Review Article

Efficiency and Future Significance of CTLA-4 and PD-1 Checkpoints Blockade Immunotherapy

Ashish Shivram Ramteke*
Visvesvaraya National Institute of Technology, Nagpur 440 010, India.
***Corresponding author's E-mail:** asramteke1992@gmail.com

ABSTRACT

The CTLA-4 (cytotoxic T-lymphocyte associated antigen 4) and PD-1 (programmed death protein 1) checkpoints are immune negative regulators of T-cell activity. Blockade of these checkpoints, resulting in raised activation of the immune system, has showed to new immunotherapies for melanoma and other cancers. Ipilimumab, an CTLA-4 blocker, is approved for the treatment of advanced melanoma. PD-1 blockers, nivolumab and pembrolizumab are approved to treat patients with advanced melanoma. Additionally, ipilimumab and nivolumab combination has been suggested in patients with BRAF metastatic melanoma. PD-1 and CTLA-4 roles in blocking immune responses, involving anticancer responses, are largely recognizable. CTLA-4 regulate T-cell proliferation primarily in lymph nodes, early in an immune response, although PD-1 suppresses T-cells, primarily in peripheral tissue, later in an immune response. The clinical outlines of therapeutic agents blocking these checkpoints may differ based on their mechanized variations.

Keywords: CTLA-4, PD-1, PD-L1, Immune Checkpoint, Cancers, T-cells.

INTRODUCTION

A major requirement of the immune system is to distinguish between self and non-self. Process center is recognition and binding of a T-cell receptor (TCR) to an antigen displayed in the major histocompatibility complex (MHC) on the surface of an antigen presenting cell (APC). Several other factors then impact whether this binding outcomes in activation of T-cell or anergy.[1] The life of a T-cell initiates in the thymus, where immature cells proliferate and create a broad reserve of TCRs by recombination of the TCR gene segments. A selection events then initiates, and T-cells with strong reactivity to self-peptides are neglected in the thymus to prevent autoreactivity in a process called central tolerance.[2]

T-cells with inadequate MHC binding face apoptosis, but those that can fragile respond to MHC molecules and self-peptides are not neglected and are delivered as naive T-cells to circulate by the blood, spleen, and lymphatic organs. There they are revealed to professional APCs presenting foreign antigens when infection occur or mutated self-proteins in the time of malignancy. Certain TCRs may have specificity that is cross-reactive to self-antigens. Various immune checkpoint pathways regulate activation of T-cells to prevent autoimmunity at multiple stages during an immune response.[3]

The PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T-lymphocyte associated antigen 4) immune checkpoint pathways are considered to regulate at various stages of an immune response. CTLA-4 is the leader of the immune checkpoint blockers, as it terminates possibly autoreactive T-cells at the primary stage of naive T-cell activation, generally in lymph nodes. The PD-1 pathway operates earlier activated T-cells at the later stages of an immune response, particularly in peripheral tissues.[4]

A fundamental concept in cancer immunotherapy is that cancer cells, which would normally be recognized by T-cells, have developed routes to elude the host immune system through taking advantage of peripheral tolerance. blocking of the immune checkpoint pathways has directed to the approval of some new therapeutics such as ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1), and nivolumab (anti-PD-1). There are major similarities and differences in these pathways, with indications for cancer immunotherapy.[5]

2. CTLA-4 immune checkpoint

T-cell activation is a complex process that requires stimulatory signal. TCR binding to MHC gives specificity to T-cell activation, but further costimulatory signals are needed. Binding of B7-1 (CD80) or B7-2 (CD86) molecules on the APC with CD28 molecules conducts to signaling the T-cell. Adequate levels of CD28:B7-1/2 binding shows to proliferation of T-cells, increased T-cell survival, and differentiation by the production of cytokines like interleukin-2 (IL-2), raised the energy metabolism, and upregulation of cell survival genes.[6]

