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**Cancer immunotherapy by targeting immune checkpoint receptors**

Isakov N. Immune checkpoint-targeted cancer immunotherapy

Noah Isakov

**Noah Isakov,** Department of Microbiology, Immunology and Genetics, Ben-Gurion University, Beer Sheva 84105, Israel

**ORCID number:** Noah Isakov (0000-0002-1412-0957).

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**Corresponding author to: Noah Isakov, PhD, Professor,** Department of Microbiology, Immunology and Genetics, Ben-Gurion University, POB. 653, Beer Sheva 84105, Israel. noah@bgu.ac.il

**Telephone:** +972-8-6477267

**Fax:** +972-8-6477626

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**Abstract**

The immune system plays a pivotal role in defending our body from invading pathogens and in surveillance against cancer. While most cells that acquire mutations are being detected and destroyed by immunocytes, a small number of transformed cells succeed in evading immune destruction by inhibiting immune checkpoint regulatory pathways leading to suppression of anti-cancer immune responses. Under normal conditions immune checkpoint receptors maintain self-tolerance, prevent immunopathology, and regulate overall immune homeostasis. However, their skewed activation by cancer cells may lead to suppression of nascent anti-tumor immunity and promotion of tumor growth. Discovering the role of immune checkpoints in cancer diseases and understanding their mode of operation has led to the development of new and novel strategies for cancer immunotherapy, which are based on the intervention or blockade of immune checkpoint-regulated pathways. Clinical studies demonstrated that immune checkpoint co-inhibitory receptor-blocking antibodies were able to revert the tumor-induced immunosuppression and to augment the overall anti-tumor immunity. These antibodies induced durable clinical responses and unprecedented therapeutic benefits in multiple types of malignancies. Although immune checkpoint inhibitors have revolutionized cancer therapy, the clinical benefits of these drugs were limited to subsets of cancer patients and treatments were frequently associated with a unique spectrum of toxicities, termed immune-related adverse events. Future discoveries of novel immune checkpoint receptors, identification of new prognostic and predictive biomarkers, and improvement of combination therapies are likely to boost the success rate of cancer immunotherapy and increase the survival rates of patients with different types of cancers.

**Key words:** Immune checkpoint; Immunotherapy; Cancer; Autoimmune diseases; T lymphocytes

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**Core tip:** The major functions of immune checkpoint receptors are to maintain self-tolerance, prevent immunopathology, and regulate overall immune homeostasis. However, skewed activation of these receptors by cancer cells may lead to suppression of nascent anti-tumor immunity and promote tumor cell growth. Clinical studies demonstrated that blocking of inhibitory immune checkpoint receptors induced durable clinical responses and unprecedented therapeutic benefits in multiple types of malignancies. The present Editorial addresses some of the major immune checkpoint receptor targets in cancer immunotherapy, discusses some of the side effects and limitations in their utilization, and highlights some of the future challenges in the field.

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**INTRODUCTION**

T lymphocytes include several functionally-distinct cell populations that are essential for combating virally-infected and neoplastic cells. A large fraction of the T cells in healthy individuals are naive resting cells that require antigen priming in order to acquire effector functions. The priming process involves the engagement of multiple T cell surface receptors that activate signaling pathways leading to T cell proliferation, differentiation and acquisition of effector functions. A signal from the T cell antigen receptor (TCR) is sine qua non for the activation process, as it provides the very early activation step and ensures that only T cell clones which are specific to the appropriate antigen will be expanded. The TCR interacts with a peptide antigen-loaded major histocompatibility complex (MHC) molecule on the surface of antigen presenting cells (APC). T cell activation and responsiveness are carefully regulated by a number of accessory co-stimulatory and co-inhibitory cell surface proteins, termed immune checkpoint receptors that can be triggered by cognate ligands on the surface of APC or target cells. The balance between signals provided by the TCR and the immune checkpoint receptors determines the cell’s ability to respond and differentiate into a fully active immunocyte.

The immune checkpoint receptors are implicated in immune response initiation and the regulation of their intensity and duration. Two of the best studied T cell-specific co-inhibitory receptors are the cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and programed cell death-1 (PD-1). The main function of these co-inhibitory receptors is to maintain self-tolerance and prevent autoimmunity. Furthermore, they also participate in the termination of T cell-mediated immune responses and limit collateral tissue injuries during anti-microbial immune responses.

