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Reinvigorating Exhausted T Cells by Blockade of the PD-1 Pathway

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Abstract

T-cell exhaustion due to persistent antigen stimulation is a key feature of chronic viral infections and cancer. Programmed cell death-1 (PD-1) is a major regulator of T-cell exhaustion, and blocking the PD-1 pathway restores T-cell function and improves pathogen control and tumor eradication. Immunotherapy targeting the PD-1 inhibitory receptor pathway has demonstrated significant antitumor activity. Recently, antibodies blocking PD-1 have been approved for use in cancer patients. In this review, we summarize the role of the PD-1 pathway in chronic infection and cancer and the therapeutic potential of PD-1-directed immunotherapy in patients with chronic infection or cancer.

Keywords

cancer; chronic infection; immunotherapy; programmed cell death-1; T-cell exhaustion

I. PERSPECTIVES

Memory T cells are generated when acute infections are cleared by the immune system. These cells rapidly reactivate effector functions upon antigen re-encounter and persist long term via homeostatic proliferation, independently of antigen.[1, 2] These key properties of memory T cells allow them to provide long-term protective immunity. In contrast, chronic infections where the virus persists result in exhaustion of the T cells, which are then unable to bring the infection under control.

Prolonged antigen stimulation and inflammation lead to loss of effector functions of virus-specific CD8 T cells in a progressive and hierarchical manner, even resulting in clonal deletion.[3, 4] This process, originally found in chronic viral infections, was termed T-cell exhaustion and has since been demonstrated to be a common feature of many chronic infections and cancer.[1, 5] Exhausted T cells, characterized by defects in effector functions and elevated and sustained expression of inhibitory receptors, are distinctly different from functional effector or memory cells.[6, 7] Although complex immunosuppressive mechanisms, including both intrinsic and extrinsic factors, can contribute to the establishment and

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maintenance of the persistent infection and T-cell dysfunction, PD-1 (CD279),[8] an inhibitory receptor of CD28 family, is well known to play a major role in regulating T-cell exhaustion. In this review, we summarize the role of the PD-1 pathway in regulating T-cell exhaustion in chronic infection and cancer and discuss the therapeutic potential of PD-1-directed immunotherapy to treat patients who are chronically infected or have cancer.

II. THE ROLE OF THE PD-1 PATHWAY IN T-CELL EXHAUSTION

PD-1 is expressed in various hematopoietic cells including T cells, B cells, natural killer (NK) cells, NK T (NKT) cells, monocytes, macrophages, and dendritic cells (DCs) following their activation.[9] PD-1 binds to its two ligands: programmed cell death 1 ligand-1 (PD-L1; B7-H1; CD274)[10, 11] and PD-L2 (B7-DC; CD273),[12, 13] both of which are B7 family members. PD-L1 is constitutively expressed in a wide range of cells including hematopoietic and nonhematopoietic cells. In contrast, PD-L2 expression is restricted to professional antigen presenting cells (APCs; monocytes, macrophages, and DCs) and a certain subset of B cells. Inflammatory cytokines such as interferons (IFNs; α, β, and γ) are potent regulators of both PD-L1 and PD-L2 expression.[9, 14] The function of PD-1 is best characterized in T cells. Its expression is induced by activation-driven T-cell receptor (TCR) signaling and further up-regulated by cytokines.[14] Upon engagement of PD-1 with its ligands, the SH2-domain containing tyrosine phosphatase 1 (SHP-1) and SHP-2 are recruited to the phosphorylated immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic domain of PD-1. Recruitment of SHP-1 and SHP-2 inactivates proximal effector molecules such as ZAP70 and phosphatidylinositol-3-kinase (PI3K), attenuating TCR- and CD28-mediated signaling (Figure 1).[15–17]

Our previous finding that PD-L1 has a differential role in hematopoietic cells and nonhematopoietic cells in regulating the T-cell response suggests a model for which PD-1/PD-1 ligand (PD-L) interaction operates.[18] In chronic LCMV infection, PD-L1 deficiency in hematopoietic cells enhanced the T-cell response in terms of both magnitude and function. In comparison, PD-L1 deficiency in nonhematopoietic cells had no effect on the T-cell response but resulted in better virus control. This indicates that the PD-1 pathway restrains T cells from killing virus-infected cells as well as attenuating T-cell activation. The PD-1/PD-L1 interaction between T cells and infected cells (or cancer cells) inhibits target cell elimination by T cells. Abrogating this interaction releases the brake on T cells and promotes their effector functions, killing of target cells (Figure 2). Therefore, the PD-1 pathway negatively regulates T cells during priming and also the effector phase when T cells act on the target cells. This presumably results in more profound “rescue” effects by the blockade of PD-1 than do other inhibitory receptor blockades.