While binding of CTLA-4 to B7 does not produce a stimulatory signal, CTLA-4 is a CD28 homolog with higher binding affinity for B7. This competition may prevent the costimulatory signal generally provided by CD28:B7 binding (**Fig. 1**). The comparative amount of CD28:B7 vs CTLA-4:B7 binding dictates whether a T-cell will undergo activation or anergy.[7] Moreover, certain proof concludes that CTLA-4 binding to B7 may actually produce inhibitory signals that neutralize the stimulatory signals from CD28:B7 and TCR:MHC binding. Suggested mechanisms for

such inhibitory signals involve direct blocking at the TCR immune events, CD28 inhibition or it's signaling pathway, or increased mobility of T-cells presenting to decreased capability to interact with APCs.[8]
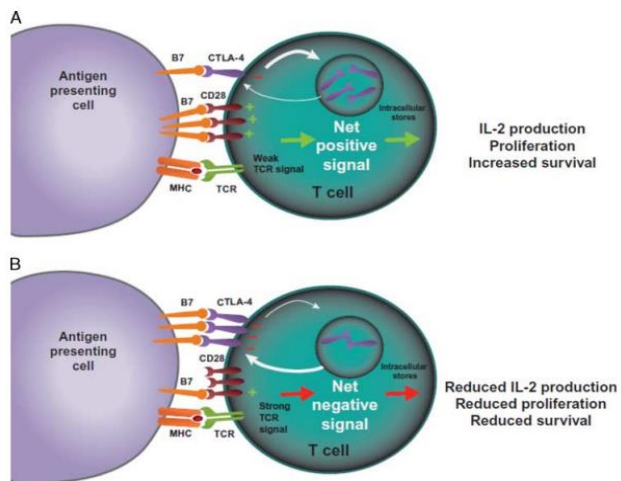
Figure 1: CTLA-4 mediated T-cell blockade (**A**) expression of positive signal, (**B**) expression of negative signal.

CTLA-4 itself is theme to regulation, specifically through localization within the cell. In resting naive T-cells CTLA-4 is situated generally in the intracellular compartment. Quicken signals coming from TCR and CD28:B7 binding create upregulation of CTLA-4 on the cell surface by process exocytosis involving of vesicles. This process regulates in a graded feedback loop whereas stronger TCR signaling evokes more CTLA-4 translocation toward the cell surface. Net negative signal by CTLA-4:B7 binding, full T-cells activation is inhibited by blocking of IL-2 production and cell cycle progression.[9]

Furthermore, CTLA-4 is involved in other features of immune control. Regulatory T-cells (Tregs) regulate functions of the effector T-cells, and thus are major players in maintaining peripheral tolerance. Differ from effector T-cells, Tregs basically presents CTLA-4, and this is concluded to be an important for their suppressive functions. In animal models, genetic CTLA-4 deficiency in Tregs decreased their suppressive functions. Tregs are control effector T-cells by downregulation of B7 ligands on APCs, proceeding to reduced CD28 co-stimulation (**Fig. 2**).[10]
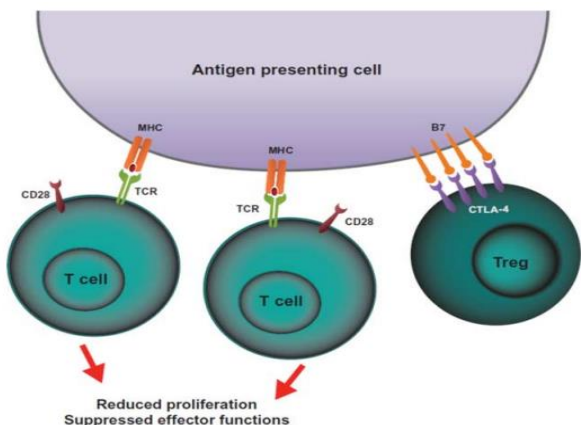
Figure 2: CTLA-4 mediated blockade of regulatory T-cell.