Recent studies demonstrated that different types of cancers utilize the immune checkpoints to their own benefit. By activating co-inhibitory receptors these tumors can suppress T cell-mediated functions and promote their own escape from immune destruction. The appreciation of the regulatory role and the mode of operation of immune checkpoints in cancer diseases have led to the development of new strategies for cancer immunotherapy based on immune checkpoint blockade. Multiple clinical studies demonstrated that immune checkpoint blocking antibodies can be highly efficient in inducing durable clinical responses in different types of malignancies.

Nevertheless, the clinical benefits of these antibodies were limited to subsets of cancer patients and treatments were frequently associated with a unique spectrum of toxicities*,* termed immune-related adverse events.

***CTLA-4: Where it all begins?***

CTLA-4 (CD152) is the prototypic immune checkpoint receptor and the most studied in the context of cancer immunotherapy. It is a disulfide-linked homodimeric transmembrane glycoprotein expressed exclusively on T cells and participates in the repression of T cell proliferation, cell cycle progression and cytokine production[[1](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_1),[2](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_2)]. The CTLA-4 protein is highly homologous to the co-stimulatory molecule CD28 and both receptors utilize the same ligands, CD80 (B7.1) and the CD86 (B7.2) that are expressed on the surface of APC[[3-5](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_3)].

CD28 costimulation is fundamental for maximal activation of T cells. It promotes T cell expansion via interleukin-2 (IL-2)-pendent and independent mechanisms[[6](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_6)], but requires a primary signal (signal 1) in the T cell which is generated by TCR engagement with MHC-bound cognate antigens on APC (Figure 1).

In contrast to CD28, which is constitutively expressed on both resting and activated T cells, CTL4 is only marginally expressed on the outer surface of resting T cells and localizes predominantly in intracellular stores. In response of memory and regulatory T cells to antigen stimulation, CTLA-4 is temporarily being transported to the outer cell membrane[[7](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_7)]. At this stage, *CTLA*-*4* is capable of out-competing CD28 for ligand binding and induces the suppression of T cells via several different and independent mechanisms[[8-10](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_8)]. One mechanism relates to the fact that CTLA-4 has a higher binding affinity for both ligands. Its transient expression on the surface of T cells prevents CD28 from interaction with CD80/CD86 and deliver costimulatory signals[[11](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_11)]. Another mechanism reflects the ability of CTLA-4 to directly deliver inhibitory signals to effector T cells through its cytoplasmic tail which associates with signaling proteins[[12](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_12)]. Furthermore, CTLA-4 can suppress T cell responses by upregulating the activity of Treg cells. The engagement of CTLA-4 on Treg delivers activatory signals that enhance their suppressive activity and inhibit antitumor immunity[[8](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_8),[13](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_13)].

The role of CTLA-4 in maintaining T-cell activation under control is well demonstrated in studies of CTLA-4-deficient mice generated by homologous recombination. These mice suffer from a CD4+ T cell-mediated lymphoproliferative disease that is driven by excessive CD28 signaling[[14-16](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_14)]. CTLA-4-deficient mice also exhibit an impaired differentiation of Treg cells resulting in a massive lymphoproliferation, splenomegaly and lymphadenopathy, including multiorgan lymphocyte infiltration and tissue destruction[[17](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_17)]. These results substantiate the inhibitory role of CTLA-4 in the regulation of both Th and Treg development and function and in the maintenance of immune homeostasis.

Studies on the biological role of CTLA-4 and its involvement in the suppression of T cell functions laid the groundwork for the development of new strategies for cancer immunotherapy, which are based on CTLA-4 blockade[[13](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_13)].

The most studied CTLA-4 blocking antibody that was used in clinical trials is Ipilimumab (Yervoy®, Bristol-Myers Squibb) which showed significant survival benefit for patients with advanced metastatic melanoma. Ipilimumab was the first anti-immune checkpoint antibody to be approved by the United States Food and Drug Administration (FDA) for the treatment of melanoma, in early 2011[[18](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_18),[19](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_19)]. Ipilimumab enhances antitumor immunity by blocking the negative effects of CTLA-4 and augmenting effector cytotoxic T cell functions.

Clinical trials with a second anti-CTLA-4 antibody, tremelimumab )ticilimumab, CP-675,20, Pfizer and AstraZeneca), demonstrated its effectiveness in patients with melanoma[[20](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_20)], refractory metastatic colorectal cancer[[21](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_21)], and hepatocellular carcinoma[[22](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_22)].

