressed on tumor cells, which is identified to contribute to the tumor immune escape.⁶

The two independent phosphorylation sites in the C and N-terminal amino acid residues of PD-1 are the immunoreceptor tyrosine-based inhibitory motif (ITIM) and the immunoreceptor tyrosine-based switch motif (ITSM).⁷ ITSM is important for PD-1 to exert its immunosuppressive function. After PD-1 binding with PD-L1, the ITSM is phosphorylated to activate intracellular pathways to exert immunosuppression activities. However, the inhibitory mechanism of PD-1/PD-L1 axis differs between T and B cells.⁶ In T cells, when PD-1 interacts with PD-L1, SHP-1/2 are recruited to ITSM which immediately dephosphorylates the TCR activation signals ZAP70 and CD3 δ ,

leading to downstream PI3K/Akt pathway repression and then decreases the cell apoptosis-related gene Bcl-x1 and promotes T cell apoptosis.⁸ In addition, PD-1/PD-L1 axis can inhibit Ras/MEK/ERK pathway to repress T cell proliferation.⁹ Alternatively, PD-1/PD-L1 pathway impairs the cytokine secretion released by T cells.⁸ While in B cells, following PD-1 activation, SHP-2 is recruited to the C-terminal of PD-1 to dephosphorylate BCR pathway molecules, such as Ig α / β and SyK, therefore inhibiting PI3K, ERK and PLC γ 2 pathway, leading to Ca²⁺ disorder and B cell growth stagnation.^{10,11}

Regulatory T Cell

Treg is a highly immunosuppressive subpopulation of CD4⁺ T cells, characterized by transcription factor forkhead box P3 (Foxp3). Tregs were previously identified as CD4⁺CD25⁺ T cells and are confirmed to inhibit T cell immunity to avoid damage from the extreme T cells activation. Thereby, Tregs play a crucial role in the maintenance of immune homeostasis and self-tolerance.¹² Tregs can be separated into two subsets: natural/thymic Tregs (nTregs) and peripherally induced Tregs (iTregs) according to the sites where they are generated.¹³ nTregs are generated in the thymus and they are mitotically quiescent in basal conditions.¹⁴ nTregs need antigenic stimulation to expand while they do not necessitate TCR engagement to exert their inhibitory activity.¹⁵ In contrast, iTregs are generated from peripheral CD4⁺Foxp3⁻ naive T cells after antigenic stimulation; these naive T cells receive TCR activation via MHC and express Foxp3 through the participation of CD25, leading to Treg differentiation.^{16–18}

Tregs execute their immunosuppressive abilities by multiple mechanisms.¹² First, Tregs consume IL-2 from the TME through their high-affinity IL-2 receptor α chain (CD25) to repress T cell proliferation and activation; second, CTLA-4 expressing Tregs will bind to and decrease the costimulatory molecules (CD80 and CD86) on APCs with greater avidity compared with CD28, depriving stimulatory signal to T cells; besides, Tregs secrete inhibitory cytokines (TGF- β , IL-10 and IL-35) to create an immunosuppressive environment, impairing the antitumor effects mediated by CD4⁺ T cells, CD8⁺ T cells and NK cells; at last, Tregs induce direct killing of responder T cells or APCs by producing granzymes and perforin.¹⁹ Therefore, Tregs will facilitate tumor progression by the above immunosuppressive mechanisms. The increased Tregs infiltrated into tumor islets and the downregulated ratio of CD8⁺ T cell to Treg are associated with the poor prognosis in

various cancers.¹² Treg depletion will promote the antitumor immunity and augment the efficacy of the current cancer treatment, such as chemoradiotherapy and immune checkpoint blockade.

The Role Of PD-1/PD-L1 Axis In Treg Development And Function

Francisco et al had reported that PD-L1-coated beads could induce Tregs in vitro, PD-L1 increased Foxp3 expression and enhanced the immunosuppressive ability of Tregs. Moreover, PD-L1 could convert naive CD4⁺ T cells to Tregs through the downregulation of Akt, mTOR and ERK2 and the simultaneous upregulation of PTEN.²⁰ Thereby, PD-1/PD-L1 axis exerts crucial role in regulating Treg Development and Function.