3. PD-1 immune checkpoint

PD-1 is the member of costimulatory receptors family B7/CD28, whereas it balances T-cell activation by binding to its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2). PD-1 binding blocks the T-cell proliferation, and interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-2 production, and also decreases T-cell survival (**Fig. 3**).[11] While T-cell experiences concurrent PD-1 and TCR binding, PD-1 created signals prevent major phosphorylation TCR signaling intermediates and stops primarily TCR signaling and also reduces T-cells activation. PD-1 expression is a trademark of "exhausted" T-cells that have competent high levels of stimulation or reduced help of CD4^{+} T-cell. This happens during cancer and chronic infections, is presented by T-cell dysfunction, proceeding in deficient control of cancers and infections.[12]
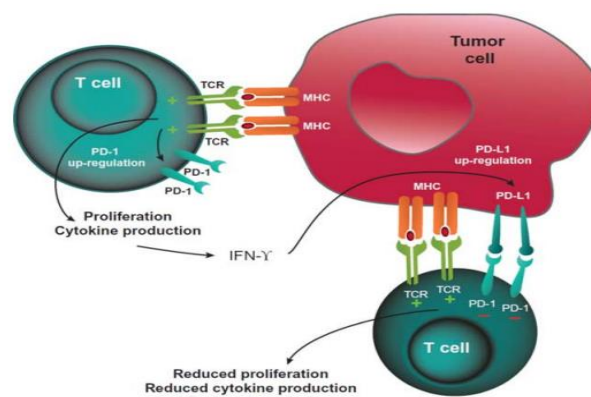
Figure 3: PD-1 mediated blockade of T-cell.

While PD-1 and CTLA-4 binding have same negative effects on T-cell activity, the scheduling of down-regulation, the organize signaling mechanisms, and the anatomic sites of immune inhibition through these immune checkpoints differ. Dissimilar CTLA-4, which is pinched to T-cells, PD-1 is more widely presented on activated T-cells, B-cells, and myeloid cells. While CTLA-4 functions throughout the primarily phase of T-cell activation, PD-1 functions throughout the effector phase, mostly in peripheral tissues.[13]

The administration of PD-1 ligands varies from those for CTLA-4, whilst B7 ligands are presented through APCs, generally reside in lymph nodes or spleen. PD-L1 is expressed on white blood cells, non-hematopoietic cells, and in non-lymphoid tissues, and may be introduced on parenchymal cells through inflammatory cytokines (IFN-γ) or cancerous signaling pathways. PD-L1 expression is found on cancer cells, and is associated with an increasing in tumor-infiltrating lymphocytes (TILs).[14]

PD-L2 is primary expressed on dendritic cells and monocytes, but may be induced on a broad difference of other immune cells and non-immune cells, depending upon microenvironment. PD-1 has a higher binding affinity for PD-L2 beside PD-L1, and this difference can be responsible for various contributions of these ligands to

immune responses. Due to PD-1 expressed in peripheral tissues, interactions with PD-L1/PD-L2 are concluded to maintain tolerance in locally infiltrated tissues.[15]

While the plurality of ligands for PD-1 shows to differs in biological effects, depending on which ligand is bound. The model illustrated opposing roles of PD-L1 and PD-L2 signaling in activation of NK cells. Blocking of PD-L2 binding conducted to promoted Th2 activity, since PD-L1 binding to CD80 has been demonstrate to block T-cell responses. These biological effects are likely to contribute to variations in activity and toxicity between antibodies directed at PD-1 as opposed to those directed at PD-L1, and thus have capable therapeutic strategies.[16]

However, regulatory T-cells express PD-1 and CTLA-4, the function of PD-1 expression on these cells remains unclear. PD-L1 has been illustrate to contribute to the conversion of naive CD4+ T-cells to Treg cells and also to inhibit T-cell responses by promoting the initiation and conservation of Tregs. Compatible with these outcomes, PD-1 inhibited can reverse Treg mediated suppression of effector T-cells in-vitro.[17]

PD-1 binding with its ligands decreases the magnitude of the immune response in T-cells that are already attracted in an effector T-cells response. This leads in a more restricted spectrum of T-cell activation compared to CTLA-4 blockade, which can elaborate the apparently lower incidence of immune mediated adverse events associated with PD-1.[18]
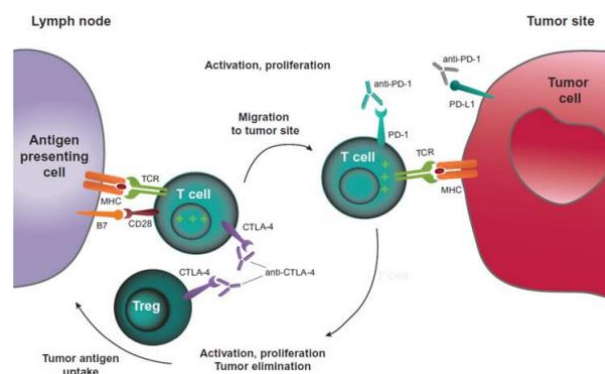
4. Significance of CTLA-4 and PD-1 checkpoints inhibition

