Programmed Cell Death Protein-1 and Other Inhibitory Receptors Expressed by Regulatory T Cells as a Restraining Factor of Checkpoint Therapy

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SUMMARY

The effectiveness of anti-PD-1/PD-L1 targeted therapies focused on the antitumor immune response restoration in the treatment of melanoma and several other tumors has renewed trust in immunotherapy potential. Despite inspiring enthusiasm that led both to the expansion of indications for anti-PD-1/PD-L1 monoclonal antibodies and to an explosive growth in trials of new immune checkpoint inhibitors, a number of unresolved problems remain: relatively low response rates to existing drugs, development of acquired resistance, tumor progression and immune-mediated adverse events. Both the response to anti-checkpoint therapy and possible adverse reactions are based on quantitative and functional changes in malignant cell clones, tumor microenvironment and immune cells. An indispensable role in these interactions is played by regulatory T cells (Tregs), a heterogeneous population of CD4$^+$ T lymphocytes capable of suppressing the immune response. It is known that, like conventional T cells, Tregs up-regulate several checkpoint receptors, including PD-1, TIM-3, LAG-3. However, the biological relevance of such expression and the consequences of Treg checkpoint blockade are vague, as data from in vitro and clinical observations are contradictory. Here, we reviewed the current understanding of inhibitory checkpoint receptor expression by Treg populations and their relationship with the effects of treatment with checkpoint inhibitors.

Keywords: *regulatory T cells; type 1 regulatory T cells; checkpoint inhibitors; PD-1; TIM-3; LAG-3; TIGIT.*

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INTRODUCTION

In September 2014, the U.S. Food and Drug Administration (FDA) approved the first anti-PD-1 monoclonal antibody drug, pembrolizumab, for the treatment of advanced melanoma (1), marking the beginning of "an era of checkpoint inhibitors" in oncology and renewing a fading trust in immunotherapy potential. Clinical trials and real-world results have shown that both anti-PD-1 monoclonal antibodies pembrolizumab and nivolumab and anti-PD-L1 drugs atezolizumab and durvalumab are generally safe, easy-to-use and effective in the treatment of advanced melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, breast cancer, ovarian cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma and other solid tumors (2), as well as classical Hodgkin lymphoma (cHL) and certain non-Hodgkin's lymphomas (NHL) (3, 4). Objective response rates range from 15–30% for most solid tumors to 73–85% for cHL (2, 3).

The mechanism of action of anti-PD-1/PD-L1 monoclonal antibodies is fundamentally different from traditional antitumor targeted therapy drugs, which aimed to deplete or impair the function of tumor cells (anti-Her2, anti-EGFR, anti-CD20 monoclonal antibodies, etc.), or to neutralize soluble pro-tumor factors (anti-VEGF, anti-IL-6 drugs). Programmed cell death-1 receptors are up-regulated by activated T cells following antigen encounter, while their ligands PD-L1 and PD-L2 are expressed by many immune and non-immune cells at the site of inflammation of any origin. PD-1/PD-L1 interaction limits T cell immune response and normally is involved in the protection of surrounding tissues, as well as in the maintenance of peripheral tolerance. Various tumors exploit the PD-1/PD-L1 axis to escape from immune surveillance: tumor and immune suppressive cells broadly express PD-L1 in the microenvironment to inhibit the functional activity of PD-1-positive tumor-infiltrating T cells. Anti-PD-1 or anti-PD-L1 monoclonal antibodies abrogate the reception of an inhibitory signal by T cells, which can lead to the restoration of their proliferative, cytokine-producing and cytotoxic functions against tumor cells. A continuously growing pool of observations demonstrates the possibility of achieving substantial regression of the tumor burden and increased survival rates even in the advanced stages of certain malignancies (2–4). The initial success of anti-PD-1/PD-L1 drugs led to a surge of interest in other checkpoint receptors – T cell immunoglobulin and mucin domain 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT), and an explosive growth in research on their inhibitors (5).