The immunoregulatory roles of PD-1 are responsible for limiting excessive T-cell activation to prevent immune-mediated tissue damage. However, prolonged TCR stimulation and PD-1 expression lead to T-cell dysfunction, and pathogens or cancer cells exploit the PD-1 pathway to persist and resist immune response. Therefore, the PD-1 pathway is an important determinant of the outcome of the T-cell response, regulating the balance between effective host defense and immunopathology, implicating the potential for manipulating the PD-1 pathway against various human diseases.

During chronic infection and cancer, expression of both PD-1 and PD-1 ligands is abundant; continuous antigen stimulation maintains high levels of PD-1 expression on antigen-specific T cells and the expression of PD-1 ligands is also up-regulated by inflammatory stimulation. PD-1–mediated T-cell dysfunction strongly dampens antiviral or antitumor immune response. The effect of interfering with the PD-1 pathway on the restoration of T-cell function has been shown in many animal models and human diseases. Recently, clinical trials targeting the PD-1 pathway have revealed very promising results. Many preclinical studies of PD-1 pathway blockade in chronic viral infections and clinical trials in many different cancers are currently ongoing.

III. THE THERAPEUTIC POTENTIAL OF INHIBITING PD-1 SIGNALING IN CHRONIC VIRAL INFECTION

The dominant role of PD-1 in regulating T-cell exhaustion was first described by our group in a mouse model of chronic LCMV infection. In this model, we found that exhausted CD8 T cells had increased PD-1 expression. Furthermore, blockade of the PD-1 pathway restored effector functions of LCMV-specific CD8 T cells and significantly reduced viral load.[19] This finding has been further extended to other types of chronic infections in mice, nonhuman primates, and humans.

In HIV infection, PD-1 expression on HIV-specific CD8 T cells was correlated with impairment of CD8 T-cell function, high viral load, disease progression, and reduced CD4 count. In vitro blockade of PD-1 on HIV-specific CD8 and CD4 T cells enhanced proliferation, cytokine production, and survival.[20, 21] Recently, the effect of blocking PD-1/PD-L interactions on HIV disease progression has been shown in vivo using the humanized mouse model of chronic HIV infection. In vivo administration of anti-PD-L1 antibody increased both CD4 and CD8 T cells that could suppress viral replication in HIV-1 chronically infected mice, showing a reduction in the HIV plasma viral load.[22] In addition to HIV, the role of the PD-1 pathway has been investigated in other chronic viral infections such as HCV. In the initial stage of HCV infection, most HCV-specific T cells expressed PD-1. In patients that resolve this disease, PD-1 expression on these cells was reduced, whereas chronically infected patients maintained a high level of PD-1 expression and HCV-specific CD8 T cells remained dysfunctional. In vitro blockade of the PD-1 and PD-L interaction enhanced the proliferation and function of HCV-specific CD8 T cells.[23, 24] One recent report demonstrated the impact of interrupting PD-1 signals in chronically HCV-infected chimpanzees. Following PD-1 blockade, one of the three animals had significantly reduced HCV viremia that was associated with restored intrahepatic CD4 and CD8 T-cell response. It has been suggested that preexisting virus-specific T cells are likely to be essential for the success of PD-1 blockade therapy in this model.[25] Blocking the PD-1 pathway was also found to promote an antiviral immune response in simian immunodeficiency virus (SIV) infection of rhesus macaques. Proliferation and polyfunctionality of SIV-specific CD8 T cells were augmented upon PD-1 blockade, and improved antiviral immunity was followed by viral load reduction and prolonged survival of chronically infected rhesus macaques.[26] Together, these preclinical studies show that PD-1 expression on virus-specific T cells is correlated with their functional defects, and

interrupting PD-1 signaling can reverse this decline. The fact that exhaustion is reversible and not an untreatable state indicates powerful therapeutic potential for manipulating the PD-1 axis to reinvigorate dysfunctional T cells in chronic viral infections.

