Chapter 2

Immune Checkpoint Blockade and Immune Monitoring

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Additional information is available at the end of the chapter

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Abstract

The concept of immunological surveillance, a monitoring process in which the immune system detects and destroys by several effector mechanisms, virally infected and neoplastic transformed cells in the body, was developed more than 50 years ago. Based on current research, it is clear that the immune system can recognize and eliminate transformed cells. An increasing number of studies has investigated the immune system in cancer patients and how it is prone to immunosuppression, due in part to the decrease of lymphocyte proliferation and cytotoxic activity. Such weakened immune system is then unable to fully accomplish its role in immunological surveillance, allowing nascent transformed cells to escape the selective pressure of the immune system. The main goal of cancer immunotherapy has been to reawaken the immune system from a suppressive slumber to enable it to attack cancer cells once again. As the results from the last 10 years attest, cancer immunotherapy is the best strategy to restore the activity of the immune system and unleash its potential to destroy cancer cells in cancer patients. This chapter aims to discuss the recent findings on immune monitoring studies and the use of immune checkpoint inhibition in cancer immunotherapy.

Keywords: immune checkpoint blockade, immunotherapy, immune monitoring

1. Introduction

Throughout the evolutionary process, the immune system has developed mechanisms to protect the living beings against infections by different microorganisms, viruses, and parasites. A notorious question in immunology has been whether an immune response could also be raised against transformed cells. Researchers have indeed, for a long time, studied if cancer prevention could be a primary function of the immune system. The concept of immunological surveillance, a monitoring process in which the immune system detects and destroys virally infected and neoplastic transformed cells, was elaborated more than 50 years ago by Lewis Thomas and Sir Frank Macfarlane Burnet [1–4]. Back in history, William B. Coley in 1893, an American bone surgeon and a pioneer in cancer immunotherapy, created a purified lysate of multiple bacteria to treat a young patient who had developed an inoperable sarcoma. As a result of the treatment, the patient had a complete remission. As head of the Bone Tumor Service at Memorial Hospital in New York, Dr. Coley would still inject more than 1000 cancer patients with bacterial products, then called Coley's toxins, which later were used by several physicians in several patients with bone and soft tissue sarcomas, reporting some excellent results [5]. In fact, the concoction initially called Coley's Toxin contained heat-killed *Streptococcus* combined with live *Serratia marcescens*. To Dr. Coley, the infection that he produced could contribute to shrinking malignant tumors. In 1909, the German biochemist Paul Ehrlich, winner of the Nobel Prize in Physiology and Medicine and father of chemotherapy, introduced the word Zauberkugel (the magic bullet) to describe antibodies. Generations of scientists interpreted the magic bullet as a compound that would target a single critical oncoprotein [6]. Unfortunately, at that time, the pioneering work led by Coley and Ehrlich could not explain the underlying mechanisms that activated the immune system to recognize and kill cancer cells. Nowadays, their ideas and the early experiments, which were aimed at fighting cancer and infectious diseases, serve to inspire new generations of researchers to develop compelling strategies of targeted therapeutics and immunotherapy. However, the role of the immune system in cancer recognition faced a shadowy period of disbelief mainly due to the difficulty in reproducing tumor regression in different types of cancer using Coley's toxin [7], the extremely toxic treatment [8], rejection of transplantable tumors [9], and the fact that thymic selection removed autoreactive T cells. These shreds of evidence led the scientists to believe that the role of the immune system as a primary strategy for recognizing cancer cells was minimal. With the advent of studies on cellular and molecular biology after the 1980s, several experiments were carried out to demonstrate that the immune system could efficiently act against cancer initiation and development. The fact that autoreactive T cells can escape from thymic selection [10], the discovery of tumor-associated antigens—TAAs [11], tumor antigen cross-presentation by dendritic cells to T lymphocytes [12], and the high frequency of cancer development in immunodeficient mice (STAT−/−, IFN−/−, RAG−/−, TCRβ−/−, TCRδ−/−, perforin−/−) [13] have considerably strengthened the concept of a protective immune system in the last decades. **Figure 1** displays a short chronological timeline of discoveries and progresses in cancer immunotherapy.

Hanahan and Weinberg on defining critical aspects of cancer development and progression, described a set of biological capabilities defined as "hallmarks of cancer." In their conceptualization, there are eight hallmark capabilities that are common to many, if not most forms of human cancer: sustained cell proliferation, evasion from growth suppressors, cell death resistance, replicative immortality, angiogenesis, tissue invasion and metastasis, deregulation of cellular energetics/metabolism, and avoidance of immune destruction [14, 15]. The primary goal of cancer immunotherapy has been to reawaken the immune system from a suppressive slumber to enable it to attack cancer cells once again. The fundamental principles that orchestrate cancer immunology and cancer immunotherapy can be described by immune surveillance, immune editing, and immune tolerance. A rapid increase in understanding the mechanistic

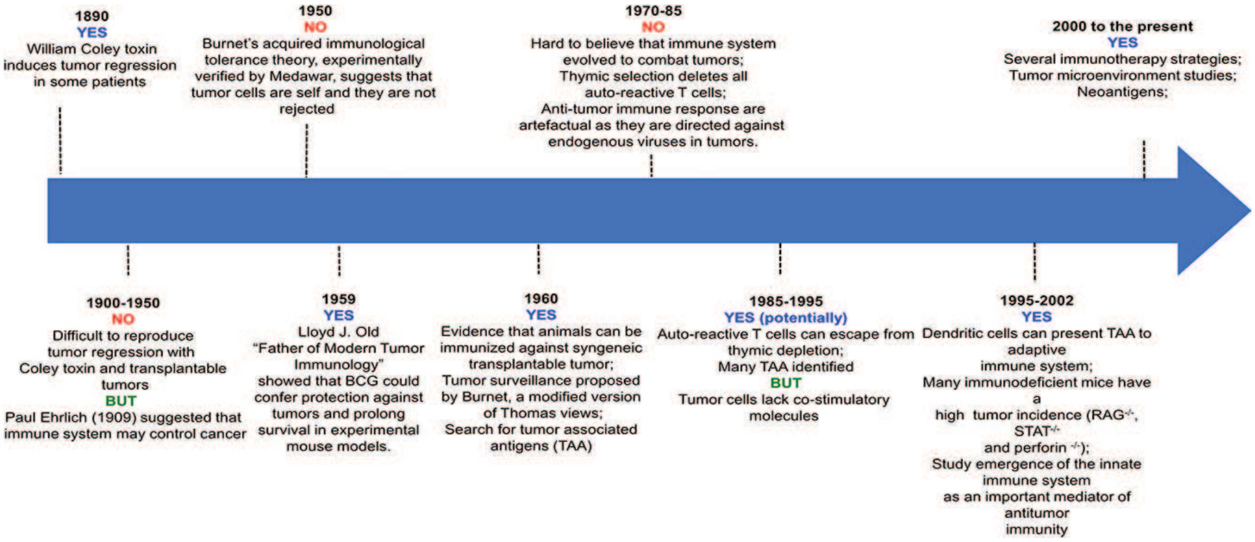

Figure 1. Is there an immune response to a malignant tumor? There was a time when the immune system was not recognized as having a protective role against developing cancer. That was until Burnet named the talent of immune system to detect tumor cells and destroy them as immune surveillance. Tumor surveillance by the immune system was, however, difficult to be practically shown.

pathways of these principles has led to clinical success in the treatment of cancer. In 2001, Robert Schreiber and Lloyd J Old (considered the father of Tumor Immunology) demonstrated that T lymphocytes and IFN-γ helped to inhibit the development of spontaneous and carcinogen-induced tumors in mice genetically deficient in RAG2 [16]. A few tumor cells escaped, however, from detection and eventually gave rise to tumors. Such selective evasion mechanism in a small number of tumor cells became known as immunoediting. In fact, the tumor cells that escaped became less immunogenic than the starting population and were no longer recognized by the immune system. At the time, the researchers wondered how these tumor cells have learned to outwit the immune attack.