PD-1/PD-L1 Axis Is Involved In Treg Expansion Through Modulating Notch Pathway

Notch pathway is crucial for modulating the generation and function of multiple cells, which composed of four Notch receptors (Notch1–4) and five ligands, including DLL family (DLL1, DLL3 and DLL4) and Jagged family (Jag1 and Jag2).²¹ Following receptor–ligand binding, the Notch intracellular domain (NICD) is cleaved by γ -secretase and binds to the transcription factor RBPJ κ , thereby activating the downstream transcription effectors.²² Notch pathway regulates the differentiation and activation of T cells, enhances Treg cells survival and function through activating PI3K/Akt/mTOR pathway. In addition, the differentiation and function of Tregs require Notch pathway involvement; DLL and Jagged ligands are able to promote Treg expansion.²² Stimulating CD4⁺ T cells with Jag1 promoted Treg generation and function, and the possible mechanism could be promoting RBPJ κ recruitment to Foxp3 promoter or inducing the Notch target gene, Hey1.^{23,24} Meanwhile, Jag1 knockdown in mesenchymal stromal cells resulted in decreased Tregs in asthmatic murine model.²⁵

In addition, Mathieu M et al confirmed the occupancy of RBPJ κ with NICD and the Pdcd1 promoter at RBPJ κ -binding sites by chromatin immunoprecipitation. They found inhibiting Notch pathway resulted in the downregulation of PD-1 which was related with the impaired NICD/RBPJ κ complex on Pdcd1 promoter.²⁶ Pan T et al also discovered that Notch pathway and PD-1 expression were upregulated in LPS-tolerant THP-1 cells and septic shock patients, and the inhibition of Notch pathway significantly

decreased PD-1 in LPS-tolerant THP-1 cells.²⁷ Therefore, Notch pathway is a critical regulator of PD-1 regulation. Furthermore, the overexpression of Jag1 on DCs increased PD-L1 expression and the coculture of CD4⁺ T cells with these Jag1-DCs promoted Treg generation, while PD-L1 blockade partially reduced Treg expansion.²⁸ The above findings confirmed the important role of PD-1 in the induction of Treg expansion through modulating Notch pathway.

PD-1/PD-L1 Axis Maintains Treg Phenotype Via Asparaginyl Endopeptidase Downregulation

In tumor microenvironment, PD-L1 expression is coincident with the upregulated intra-tumor Foxp3⁺ Tregs, indicating PD-L1 may be responsible for sustaining Foxp3 expression in CD4⁺ T cells.^{29,30} Following PD-L1 binds to PD-1 on T cells, SHP-1/2 are recruited to result in the dephosphorylation of STAT, destabilizing the transcriptional molecules of Th1 cell, therefore PD-1 is involved in the regulation of Th1 cells.^{31,32} Recently, Stathopoulou C et al demonstrated that PD-1 inactivated asparaginyl endopeptidase (AEP) which is an endolysosomal protease involved in the antigen processing in DCs and responsible for destabilizing Foxp3 in Tregs; AEP deficiency increased the number and frequency of Treg in melanoma and graft-versus-host disease (GVHD). PD-1 could enhance Foxp3 expression in Th1 cells and that could be reversed in the presence of PD-L1 blockade; moreover, PD-L1 inhibitor could not restore Th1 cell conversion and Treg induction in the absence of AEP.³³ Therefore, PD-1 is able to impart regulatory ability to Foxp3⁺ Th1 cells and Tregs through inactivating AEP.