A therapeutic response to anti-PD-1/anti-PD-L1 monoclonal antibodies partially depends on both the tumor mutational burden and PD-L1 up-regulation by malignant cells and immune and stromal cells in the tumor microenvironment (6–8). According to a meta-analysis of 45 publications by Lu S. et al., next-generation sequencing and immunohistochemistry, assessing the mutational load and PD-L1$^+$ cells in tumor samples, respectively, had comparable accuracy in predicting responses to anti-PD-1/PD-L1 therapy (area under the curve in ROC analysis of 0.69 and 0.65, respectively) (9).

The most obvious connection between the effectiveness of anti-PD-1 therapy and PD-L1 expression was

observed only for B cell lymphoproliferative disorders. Significant improvements in the survival rates with checkpoint inhibitors have been achieved in cHL, primary mediastinal B-cell lymphoma, primary central nervous system lymphoma and primary testicular lymphoma. In these diseases, PD-L1 overexpression in tumor samples has been described in 43–87% of patients, usually associated with *PD-L1/PD-L2* genetic alterations (polysomy, copy gain, amplification) (10–12). In other B cell lymphomas, elevated levels of PD-L1$^+$ cells and/or increased *PD-L1/PD-L2* gene alterations were observed much less frequently, and in most cases, an objective response to anti-PD-1/PD-L1 therapy was not achieved in clinical trials (11, 12).

The main adverse events identified during the clinical trials and real-world use of anti-PD-1/PD-L1 antibodies are the development of secondary resistance, immune-mediated adverse reactions and tumor progression (13–15). Both the response to anti-checkpoint therapy and the possible adverse effects arise on quantitative and functional changes during interactions within malignant clones, tumor microenvironment and immune cells. Among the latter, regulatory T cells (Tregs) play a considerable role. Here, we reviewed the current understanding of the inhibitory checkpoint receptor expression by Treg populations and their relationship with the effects of the treatment with checkpoint inhibitors.

The clinical significance of regulatory T cells in tumor growth

The involvement of Tregs in carcinogenesis, tumor progression and metastasis, as well as molecular and cellular mechanisms engaged in these processes, has been repeatedly described in the literature (16, 17). Here we represent only the data that is substantial for the review.

Regulatory T cells are a heterogeneous population of CD4$^+$ T helper cells capable of suppressing the immune response. The most studied are natural Tregs of thymic origin, generally identified as CD4$^+$CD25^{hi}CD127$^-$FOXP3$^+$ T lymphocytes; additional surface and intracellular markers (CD45RA, CD39, CTLA-4, Helios) can be used to assess the functional activity (18). It should be emphasized that apparently all Treg markers can be transiently up-regulated by CD4$^+$ T helper cells at different stages of activation, differing at least in expression density and combination of antigens, including the mutually exclusive expression of CD25 and CD127, transcription factors FOXP3 and Helios and ectonucleotidase CD39 (16, 18–20).

Inducible or peripheral Tregs can be generated in vitro from conventional CD4+ T cells under the influence of the transforming growth factor β (TGFβ), through direct cell-to-cell contact with several leukemic and NHL cell lines, and distinct still unexplored mechanisms (21, 22). Currently, type 1 regulatory T cells (Tr1), producing the suppressor cytokine interleukin-10 (IL-10) and, possibly, TGFβ, are being actively studied (23). The pivotal physiological function of Tregs is to maintain peripheral tolerance to autoantigens through both antigen-dependent and nonspecific suppression of immune cells. Normally, nTreg frequency is 2–10% of the circulating CD4$^+$ T cells (16).