Additional clinical trials using a variety of CTLA-4 blocking drugs, either alone or in combination therapy, are being conducted on patients with in a wide range of tumors. Combination of anti-CTLA-4 antibodies with immunotherapy, chemotherapy, or radiotherapy was found to improve long-term survival of cancer patients suffering from different types of malignancies[[23-26](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_23)].

***PD-1/PD-L1 pathway***

The PD-1 immune checkpoint receptor is expressed on activated T cells and helps to preserve self-tolerance and the prevention of autoimmunity. It was initially cloned in 1992 in a search for molecules involved in the negative selection of thymocytes that undergo programed cell death[[27](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_27)]. The role of PD-1 as an immune checkpoint became clear in 2000 upon identification of its physiological ligand, the programed cell death ligand-1 (PD-L1), which is expressed on APC and on some types of tumor cells[[28](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_28)]. These tumors can co-opt the PD-1/PD-L1 pathway by upregulating PD-L1 expression as a mechanism of immune resistance[[29](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_29)]. Preclinical studies in animal models demonstrated that targeting the PD-1/PD-L1 pathway can serve as another promising immunotherapeutic strategy for augmenting the endogenous T cell antitumor immunity[[30](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_30)].

***PD-1***

In addition to its fundamental role in maintaining self-tolerance and preventing autoimmunity, the PD-1 (CD279) immune checkpoint receptor is also critical for terminating immune responses. Its absence or down-regulation may cause tolerance breakdown or induction of autoimmune responses. PD-1 is expressed on activated T lymphocytes and upon engagement with its ligand, it delivers signals that counteract the TCR-induced signals and inhibit IL2 production and T cell proliferation.

PD-1-mediated intervention with the TCR-coupled signals involves the inhibition of ZAP70 binding to CD3, which down-regulates ZAP70 phosphorylation and catalytic activity. This occurs concomitantly with a reduction in the phosphorylation and activity of protein kinase C-theta (PKCθ), which attenuates the NF-κB and AP-1 transcription factors[[31](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_31)]. As a result, PD-1 signals can promote apoptosis of antigen-specific effector T-cells, and induce opposite effects on Treg cells leading to their exemption from programmed cell death[[32](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_32),[33](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_33)]. One possible explanation for these contrasting effects might rely on the spatially-regulated distinct functions of PKCθ in these two cell type[[34-36](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_34)]. Thus, PKCθ recruits to the center of the immunological synapse of activated effector T-cells[[34](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_34),[35](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_35)] but is sequestered away from the immunological synapse of Treg, and concentrates in the opposite pole[[36](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_36)]. It is highly probable therefore that activation of PKCθ in the temporally-organized distinct subcellular structures will result in PKCθ-mediated phosphorylation of different substrate proteins that regulate distinct biological processes.

Although signaling through PD-1 helps maintain homeostasis in the immune system, PD-1-induced signals in cancer patients may facilitate tumor progression by suppressing effector T cell functions, in general, and the induction of effective anti-tumor immunity in particular[[37](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_37)].

The realization that PD-1 blockade might help boost the immune system in cancer patients has led to the development of an array of PD-1 targeting drugs which have already demonstrated remarkable clinical success in experimental models and in cancer patients.

The first anti-PD-1 monoclonal antibody to gain the FDA approval, in December 2014, was nivolumab (Opdivo®, Bristol-Myers Squibb) which was used in a clinical trial (CA209037) in patients with metastatic melanoma that deteriorated after treatment with CTLA-4-blocking antibodies. The overall objective response rate (ORR) of the PD-L1+ melanoma patients was at the range of 0%-40%. In 2015, nivolumab received FDA approval for the treatment of patients with metastatic squamous non-small cell lung cancer (NSCLC), and metastatic renal cell carcinoma (RCC), whereby the treatment extended the overall survival rate of cancer patients in the two study groups.

In recent years, nivolumab received additional FDA approval for the treatment of a wide range of cancers.

A combination therapy of nivolumab and ipilimumab, which simultaneously target PD-1 and CTLA-4, was also used in clinical studies and found to improve the outcome of patients with melanoma[[38-40](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_38)] and other types of solid tumors[[41-43](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_41)].