Immunoediting consists of three well-established and orchestrated following processes called the "3Es" [17]. The first phase is the "elimination," in which the immune responses (innate and adaptive) can recognize and destroy tumors. Normal cells can prevent or inhibit malignant transformation through the expression of intrinsic tumor suppressors genes such as p16, p53, BRCA (breast cancer type 1 susceptibility protein), and APC (adenomatous polyposis coli). Chemical, physical, and biological factors can induce neoplastic transformation and consequently the expression of several tumor antigens, which can be captured, processed, and presented by dendritic cells and macrophages via MHC/peptides to T naïve cells. Immune cells such as CD4+, CD8+, NKT, and γδ T cells, as well as NK cells, and the cytokines released in this environment, such as IFN-y, IFN-α, IFN-β, and perforin, are responsible for tumor cell killing. The genetic instability and/or immune selection allow the transformed cells to resist to the immune response, starting the second phase, called "equilibrium," in which transformed cells that had survived to the immune surveillance phase are in dynamic equilibrium (growing cancer cells = dying cancer cells). When transformed cell variants selected in the second phase start a clonal growth in an immunologically controlled environment mainly due to the reduction of cancer immunogenicity followed by the immune exhaustion profile on T cells (PD-1, TIM-3, LAG-3, and TIGIT), and then, the third phase of immunoediting called "escape" begins [18]. Classic mediators of immune escape include downregulation of co-stimulatory molecules (B7 molecules), antigen loss by downregulation of MHC molecules, increased resistance to apoptosis induction, and cell-mediated cytotoxicity due to the overexpression of antiapoptotic proteins such as FLIP and BCL-X, mutated Fas and TRAIL [19], expression of T cell inhibitory molecules such as PD-L1, B7-H3, HLA-G, HLA-E, and B7-x by cancer cells, tumor stromal cells, and APCs [20]. The presence of CD4 + CD25 + Foxp3 + T regulatory cells, IL-10 secreting T cells, M2 macrophages, immature dendritic cells, and myeloid-derived suppressor cells (MDSC) into the tumor or draining lymph nodes is decisive to maintain the immunosuppressive environment [21, 22]. Clearly, without a doubt, the immune system plays a dual role in the multifaceted interactions between cancer cells and the host.

Based on current immunological findings, the role of the immune system in the recognition and elimination of transformed cells is beyond any doubt. Numerous studies have investigated the immune system in cancer patients undergoing immunosuppression, mainly due to a decrease of lymphocyte proliferation and cytotoxic activity [23–27]. In this circumstance, the immune system becomes weak, inactive, or inefficient. Currently, immunotherapy includes several strategies for restoring cancer patient's immune system in an attempt to harness and to destroy cancer cells specifically. This chapter discusses the recent findings on immune checkpoint function and the immune monitoring studies in cancer immunotherapy.

2. T cell activation and immune checkpoint blockade

Currently, the pathways that preclude the complete and responsive immune response to cancer cells are better understood. Since CTLA-4 (cytotoxic T lymphocyte-associated protein 4) has a marked structural homology to CD28, and because it was unknown whether the antibodies used were agonistic or antagonistic, it was not clear whether CTLA-4 had an analogous function as a secondary co-stimulatory agent [28] or an opposing role as a dampener of T cell activation [29, 30]. Only the data from CTLA-4 knockout animals definitively revealed the inhibitory function of CTLA-4 [31, 32].

Cancer immunotherapy has been declared the breakthrough of the year, in 2013. The ecstasy is fundamentally grounded on the clinical success of antibodies that modulate immune checkpoints mainly by targeting CTLA-4 and PD-1 (programmed cell death protein 1). Immune responses are tightly regulated by a remarkable system with checkpoints that control either positively or negatively the magnitude of the immune response. Several immune checkpoint molecules expressed on T cells can promote activation of naïve T cells (stimulatory checkpoint pathway) or otherwise inhibit this activation by restraining T cell activation and extension of the immune response (inhibitory checkpoint pathway), thus regulating the homeostasis, magnitude of inflammation, and tolerance [33]. Positive co-stimulatory molecules on T cells such as CD28, 4-1BB, OX-40, ICOS, CD2, and CD226 (DNAM-1) allow for T cell activation, proliferation, and cytokine production. In contrast, negative signals, mediated by LAG-3, CTLA-4, PD-1 and PD-L1, VISTA, B7-H3, CD96, and TIGIT, downregulate T cell activation. These molecules are critical to prevent autoimmunity and protect healthy tissues from immune activation. Finally, signals provided by pro-inflammatory cytokines mainly IL-12, IL-21, and type I interferons (IFN-α/β) are necessary for T cell response [34]. Blocking CTLA-4 and PD-1 using monoclonal antibodies represents the innovative concept in cancer therapy owing to (a) these molecules entirely ignore the tumor cells—they rely solely on the immune system; (b) they are not used to activate the immune system against a particular cancer but to neutralize inhibitory molecules that block a positive antitumor T cell response [35].

The immune system capacity to detect and destroy abnormal cells may prevent the development of many cancers. Cancer cells arise from normal cells, driven by mutations that lift brakes on cell proliferation. As an evolutionary process, tumor cells appropriate regulatory immune checkpoints to evade elimination. To keep growing, tumor cells take advantage from a sophisticated and dynamic bionetwork called the immune microenvironment. The microenvironment, in addition to tumor cells and infiltrating immune cells, contains epithelial cells, lymphatic and vascular vessels, cytokines, and chemokines [36]. Targeting the microenvironment for more efficient cancer immunotherapy in solid cancer has been a research objective in the last decade.

3. Monoclonal antibodies and novel checkpoint inhibitors in cancer immunotherapy

Monoclonal antibodies have had a considerable impact on the care of patients with cancer in the last 30 years. The initial report introducing the monoclonal antibody technology (hybridoma technique) arose from an article published by Köhler and Milstein in 1975, which caused a tremendous impact on laboratory research [37–39]. Sometime later, Kohler and Milstein won the Nobel Prize in Physiology or Medicine in 1984 awarded jointly to Niels K. Jerne. From then on, the new monoclonal antibodies aimed at cancer cell proteins such as CD20 (Rituximab, Ocrelizumab, Veltuzumab, Ofatumumab, and Obinutuzumab), HER-2 (Trastuzumab and Pertuzumab), EGFR (Cetuximab and Panitumumab), VEGF (Bevacizumab and Ramucirumab), GD2 ganglioside (Dinutuximab) or the immune cell surface inhibitors, PD-1 (Nivolumab, Pembrolizumab, and Pidilizumab), CTLA-4 (Ipilimumab and Tremelimumab), and PD-L1 (Atezolizumab, Avelumab, and Durvalumab). More effective immune responses can be achieved by modifying those monoclonal antibodies when they have failed, mainly due to the heterogeneity of epitope expression, the delivery to tumor cells, and antigenic modulation [40]. Several monoclonal antibodies have been conjugated to cytotoxic agents (mAb drug conjugates —ADCs). Some examples of ADCs include Ado-trastuzumab (anti-HER2 conjugated with emtansine), Gentuzumab ozogamicin (anti-CD33 conjugated with calicheamicin), Brentuximab vedotin (anti-CD30 conjugated with vedotin), immunotoxins (Moxetumomab pasudotox, Denileukin diftitox, DT2219, Resimmune, and SL-401), and radionuclides (131I-tositumomab and Y-ibritumomab) [41].