Preclinical investigations leading decreased cancer growth and enhanced survival with CTLA-4 or PD-1 inhibition provide the rationale for immune checkpoint blocked for cancer treatment. Monoclonal antibodies that inhibit PD-1, PD-L1 and CTLA-4 are now approved for melanoma, lung cancer, and also in the development for kidney cancer, prostate cancer, and head and neck cancer (**Table 1**).[19]

Table 1: CTLA-4 and PD-1/PD-L1 checkpoint blockers approved.

Target	Drug	Status	Organization
CTLA-4	Ipilimumab	*Phase II*: gastric, cervical, pancreatic, and colorectal cancer *Phase III*: lung, and kidney cancer	Bristol-Myers Squibb
CTLA-4	Tremelimumab	*Phase II*: lung cancer	MedImmune/ AstraZeneca
PD-1	Pembrolizumab	*Phase II*: hematologic malignancies, glioblastoma, colorectal, Merkel cell, and pancreatic cancer *Phase III*: head & neck, gastric, and lung cancer	Merck
PD-1	Nivolumab	*Phase II*: hematologic malignancies, cervical, colorectal, and pancreatic cancer *Phase III*: head & neck, kidney, gastric, glioblastoma, and lung cancer	Bristol-Myers Squibb
PD-L1	Pidilizumab	*Phase II*: hematologic malignancies, and kidney cancer	CureTech
PD-L1	Durvalumab	*Phase II*: glioblastoma, and colorectal cancer *Phase III*: lung, and head & neck cancer	AstraZeneca/ MedImmune
PD-L1	Atezolimab	*Phase II*: kidney cancer *Phase III*: bladder, and lung cancer	Roche

The mechanism through which anti-CTLA-4 antibodies introduce an anticancer response is uncertain, whilst research ongoing concludes that CTLA-4 inhibition affects the immune priming phase through supporting the activation and proliferation of a higher number of effector T-cells, nevertheless of TCR specificity, and also through reducing Treg mediated suppression of T-cell responses (**Fig. 4**). An increase in the patient diversity of the peripheral T-cell pool following CTLA-4 inhibition with melanoma has recently been investigated.[20]

Figure 4: CTLA-4 and PD-1/PD-L1 checkpoint inhibition.

An ipilimumab investigations with melanoma or prostate cancer patients provided proof that onset T-cell background can also be significance. As quickly turnover of the T-cell repository on primarily treatment was shown, and it continued to evolve with some treatment.[21] Both expansion and loss of individual T-cell ancestor were reported, but there was a net raise in TCR diversity. Overall survival was associated with the maintenance of clone process in high frequency at beginning. In patients with little overall survival, treatment numbers of these highest frequency clones decreased.[22]

These investigations conclude that effective CTLA-4 inhibition can depend on the capability to maintain preexisting passionately T-cells with relevance to the anticancer response. PD-1 inhibition implements at the effector phase to restore the immune function in the periphery that have been turned off following high levels of antigen exposure.[23] The ligands for PD-1 can be expressed by cancer cells and cancer infiltrating immune cells. PD-L1 expression on cancer cells differentiate through cancers, but appears to be specifically abundant in melanoma, non-small cell lung cancer (NSCLC), and ovarian cancer.[24]

Recently, PD-L1 expression on cancer cells was shown to be importantly associated with PD-1 expression on TILs, and also mainly associated with PD-L2 expression when this ligand was expressed. In the similar investigation, cancer PD-L1 expression was the single factor most closely related with response to anti-PD-1 inhibition, since PD-L1 expression on TILs was not associated with response.[25] Other investigation observed that patient response to anti-PD-L1 blockade was strongest when PD-L1 was expressed by cancer infiltrating cells. PD-L1 blocking relevantly, as distracted to PD-1 blockade, will inhibit PD-1:PD-L1 while preserving PD-1:PD-L2 interactions.[26]