Currently, one clinical trial has been reported on PD-1 blockade in chronic viral infection. Anti-PD-1 antibody (BMS-936558, a fully human monoclonal antibody targeting PD-1) was used to treat patients chronically infected with HCV. Following a single infusion, suppression of HCV replication was observed in 11.1% of patients (5/45).[27] Also in this trial, one patient who previously did not respond to IFN-α therapy had undetectable viral load for at least 1 year following administration of the anti-PD-1 antibody. This promising result warrants further exploration of PD-1 blocking agents for therapeutic use in human chronic viral infection.

IV. THE PD-1 PATHWAY IN ANTITUMOR IMMUNITY AND PD-1-DIRECTED CANCER IMMUNOTHERAPY

PD-1 and PD-L1 interaction in the tumor environment is a mechanism used by the tumor to resist destruction by the immune system. PD-L1 is expressed by many types of cancer cells and up-regulated by various inflammatory stimuli in the tumor environ-ment.[28, 29] Myeloid cells in tumors were shown to express PD-L1 and mediate inhibition of T cells.[30] Tumor-infiltrating T cells express high levels of PD-1 due to prolonged exposure to the tumor antigen and immunosuppressive environment and exhibit similar functional and phenotypic properties as the exhausted T cells in chronic infection. This includes defects in effector cytokine production and up-regulated expression of inhibitory receptors.[31–33] Currently, the prevailing mechanism underlying the PD-1/PD-L1 axis in tumor sites is that the interaction of PD-L1 on tumor cells with PD-1 on tumor-infiltrating lymphocytes (TILs) delivers negative signals and inhibits antitumor T-cell response, facilitating tumorigenesis.

The role of PD-1 in tumor immune evasion was first shown when P815 tumor cells were transfected with PD-L1 and they became less susceptible to cytotoxic T-cell-mediated killing. This report also showed that the growth of PD-L1$^+$ myeloma cells was completely suppressed in syngeneic PD-1–deficient mice, whereas rapid tumor growth was observed in wild-type littermates.[34] Multiple in vivo mouse studies have shown that the PD-1/PD-L1 interaction inhibits antitumor immunity, and abrogating this interaction enhances the T-cell-mediated antitumor response, leading to tumor regression.[29, 34, 35] Encouraging results from preclinical studies and the therapeutic potential of blocking the PD-1 pathway have led to clinical development of several blocking antibodies against PD-1 or PD-L1. Currently, the results of clinical trials targeting the PD-1 pathway are very promising. Blockade of the PD-1 pathway using either anti-PD-1 or anti-PD-L1 antibodies has revealed high clinical response rates and was effective in patients with advanced cancer including metastatic melanoma, non-small cell lung cancer (NSCLC), renal cell cancer (RCC), bladder cancer, Hodgkin's lymphoma, head and neck cancer, and breast cancer[36–60] (Table 1). Clinical responses tended to be durable and were accompanied by less adverse effects than those seen with ipilimumab, a CTLA-4 blocking antibody used for treating metastatic melanoma. Recently, the Food and Drug Administration (FDA) approved two anti-PD-1 antibodies,

pembrolizumab (Merck) and nivolumab (Bristol-Myers Squibb), for the treatment of unresectable or metastatic melanoma.

Consistent with the concept that the tumor evades host immune response through engagement of PD-L1 with PD-1 on T cells, early studies suggested a correlation between PD-L1 expressed by the tumor and poor prognosis. However, several studies indicated a lack of correlation or even a positive association of PD-L1 expression on tumor cells with lymphocyte infiltration and better prognosis.[28] A recent study reported a negative feedback loop, whereby activated T cells infiltrating the tumor environment produce proinflammatory cytokines, such as IFNγ, that induce the up-regulation of PD-L1 on tumor cells. PD-L1 interaction with PD-1 on tumor-infiltrating T cells suppresses T-cell functions.[61] Therefore, PD-L1 expression in tumor cells possibly indicates preexisting immune responses.

Based on the mechanism of PD-1/PD-L1 expression, PD-L1 expression by tumor cells has been suggested as a biomarker for predicting the clinical response to PD-1 blockade therapy. Several clinical studies evaluated a correlation between tumor-associated PD-L1 expression and the clinical response to PD-1 blocking agents, and there seemed to be a trend of positive association. However, tumor expression of PD-L1 is apparently not an absolute biomarker because not all patients with PD-L1+ tumors respond to PD-1 blockade, and some patients with PD-L1-(PD-L1 negative) tumors are still responsive to PD-1 therapy.[62, 63] Considering the inducible nature of PD-L1 and the fact that many other PD-1/PD-L interactions are possibly affected by PD-1 pathway blockade along with tumor cells and TIL interactions, tumor PD-L1 expression as a single marker is not an optimal biomarker of the response to PD-1-targeted immunotherapy. Therefore, it is imperative to identify reliable biomarkers to select patients who can benefit from this therapy.