PD-1/PD-L1 Axis Converts Th1 Cells Into Tregs

Amarnath S et al have confirmed that the irradiated myeloid tumor cells K562 or conventional T cells overexpressing PD-L1 could convert Th1 cells into Tregs. PD-1/PD-L1 axis reduces STAT activation in Th1 cells via the phosphatase of the SHP-1/2, promotes Th1 cells to convert into Tregs, therefore inhibiting human-into-mouse xenogeneic GVHD (xGVHD). Moreover, pretreating Th1 cells with SHP-1/2 inhibitors or anti-PD-1 siRNA abrogated PD-L1-induced Treg phenotype conversion and recovered Th1 cell capacity to induce fatal xGVHD.³¹ Thereby, converting Th1 cells to Tregs through PD-1/PD-L1 pathway provides a potential method to inhibit GVHD after transplantation and paves the way for strengthening T cell immunity to infection and cancer.

Endothelial Cells Enhance Treg Function Via PD-1/PD-L1 Axis

The immunosuppressive function of Treg is modulated by plenty of mechanisms, including direct engagement with cells through costimulatory signals via OX40, PD-1 and their ligands and indirectly by cytokine through TGF- β and IL-10.³⁴ Endothelial cells (ECs) exert important effect on immunocyte recruitment and activation. Recently, Lim WC et al have confirmed that ECs could promote the concurrent proliferation of Teff cells and Tregs when they were cocultured with anti-CD3/28 antibodies, purified Tregs were activated to suppress Teff cell proliferation after pre-cultured with ECs; meanwhile, the expression of PD-1 on Treg was upregulated, as well as the PD-L1 and PD-L2 expression on activated ECs.³⁴ Wang C et al also demonstrated that after cocultured with rapamycin-treated HUVECs, the suppressive ability of Tregs was enhanced via the upregulated PD-L1 and PD-L2.³⁵ This ability of ECs to strengthen Treg activity will provide novel targets to increase Treg function during inflammatory disease.

Tregs Suppress Autoreactive B Cells Via PD-1/PD-L1 Axis

Previous research has demonstrated the immunosuppression induced by Tregs required the PD-1 expression on autoreactive B cells and the two PD-1 ligands expression on Tregs.³⁶ Tregs utilized PD-1 ligands to directly suppress B cell activation and then inhibited antibodies production, as well as impairing B cell proliferation and inducing B cell apoptosis. Okamura T et al also demonstrated that Tregs were modulated by early growth response gene 2 (Egr2), a zinc-finger transcription factor which plays an important role in the maintenance of T cell anergy via inhibiting T cell activation.³⁷ Treg secreted TGF- β 3 in an Egr2-dependent manner to impair B cell immunity by suppressing antibody production in lupus mouse model. Moreover, the immunosuppression mediated by TGF- β 3 expressing Treg required PD-1 expression on B cells.³⁸

The Potential Mechanisms Of PD-1/PD-L1 Blockade Resistance Related With Tregs

Apoptotic Tregs Release Adenosine To Induce Checkpoint Blockade Resistance

Oxidative stress is a metabolic character of TME; poor glycolysis is able to affect Teff cell function^{39,40} and free oxygen species can induce Treg apoptosis by targeting

mitochondria. Maj T et al have reported that adenosine might contribute to the immunosuppression capacity of apoptotic Tregs. They found Tregs could secrete ATP and convert it into adenosine via ectoenzymes CD73 and CD39, as well as the A2A receptor signaling pathway, and the apoptotic Tregs were more efficient than live Tregs to suppress T cell activation via adenosine production. More importantly, they suggested that the therapeutic effect of PD-L1 antagonist was eliminated by apoptotic Tregs in mouse tumor models.⁴¹ Therefore, Treg apoptosis induced by oxidative stress in tumor environment is a potential mechanism of tumor immune evasion, and the immunosuppression mediated by apoptotic Tregs may result in checkpoint blockade resistance.