Presently, several efforts are being made to design effective combinations of immunotherapeutic mAbs and new agents that target particular pathways and to reach synergistic effects in the

Molecule	**Expression status**	**Function**	**Cognate ligand**	**Mechanism of action**	**Tested in (Cancer types)**	**Blocking antibodies**
CTLA-4 (CD152)	Upon activation of naïve T cells (CD4+ and CD8+); Constitutively expressed on suppressive T regulatory cells (CD4+Foxp3+ T regs; dendritic cells, monocytes, macrophages and B cells	Critical for initial activation of T cells in secondary lymphoid organs. Effector function, cell growth, survival and memory	CD80 (B7-1) CD86 (B7-2)	Outcompeting CD28 and by recruiting phosphatases to the cytoplasmic domain	Advanced and Metastatic melanoma	Ipilimumab and Tremelimumab
PD-1 (CD279)	Inducible on naïve T cells upon activation; Constitutively expressed on T regs; monocytes, macrophages, and B cells	Critical in regulating peripheral T cells tolerance. Effector function, cell growth, survival and memory	PD-L1 PD-L2	Reduces the signal downstream of TCR stimulation leading to a decreased activation and cytokine production; Induce genes that reduce T cell proliferation; decrease anti-apoptotic proteins and increase pro apoptotic	Advanced melanoma, metastatic melanoma, Prostate, Colorectal, Non–small cell lung cancer (NSCLC), Renal cell carcinoma (RCC)	Nivolumab and Pembrolizumab
TIM-3	Dysfunctional CD8+ tumor-infiltrating lymphocytes (TILs) also referred to as T cell exhaustion, intra-tumor Treg NK cells, monocytes, macrophages and dendritic cells	Effector function, cell growth, survival and memory	Galectin-9 Ceacam1 HMGB1 PtdSer	Causes negative signals on T cells resulting in apoptosis of Th1 and CD8+ cells	Tested in solid tumors and leukemia	TRS-022, LY3321367 and MBG453
LAG-3 (CD223)	T cells, NK cells, B cells, monocytes, macrophages, endothelial cells and dendritic cells Also, expressed on cancer cells	Effector function, cell growth, survival and memory	MHCII (Higher affinity than CD4)	Homologue of CD4+ Negative regulatory function on T cells; Mediate a profile of exhaustion in combination with PD-1 and TIM-3 on CD8+ T cells	Tested in Advanced renal cell carcinoma	IMP321 BMS-986016 MK-4280 GSK 2831781 LAG525
TIGIT	T cells and NK cells	Cell growth and effector function	Interacts with members of Poliovirus receptors family (PVR)	Acts as a functional ligand inducing a tolerogenic phenotype in dendritic cells, resulting in elevated IL-10 and reduced IL-12. A regulatory	Locally advanced or Metastatic solid tumors	OMP313M32 COM701

Molecule	Expression status	Function	Cognate ligand	Mechanism of action	Tested in (Cancer types)	Blocking antibodies
			DNAM-1 (CD226) CD96 PVRL 2 (CD112) PVR (CD155) Other nectins	role of TIGIT in modulating the signaling pathway, which facilitates M2-polarization, a class of immunosuppressive tumor associated macrophages that arise in response to Th2 cytokines; Like PD-1, LAG-3 and TIM-3 can be expressed by exhausted CD8+ T cells		
BTLA (CD272)	Dendritic cells, monocytes, macrophages, T cells (Th1) and B cells	Effector function, cell growth, survival and memory	HVEM	Display T cell inhibition	-	-

Data taken from: [20, 22, 30–33, 35, 47, 50, 76–78, 89–94, 100, 107, 109, 110, 112, 114, 116, 118, 124, 127, 130, 137, 145].

Table 1. Targeting potential co-inhibitory molecules which may contribute to improve the immune checkpoint blockade immunotherapy.

Molecule	Expression status	Function	Receptor	Mechanism of action	Tested in (Cancer types)	Blocking antibodies
PD-L1	NK cells, endothelial cells, stromal cells, epithelial cells and B cells	Regulates the development, maintenance and functions of T cells	PD-1	PD-L1/PD-1 on T cells provides a signal that prevents TCR-mediated activation of IL-2 production and T cell proliferation. The pathway involves inhibition of ZAP70 phosphorylation and its association with CD3ζ; PD-L1/PD-1 attenuates PKC-θ activation loop phosphorylation necessary for the activation of transcription factors NF-κB and AP-1, and for production of IL-2; PD-L1/PD-1 also	Bladder, non–small cell lung cancer, melanoma, breast, ovarian and pancreas	Atezolumab; Avelumab; Durvalumab

Molecule	Expression status	Function	Receptor	Mechanism of action	Tested in (Cancer types)	Blocking antibodies
				contributes to ligand-induced TCR down-regulation during antigen presentation to naive T cells		
PD-L2	Initially believed to be restricted to macrophages and dendritic cells; PD-L2 expression can be induced on a wide variety of other immune cells and nonimmune cells depending on microenvironmental stimuli	Regulates the development, maintenance and functions of T cells	PD-1	The same effect as above	Melanoma, Renal cell carcinoma, non-small lung cancer cell, bladder and head and neck	AMP-224 CA-170
B7-H3 (CD276)	Initially was believed to co-stimulate the immune response, but recent studies have shown that it has a co-inhibitory role on T-cells, contributing to tumor cell immune evasion; It has been found to be inducible on T cells, NK cells, and APCs; Broadly expressed on osteoblasts, fibroblasts, and epithelial cells, as well as in liver, lung, bladder, testis, prostate, breast, placenta, and lymphoid organs.	Regulates the development, maintenance and functions of T cells; Also, this molecule influences cancer development and progression beyond the immune regulatory roles	TLT-2 receptor on activated T cells	It is a member of the B7 family	Melanoma, Renal cell carcinoma, non-small lung cancer cell, bladder and head and neck, prostate, breast,	MGD009
B7-H4	mRNA is largely expressed in the peripheral tissues; Protein expression is restricted to activated B cells, T cells, and monocytes.	Regulates the development, maintenance and functions of T cells; Also, this molecule influences cancer development and	To date, the cognate receptor of B7-H4 on activated T cells remains unclear, although BTLA has been	It is a member of the B7 family	Non–small cell lung cancer, ovarian cancer, prostate cancer, breast cancer, and renal cancer	-

Molecule	Expression status	Function	Receptor	Mechanism of action	Tested in (Cancer types)	Blocking antibodies
		progression beyond the immune regulatory roles	reported as a possible receptor.			
Galectin-9	Cancer cells and MDSC	Regulates the development, maintenance and functions of T cells; Also, this molecule influences cancer development and progression beyond the immune regulatory roles	Loss of galectin-9 expression is closely associated with metastatic progression	A family of beta-galactosidase-binding proteins implicated in modulating cell-cell and cell-matrix interactions.	Several cancer cells	-

Data taken from: [20, 22, 30–33, 35, 47, 50, 76–78, 89–94, 100, 107, 109, 110, 112, 114, 116, 118, 124, 127, 130, 137, 145].

Table 2. Targeting cancer ligands which may contribute to improve the immune checkpoint blockade immunotherapy.