V. COMBINATION THERAPY WITH PD-1 PATHWAY BLOCKADE

Because PD-1 plays a critical role in T-cell exhaustion, the efficacy of other immunotherapies attempting to restore the function of exhausted T cells might be enhanced by simultaneously blocking PD-1 signaling. In addition, combinations with other therapeutic strategies may enhance treatments targeting PD-1. It has been shown that PD-1 blockade rescues the less exhausted CD8 T cells expressing intermediate levels of PD-1, whereas exhausted cells with high levels of PD-1 respond poorly and are unlikely to be reversed by the treatment.[64] Several studies have shown that a certain level of preexisting antigen-specific T cells is essential to better respond to blockade of the PD-1 pathway. Therefore, combining PD-1 pathway blockade with other therapies that possibly stimulate T-cell responses or interrupt other negative signaling pathways could generate a synergistic effect.

Therapeutic vaccination in chronic infection or cancer has been shown to have limited efficacy due to T-cell exhaustion.[65] In chronic viral infection, immunization with recombinant vaccinia virus vectors encoding LCMV glycoprotein (rVV-LCMV GP33) had a minimal effect on enhancing CD8 T-cell response, but combined PD-L1 blockade significantly improved LCMV-specific CD8 T-cell response and virus control.[66] Furthermore, PD-L1 blockade markedly enhanced antitumor T-cell response driven by granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting tumor cell

immunotherapy in mouse models of melanoma and colon carcinoma.[67] This result indicates that blocking PD-1 signaling can enhance the efficacy of therapeutic vaccination. Currently, clinical trials assessing the efficacy of multipeptide melanoma vaccines in combination with PD-1 blockade are ongoing (NCT01176474, NCT01176461) and the effect of combining dendritic cell-based tumor vaccines with PD-1 blockade is being tested in several types of cancer including RCC and multiple myeloma (NCT01067287, NCT01441765, NCT01096602).

The severity of T-cell exhaustion has been shown to be correlated with coexpression of multiple inhibitory receptors including PD-1, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin- and mucin-domain-containing molecule-3 (TIM-3), CD160, and 2B4.[68] During chronic LCMV infection, Tim-3 or LAG-3 blockade alone had a minimal effect on rescuing virus-specific CD8 T cells, but combining with PD-1 pathway blockade synergistically improved LCMV-specific CD8 T-cell response and virus control.[68, 69] In addition, in murine cancer models, PD-1 pathway blockade in combination with blocking Tim-3, LAG-3, or CTLA-4 was more effective in restoring antitumor immunity and promoting tumor regression than targeting either pathway alone.[70–72] An additive or synergistic effect on rescuing T cells by combined blockade of different inhibitory receptors indicates their nonredundant roles and the complex regulatory mechanisms underlying T-cell dysfunction. Recently, a clinical evaluation of PD-1 and CTLA-4 combination blockade reported a higher rate of clinical response than single therapy in patients with advanced melanoma.[42] Dual blockade of PD-1 and LAG-3 is being tested in solid tumors (NCT01968109).

The effect of manipulating stimulatory or inhibitory cytokines can be enhanced when combined with PD-1 pathway blockade. IL-10 is an immunosuppressive cytokine involved in T-cell exhaustion and blocking the IL-10 signal leads to restoration of T-cell function and viral clearance in chronic LCMV infection.[73] Combined blockade of the IL-10 receptor and PD-1 pathway further enhanced virus-specific T-cell response and virus control during chronic LCMV infection.[74] Previously, we found that administration of IL-2, an immunostimulatory cytokine, during chronic infection resulted in the rescue of exhausted T cells and better viral control.[75] Combined IL-2 treatment and PD-1 pathway blockade had a synergistic effect on augmenting virus-specific CD8 T-cell response and reducing viral load in chronic LCMV infection.[76] IL-21 enhances cytolytic activity of CD8 T cells and NK cells and recombinant IL-21 (rIL-21) administration has demonstrated potent antitumor activity.[77] In preclinical murine tumor models, rIL-21 administration combined with PD-1 blockade further enhanced antitumor responses.[78] Clinical evaluation of combination treatment with rIL-21 and the anti-PD-1 antibody has recently been performed in advanced or metastatic solid tumors.[79]