In August 2016, pembrolizumab (Keytruda®, Merck Sharp and Dohme Corp.) was the second anti-PD-1 antibody that received FDA approval for the treatment of metastatic head and neck squamous cell carcinoma (HNSCC) patients. Several clinical studies are being conducted with pembrolizumab in patients with head and neck cancer (NCT02454179, NCT02707588) and other cancers, in addition to multiple clinical studies that evaluate efficacy of newly designed PD-1-targeting drugs in a wide range of cancers.

**PD-L1**

The PD-L1 [CD274; also known as B7 homolog 1(B7-H1)] is a 40kD type I transmembrane protein that serves as a ligand for PD-1[[44](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_44)]. Under normal physiological conditions, PD-L1 plays a vital role in the regulation of Treg functions and suppression of immunity during pregnancy and autoimmune diseases[[45](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_45),[46](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_46)].

Upregulation of PD-L1 on APC delivers inhibitory signals to PD-1+ T cells, which control immune responses at bay by terminating them once antigens have been eliminated.

However, some tumors can disrupt this equilibrium and manipulate the PD-1/PD-L1 checkpoint pathways by expressing PD-L1 and delivering signals that turn-off effector T cells[[47](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_47)].

Observations that support this mechanism were made in several studies when high expression of PD-L1 on the tumor cells correlated with reduced immune responses, increased tumor aggressiveness and relatively poor survival[[48](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_48),[49](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_49)].

Checkpoint inhibitors that target PD-L1 can therefore prevent its interaction with PD-1, restore T-cell activation, and amplify antitumor immunity[[47](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_47),[50](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_50)].

The first FDA-approved anti-PD-L1 antibody, atezolizumab (Tecentriq®, Genentech), was authorized in 2016 for the treatment of metastatic NSCLC and advanced and metastatic urothelial carcinoma[[50](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_50),[51](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_51)]. The clinical results indicated that tumors with high frequency of PD-L1+ tumor infiltrating immune cells demonstrated particularly high response rates.

Two additional anti-PD-L1 antibodies, avelumab (Bavencio®, Merck KGaA, Pfizer and Eli Lilly) and durvalumab (Imfinzi®, AstraZeneca) received FDA approval in 2017 for the treatment of Merkel-cell carcinoma and advanced bladder cancer, respectively. Additional PD-L1 blocking antibodies are currently being evaluated for the treatment of a wide range of cancers in multiple clinical trials[[52-54](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_52)].

***Additional T cell-associated co-inhibitory pathways***

Cancer immunotherapies directed against the CTLA-4- and PD-1/PD-L1 co-inhibitory receptors exhibited undisputed efficacy in selected types of cancer diseases, but many patients were nonresponsive to these therapies, and several tumor types are refractory to these therapies. To increase the repertoire of drug targets in different cancer diseases, scientists are searching for additional immune checkpoint receptors and testing their usefulness as drug targets in cancer immunotherapy.

The T cell immunoglobulin and ITIM domain (TIGIT) protein is a novel inhibitory receptor discovered in a genomic search for genes expressed in T cells. Their protein domain structure represents a potential inhibitory receptor[[55](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_55)]. The expression mechanism of TIGIT on the outer surface of T cells is somewhat similar to that of CTLA-4, as it is upregulated by T cell activation and is transient in nature.

Other inhibitory receptors are constitutively expressed on “exhausted’ T cells in patients with chronic cancer[[56](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_56)], including the lymphocyte activation gene 3 (LAG3; CD223) protein and the T cell immunoglobulin and mucin-containing molecule-3 (TIM-3; CD366).

The newly identified inhibitory receptors are being analyzed for their effectiveness as blocking antibody targets, while other surface proteins, such as the CD137, CD27, ICOS, and GITR activating receptors, are being evaluated for their effectiveness as agonistic targets that amplify anti-cancer immune responses[[57](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_57)].