The suppressive potential of Tregs is fully realized under conditions of tumor growth. Malignant and stromal cells actively promote Treg migration into the tumor microenvironment via chemokine secretion. Tumor metabolic changes – hypoxia, lactate and fatty acid excess, deficiency of glucose and tryptophan, increased levels of kynurenine and suppressive cytokines, – are favorable for Treg expansion and the functional activity. Regulatory T cell populations in the tumor microenvironment generally exert a higher suppressive potential compared to the circulating counterparts (16, 22). For many solid tumors and hematological malignancies, an increase in Treg counts has been described in peripheral blood, draining lymph nodes and the tumor microenvironment; the proportion of Tregs obtained from the tumor samples can account for up to 50% of CD4$^+$ T cells. In most observations, increased Treg frequencies are associated with advanced tumor stages, declined responses to therapy, and poor prognosis (16, 24, 25). It should be noted that several publications have not found a link between the Treg counts of these cells and a worsening prognosis. Besides, in animal models of breast cancer and prostate cancer, a restraining effect of Tregs on tumor growth was revealed, due to the control of inflammation and proliferative activity of malignant cells (21, 25–27).

Regulatory T cells are generally less sensitive to radiotherapy and cisplatin compared to the effector T cells (28, 29). Attempts to deplete Tregs with the anti-CD25 monoclonal antibody daclizumab and IL-2-conjugated diphtheria toxin did not achieve significant results (30, 31). Anti-CTLA-4 targeted therapy may be associated with both a decrease and an increase in Treg counts in the tumor microenvironment (32, 33). Studies of cyclophosphamide, the anti-CCR4 monoclonal antibody mogamulizumab and new targeted medications (anti-CD39, anti-GITR, etc) against Tregs are ongoing; nevertheless, the isolated depletion of these cells appears to be unfeasible due to the lack of specific surface markers (28, 34, 35).

It is noteworthy to mention that a considerable proportion of immune and non-immune cells, apart from Treg populations, exhibit suppressive and/or pro-tumor activity: myeloid-derived suppressor cells, tumor-associated M2 macrophages, tolerogenic dendritic cells, N2-neutrophils, mast cells, and stromal cells. Currently, the revival of interest in Tregs is due to the fact that, like effector T lymphocytes, Tregs up-regulate several checkpoint receptors, including PD-1 and TIM-3. However, both the biological relevance of such expression and clinical consequences of the Treg checkpoint blockade are vague.

The expression of checkpoint receptors on effector T cells

Effector T cells up-regulate inhibitory receptors PD-1, TIM-3, LAG-3, TIGIT after activation through the T cell receptor (TCR); the feasibility of PD-1 and TIM-3 expression upon stimulation with homeostatic cytokines IL-2, IL-7, IL-15, IL-21 has also been demonstrated (36–38). Thus, during transient antigenic stimulation, checkpoint receptor expression, especially PD-1, is a hallmark of T cell activation. In cases of continuous antigen persistence and, accordingly, long-term TCR stimulation (chronic viral infections, tumor growth, more than two weeks of stimulation *in vitro*), activated T cells turn into a state of T cell exhaustion. The latter is characterized by cytotoxicity attenuation, a decline in cytokine production (tumor necrosis factor (TNF), IL-2, interferon-γ (IFNγ)) and diminished proliferative activity, as well as a stable expression of inhibitory checkpoint receptors. The co-expression of two or more inhibitory molecules is associated with later stages of T cell exhaustion. In addition to prolonged contact with tumor antigens, the development of T cell exhaustion is facilitated by metabolic changes in the tumor microenvironment unfavorable for the T cells: glucose deficiency, elevated levels of lactate, cholesterol and fatty acids, high concentrations of suppressive molecules (arginase-1, adenosine, indoleamine 2,3-dioxygenase) and cytokines (IL-10, IL-35, TGFβ). Malignant cells and the tumor microenvironment express checkpoint receptor ligands (PD-L1/PD-L2, galectin-9, galectin-3, CD155) to escape immune surveillance. T-cell exhaustion is reversible in early stages; anti-PD-1/PD-L1 targeted therapy is one method to "reset" exhausted T cells (39–41).

In addition to T cell exhaustion, checkpoint receptor expression has been described for other dysfunctional states of effector T cells: anergy and senescence (40, 42–44).