TGF- β Restricts T Cell Infiltrate Into Tumor Parenchyma

In order to identify the major determinants of the clinical response after PD-L1 blockade treatment, Mariathasan S et al examined the tumors from metastatic urothelial cancer patients who were treated with atezolizumab and they concluded that the outcome of PD-L1 inhibitor was related with three biological indicators: high neoantigen or tumor mutation burden, pre-existing CD8⁺ Teff cells and TGF- β pathway in fibroblasts. They found CD8⁺ T cells were excluded from the tumor parenchyma but were detected in the collagen and fibroblast rich peritumoral stroma. In addition, the combinatorial blockade of TGF- β and PD-L1 repressed TGF- β pathway in peritumoral stromal fibroblasts, facilitated CD8⁺ Teff cell to infiltrate into tumor islets, generating robust anti-tumor immunity and tumor regression.⁴² This study suggested that TGF- β might reprogram TME through inhibiting T cell infiltration to impair the anti-tumor immunity restored by PD-L1 blockade.

PD-1 Blockade Enhances TGF- β /Smad3 Signal Pathway Within Tumors

Dodagatta-Marri E et al have found PD-1 blockade monotherapy could lead to the skewing of the Teff/Treg balance in favor of Tregs, thus restraining the anti-tumor effect. They also demonstrated anti-PD-1 activated TGF- β /Smad3 pathway within tumor cells. Furthermore, the increased Smad3 might promote tumor cell epithelial-to-mesenchymal transition (EMT) towards myofibroblast phenotype, reduce antigen presentation activity, and affect cytokine production and extracellular matrix profiles to inhibit

immuno-surveillance.⁴³ As Treg is a main source of TGF- β , PD-1 blockade might strengthen TGF- β activity through Tregs activation. Alternatively, anti-PD-1 could promote Treg induction indirectly, in response to TGF- β secreted by CD4⁺ Th cells⁴⁴ and immunosuppressive myeloid cells. Moreover, the combined blockade of PD-1 with TGF- β promoted anti-tumor activity, and the cooperation between anti-PD-1 and anti-TGF- β was mediated partially through the involvement of Tregs.

Combination Therapy

Since PD-1/PD-L1 blockade initiates an incomplete restore of exhausted T cells and the high proportion of intra-tumoral Tregs is related with the poor response in various cancers, targeting Tregs may significantly enhance the therapeutic effect of PD-1/PD-L1 blockade therapy.

Anti-CTLA-4

T cell activation is a complicated process which requires more than one stimulatory signal,⁴⁵ in addition to TCR bind with MHC to activate T cells, further costimulatory signals are needed. The binding of CD28 on the T cells with B7-1 (CD80) or B7-2 (CD86) on the APCs leads to T cell proliferation, differentiation and survival. CTLA-4 is a CD28 homolog with a stronger affinity with B7;^{46,47} the binding of CTLA-4 with B7 induces inhibitory signals that neutralize the stimulatory signals mediated by CD28/B7 binding. The ratio of CD28/B7 binding versus CTLA-4/B7 binding determines T cell activation or anergy.⁴⁸ Tregs constitutively express CTLA-4 which is critical for their suppressive abilities. Similar to PD-1/PD-L1 axis, CTLA-4 inhibits T cell proliferation and survival, as well as reducing cytokine production. But differed from PD-1/PD-L1 pathway, CTLA-4 blockade inhibits the activation stage of T cell in lymph nodes when Tregs remove B7 from the APCs surface,⁴⁹ while PD-1 antagonists mostly influence the effector stage of immunity.⁵⁰

Although CTLA-4 and PD-1 blockades can prolong cancer patient survival when compared with the commonly used chemotherapies, CTLA-4 and PD-1 inhibitors are not effective in all patients and some patients who respond primitively will relapse eventually. Besides, it is noteworthy that anti-CTLA-4 and anti-PD-1 therapy will cause the upregulation of other inhibitory receptors,⁵¹ and CTLA-4 inhibitors appear to produce more side effects when compared with PD-1 blockade.⁵² These findings highlight the need for combined immune checkpoint blockade treatment. Compared to monotherapy, combination treatment of

CTLA-4 and PD-1 inhibitors has superior efficacy in melanoma and non-small cell lung cancer.⁵¹