Adoptive transfer of T cells is an effective immunotherapeutic approach to restore the antiviral or antitumor immune response. However, under the influence of continuous antigen exposure, transferred cells become dysfunctional. Therefore, blocking the PD-1/PD-L interaction can further augment the therapeutic efficacy of adoptively transferred cells. Our recent work has shown that in chronic LCMV infection, the therapeutic effects of naïve antigen-specific CD4 T-cell transfer were further enhanced by blocking the PD-1 pathway,

resulting in greater functionality of LCMV-specific CD8 T cells and in a further reduction of viral load.[80] In tumor mouse models, combined therapy of adoptive cell transfer and PD-1 pathway blockade has shown a synergistic effect on tumor regression than single treatment. Blocking PD-1 increased the number of transferred cells at the tumor site, and this was associated with greater T-cell proliferation and increased expression of IFNγ and the IFNγ-inducible chemokine at the tumor site, facilitating immune cell infiltration.[81]

IFNα has potent antitumor effects, but at the same time it can induce expression of PD-1 and its ligands. Preclinical studies have shown that the combination of IFNα therapy or IFNα-transduced cancer vaccines with PD-1 pathway blockade further enhances antitumor immunity in tumor-bearing mice.[82, 83] This combination therapy suggests a promising candidate for cancer treatment, and combined treatment with IFNα-2b and the anti-PD-1 antibody is being tested or planned in patients with melanoma or RCC (NCT02089685, NCT02112032, NCT02339324). Furthermore, the combination of PD-1 blockade and the treatment with an antiangiogenic agent blocking the vascular endothelial growth factor (VEGF)-VEGF receptor 2 (VEGFR2) interaction has revealed a synergistic antitumor effect in a murine cancer model. [84] Based on the synergistic effects found from preclinical studies, many combination therapies are being evaluated for cancer patients.

VI. CONCLUSION

Cancer clinical trials targeting the PD-1 pathway have achieved a very high rate of antitumor response. Currently, monotherapies targeting PD-1 or PD-L1 and combination therapies with various immunotherapeutic strategies, including checkpoint inhibitors, tumor vaccines, chemotherapy, and antiangiogenic agents, are being evaluated in different types of cancer. It is important to assess different combination therapies for those who do not respond to PD-1 blockade therapy. The clinical evaluation of the PD-1 blocking agents is currently focused on cancer treatment, but the therapies targeting the PD-1 pathway also have potential for treating chronic infections. Still, the molecular mechanisms associated with the PD-1 pathway regulating T-cell exhaustion and the way in which PD-1 signaling is altered upon blocking the PD-1/PD-L interaction to restore exhausted T cells remain to be determined. It is also essential to identify the predictive biomarkers to personalize the therapy.

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ABBREVIATIONS

HCV	Hepatitis C virus
HIV	human immunodeficiency virus
LAG-3	lymphocyte-activation gene-3
LCMV	lymphocytic choriomeningitis virus
PD-1	programmed cell death-1