T cell anergy is a state of unresponsiveness to antigenic stimulation that occurs in case of inadequate antigen presentation (without a co-stimulatory signal). The possibility to avoid immune surveillance during tumor growth through the induction of T cell anergy, as well as the expression of PD-1 and LAG-3 in this state, has been described in murine models (40, 42). Besides, in a mouse model, a higher proportion of anergic $CD8^+PD\text{-}1^+CD38^{hi}$ T cells were associated with the resistance to anti-PD-1 therapy (43). In humans, the involvement of T cell anergy in escaping antitumor immune surveillance, the phenotype of anergic T cells, and its role in the development of resistance to therapy (including checkpoint inhibitors) are currently unknown.

T cell senescence is an apparently irreversible state of reduced proliferative activity (cell cycle arrest) of terminally differentiated effector T cells under conditions of repeated antigenic stimulation and/or exposure to damaging factors (40). Telomere-dependent senescence occurs in the case of repeated exposure to an antigen and multiple clonal expansion, causing telomere shortening (months to years from the first antigen presentation). Telomere-independent (stress-induced) senescence emerges following the exposure to DNA-damaging agents (reactive oxygen species, radiation, chemotherapy drugs). Both types are characterized by a pronounced decrease in proliferative activity and cytotoxicity, but a preserved or increased production of pro-inflammatory (IL-6, IL-8, IFNγ, TNF) and anti-inflammatory (IL-10, TGFβ) cytokines. Senescent T cells up-regulate checkpoint receptors TIM-3 and TIGIT, while the expression of PD-1 and LAG-3 remains a matter of debate. Higher levels of circulating senescent CD8+ T cells have been associated with the resistance to anti-PD-1/PD-L1 targeted therapy in melanoma and NSCLC (40, 44, 45).

Thus, effector T cells express PD-1 and other inhibitory checkpoint receptors both during activation and under various dysfunctional states (reversible or not). The goal of targeted anti-checkpoint therapy is to potentially restore an antitumor T cell immune response.

The expression of PD-1 and other inhibitory checkpoint receptors on regulatory T cells

Human Treg populations express the same inhibitory checkpoint receptors as effector T cells. Natural $CD4^+CD25^{hi}FOXP3^+$ Tregs appear to up-regulate PD-1, TIM-3, LAG-3, TIGIT after activation through TCR. The surface expression of these molecules is associated with a pronounced suppressive activity, and higher counts of checkpoint-positive Tregs are observed in the tumor microenvironment (46–52). The mentioned checkpoint receptor expression (separately) has also been described for IL-10-producing Tr1 (23, 49, 50, 52, 53).

The role of checkpoint receptors in the Treg function remains the subject of scrutiny, and the accumulated data are extremely contradictory.

Early experimental studies showed that PD-L1–PD-1 interaction disrupts the Treg functional activity. The suppressive potential of $CD4^+FOXP3^+$ T cells was diminished upon the treatment with PD-L1 in vitro (54). In a recent study by Kamada T. et al., the murine model PD-1-deficient Tregs showed strong suppressive and proliferative potential; besides, the nivolumab treatment in vitro increased the immunosuppressive activity of the $CD4^+CD25^{hi}CD45RA^-PD\text{-}1^+$ T cells obtained from healthy donors (55). Chen X. et al. did not find evidence of such inhibitory role of PD-1 in a mouse model but concluded that this receptor is crucial for the conversion of effector T cells into induced Tregs (56).

Chronic antigen exposure appears to exert Treg dysfunction, an analogue of T cell exhaustion. In the detailed study of Lowther D. et al, the authors showed a diminished inhibitory activity of $PD\text{-}1^{hi}$ Tregs isolated from peripheral blood and tumor samples of glioblastoma multiforme patients and peripheral blood of healthy individuals. Using RNA sequencing and cytometry by the time of flight, molecular signatures associated with T cell exhaustion were identified in $PD\text{-}1^{hi}$ Tregs. Interestingly, the co-expression of PD-1, TIM-3 and LAG-3 by exhausted Tregs was noted, which is also a hallmark

of the profound dysfunction for effector T cells (57).