Anti-LAG-3

LAG-3 (CD223) is expressed on a variety of T cells, including activated CD4⁺ T cells, CD8⁺ T cells and Tregs. LAG-3 binds to MHC-II on APCs with a stronger avidity than CD4,⁵³ and LAG-3 interacts with MHC-II to inhibit the binding of MHC to TCR and CD4, therefore repressing TCR pathway in immunity. LAG-3-expressing Tregs use an ITAM-regulated suppressive pathway to inhibit DCs function, repress T cell proliferation, promote T cell apoptosis and induce T cell exhaustion.⁵⁴ Moreover, tumor-infiltrating LAG-3⁺ Tregs secrete more immunosuppressive cytokines to augment Tregs function, such as TGF- β and IL-10.

As a co-inhibitory receptor, LAG-3 will be a promising immune checkpoint to induce T cell exhaustion. Recent studies have reported that LAG-3 facilitated Treg differentiation and LAG-3 blockade impaired Treg induction.⁵⁵ LAG-3 knockout was more effective than anti-LAG-3 monoclonal antibody in tumor control; this finding could be related with the incomplete blockade of anti-LAG-3 mAb or explained by the fact that LAG-3 expression induces robust immune suppression in the early phase of tumorigenesis that can only be reversed partially in late stage of tumor progression through mAb blockade.⁵⁶ Harris-Bookman S et al also suggested LAG-3 inhibitors should be adopted early in treatment. Furthermore, anti-LAG-3 alone or in combination with PD-1 inhibitor was efficacious at glioblastoma eradication, their combination could be more effective than LAG-3 blockade was used at an early time point.⁵⁶ In addition, the combined blockade of LAG-3 and PD-1 has been reported to eradicate murine fibrosarcoma, melanoma and colorectal adenocarcinoma effectively, especially the most established tumors which are mostly resistant to monotherapy.⁵⁴

Anti-GITR

GITR is expressed on Tregs at high levels and on naive T cells at low levels. As its ligand, GITRL is expressed on activated APCs, including DCs, activated B cells and macrophages. The function of GITR pathway is cell type and context dependent. GITR is constitutively expressed during T cell development and exerts an important role in nTreg differentiation and expansion in the thymus. While in the periphery, after TCR stimulation, the binding of GITR with GITRL will promote T cell activation via

CD25 upregulation, induce IL-2 and IFN- γ production and facilitate T cell proliferation. The function of GITR on Treg is complicated; in general, GITR pathway promotes Treg expansion, attenuates Treg immunosuppressive effect and induces Teff cells nonresponse to Treg suppression.⁵⁷ DTA-1 (monoclonal antibody reacts with GITR) treatment was confirmed to induce the loss of Foxp3 within the intratumoral Tregs.⁵⁸

Several studies have demonstrated that blocking PD-1/PD-L1 axis to overcome T cell exhaustion could strengthen GITR-targeted therapy.⁵⁷ Wang B et al have conducted single-cell RNA sequencing for over 2000 intratumoral CD8⁺ T cells in mouse after GITR and PD-1 antibodies administration and confirmed that this combinatorial treatment synergistically improved CD8⁺ T cell function; PD-1 blockade restored the critical homeostatic regulators CD226 through inhibiting SHP-2 dephosphorylation of the CD226 intracellular domain, while GITR agonism weakened T cell ITIM domains and immunoreceptor with Ig expression. The combined treatment not only increased CD8⁺ T cell function but also generated proliferative Teff cells in CD226-dependent manner.⁵⁹ Synergistic therapy aimed at activating costimulatory receptor GITR and inhibiting PD-1 to generate robust T cell activation is being assessed in preclinical researches for metastatic melanoma and other solid tumors.^{57,60} In these studies, GITR or PD-1-targeted monotherapy showed limited therapeutic effect and synergistic therapy could reactivate exhausted tumor-infiltrating CD8⁺ T cells to induce long-term survival in breast and ovarian cancer mouse models.⁶¹