***TIGIT***

TIGIT (also known as WUCAM, Vstm3, VSIG9) is part of the CD28 family-like receptors that are expressed on T cells and various other hematopoietic cells[[55](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_55),[58](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_58),[59](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_59)]. The TIGIT agonists include CD155 (poliovirus receptor-PVR) and CD122 (PVRL2, nectin-2), which are expressed by immune and non-immune cells, as well as tumor cells[[55](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_55)]. TIGIT is a coinhibitory receptor with a role in tolerance induction and autoimmunity. In general, TIGIT-/- mice were found to be more sensitive than wild-type mice to immunization with myelin oligodendrocyte glycoprotein (MOG) peptide, and develop more severe experimental autoimmune encephalomyelitis (EAE). In contrast, TIGIT transgenic mice are less sensitive to immunization with MOG and develop reduced symptoms of EAE[[58](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_58),[60](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_60)]. TIGIT-/- mice develop severe autoimmune responses in a model of collagen-induced arthritis and graft-versus-host disease, similar to the effects induced by anti-TIGIT blocking antibodies[[58](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_58)], indicating that TIGIT functions as an immunoreceptor that downregulates T cell-mediated immunity.

The mechanism of action of TIGIT and receptor, the CD226, is reminiscent of that of the CTLA-4/CD28 receptor pair, with TIGIT fulfilling the role of the coinhibitory receptor that counterbalances costimulation mediated by CD226. Furthermore, the TIGIT/CD226 expression kinetics are similar to those of CTLA-4/CD28, with the coinhibitory receptor being expressed only following T cell activation, in contrast to the costimulatory receptor which is constitutively expressed on resting T cells[[55](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_55),[58](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_58),[59](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_59)].

The expression of TIGIT on peripheral blood lymphocytes and in lymphoid organs of tumor bearing mice is relatively poor, but it is highly expressed on tumor infiltrating lymphocytes (TILs), including Treg, both in mouse and humans[[61](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_61),[62](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_62)].

The observations that TIGIT may impose negative effects on anti-tumor responses were made in studies in which growth of tumors in TIGIT-deficient mice were retarded, compared to the growth in wild type mice[[61](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_61)], and that co-blockade of TIGIT and PD-1 had an additive positive effect on lymphoid cell functions in melanoma patients[[63](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_63)]. Further studies revealed that progression of multiple myeloma (MM) in mice and humans was associated with high expression levels of TIGIT in CD8+ T that exhibited reduced functions, while targeting of TIGIT with monoclonal antibodies increased the effector function of the CD8+ T cells and prolonged the survival of the MM patients[[64](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_64)].

In another set of studies, TIGIT, but not CTLA-4 or PD-1, was found to be associated with NK cell exhaustion in mice and humans with colon cancer[[65](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_65)]. Blockade of TIGIT prevented NK cell exhaustion and promoted NK cell-dependent and T cell-mediated anti-tumor immunity[[65](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_65)].

The knowledge accumulated thus far on the immune-inhibitory role of TIGIT in tumor bearing mice and human cancer patients suggests that future designed TIGIT-directed blocking strategies might lead to the development of highly effective and improvedanti-cancer therapies.

A phase I clinical trial using anti-TIGIT monoclonal antibodies (MTIG7192A) is in progress in patients with advanced or metastatic tumors (NCT02794571).

Another phase I clinical trial (NCT03119428) in patients with locally advanced or metastatic solid tumors investigates the safety and pharmacokinetics of OMP-313M32 antibody that blocks TIGIT binding to PVR.

***TIM-3***

TIM-3 [also known as Hepatitis A virus cellular receptor 2 (HAVCR2)] belongs to the TIM family of cell surface receptor proteins which consists of eight members (TIM-1-8) in mice and three members (TIM-1, TIM-3, and TIM-4) in humans[[66](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_66)]. The individual TIM proteins differ in molecular structure and expression patterns, as well as in their regulatory functions and impact on T-cell responses[[66](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_66)]. TIM-3 is expressed on T cells and additional hematopoietic cell types[[67-69](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_67)] and utilize the C-type lectin galectin-9 as its ligand.

Ligation of the TIM-3 receptor in vitro initiates signals that suppress T cell responses, and in vivo administration of anti-TIM-3 antibodies increase the severity of clinical symptoms in a mouse model of experimental autoimmune encephalomyelitis (EAE)[[70](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_70)]. Since EAE is a T helper 1 (Th1)-dependent autoimmune disease, it has been suggested that Tim-3 plays a role in the induction of autoimmune diseases by regulating macrophage functions[[70](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_70)].

Additional studies demonstrated that both Tim-3–deficient mice and normal mice treated with a TIM-3–Ig fusion protein exhibited defects in the induction of antigen-specific tolerance[[71](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_71),[72](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_72)]. These studies and others indicated that Tim-3 is an immune checkpoint receptor that functions to specifically limit the duration and magnitude of T cell-mediated immune responses and contributes to the overall maintenance of immune tolerance[[71](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_71),[72](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_72)].