The currently known ligands for LAG-3 are major histocompatibility complex class II (MHC II) molecules, fibrinogen-like protein-1, α-synuclein, galectin-3 and lymph node sinusoidal endothelial cell C-type lectin, widely expressed on immune and non-immune cells, stromal microenvironment and tumor cells (58). By contrast, LAG-3$^+$ Tregs seem to be relatively infrequent. According to the data provided by Camisaschi C. et al, the median values of LAG-3-expressing subset among circulating CD4$^+$CD25^{hi}FOXP3$^+$ Tregs were about 3.6 % in healthy donors and 5.8 % in cancer patients, while LAG-3$^+$ cells among Tregs in metastasis-infiltrating lymphocytes and tumor-invaded lymph nodes were 9–10 % (calculated from the data provided in the Supplementary materials for (50)). Chen K. et al recently evaluated the mean frequencies of LAG-3$^+$ cells about 15% of the donor CD4$^+$CD25$^+$FOXP3$^+$ Tregs and proposed a limitation of Treg and Tr1 functions following LAG-3 stimulation (59). Camisaschi C. et al also showed that substantial proportions (≈ 50 %) of LAG-3$^+$ CD4$^+$CD25^{hi}FOXP3$^+$ Tregs in cancer patients produce IL-10 and TGF-β (50). LAG-3 expression by IL-10 producing Tr1 (CD25$^{low/-}$FOXP3$^{low/-}$) were confirmed in other publications with ≈2–5 % frequencies among CD4$^+$ T cells of healthy controls (60).

A substantial part of Tregs – up to 50% or more – up-regulate TIM-3 in the tumor microenvironment (52, 61, 62). In most publications, TIM-3$^+$ Tregs and TIM-3$^+$ Tr1 are presented as activated cell subsets with a strong suppressive potential, even in cases of the co-expression of PD-1(47, 52, 61–64). Only the above mentioned study of Lowther D. et al. indicated an impaired activity of tumor infiltrating TIM-3$^+$ PD-1^{hi} Tregs (57).

The interaction between TIM-3 and its ligand galectin-9 abrogates effector T cell activation but appears to ameliorate the Treg inhibitory functions (64). The IM-3 and/or PD-1 blockade disrupts the Treg suppressive capacity and generally ameliorates the anti-tumor immune response in vitro (63, 64) and in mouse models (62). However, according to the available publications, the role of TIM-3 on Tregs seems to be restricted to receiving signals, without any active direct or indirect suppressive functions (e.g., unlike CD39, CD25, CTLA-4 expression, or IL-10 production).

The inhibitory checkpoint molecule TIGIT competitively shares its ligands CD155 and CD112 expressed by DCs with activating receptor CD226 similarly to CTLA-4/CD28–CD80 and CD86 axis (65). Concerning Tregs, TIGIT is of great interest, as FOXP3, a pivotal Treg transcription factor, directly targets its gene, and TIGIT-expressing nTregs are considered a highly suppressive and activated population in murine models, healthy donors and cancer patients (49, 51, 66–69).

The mean relative counts of TIGIT$^+$ cells in CD4$^+$CD25^{hi}FOXP3$^+$ or CD4$^+$FOXP3$^+$Helios$^+$ Tregs were reported as approximately 77–84 % in the peripheral blood of healthy donors and melanoma patients (67, 68). Tumor-infiltrating CD4$^+$CD25$^+$ or CD4$^+$CD25^{hi}FOXP3$^+$ Tregs were TIGIT-positive in about 78 and 99 % specimens obtained from follicular lymphoma and metastatic melanoma patients, respectively (51, 68).