Molecule	Expression status	Function	Cognate ligand	Mechanism of action	Tested in (Cancer types)	Agonist antibodies
CD28	T cells	Priming, survival, cell growth and memory	CD80 (B7-1) CD86 (B7-2) ICOS-L (human)	Provide co-stimulatory signals required for T cell activation and survival. In addition to the T-cell receptor (TCR) can provide a potent signal for the production of various interleukin such IL-2, IL-4, IL-6, IL-13 and IFN-y	Solid tumors	Theralizumab (TGN1412)
CD27	T cells, NK cells and B cells	Priming, survival, cell growth, differentiation and memory	CD70	Transduces signals that promote the activation of NF-κB and MAPK8/JNK	Glioma	IMA950
ICOS (CD278)	Is not constitutively expressed on resting T	Priming, survival, cell	ICOSL	Induce the recruitment of	Advanced solid tumors	JTX-2011 GSK3359609IV

Molecule	Expression status	Function	Cognate ligand	Mechanism of action	Tested in (Cancer types)	Agonist antibodies
	cells; Rapidly induced following TCR cross-linking and/or CD28 co-stimulation on T cells and NK cells	growth, differentiation and memory		phosphatidylinositol 3-kinase (PI3K) culminating in the activation of Akt; Promotes the recruitment of p50α and p85α regulatory subunits of PI3K, in conjunction with recruitment of the p110δ catalytic subunit		
4-1BB (CD137)	Barely expressed levels on naïve T cells; Expressed by activated T cells, but to a larger extent on CD8 than on CD4 T cells.	Survival, cell growth, differentiation and memory	4-1BBL (CD137L)	A member of TNF receptor family; Delivers polyubiquitination signals via TNFR; inhibits apoptosis, enhances proliferation and effector functions; Alternative NF-κB activation	Lymphomas	PF-05082566
OX40 (CD134)	Expressed on activated CD4, T regs and CD8 T cells as well as in a number of other lymphoid and non-lymphoid cells; Low expression in naïve effector T cells, but rapidly upregulated upon TCR ligation; Additionally, suppresses the differentiation and activity of Treg	Survival, cell growth, differentiation and memory;	OX40L	Binds to TRAF2, 3 and 5 as well as PI3K; TRAF2 is required for survival via NF-κB and memory cell generation whereas TRAF5 seems to have a modulatory role (as knockouts have higher levels of cytokines and are more susceptible to Th2-meditated inflammation; Appears to be more potent costimulator of CD4+ T cells (both Teff and Treg) than for CD8 + T cells	Advanced solid tumors	PF-04518600 MEDI0562 MOXR0916
GITR	Expressed in several cells and tissues including B cells, T lymphocytes, NK cells and antigen-presenting cells (APC); It is upregulated by responder T cells ($CD4^{+}CD25^{-}$ T cells or $CD8^{+}CD25^{-}$ T cells)	Cell growth, differentiation and effector function	GITRL	It is a member of the TNFR superfamily; GITR signaling is mediated through the activation of NF-kB and members of the MAPK pathway, including ERK, p38 and Jnk; Up regulation of Bcl-XL expression on CD8+	Advanced solid tumors	MEDI1873

Molecule	Expression status	Function	Cognate ligand	Mechanism of action	Tested in (Cancer types)	Agonist antibodies
TNFRSF25 (DR3, Apo-3, LARD, TRAMP)	Expressed almost exclusively by lymphocytes (CD4+, CD8+, NK and NKT)	Survival, proliferation and effector functions	TL1A	The most recently identified TNF member; Transduces signals that promote the activation of NF-κB	Not tested yet	-

Data taken from: [20, 22, 30–33, 35, 47, 50, 76–78, 89–94, 100, 107, 109, 110, 112, 114, 116, 118, 124, 127, 130, 137, 145].

Table 3. Targeting potential co-stimulatory molecules which may contribute to improve the immune checkpoint blockade immunotherapy.

inhibition of tumor growth and development. After plenty of clinical trials and preclinical models, it is clear now that several inhibitory receptors may need to be blocked so that full T cell activation and antitumor immunity can be achieved. Blocking some T cell inhibitory receptors such as TIM-3 (T cell immunoglobulin mucin domain 3), LAG-3 (lymphocyte-activation gene 3), TIGIT (T cell immunoglobulin and ITIM domain), BTLA (B and T lymphocyte attenuator), VISTA (immunoglobulin suppressor of T cell activation), B7-H3, and B7-H4 has emerged as new target for immune checkpoint blockade strategies (**Tables 1** and **2**). In contrast, inducing T cell activation by mAbs directed to co-stimulatory molecules such as CD27, CD28, ICOS, OX-40, 4-1BB, and GITR has been successfully used as a cancer immunotherapy strategy against several types of cancer (**Table 3**) [33, 42–49].

4. Checkpoint blockade and neoantigens

The conventional treatment of patients with several cancer types involves in most cases, surgery, radiation, and chemotherapy. There is a crucial need to develop new therapies for cancer treatment. Some strategies for cancer immunotherapy including cytokines, signal transduction inhibitors, oncolytic viruses, bispecific antibodies, monoclonal antibodies, dendritic cells, engineered T cells, drug conjugates, radioimmunotherapy, angiogenesis inhibitors, and therapy with targeted toxins are currently increasing the perspectives of treating cancer patients [50]. Nevertheless, despite the recent achievements of these therapies, not every patient responds to immunotherapy and even the responders often experience toxic effects [51]. Moreover, there is a rising need to identify potential biomarkers, especially in immune cells, which could predict whether the cancer patient will respond or not to particular immunotherapy, such as immune checkpoint blockade, for example. Also, we need to improve our knowledge of the fundamental mechanisms and the elegant interface between the immune system and cancer. For example, dacarbazine has for decades been considered the gold standard for the treatment of metastatic melanoma. Immunotherapy, however, has extended the list of options available for the treatment of metastatic melanoma, and its success has been supported by studies using immune checkpoint blockade, as with anti-CTLA-4 and anti-PD-1 [52]. Currently, the factors that preclude a completely effective immune response to cancer are better characterized. Poor immunogenicity is found in several tumors, which can be explained due to the lack of co-stimulatory factors that provide signals to fully activate T cells, mainly CD28 molecules [53]. Inhibitory molecules that repress T cell activation can also be present in the tumor microenvironment. The idea of immune checkpoint blockade and consequently the renaissance of cancer immunotherapy emerged when James Allison's group questioned why T cells were not being able to attack cancer cells effectively. Allison decided to look at a biological molecule called CTLA-4. The first evidence exhibiting the potential effect of anti-CTLA-4 arose from an article published by his group in 1996. In this article [54], the authors showed that the injection of a blocking CTLA-4 agent in tumor-bearing mice led to the rejection of pre-established tumors, including the rejection to the second exposure of tumor cells, when compared with naïve controls. The sequence of experiments published in this paper paved the route to a new perception in cancer immunotherapy—the immune checkpoint blockade [55]. Bristol Myers Squibb (BMS) sponsored the clinical trials with the anti-CTLA-4 antibody under the name Yervoy. In 2010, the results of the first phase-III clinical trial with Ipilimumab were published in the New England Journal of Medicine [56]. This paper provided evidence that Ipilimumab can significantly prolong the lives of patients with metastatic melanoma. A subset of patients under treatment exhibited permanent beneficial effects, and in some cases, their cancer was apparently "cured." Ipilimumab was the first therapy to provide durable remissions in a fraction of patients with metastatic melanoma in 30 years of exhaustive clinical research to show improved quality-of-life and overall survival (OS) [57]. The outcomes of a randomized clinical trial in patients with metastatic melanoma without BRAF mutation were reported by Robert et al. [58]. In this study, the authors compared the benefits of anti-PD-1 (Nivolumab) and dacarbazine therapy. The treatment with anti-PD-1 enhanced the overall survival, as compared with dacarbazine (objective response rate, 40 vs. 14%), in patients with advanced melanoma [58]. In a randomized, double-blind clinical trial, the results of the combination of anti-PD-1 (Nivolumab) and anti-CTLA-4 (Ipilimumab), as reported by Postow et al., achieved a considerably higher objective rate and longer progression-free survival when compared with Ipilimumab monotherapy as a first-line treatment in patients with advanced melanoma [59]. It is not new that the dual blockade using anti-CTLA-4 and anti-PD-1 improves antitumor responses by a complementary and distinctive mechanism [52]. The anti-CTLA-4 therapy acts improving the priming phase, whereas anti-PD-1 acts helping the effector phase [60]. Using a murine melanoma model, Curran et al. showed that the combination of anti-CTLA-4 and anti-PD-1 was more than twice as efficient as either therapy alone in generating an effector immune response against B16 melanomas. In this preclinical study, the authors showed that the dual immune blockade was able to expand the effector T cell infiltration and decrease the regulatory T cells and myeloid cell profile [61]. In another preclinical study, Selby et al. evaluated the dual blockade in murine colon adenocarcinoma model. The authors concluded that the concurrent therapy with anti-CTLA-4 and anti-PD-1 caused a synergic effect in the antitumor activity [62].