TIM-3 is expressed on a large fraction of T cells in cancer patients where it is upregulated predominantly in tumor-infiltrating lymphocytes[[73-75](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_73)]. In addition, expression of TIM-3 was found on various types of cancer cells where increased expression was frequently associated with disease progression and shorter survival[[73-75](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_73)].

Preclinical studies in cancer patients demonstrated that TIM-3+ T cells exhibited the most suppressed phenotype and were among the most severely exhausted lymphocytes[[76](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_76),[77](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_77)].

In addition, a large fraction of the TIM-3+ T cells in the cancer patients coexpressed PD-1, and the dual receptor-expressing T cells exhibited greater immune function impairments compared to T cells that expressed PD-1 alone[[76-78](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_76)].

These investigations and additional studies support the assumption that a cooperation exists between TIM-3- and PD-1-coupled signaling pathways, which induce severe T cell anergy, or active T cell suppression in cancer diseases.

In contrast to CTLA-4 and PD-1, TIM-3 does not utilize immunoreceptor tyrosine-based inhibition motifs (ITIM) or immunoreceptor tyrosine-based switch motifs (ITSM) to transduce its signals. It is suggested therefore that TIM-3 is unlikely to be functionally redundant with other ITIM/ITSM-containing checkpoint receptors and that targeting of TIM-3 might have additive or synergistic effects with those induced by anti-CTLA-4 or PD-1 antibodies.

In agreement with this hypothesis, targeting the TIM-3 pathway was found to have modest antitumor effects in various experimental models and preclinical cancer studies[[79](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_79),[80](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_80)]. However, combination of anti-TIM-3 and anti-PD-1 monoclonal antibodies had a synergistic effect in suppression of tumor growth[[73](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_73),[76](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_76),[81](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_81),[82](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_82)].

Multiple clinical trials (NCT03489343; NCT02817633; NCT03099109; NCT03066648) using a variety of anti-TIM-3 monoclonal antibodies (such as, LY3321367, MBG453 and TSR-022) alone, or in combination with anti-PD-1 or anti-PD-L1 antibodies are currently in progress in different types of leukemia and solid tumors.

***LAG3***

LAG3 is a CD4-related 70 kDa type I transmembrane glycoprotein that is expressed on activated CD4+ and CD8+ T lymphocytes[[83](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_83)]. It binds MHC-II with a high affinity compared to CD4, suggesting that it might function as a CD4 competitor[[84](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_84)] and negatively regulate TCR-induced signals leading to T cell activation[[85](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_85),[86](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_86)].

A negative regulatory role of LAG3 was demonstrated in human peripheral blood cells, when anti-LAG-3 blocking antibody combined with superantigen stimulation led to increased proliferation of CD4+ and CD8+ T cells[[87](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_87)]. LAG3 was found to synergize with PD-1 in downregulating T cell functions and promoting immune evasion by cancer cells[[88](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_88)]. Furthermore, dual anti-LAG3/anti-PD-1 antibody treatment cured most mice of established fibrosarcoma tumors that were otherwise resistant to a single antibody treatment[[88](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_88)].

Several LAG3-targeted therapies are currently at various stages of preclinical and clinical development. Combination therapies of anti-LAG-3 and anti-PD-1 antibodies showed promising results in melanoma patients who were relapsed or refractory to anti-PD-1/PD-L1-Antibodytherapy.

Multiple clinical trials (NCT02658981; NCT03489369; NCT02061761; NCT03250832) using a variety of LAG3 blocking reagents [such as, TSR-033, Sym022, and BMS-986016 (relatlimab) monoclonal antibodies, or a soluble LAG-3Ig fusion protein (IMP321)] alone, or in combination with a variety of other drugs are currently in progress in different types of hematological malignancies and solid tumors.

***Adverse effects of immune checkpoint therapy***

Although immune checkpoint targeting has shown a great deal of promise in treating a wide range of cancers, drug-induced side-effects, termed “immune-related adverse events’’, (irAEs) have been observed in numerous patients due to non-tumor-specific activation of T cells by the immune checkpoint blocking Abs.