Contrariwise, the TIGIT up-regulation on Tr1 (or IL-10-secreting Tregs) is a topic of discussion, and few publications showed an increased *IL-10* gene expression in CD4$^+$FOXP3$^+$TIGIT$^+$ Tregs (49, 66), and a substantial IL-10 production by CD4$^+$CD25$^+$CD127$^{-/lo}$TIGIT$^-$CD226$^+$ Tregs (67). Moreover, Gagliani N. et al demonstrated the expression of CD226, a competitive antagonist of TIGIT, by CD4$^+$CD25$^{low/-}$FOXP3$^{low/-}$ Tr1 (60).

The TIGIT–CD155 pathway strongly attenuates the effector T lymphocyte and natural killer cell activity. Simultaneously, the TIGIT signaling ameliorates suppressive functions, especially against Th1 and Th17 cell immune responses, migration and stability of Tregs (49, 66, 68), while the stimulation of CD226 on Tregs seems to hinder their inhibitory potential (68).

Conflicting and often mutually exclusive data regarding the functions of PD-1 and other inhibitory receptors expressed by Tregs greatly complicate the prediction of the checkpoint inhibitor therapy effects, mediated by these cell populations.

Anti-PD-1 targeted therapy and regulatory T cells

Experimental studies indicate a stimulatory effect of anti-PD-1/anti-PD-L1 monoclonal antibodies on the proliferative and suppressive potential of Tregs isolated from healthy donors (55), as well as in the murine tumor models (70).

Experimental observations have been confirmed by recently published clinical data. Comparing the tumor samples obtained from gastric cancer patients before and after nivolumab therapy, Kamada T. et al. found a marked increase in proliferating PD-1$^+$ Tregs in four individuals with tumor progression after anti-PD-1 therapy, while in 32 patients without progression, a decrease in the proliferation of these cells was observed (55). Van Gulijk M. et al. described the activation of suppressor genes in tumor-infiltrating Tregs after anti-PD-1 therapy in ten patients with non-melanoma skin tumors and NSCLC who did not respond to the therapy. The researchers also showed an increase in proliferating PD-1$^+$ Tregs in the peripheral blood of patients with small cell lung cancer (n=7), NSCLC (n= 21) and mesothelioma (n=15) after two weeks of anti-PD-1/PD-L1-containing therapy compared with baseline values. In the patients with NSCLC and mesothelioma who did not respond to the therapy (n=11 and n=9, respectively) with nivolumab or pembrolizumab, the increased proliferation of PD-1$^+$ Tregs was accompanied by a significant increment in their relative counts (70). Kumagai S. et al. found that the imbalance between tumor-infiltrating PD-1$^+$ cytotoxic CD8$^+$ T cells and the PD-1$^+$ Tregs towards an increase in the latter population before anti-PD-1 therapy in the patients with NSCLC and gastric cancer (seven of 15 and 19 of 24 individuals, respectively) is a predictor of nonresponse to the treatment (71).

Thus, the blocking of PD-1/PD-L1 signaling unleashes both the anti-tumor T cell immune response and Treg mediated immune suppression. A risk factor for progression during the treatment with anti-PD-1 monoclonal antibodies appears to be a relative increase in tumor-infiltrating PD-1$^+$ Tregs (Figure 1). An isolated up-regulation of the PD-1 on the Tregs, but not on cytotoxic T cells, could result, for example, from elevated lactate levels in the tumor microenvironment (72). Probably, other factors could also initiate and maintain the Treg functional activity (16, 22), but the majority of exact molecular mechanisms that determine the checkpoint expression by Treg remain unknown.

Other checkpoint inhibitors and regulatory T cells

In addition to anti-PD-1/PD-L1 drugs, the first anti-LAG-3 monoclonal antibody is currently approved for use, and TIM-3 and TIGIT inhibitors are undergoing clinical trials.

In clinical trials, the combination of the LAG-3 inhibitor relatlimab and nivolumab for the treatment of unresectable and metastatic melanoma was associated with improved progression-free survival compared with nivolumab monotherapy (73). Relatlimab (in combination with nivolumab) was approved by the FDA in March 2022. Due to the relatively limited use, there is no data on the effect of anti-LAG-3 therapy on Treg expansion and the subsequent development of adverse reactions. The results of experimental studies are contradictory; the inhibition of Treg functions has been described both with the stimulation and blockade of the LAG-3 (48, 59). Numerous (>60) clinical trials are investigating the possibility of expanding the indications for relatlimab, as well as new anti-LAG-3 antibody medications (74), and a presumable Treg involvement should be considered in the coming years.