In the vast majority of tumors, the combination of the presence of cytotoxic lymphocytes, Th1 profile, and mature dendritic cells (DC) restrained at the tertiary lymphoid structures, are associated with an excellent clinical outcome [63]. Nonetheless, recent findings show that the increase of CD8+ T cell infiltration does not always correlate with a good prognosis in cancer, as it could be seen in Hodgkin lymphoma, diffuse large B cell lymphoma, renal cell carcinoma (ccRCC), lung metastases from ccRCC, and non–small cell lung cancer (NSCLC) in which different densities of cytotoxic lymphocytes may or may not correlate with good prognosis [64–68]. These effects could be explained due to the expression of several negative immune checkpoints such as CTLA-4, PD-1, BTLA, TIM-3, LAG-3, VISTA, and TIGIT in infiltrating T cells or its ligands on tumor cells such as PD-L1, PD-L2, B7-H3, B7-H4 and HVEM that are fundamental to immune escape in cancer [51]. In fact, the nature of the interaction between the immune system and the tumor allows for the clinician to predict patient's prognosis and further guide immunotherapeutic strategies. One of the most meaningful challenges to the triumph of cancer immunotherapy is the relatively small percentage of responding patients. The leading causes of resistance to cancer immunotherapy, especially to the immune checkpoints blockade, could be explained by the failure of the T cells to become fully activated. Severely immune-compromised patients, low mutational neoantigen rates, inhibitory molecules, and the tumor microenvironment are considered crucial to dampening T cell activity [69]. Indeed, the resistance could also be induced by immunotherapy. After recognizing the antigen, the tumor-infiltrating lymphocytes (TILs) become activated, and then they start to produce IFN-γ. As a result of this activation, the IFN-γ released can promote the expression of PD-L1 on the tumor cells and increased IDO (indoleamine 2,3 dioxygenase) and CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) [70–72].

Starting with the comprehension that cancer is a genetic disease, the design of personalized molecularly targeted therapies, seems a rational step to endeavor. Resistance to several of these therapeutic agents such as Vemurafenib, Imatinib, Nilotinib, Erlotinib, and Trastuzumab is the main issue focused on current cancer research [73]. Transformed cells that may express, for instance, high levels of BRAF mutations, BCR-ABL, EGFR, and HER2 must be discerned from nontransformed cells. Through natural selection transformed cells, submitted to molecularly targeted therapies, have learned to escape from these therapies. Alterations in the drug target, activation of pro-survival pathways, and ineffective induction of cell death are some examples [74]. Consequently, there is a critical demand to develop new therapies for cancer treatment. The role of the immune system and its importance in conferring protection against transformed cells have been extensively discussed in this book. As the results from the last 10 years attest, cancer immunotherapy is the best strategy to restore the activity of the immune system and unleash its potential to destroy cancer cells in cancer patients. The absence of an immunocompetent system revealed the increase in the susceptibility to carcinogens induced in spontaneous cancer [75]. The genetic landscape of the antigens that allow the immune system to discriminate between cancer cells from nontransformed cells remains unclear. Not all antigens can elicit an effective immune response. A tumor rejection is defined by how satisfactory an immune response can act against a specific tumor antigen and how this response would impact on tumor growth [76].

Deep sequencing and DNA libraries have profoundly contributed to cancer immunology and immunotherapy, mainly by the characterization of neoantigens that arise from tumor-specific mutations [76]. As cancer cells divide, they accumulate mutations that result in altered or novel peptide sequences specific to the tumor cell. Distinguished as neoantigens, these tumor-specific antigens could be the key to developing successful cancer therapies [77]. The exome-based cancer is, indeed, a crucial approach to determine the T cell reactivity against cancer neoantigens [78]. Preclinical models conducted by Castle et al. and Matsushita et al. provided the original evidence for the cancer exome-based method that could be used to identify neoantigens and interrogate about the T cell reactivity [79, 80]. One of the reasons why the immune checkpoint blockade, especially by anti-CTLA-4 and anti-PD1, successfully works on melanoma and lung cancer patients is the potential formation of a neoantigen repertoire [81]. Melanoma and lung cancer cells have a mutational rate above 10 somatic mutations per megabase (Mb) of encoding DNA, unlike astrocytoma, thyroid, medulloblastoma, neuroblastoma, glioblastoma, myeloma, ovary, thyroid, pancreas, and prostate cancers, which occasionally have one mutation per megabase [82]. That could explain why the effectiveness of the immune checkpoint blockade is not impressive in those tumors that have few somatic mutations and consequently a poor neoantigen repertoire. Such evidence together with assumptions about the tumor microenvironment, immune privilege, and the expression of negative immune checkpoints lead to an insufficient T cell activity [83, 84] and consequently cancer progression.

Several groups are trying to develop novel approaches so that the effect of the immune checkpoint blockade could be augmented in patients with few somatic mutations. To this end, the researchers are focusing their attention on the mechanisms involved in the antitumor response [85]. Preclinical models suggest that an effective antitumor response is obtained when Ipilimumab and Nivolumab induce lymphocyte responses to neoantigens expressed on the individual tumor [86]. If so, a therapeutic approach could be the combination of the immune checkpoint blockade with peptide vaccines. Since the majority of mutations are patient specific, this new approach could lead the way favoring personalized immunotherapy, combining immune checkpoint blockade with cancer vaccines containing a cocktail of peptides corresponding to neoantigens known to be expressed in a given patient's tumor cells.

In prostate cancer and gliomas, for example, the challenge for developing effective immunotherapy is discouraging. Although prostate cancer had the first adult solid tumor-approved vaccine (Sipuleucel-T), which prolongs survival, it was difficult to go beyond that [87]. High-grade gliomas such as DIPG (Diffuse Intrinsic Pontine Glioma) are destructive and incurable cancers, representing the main cause of pediatric brain tumor death. Growing diffusely in the ventral pons, DIPG causes disabling neurologic symptoms that gradually abolish the coordination of the face, pharynx, and body. Unfortunately, surgical resection is not a feasible option, radiation therapy results in just temporary stabilization of symptoms, and several chemotherapy trials developed for adult glioma have not been successful to date [88]. In both scenarios, the challenges that may account for this negative outcome could be (a) there are no immune-related biomarkers that can monitor efficacy in easily accessible tissues; (b) immunologic changes within the peripheral blood have been relatively unhelpful; (c) there is a disease stage; *d)* the immunotherapy efficacy may be therapy-specific (i.e., immune checkpoint therapies are more effective in cancers with high mutation rate, whereas vaccines can be more effective early in tumor progression [89]. As a basis for future research in cancer immunotherapy, immunological pathways in response to monotherapy versus combination therapy need to be assessed in the context of clinical outcome. Novel predictive and prognostic biomarkers have been identified for immune monitoring and clinical correlation in several types of cancer [90–92].