The capability to distribute a more targeted signal with less toxicity, as self-tolerance mediated by PD-1:PD-L2 interactions should be conserved. Moreover, as PD-L1 is known to bind CD80 and PD-1 to deliver inhibitory signals to T-cells, PD-L1 blockade with an appropriate antibody could prevent PD-L1 reverse signaling and its leading T-cell down-regulation through CD80.[27] PD-L1 antibody could also terminate the PD-L1:CD80 combination on other cells where they are co-expressed, like dendritic cells. The variations in time, location, and unnecessary effects of their actions conclude that CTLA-4 and PD-1 targeted therapies have the capable for additive or combine effects in the advanced malignancies treatment.[28]

More proof that supports the conclusions that various role of each immune checkpoint outline that investigated the biological effect of PD-1 and CTLA-4 inhibition in patients ongoing single or combine treatment. CTLA-4 blockade introduced a proliferative signal found primarily in a subset of memory T-cells, whilst PD-1 blockade associated with variations in genes to be involved in cytolysis and natural killer cell function.[29] Dual inhibition showed to non-overlapping variations in gene expression. While the treatments produced various effects on levels of circulating cytokines. This investigation reports that PD-1 and CTLA-4 inhibition showed to different patterns of immune activation.[30]

5. Efficiency and specific response with checkpoint blockers

CTLA-4 inhibition with ipilimumab was the first treatment in patients to continue overall survival with advanced melanoma in adventitious pattern. Investigation of long-term survival report pooled over various phase II and III trials illustrated that the survival curve starts to plateau at about 3 years with survival rates (22 %, 26 % and 20 % respectively) in all patients with adequate follow-up, in treatment of naive patients as well as early treated patients. Compatible with its survival advantages, CTLA-4 inhibition is associated with secure responses in a proportion of patients treated compare to several responses reported to last 3 years.[31]

Nowadays, PD-1 inhibition has been seen to enhance progression free survival with metastatic melanoma and earlier treated metastatic squamous and non-squamous patients. The prolonged follow-up records available show that highly substantial responses may occur with PD-1 inhibition in patients with melanoma or renal cell carcinoma. The response rates with PD-1 checkpoint inhibition were higher than CTLA-4 inhibition in advanced melanoma (33-34 % vs 12 %) patients in a phase III head-to-head trial of pembrolizumab vs ipilimumab. This case estimated higher 1 year survival rates with pembrolizumab vs ipilimumab (68-74 % vs 58 %).[32]

Due to immune checkpoint blockers work through proceed efficacious anticancer immune response patterns may vary from those shown with chemical therapeutics or targeted agents. Detained or unfamiliar responses can be related to differs in the kinetics and efficiency of particular patient's individual immune system, and also its interaction with cancers and metastases.[33] The beginning raise in target lesion cancer volume could be due to correct cancer growth before the generation of valuable anticancer response. Contrarily, speedy activation of an anticancer immune response could responsible to inflammation and an influx of immune cells into the cancer site, which could act as cancer progression.[34]

Clinical trials of ipilimumab, around 10 % of patients were primarily characterized as having progressive disease, but subsequently had favorable survival. Patients (4-8 %) with advanced melanoma receiving nivolumab or pembrolizumab in clinical trials had unusual responses that did not meet Response Evaluation Criteria in Solid Tumors criteria, but were nonetheless associated with patient advantages. Unfamiliar response patterns have also been reported in patients with lung cancer receiving PD-1 checkpoint blockers. These unconventional responses have directed to the development of modified response criteria known as immune related response criteria.[35]