PD-L programmed cell death-1 ligand

PD-L1 programmed cell death-1 ligand-1

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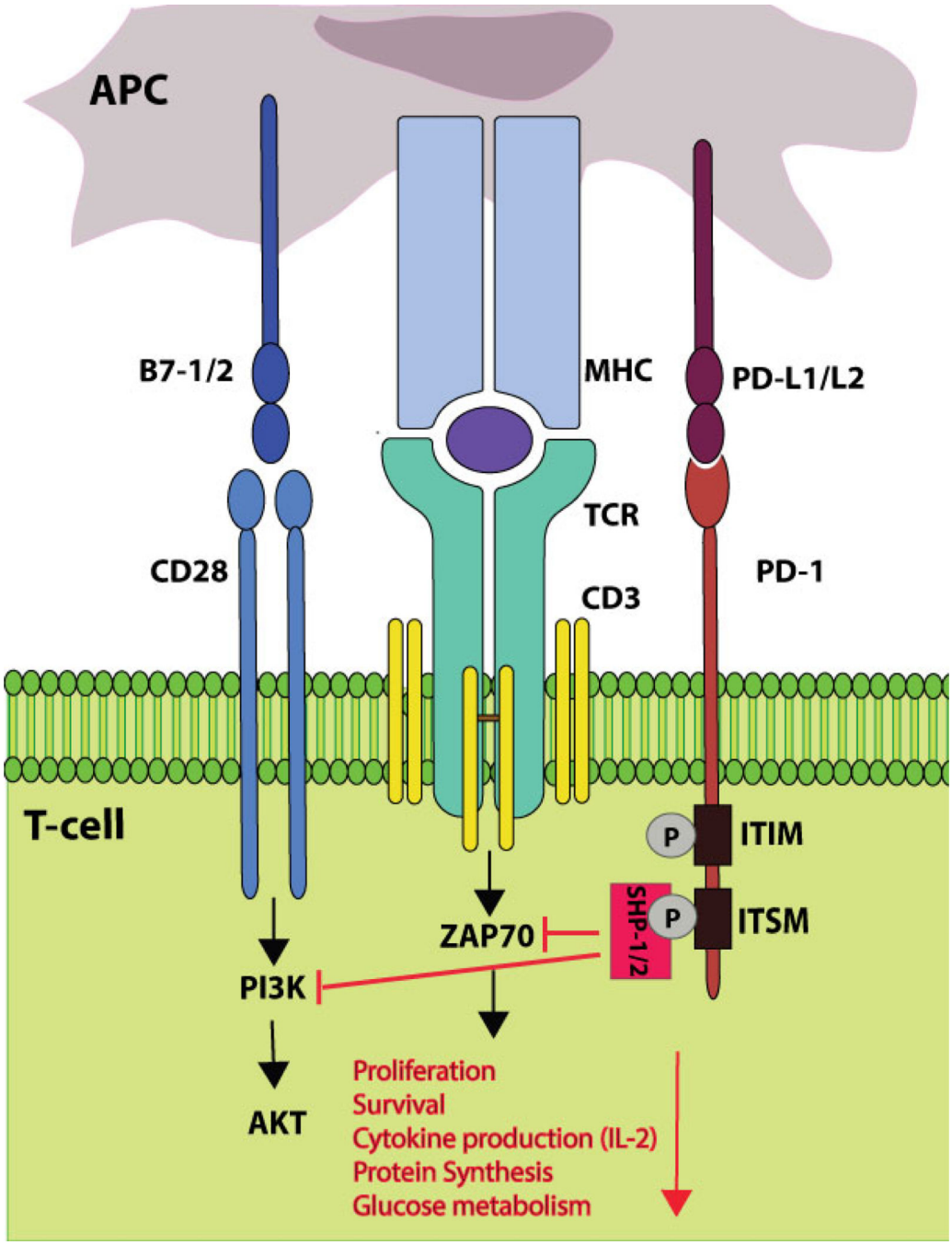

FIG. 1.

PD-1 signaling. PD-1 contains two tyrosine-based signaling motifs in the cytoplasmic domain: an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an ITSM. Upon engagement by PD-L1 or PD-L2, PD-1 is phosphorylated at both tyrosine residues. Phosphorylated ITSM recruits SHP-1 and SHP-2 that dephosphorylate effector molecules such as ZAP70 and PI3K activated by TCR and CD28 signaling. As a result, PD-1 signaling inhibits T-cell proliferation, survival, cytokine production, protein synthesis, and glucose metabolism.