5. Cancer immune monitoring

Immune monitoring studies have supported the hypothesis that combining immunotherapy and standard treatment or their use as monotherapy can benefit patients developing different types of cancer. Analyzes involved ligands, infiltration quality, co-stimulatory/inhibitory profile, and microenvironment. Several assays such as whole exome sequencing (WES), protein array, flow/mass cytometry (CyTOF), multicolor immunohistochemistry (IHC), Multiplexed Ion Beam Imaging (MIBI), Systematic evolution of ligands by exponential enrichment (SELEX), epigenetic modification, and B/T cell receptor repertoire sequencing have been used to pursue potential biomarkers and contribute for the future of cancer immunotherapy [93–96]. Also, these studies have the potential to elucidate immunological mechanisms of antitumor responses, monitor disease progression, evaluate the therapeutic effect, identify candidates for immunotherapy, and serve as prognostic markers of clinical outcome. As discussed above, neoantigens expressed on cancer cells can elicit cellular and humoral immune responses, and they also can be identified to develop immunotherapies [97]. Patient serum and tissue samples can be analyzed to determine candidate tumor-associated neoantigens or genes that evoke cellular and humoral immune responses in cancer patient [98]. Since fresh tumor samples from cancer patients are not always possible to obtain, several clinical studies are undertaken on peripheral blood samples. The successfulness of anti-CTLA-4 and anti-PD-1 in the clinic has stimulated further studies on other molecules that can be targeted. There are several known checkpoint molecules, and their evaluation has progressed to clinical trials. Immunophenotyping studies using the approaches quoted above, examine for instance, the activation or exhaustion of the T cell markers (CD28, CD27, ICOS, OX40, GITR, 4-1BB, PD-1, CTLA-4, LAG-3, TIGIT, TIM-3, BTLA, and VISTA) and the tumor microenvironment ligands (PD-L1, PD-L2, ICOSL, OX-40L, 4-1BBL, Galectin, B7H3, and B7H4). T cell populations including but not limited to CD4 cells, CD8 cells, NK cells, and their subpopulations such as activated T cells, MDSCs, and Tregs have been analyzed in several immune monitoring studies [99–103]. Serum cytokines, chemokines, and angiogenic factors have also been investigated by ELISA, ELISPOT, or other relevant multiplex-based protein assay methods [104, 105]. By questioning the efficacy and even the possible failure, the potential of using immune monitoring studies in cancer prognosis, prediction of treatment efficacy, immune tolerance, and disease progression have contributed to the improvement of the immune-related response criteria (irRC) [106].

Currently, immune checkpoint blockade therapies represent the breakthrough in cancer therapy and have led to robust antitumor responses and clinical benefit in a large number of patients with cancer, but, despite the outstanding achievement of clinical applications of the checkpoint blockade, the efficacy of these therapies differ critically across individual patients and among different tumor types [107, 108]. There is an urgent need to find potential biomarkers that could predict whether cancer patients would respond to the immune checkpoint blockade [109].

Fan et al. using Ipilimumab in a cohort of patients with bladder cancer showed the ICOS molecule (Inducible T cell co-stimulator) to be selectively upregulated in intratumor CD8+ and CD4+ T effector cells [110]. This particular clinical trial indicated the ICOS/ICOSL pathway as relevant for antitumor immune responses in bladder cancer patients under Ipilimumab treatment. Liakou et al. showed that Ipilimumab therapy led to an increase in IFN-y secretion by T cells [111]. It is well-established that melanoma cells are sensitive to IFN-y and quite often some cells containing defective IFN-y signaling genes may be resistant to IFN-y mediated growth inhibition and apoptosis. In order to investigate the reasons determining responders or nonresponders to Ipilimumab therapy, Gao et al. evaluated from whole exome sequencing data the genomic alterations in the family genes of IFN-y pathways in melanoma tumors [112]. The authors encountered significantly more somatic mutations, including copy-number alterations (CNAs) and single-nucleotide variants (SNVs) in nonresponders. Since their results suggested that CNAs in genes of the IFN-y pathways in melanoma patients could predict initial resistance to Ipilimumab, the authors also evaluated data on a total of 367 patients with metastatic melanoma in the TCGA (The Cancer Genome Atlas) database. About 36% of patients had CNAs in the IFN-y pathway genes and had significantly shorter overall survival when compared with the wild-type tumor genes. In order to explain the acquired resistance to PD-1 blockade (Pembrolizumab) treatment, Zaretsky et al. compared melanoma tissues from the baseline with the tumors that had relapsed months to years. As a result, the authors found new JAK1/2 loss-of-function mutations and truncating mutations in the beta-2 microglobulin (B2M) gene. These two studies are closely related to the melanoma development, progression, and primary resistance to anti-CTLA-4 and anti PD-1 [113, 114].

Immune checkpoint blockade seems to be a promising approach for patients with orphan types of cancer like squamous cell carcinoma (SCC). This type of cancer is rare and is caused by Human Papillomavirus (HPV) infection. Until now, there is no consensus treatment for the metastatic form. Morris et al. evaluated tissues from patients who received at least one dose of Nivolumab. As a result, the authors found an objective response in 24% of patients with metastatic SCC. Immunohistochemistry and flow cytometry of baseline biopsies showed a link between the therapy responses and the presence of an activated inflammatory profile in the tumor. Tumors from the responders had more activated effector T cells at baseline than nonresponders. The authors also showed a high expression of PD-1/PD-L1 and higher co-expression of inhibitory molecules such as LAG-3 and TIM-3 in baseline tissues among responders than in nonresponders [115], indicating a previous activation profile in those cells before the treatment. These results were consistent with other solid tumors such as melanoma [56]. The expression of immune molecules in pretreatment biopsies has been described to correlate with response rates in patients with melanoma and other types of cancer, but a fundamental class of biomarkers has not been identified. It seems that PD-1/PD-L1 and inhibitory molecules may serve as an indirect biomarker of acquired immune resistance in response to tumor antigen-specific T cell infiltration [116]. Gao et al. identified additional immune-inhibitory paths in the prostate tumor microenvironment in patients untreated and treated with Ipilimumab. Under the Ipilimumab therapy, there was an increase in immune cells infiltration, including macrophages expressing PD-L1 and VISTA both acting as suppressors of T cell function. Their data advocated that VISTA could represent another inhibitory mediator after immune checkpoint blockade therapy [117]. Genomic and cellular tools to determine several immune signatures in longitudinal biopsies collected at multiple time points during anti-CTLA-4 followed by an anti-PD-1 blockade of melanoma progression were used by Chen et al. [109]. At the baseline, there was no change in any of the measured biomarkers (CD45RO, CD20, CD57, CD68, Foxp3, Granzyme B, PD-1, LAG-3, CD14, CD33, CD163, and CD206), comparing responders and nonresponders to the CTLA-4 blockade. During the treatment, however, there was a significantly higher density of CD8+ T cells in responders than in nonresponders. Furthermore, a higher expression of CD45RO, CD20, CD57, Foxp3, and Granzyme B was observed in responders versus nonresponders in the CTLA-4 blockade arm. Together, these data are relevant in the attempt to identify biomarkers of response and resistance to the immune checkpoint blockade while offering a mechanistic understanding of PD-1 blockade as associated to enhanced cytotoxic activity, antigen processing, and IFN-y pathway [109].

Anagnostou et al. performed a comprehensive study using a genome-wide sequence of protein-coding genes and T cell receptor clonotype analysis followed by functional assays of autologous T cell activation of non–small cell lung cancer in patients that demonstrated initial response and in those patients who experienced checkpoint blockade resistance (anti-CTLA/-anti-PD-1). The authors found a relationship between the acquired resistance and the loss of mutations encoding putative tumor-specific neoantigens. In the tumor samples analyzed at the time of acquired resistance, the authors also found that the majority of eliminated mutations were in genes typically expressed at high levels in lung cancer, which encoded neoantigens that were predicted to either confer high-affinity MHC binding or affect TCR contact residues [118].