6. Molecular biomarkers

A discomfort with ipilimumab has been the incapability to predict perspective which patients are most possible satisfaction from medication. The down level of inductive CTLA-4 and the widespread expression of its B7 ligands are useless as anticipating biomarkers.[36] Retroactive investigations have scrutinized various biomarkers associated with response, involving upregulation of T-cell activation maker inducible co-stimulator, development of polyfunctional T-cell response and also absolute lymphocyte count. Corporation between cancer mutational load and clinical advantages with CTLA-4 inhibition has been seen, but was not sufficient alone to predict patients who are probably respond against treatment.[37]

Although, work scrutinizing cancer neoantigens has seen satisfy, with the recognition of a neoantigen signature peptide situate in cancers that corresponded with overall survival of independently treated with CTLA-4 inhibitor. Comparatively, the upregulation of PD-1 on exhausted cells and ligands on cancer cells can offer the possible for identifying patients responsive to PD-1/PD-L1 inhibition.[38] Initial record across cancer types recommends that patients with PD-L1 presenting cancers or infiltrating immune cells generally have a higher response rate against PD-1/PD-L1 therapy. They can have achieved survival results compared to patients with negative PD-L1 expression. Moreover, responses have been shown in patients with PD-L1 low/negative cancers, and hence these patients should be unprohibited from treatment.[39]

The ipilimumab and nivolumab combination to individual agent alone, responses in PD-L1 positive patients were same with the combine vs nivolumab alone, since PD-L1 negative patients did preferable receiving the combination. All of these outcomes are provoking, whilst more research is needed to initiate the sustainability and utility of PD-L1 expression as a prophetic biomarker.[40] Another markers response against PD-1/PD-L1 therapy have also been investigated and involve characteristics associated with PD-L1 mediated suppression of premature immunity. Mutational and higher neoantigen burden with CTLA-4 have currently been seen to be associated with efficiency in patients with PD-1 inhibitor.[41]

7. Immunological toxicity

Immune checkpoint inhibitor is associated with prospective immunologic etiologies, known as immune mediated AEs. Mainly recorded immune mediated AEs involve rashes, gastrointestinal disorders, and endocrinopathies. The overall rate of grade Z3 AEs was higher with ipilimumab (20 %) compared to phase III trial with pembrolizumab (10-13 %).[42] This could be a result of a larger magnitude of T-cell proliferation or reduced Treg mediated immunosuppression with CTLA-4 inhibition, or activation of a small number of T-cell clones with PD-1 inhibition.[43]

Hypophysitis (inflammation of the pituitary gland) is recorded in patients (2-4 %) receiving ipilimumab but in <1 % of administrating PD-1 blockers. Moreover, this differs in incidence can be unrelated to variations in immune mechanism of action, but can be elucidated through ectopic expression of CTLA-4 in the pituitary gland, priming to ipilimumab binding to endocrine cells, attended through complement fixation and inflammation. Blocking PD-L1 rather than PD-1 can outcome in a slightly variant toxicity profile.[44]

Treatment related grade 3-4 AEs were estimated in patients (4-13 %) receiving PD-L1 blockers in phase I/II across two various agents and multiple cancers. Record from comparative trials is not yet available, while the incidence of grade 3-4 treatment related AEs can down with PD-L1 blockers than with PD-1. Since, the immune mediated AEs estimated to date have been same between the two agents.[45]

8. Combined inhibition of CTLA-4 and PD-1/PD-L1 checkpoints

Inhibition of both CTLA-4 and PD-1/PD-L1 could, not only introduce proliferation of an increasing number of T-cells primarily in an immune response, but also re-establish immune responses of earlier activated T-cells that have become drained, and reduce Treg mediated immunosuppression (**Fig. 4**).[46] Preclinical investigations illustrated promoted anticancer responses by combined inhibition compared with alone, which was also reported in initial clinical phases. This complementary effect authenticates the various roles of these agents help in immune regulation.[47] An improved progression free survival and raised response rate were showed with the combination of ipilimumab and nivolumab when compared with ipilimumab alone in a scattered phase III treatment with metastatic melanoma patients.[48]