Currently, 38 clinical trials of anti-TIM-3 monoclonal antibodies are registered for the treatment of solid tumors and hematological malignancies (74). There is also no data on the possible effects of anti-TIM-3 drugs on the Treg functional activity in humans. Considering the high proportions of the TIM-3$^+$ Tregs in tumor samples, the depletion of this cell subset seems plausible. Nonetheless, the risk of TIM-3$^+$ Treg activation, like that seen with anti-PD-1 therapy, remains and requires close monitoring.

More than 20 clinical trials of anti-TIGIT monoclonal antibodies have been registered, of which three are phase III (75). According to the published data, the TIGIT$^+$

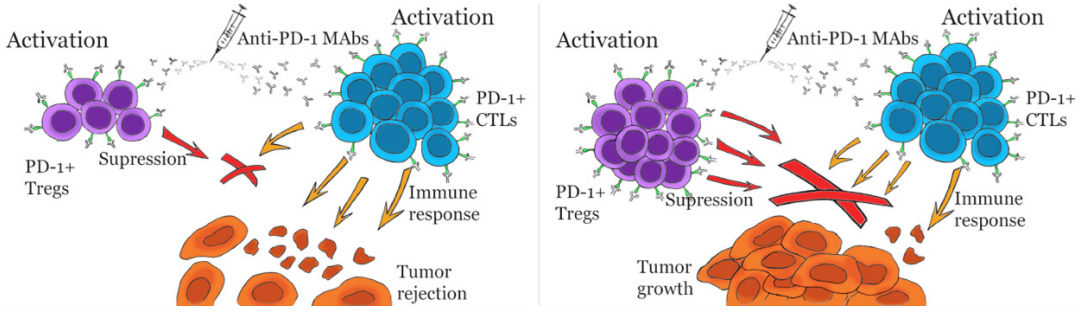

Figure 1. The anti-PD-1 treatment affects PD-1+ cytotoxic T lymphocytes/ PD-1+ regulatory T cells ratio.
The blocking of PD-1/PD-L1 signaling triggers both the anti-tumor T cell immune response and regulatory T cell (Treg) mediated immune suppression. In the case of relative abundance of PD-1$^+$ cytotoxic T lymphocytes (CTLs), Treg activation is clinically negligible (left picture). A risk factor for tumor progression during treatment with anti-PD-1 monoclonal antibodies is a relative increase in tumor-infiltrating PD-1$^+$ Tregs (right picture).