TIM-3 is a co-inhibitory immune checkpoint receptor that is highly expressed in dysfunctional CD8+ tumor-infiltrating lymphocytes (TILs) also referred to as T cell exhaustion, intra-tumor Treg cells, monocytes, macrophages, and dendritic cells [119]. It is characterized as a type I transmembrane protein that was originally described in an EAE model (autoimmune encephalomyelitis). Monney et al., in an attempt to identify novel cell surface molecules that would label IFN-y producing Th1 and CD8+ stimulated naïve T cells, found the expression of TIM-3 in these cells. Furthermore, subsequent studies showed that anti-TIM-3 antibodies exacerbated EAE [120]. Galectin-9 (C-type lectin galectin-9), Ceacam 1 (carcinoembryonic antigen cell adhesion molecule 1), HMGB1 (high-mobility group box 1), and PtdSer (phosphatidylserine) have been identified as four TIM-3 ligands [121]. Interaction with TIM-3 caused negative signals on T cells resulting in apoptosis of Th1 and CD8+ cells [122].

High levels of TIM-3 on CD8+ have been correlated with poor prognosis in tumor progression [123]. Exhausted T cells were associated with PD-1+ single positive CD8+ cells [56]. In some types of cancer as lung, melanoma, and renal cancer, resistance to these therapies has gradually been observed [124–127]. To elucidate the mechanisms of adaptive resistance, Koyama et al. analyzed the tumor microenvironment in the context of anti-PD-1 therapy in two immunocompetent mouse models of lung adenocarcinoma. In the tumor progression, following response to anti-PD-1, the authors observed upregulation of TIM-3. According to the mouse model, TIM-3 upregulation was time dependent in TILs expressing PD-1. TIM-3 blockade using anti-TIM-3 overcame the acquired resistance to the PD-1 blockade. Furthermore, the same scenario could be observed in humans. Patients who developed adaptive resistance to anti-PD-1 therapy also showed a comparable TIM-3 upregulation [119]. In patients with metastatic melanoma, Fourcade et al. found approximately 30% of NY-ESO-1–specific CD8+ T cells that expressed TIM-3 [128]. Gao et al. analyzed patients with non–small cell lung cancer (NSCLC), and approximately one-third of CD8+ tumor-infiltrating T lymphocytes (TIL) expressed TIM-3 [129]. Also, Yang et al. analyzed patients with follicular B cell non-Hodgkin lymphoma, and approximately one-third of lymph node CD4+ T and CD8+ T cells expressed TIM-3 [130]. In these three different types of cancers, TIM-3 positive T cells co-expressed PD-1 and exhibited defects in the proliferation of effector cells and cytokine production. In fact, TIM-3 labels dysfunctional T cells in multiple cancer types both in experimental models and in humans. Anti-TIM-3 antibodies have shown good results as monotherapy in some preclinical cancer models and when used in combination with anti-PD-1 antibodies [131–134]. Since TIM-3 expression has been shown to regulate Th1 and Tc1 responses negatively, Th17/T regulatory cells, innate cell activation, and T cell exhaustion, there is rational evidence for targeting TIM-3 [135]. Recently, Gefen et al. isolated an oligonucleotide aptamer ligand that blocked the interaction between TIM-3 with Galectin-9 with a high-affinity and specificity in T cells. The authors demonstrated in vitro, a reduced cell death followed by enhanced survival, proliferation, and cytokine secretion. In in vivo experiments, the aptamer postponed tumor development as monotherapy and synergized with anti-PD-1 in prolonging the survival of the tumor-bearing mice. Together, these results indicate that TIM-3 signaling exerts a secondary effect in keeping T cell immune responses in check [136].

LAG-3 (Lymphocyte-activation gene) is a reliable cancer immunotherapeutic target like TIM-3, due to its negative regulatory function on T cells and its ability to mediate a profile of exhaustion in combination with PD-1 [137]. LAG-3 is a type I membrane protein highly homologous in structure to CD4, described for the first time in 1990 as a novel protein identified on activated NK and T cells [138]. The structural motifs in CD4 and LAG-3 are highly conserved, but LAG-3 can bind to MHC class II molecules with higher affinity than CD4 [139]. As TIM-3 is a marker of IFN-producing Th1 cells, LAG-3 is a marker of IL-10 producing T regulatory cells in both mice and humans [140]. The first evidence in vitro on the role of LAG-3 inhibiting T cells was shown by Huard et al., when the authors by blocking LAG-3 increased the proliferation of human T cells [141]. Furthermore, the ectopic expression of LAG-3 on mouse CD4+ T cells reduced their proliferation [142] significantly. Unlike CTLA-4 knockout (KO) mice, which develop spontaneous lymphoproliferative diseases, mice lacking LAG-3 do not develop lymphoproliferative disorders. In the absence of LAG-3, however, T regulatory cells display a reduced activity [143]. Besides the negative regulation on T cell activation, innate cell activation, and T cell exhaustion, LAG-3 also induces the upregulation of cell surface receptors such as CD40, CD80, CD83, and CD86 in monocyte-derived dendritic cells (DCs) [144]. These facts led Quezada et al. to affirm that LAG-3 has a more complex role in immune homeostasis than just inhibiting T cell activation [145]. LAG-3 has been suggested to regulate the activity of PD-1 cells, and their co-expression has been shown in malignant mouse and human tumor cells [146]. Using murine models of B16 melanoma, MC38 colorectal adenocarcinoma, and Sa1N fibrosarcoma, Woo et al. also showed that the combinations of anti-LAG-3/anti-PD-1 antibodies inhibited tumor growth and progression besides enhancing adaptive immune responses in tumor-infiltrating lymphocytes [147].

TIGIT (T cell immunoreceptor with Ig and ITIM domains) also known as WUCAM is an inhibitory receptor, member of the poliovirus receptor (PVR/nectin family) classified as type 1 transmembrane domain, with an intracellular domain containing a canonical receptor tyrosine-based inhibitory motif (ITIM) and an immunoglobulin tyrosine tail (ITT) [148]. Yu et al [149] discovered TIGIT expressed in regulatory, memory and activated T cells. Currently, we know that TIGIT is also expressed in T regulatory and NK cells in multiple types of cancer [150]. CD155 and CD122 are TIGIT ligands, expressed in macrophages and dendritic cells [151]. TIGIT is upregulated in tumor-specific peripheral CD8+ T cells and CD8+ tumor-infiltrating lymphocytes (TILs) from patients with metastatic melanoma and TIGIT-expressing CD8+ T cells often co-express PD-1. In metastatic melanoma, Chauvin et al. showed that TILs from these patients downregulated the co-stimulatory molecule CD226. It has been shown that CD226 competes with TIGIT for the same ligand, supporting a TIGIT/CD226 imbalance in metastatic melanoma [152]. In addition to its role as a lymphocyte receptor, TIGIT acts as a functional ligand inducing a tolerogenic phenotype in dendritic cells, resulting in elevated IL-10 and reduced IL-12 [153]. A regulatory role of TIGIT in modulating the signaling pathway, which facilitates M2-polarization, a class of immunosuppressive tumor-associated macrophages that arise in response to Th2 cytokines was shown by Chen et al. [154]. The capacity of TIGIT to interfere in the tumor microenvironment by suppressing the immune response mediated by an increase of T regulatory activity, recruitment of MDSC, induction of blood vessel formation, cancer-associated fibroblasts, NK cell inhibition, and CD8+ T cell-mediated tumor killing, priming, and differentiation, suggest altogether that cancer cells upregulate TIGIT pathway to promote immunosuppression [151]. TIGIT becomes, therefore, a good candidate for the blockade in combination with anti-CTLA-4 and anti PD-1 [155–161].