The objective response rate was 58 % vs 19 %, and the median progression free survival was 11.5 vs 2.9 months for the combined and monotherapy. Combine CTLA-4 and PD-1 blockers are being reported in patients with various cancers, involving advanced NSCLC and RCC.[49] In metastatic RCC, primarily record conclude that the objective response rate is higher to combined checkpoint inhibition (38-43 %) than was shown with PD-1 blockade alone in a various trial (20-22 %). preliminary record from lung cancer trials unsatisfied raised anticancer activity with a combined inhibition in NSCLC. Since, increased anticancer activity was shown with a combined inhibition in small cell lung cancer (SCLC) vs nivolumab.[50]

CTLA-4 and PD-1 combined inhibition with the goal of increasing efficiency is highly desirable, but combined treatment could report more toxic. In patients with earlier untreated melanoma or recurrent SCLC, the occurrence of drug related grade 3-4 AEs was 54-55 % with concurrent inhibition compared with ipilimumab (24-27 %) and nivolumab alone (15-16 %).[51] Initial CTLA-4 blockade does not appear to liable patients to development of immune mediated AEs with PD-1 blockade, which can therefore support continues rather than combined treatment.[52]

CONCLUSIONS

The CTLA-4 and PD-1/PD-L1 checkpoint blockade immunotherapy down-regulate T-cell activation to maintain peripheral tolerance. Also, they may be utilized by cancers to introduce an immune suppressive state that permits the cancers to grow or develop rather of being removed through the immune system.[53] The variational patterns of CTLA-4 and PD-1 ligand expression originate mainly in lymphoid tissue and peripheral tissues. They are central to the hypothesis that CTLA-4 acts primarily in tolerance initiation, whereas PD-1 acts later to maintain long term tolerance.[54] CTLA-4 and PD-1/PD-L1 blockers may restore anticancer immune responses which controlling to long term advantage in a valuable proportion of treated patients.[55]

Expected outcomes of their mechanism of action, immune checkpoint blockers are led with immune mediated toxicities. Most of which may be cleared successfully with corticosteroids.[56] Primarily investigation recommend that inhibition of both CTLA-4 and PD-1/PD-L1 checkpoints shows to increased efficiency rather blockade alone or in sequence. This providing extra proof of the individual roles of these immune checkpoints in regulating anticancer immune responses.[57] More trials are required to confirm study and validate a combined strategy. CTLA-4 and PD-1/PD-L1 blockers are in active clinical development for multiple medications and have the prospective to revolutionize future treatment strategies for many cancer patients.[58]

Acknowledgements

REFERENCES

1. Elizabeth IB, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. American J of Clinical Oncology. 2016;29(1):98-106.

2. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008;224:166-182.

3. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol. 2005;23:515-548.

4. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182:459-465.

5. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21:137-148.

6. Chambers CA, Kuhns MS, Egen JG. CTLA-4 mediated inhibition in regulation of T-cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol. 2001;19:565-594.

7. Collins AV, Brodie DW, Gilbert RJ. The interaction properties of costimulatory molecules revisited. Immunity. 2002;17:201-210.

8. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol. 2002;3:611-618.

9. Parry RV, Chemnitz JM, Frauwirth KA. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25:9543-9553.

10. Fallarino F, Fields PE, Gajewski TF. B7-1 engagement of cytotoxic T-lymphocyte antigen 4 inhibits T-cell activation in the absence of CD28. J Exp Med. 1998;188:205-210.

11. Masteller EL, Chuang E, Mullen AC. Structural analysis of CTLA-4 function in vivo. J Immunol. 2000;164:5319-5327.

12. Schneider H, Downey J, Smith A. Reversal of the TCR stop signal by CTLA-4. Science. 2006;313:1972-1975.

13. Linsley PS, Bradshaw J, Greene J. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity. 1996;4:535-543.

14. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T-cells. J Exp Med. 1996;183:2533-2540.

15. Wing K, Onishi Y, Prieto-Martin P. CTLA-4 control over Foxp3+ regulatory T-cell function. Science. 2008;322:271-275.

16. Qureshi OS, Zheng Y, Nakamura K. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011;332:600-603.

17. Keir ME, Butte MJ, Freeman GJ. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704.

18. Wherry EJ. T-cell exhaustion. Nat Immunol. 2011;12:492-499.

19. Chen DS, Irving BA, Hodi FS. Molecular pathways: next generation immunotherapy-inhibiting programmed death ligand 1 and programmed death 1. Clin Cancer Res. 2012;18:6580-6587.

20. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol. 2004;4:336-347.

21. Zou W, Chen L. Inhibitory B7-family molecules in the tumor microenvironment. Nat Rev Immunol. 2008;8:467-477.

22. Akbari O, Stock P, Singh AK. PD-L1 and PD-L2 modulate airway inflammation and iNKT-cell-dependent airway hyperreactivity in opposing directions. Mucosal Immunol. 2010;3:81-91.

23. Huber S, Hoffmann R, Muskens F. Alternatively activated macrophages inhibit T-cell proliferation by Stat6-dependent expression of PD-L2. Blood. 2010;116:3311-3320.

24. Francisco LM, Salinas VH, Brown KE. PD-L1 regulates the development, maintenance, and function of induced regulatory T-cells. J Exp Med. 2009;206:3015-3029.

25. Wang C, Thudium KB, Han M. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014;2:846-856.

26. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. Clin Cancer Res. 2013;19:5300-5309.

27. Egen JG, Allison JP. Cytotoxic T-lymphocyte antigen 4 accumulation in the immunological synapse is regulated by TCR signal strength. Immunity. 2002;16:23-35.

28. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734-1736.

29. Hirano F, Kaneko K, Tamura H. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005;65:1089-1096.

30. Robert L, Tsoi J, Wang X. CTLA-4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res. 2014;20:2424-2432.

31. Cha E, Klinger M, Hou Y. Improved survival with T-cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Sci Transl Med. 2014;6:238-270.

32. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther. 2015;14:847-856.

33. Herbst RS, Soria JC, Kowanetz M. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563-567.

34. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252-264.

35. Das R, Verma R, Sznol M. Combination therapy with anti-CTLA-4 and anti-PD-1 leads to distinct immunologic changes in vivo. J Immunol. 2015;194:950-959.

36. Hodi FS, O'Day SJ, McDermott DF. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711-723.

37. Robert C, Thomas L, Bondarenko I. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364:2517-2526.

38. Schadendorf D, Hodi FS, Robert C. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol. 2015;33:1889-1894.

39. Farolfi A, Ridolfi L, Guidoboni M. Ipilimumab in advanced melanoma: reports of long-lasting responses. Melanoma Res. 2012;22:263-270.

40. Robert C, Long GV, Brady B. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372:320-330.

41. Larkin J, Chiarion-Sileni V, Gonzalez R. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23-34.

42. Garon EB, Rizvi NA, Hui R. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372:2018-2028.

43. McDermott DF, Drake CG, Sznol M. Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab. J Clin Oncol. 2015;33:2013-2020.

44. Topalian SL, Sznol M, McDermott DF. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32:1020-1030.

45. Wolchok JD, Hoos A, O'Day S. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15:7412-7420.

46. Motzer R, Rini B, McDermott D. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol. 2015;33:1430-1437.

47. Callahan MK, Postow MA, Wolchok JD. Immunomodulatory therapy for melanoma: ipilimumab and beyond. Clin Dermatol. 2013;31:191-199.

48. Snyder A, Makarov V, Merghoub T. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2189-2199.

49. Powles T, Eder JP, Fine GD. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515:558-562.

50. Tumeh PC, Harview CL, Yearley JH. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568-571.

51. Rizvi NA, Hellmann MD, Snyder A. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124-128.

52. Topalian SL, Hodi FS, Brahmer JR. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443-2454.

53. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol. 2012;30:2691-2697.

54. Iwama S, De RA, Callahan MK. Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med. 2014;6:230-245.

55. Curran MA, Montalvo W, Yagita H. PD-1 and CTLA-4 combination blockade expands infiltrating T-cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci. 2010;107:4275-4280.

56. Postow MA, Chesney J, Pavlick AC. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372:2006-2017.

57. Wolchok JD, Kluger H, Callahan MK. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122-133.

58. Weber JS, Kudchadkar RR, Yu B. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or naive melanoma. J Clin Oncol. 2013;31:4311-4318.

For any questions related to this article, please reach us at: globalresearchonline@rediffmail.com

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com