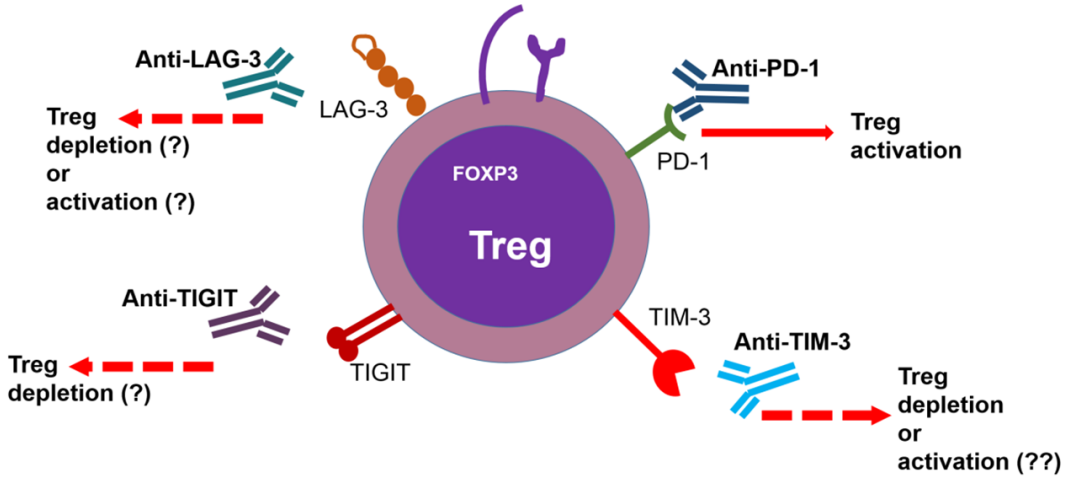

Figure 2. The possible effects of the checkpoint inhibitors on regulatory T cells.
The currently existing anti-PD-1/anti-PD-L1 monoclonal antibodies seem to stimulate the proliferative and suppressive potential of Tregs. Future anti-LAG-3 and anti-TIM-3 targeted therapy may lead both to the depletion and activation of Tregs, expressing these checkpoint receptors, while anti-TIGIT antibodies may be more promising in Treg depletion.

Tregs demonstrate high suppressive activity in the murine models and cancer patients (49, 51, 67–69). The results of experimental studies give hope for the attenuation of the Treg inhibitory potential and their depletion during anti-TIGIT therapy alone or in combination with anti-PD-1 antibodies (69, 75) (Figure 2).

CONCLUSION

Regulatory T cell populations express PD-1, TIM-3, LAG-3, TIGIT inhibitory checkpoint receptors as effector T cells. The up-regulation of these molecules is linked with a pronounced suppressive activity, and higher counts of checkpoint-expressing Tregs are observed in the tumor microenvironment. However, the data concerning the role of the checkpoint receptors in the Treg function remain contradictory.

Most murine and human in vitro findings emphasize a stimulatory effect of anti-PD-1/ PD-L1 monoclonal antibodies on the proliferative and suppressive potential of Tregs. According to recently published clinical data, a relative increase in tumor-infiltrating PD-1$^+$ Tregs appears to be a risk factor for tumor progression during treatment with anti-PD-1 monoclonal antibodies. Considering the high frequencies of LAG-3$^+$ and TIM-3$^+$ Tregs in the tumor samples, the corresponding targeted therapies may lead to the depletion of these subsets, albeit the risk of Treg activation remains. At the same time, anti-TIGIT treatment seems to be promising in hindering the Treg inhibitory potential.

The connection between possible tumor progression, resistance and adverse reactions following the existing and future anti-checkpoint therapies and changes in the counts and/or functional activity of Tregs, and other suppressor cell populations requires further rigorous investigations. These findings can be used as markers for predicting the effectiveness of checkpoint inhibitors and, in turn, serve as potential targets for future antitumor therapy.

Abbreviations

CCL: C-C motif chemokine ligand; CCR4: C-C motif chemokine receptor 4; CD: cluster of differentiation; cHL: classical Hodgkin lymphoma; CTL: cytotoxic T lymphocyte; CTLA-4: cytotoxic T-lymphocyte associated protein 4; DNA: deoxyribonucleic acid; EGFR: epidermal growth factor receptor; FDA: U.S. Food and Drug Administration; FOXP3: forkhead box P3; GITR: Glucocorticoid-induced TNF-related protein; IFNγ: interferon-γ; IL: interleukine; LAG-3: lymphocyte activation gene 3; MHC II: major histocompatibility complex class II; NHL: non-Hodgkin's lymphoma; NSCLC: non-small cell lung cancer; PD-1: programmed cell death protein-1; PD-L1: programmed cell death protein ligand-1; PD-L2: programmed cell death protein ligand-2; TCR: T cell receptor; TGFβ: transforming growth factor β; TIGIT: T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; TIM-3: T cell immunoglobulin and mucin domain 3; TNF: tumor necrosis factor; Tr1: type 1 regulatory T cell; Treg: regulatory T cell; VEGF: vascular endothelial growth factor.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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