VISTA (V-region Immunoglobulin-containing Suppressor of T Cell Activation) was discovered, characterized, and functionally defined as a novel hematopoietically restricted inhibitory ligand by Noelle's group. It is expressed primarily within the hematopoietic compartment (monocytes, neutrophil, and dendritic cells) with a low expression on CD4+, CD4+ Foxp3+ T regulatory cells, and CD8+ T cells [162]. VISTA is a type I transmembrane protein, with a single N-terminal immunoglobulin V domain and sharing structural similarities with PD-1, CD28, and CTLA-4 [163]. Remarkably, this molecule is at the same time a ligand and can function as a receptor. Wang et al. evaluated in vitro and in vivo the role of VISTA as a ligand. The authors conducted a range of experiments using VISTA-Ig fusion protein or VISTA expression on APC's. In both situations, VISTA was able to inhibit CD8+ T and CD4+ T cell proliferation and cytokine production at the early stage of activation mainly by suppression of CD25, CD44, CD69, and CD62L markers, IL-2, and IFN-y [164, 165]. *In vivo* experiments led the authors to conclude that VISTA expression in tumor cells can overcome protective antitumor immunity. To achieve this conclusion, mice were immunized with irradiated MCA105 fibrosarcoma tumor cells that do not express VISTA and were re-challenged with MCA105 overexpressing VISTA. Cancer cells expressing VISTA showed enhanced tumor growth compared to the VISTA negative parent MCA105. Furthermore, Lines et al. using VISTA-Ig fusion protein demonstrated *in vitro* that VISTA could increase the conversion of naïve CD4+ T into T regulatory cells in both human and mice [166]. The anti-VISTA monotherapy impaired tumor growth in several types of cancer (B16OVA melanoma, B16-BL6 melanoma, MB49 bladder carcinoma, and PTEN/BRAF inducible melanoma) and altered the cellular composition of the tumor microenvironment enhancing T cell responses within the tumor by cytotoxic and cytokine production such as IFN-y and TNF-alpha [167].

As a receptor, VISTA molecules on T cells have been shown to regulate their activity negatively. VISTA is a co-inhibitory receptor on CD4+ T cells because it suppresses early CD4+ T cell expansion in vivo and CD4+ T VISTA−/− cells responded more strongly than wild-type (WT) CD4+ T cells to both polyclonal and antigen-specific stimulation, leading to increased proliferation and production of cytokines such IFN-γ, TNFα, and IL-17A. The anti-VISTA monotherapy impaired tumor growth in several types of cancer (B16OVA melanoma, B16-BL6 melanoma, MB49 bladder carcinoma, and PTEN/BRAF inducible melanoma) and altered the cellular composition of the tumor microenvironment enhancing T cell responses within the tumor by cytotoxic and cytokine production such as IFN-y and TNF-alpha [167]. Their results announced a new role for VISTA molecules, as a regulator of the tumor microenvironment playing an essential function in regulating protective immunity to cancer.

The exciting development of cancer treatment recently fostered the ambition of the traditional cancer therapy to increase the median of survival from a few months to definitely announce victory against cancer. Currently, we have been able to move the median survival a little longer especially with the approval by the FDA of anti-CTLA-4, anti-PD-1, and therapeutic combinations. One must be cautious, however, because currently, only about 30% patients are responders to immunotherapy. This fact has incited for the search of new molecules, new biomarkers, and new combinations such as other checkpoint blockers, co-stimulatory molecules agonists, IDO pathway inhibition, oncolytic viruses, adoptive T cell transfer, T cell engineering, therapeutic vaccines, targeted therapy, chemotherapy, and radiotherapy in the attempt to increase the number of responders and consequently of survivors. There has been much of enthusiasm on recent news about immunotherapy in the treatment of cancer patients. Int the past year, checkpoint inhibitors have become an impotant tool for treating certain types of tumor such as non-small cell lung cancer (NSCLC) and melanoma with an increase in the median survival. Novel immunotherapeutic approaches are essential to the success in the treatment of different cancer types.

6. Conclusion

The effectiveness of monoclonal antibodies, especially the immune checkpoint blocking ones, associated to other cancer therapies and consequently with the improvement of preclinical studies and the advent of screening techniques, constitutes a unique opportunity to understand and overcome drug resistance. Not only that but also by profoundly investigating predictive biomarkers related to the different immunotherapeutic agents. As discussed in this chapter, to date, there are three types of potential biomarkers that have been studied exhaustively: (a) Immune infiltrate in the tumor; (b) high mutation profile (neoantigens); and (c) expression of PD-L1 by tumor cells or tumor cell infiltrates. Data from immune monitoring studies have provided a link between immunologic/genomic and proteomic platforms. The main goals of the immune checkpoint blockade are to either stimulate the T cells to attack cancer cells or to suspend the suppression of remaining antitumor T cells. The immune monitoring study consists in analyzing the activity of innate and adaptive cell populations like T cells, B cells, myeloid-derived suppressor cells (MDSC) and natural killer (NK) cells, which are critical in the immune response against cancer and may regulate positively or negatively T cell responses. In summary, this approach may lead to the identification of biomarkers that will predict whether immune checkpoint blockade (monotherapy or combination) would be sufficient to induce an objective response.

The most critical cell populations include the total CD4+ T and effector CD4+ T cells, T regulatory cells, total CD8+ T, naive, T central memory and T effector memory cells; MDSC (myeloid-derived suppressor cell), and B cells; and M1 and M2 macrophages have recently being studied in the context of cancer development. A great number of molecules involved in immune responses against cancer cells have been studied, such as immune checkpoint molecules on T cells, 4-1BB (CD137), CTLA-4, GITR (glucocorticoid-induced TNFR-related protein), OX-40 (CD134), TIM-3, LAG-3, PD-1, and ICOS (inducible T cell co-stimulator); cytotoxic and cytokine secreting molecules on NK cells such as 4-1BB, CD69, NKG2A, NKG2C, NKG2D, NKp30, NKp44, and NKp46; some ligands on tumor cells such as B7H3, B7H4, CD73, CD80, CD86, CD137, PD-L1, PD-L2, ICOSL, Galectin 9, MIC A/B and OX40; and the expression of transcription factors such as Bcl-6, Blimp, CD27, CD28, Eomes, Ki-67, ICOS, and c-myc. They might bring a better understanding of the immune response under immunotherapy and help us to answer why not every patient responds to immunotherapy. Immunotherapy offers at least three actions that no other modality of cancer therapy provides: specificity, memory, and adaptability. We have consistently seen that one of the principal issues of immunotherapy strategy is the enhanced proportion of responders to the immunotherapeutic agents. Combining immune checkpoint blockade with other therapies, which overcome the possible failures, may lead to synergies. That is the reason why the most broadly studied combination of checkpoint blockade agents uses the anti-CTLA-4 and anti-PD-1 monoclonal antibodies. However, a more in-depth understanding on the mechanisms of efficacy and the identification of resistance to checkpoint blockade and their agents are needed. Despite significant progress in the immune checkpoint blockade, much remains to be done. Inquiries on the responder profile, the differences between mouse models and results application to clinical studies, the relative effects on effector, T regulatory and other cells, expressing several immune checkpoints, and the comprehension regarding the differences between the immune profile in different compartments such as in the periphery versus the tumor microenvironment must be addressed. Clinical samples and immune monitoring approaches obtained at multiple time points during immune checkpoint blockade would be valuable for exploring the responsiveness and nonresponsiveness profile. Additionally, studies of immune modifications within human cancer cells and the tumor microenvironment have the potential to establish efficacy and resistance mechanisms. In this context, The Cancer Genome Atlas project (TCGA, available at https://cancergenome.nih.gov) has helped to identify some mutations in cancer cells, which increase the prospect of resistance to immunotherapy. Exciting secret waits to be unveiled.

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Conflict of interest

The authors declare there is no conflict of interest.

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