Side effects of the Immune checkpoint therapy range from mild to severe and may include fatigue, cough, fever, diarrhea, nausea, loss of appetite, skin rash, itching and nerve inflammation. In addition, the irAEs may affect multiple organs and systems including the gastrointestinal tract, liver, kidney, central nervous system, endocrine glands, and the pulmonary, cardiovascular and hematological systems.

Frequency and severity of irAEs usually correlate with the antibody dosage. The median time of onset of irAEs is about 10 wk after the start of the treatment, although it varies with respect to the affected tissues and between individual patients, and may occur even after cessation of the immune checkpoint therapy.

While the occurrence of irAEs normally indicates that immune checkpoint blockade has activated the patient’s immune system, the correlates with improved antitumor immunity remains controversial, and the general assumption is that irAEs are not required for obtaining an effective anti-tumor response.

Although the majority of irAEs are completely reversible, in cases of severe adverse events, immunosuppression using glucocorticoids or other drugs is recommended. This temporary immunosuppression was found to have no influence on the overall survival rate of the patients[[89](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_89)].

The discovery of additional biomarkers and the development of new tools for prediction of the patient’s risk to develop irAEs will facilitate the application of the full therapeutic potential of the immune checkpoint-targeting drugs.

**CONCLUSION**

Immune checkpoints consist of inhibitory and stimulatory pathways that are essential for maintaining self-tolerance and assist with the regulation of immune responses.

Coinhibitory immune checkpoint receptors are often activated in cancer cells and enable tumor progression by dampening antitumor immune responses.

Ab-mediated cancer immunotherapy based on the blockade of the signaling axis between coinhibitory receptors and their ligands has shown remarkable clinical success in patients with different types of cancers.

The most effective reagents thus far are antibodies directed against the CTLA-4, PD-1 and PD-L1 receptors, while other antibodies that react with receptors, such as TIGIT, TIM-3 and LAG3 are under evaluation.

While immune checkpoint blockade therapy has revolutionized oncology care, only a subset of the treated cancer patients shows durable responses.

One reason relates to the inherent limitations of the antibody molecules that have poor tissue and tumor penetrance and harmful Fc-effector functions that deplete immune cells. These limitations can be partially overcome by using genetically-modified antibodies that have smaller size (nanobodies; monomeric variablefragments of Camelid heavy-chain Abs) or lack their Fc portion. Another option is using smaller, non-antibody molecules with high affinity to selected immune checkpoint receptors that function as soluble agonists. Such molecules can be designed to have a higher affinity to immune checkpoint receptors, compared to those of the physiological ligands, to have superior tumor penetration due to their smaller size, and to have no effect on Fc-mediated T cell depletion[[90](file:///C:\Users\WHL\Desktop\修稿\41895\41895-Review1.docx#_ENREF_90)].

At present, the immune checkpoint therapy is aided by only a limited number of biomarkers that can accurately predict an optimal therapy for patients with different types of cancers. However, intense investigations are ongoing in order to identify new and novel biomarkers for immune checkpoint therapy that will increase the specificity and selectivity of the treatment, minimize the risk of toxicity, and serve as non-redundant targets that can maximize the potential of combination therapies.

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# Figure 1 T cells are subjected to co-stimulatory and co-inhibitory signals obtained by interaction of their plasma membrane receptors with ligands on the surface of antigen presenting cells ( dendritic cells and macrophages), as well as tumor cells. Targeting of the co-inhibitory receptors by specific antibodies block the receptor-coupled inhibitory signals and enhance T cell proliferation and cytokine production and can augment the overall antitumor T cell responses. A: A productive interaction of T cells with antigen presenting cells (APC) results in a rapid delivery of stimulator signals leading to T cell activation; B: T cell activation leads to surface of expression of cytotoxic T lymphocyte associated antigen-4 (CTLA-4). Interactions of the T cell CTLA-4 and programed cell death-1 (PD-1) co-inhibitory immune checkpoint receptors with the CD80/86 and PD-L1 ligands, respectively, on the surface of the APC, result in the delivery of inhibitory signals that downregulate T cell activation and cytokine production; C: Targeting of the CTLA-4 and PD-1 coinhibitory receptors on T cells, or the PD-1 ligand, PD-L1, on the APC (or the tumor cells), can block the inhibitory signals, increase the intensity and efficacy of anti-tumor T cell responses, and induce durable clinical responses and therapeutic benefits in cancer patients.