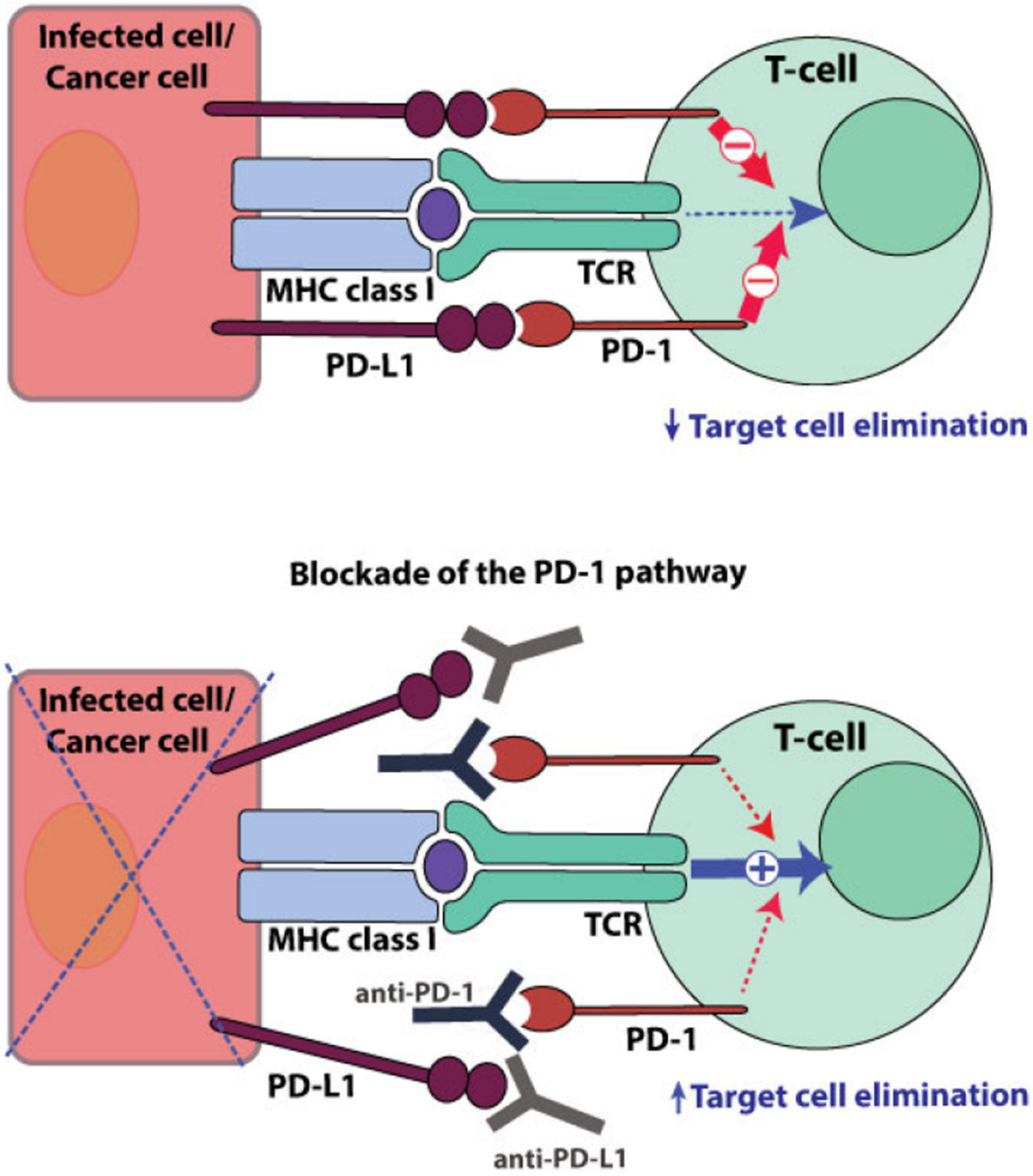

FIG. 2.

Blockade of PD-1/PD-L1 interactions between CD8 T cells and target cells. Antibody-mediated blockade of the PD-1 pathway promotes T cell-mediated elimination of target cells.

TABLE 1

Published clinical trials targeting PD-1 pathway in cancer patients.

Cancer types	Sponsor/Company	Target	References
Melanoma	BMS	PD-1	38, 40, 42, 43, 44, 49, 51, 60
		PD-L1	39
	Merck	PD-1	41, 48
	Roche/Genentech	PD-L1	45, 61
Non small-cell lung cancer (NSCLC)	BMS	PD-1	40 49
		PD-L1	39
	Merck	PD-1	58, 59
	Roche/Genentech	PD-L1	45
	AstraZeneca/MedImmune	PD-L1	54, 57
Renal cell cancer (RCC)	BMS	PD-1	38, 40, 42, 46, 49
		PD-L1	39
	Roche/Genentech	PD-L1	45
Urothelial bladder cancer (UBC)	Merck	PD-1	56
	Roche/Genentech	PD-L1	47
Hodgkin's lymphoma	BMS	PD-1	50
Head and neck cancer	Merck	PD-1	55
	Roche/Genentech	PD-L1	45, 53
	AstraZeneca/MedImmune	PD-L1	54
Triple negative breast cancer	Merck	PD-1	52
	Roche/Genentech	PD-L1	62

BMS, Bristol-Myers Squibb