Anti-CD25

CD25 is the first marker used to identify and isolate Tregs before the discovery of Foxp3 and it is constitutively expressed on Tregs and absent on naive Teff cells. Some studies have demonstrated the effect of CD25 antagonist in depleting tumor-infiltrating Tregs, but this deprivation of Tregs by CD25 blockade monotherapy only resulted in modest efficacy against established tumors and large-dose possible side effects.⁶² The mouse studies have reported that the anti-CD25 antibody partly depleted Tregs in peripheral lymphoid organs and blood, suppressed tumor growth and prolonged survival only when employed before or soon after tumor challenge; however, anti-CD25 failed to delay tumor growth or prolong survival against established tumors.⁴

Besides, several clinical researches have confirmed the potential of depleting CD25⁺ Tregs to reactivate anti-tumor

immunity, but other studies failed to support this conclusion. Because in addition to Tregs, targeting CD25 may deplete activated Teff cells which express CD25 as well, impairing the anti-tumor effect of Treg depletion.¹² Therefore, it is possible to combine anti-CD25 with immune checkpoint blockade which aimed at reinvigorating Teff cells. Recently, Arce Vargas F et al have demonstrated that the efficacy of anti-CD25 relied on the depletion of Treg in the TME and the available anti-CD25 antibodies failed to deprive intra-tumoral Tregs because of the upregulation of Fc γ RIIb within tumors. Furthermore, the Fc-optimized anti-CD25 induced significant intra-tumoral Treg depletion and synergized with PD-1 blockade to enhance the tumor repression.⁴ In addition, combining PD-1 blockade with anti-CD25 appeared to have stronger therapeutic effect compared to the CTLA-4 blockade combination.⁴

Anti-TGF- β

In addition to immune checkpoints, suppressive cytokines are good candidates for immunotherapy. Act as a pleiotropic cytokine, TGF- β is involved in multiple pathways in regulating immunity. TGF- β plays an important role in the accumulation of MDSCs and IgA class switch of B cells. It is also known to induce IL-17-producing T cells and Tregs, respectively. Besides, TGF- β is one of the immunosuppressive cytokines released by Tregs and plays a crucial role in inflammation and cell differentiation. In addition to modulating immunocyte differentiation, TGF- β exerts robust suppressive activity against NK cells and conventional T cells directly, and part of Tregs utilizes surface TGF- β to inhibit T cells via cell-cell contact. More importantly, tumor cells often overproduce TGF- β to induce immune suppression.⁶³ Lately, two studies have confirmed TGF- β secreted by TME is the major driver of immunosuppression leading to the low response to treatment in models of metastatic urothelial and colorectal cancer.^{42,64}

The increased anti-cancer effect of inhibiting TGF- β or its receptors had been confirmed in some tumor models, but TGF- β blockade did not result in promising efficacy in these researches and combination therapy should be considered.⁶⁵ Terabe M et al have demonstrated that although TGF- β blockade promoted vaccine-induced Th1 cell response in tumors and tumor-draining lymph nodes, it did not upregulate CD8⁺ T cells.⁶³ Since the response to PD-1 blockade therapy appears to be related with the number of pre-infiltrated Teff cells. Concurrent TGF- β inhibition can enhance the effect of PD-1 blockade by facilitating Teff

cells infiltrate into tumor islets, deplete suppressive immune cells and promote cytotoxic destruction of tumor cells.⁶⁶

Conclusion

Above all, PD-1/PD-L1 axis can regulate the differentiation and function of Tregs, and Tregs will influence the therapeutic efficacy of PD-1/PD-L1 blockade in turn. In consideration of the complicated relationship between PD-1/PD-L1 pathway and Tregs during the progression and treatment of cancer, the proportion and phenotype of Tregs may become the biomarkers to predict the clinical outcomes of PD-1/PD-L1 antagonists. Furthermore, combination therapy of PD-1/PD-L1 inhibition with Tregs-targeted treatment will execute synergistic anti-tumor effect, especially for those solid tumors that have poor responses to PD-1/PD-L1 blockade monotherapy.

Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest on this work.

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