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Review

Anti-PD-1/PD-L1 therapy for infectious diseases: learning from the cancer paradigm

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SUMMARY

Objectives: Immune checkpoint pathways regulate optimal host immune responses against transformed cells, induce immunological memory, and limit tissue pathology. Conversely, aberrant immune checkpoint activity signifies a poor prognosis in cancer and infectious diseases. Host-directed therapy (HDT) via immune checkpoint blockade has revolutionized cancer treatment with therapeutic implications for chronic infections, thus laying the foundation for this review.
Methods: Online literature searches were performed via PubMed, PubMed Central, and Google using the keywords "immune checkpoint inhibition"; "host-directed therapy"; "T cell exhaustion"; "cancer immunotherapy"; "anti-PD-1 therapy"; "anti-PD-L1 therapy"; "chronic infections"; "antigen-specific cells"; "tuberculosis"; "malaria"; "viral infections"; "human immunodeficiency virus"; "hepatitis B virus"; "hepatitis C virus"; "cytomegalovirus" and "Epstein–Barr virus". Search results were filtered based on relevance to the topics covered in this review.
Results: The use of monoclonal antibodies directed against the antigen-experienced T-cell marker programmed cell death 1 (PD-1) and its ligand PD-L1 in the context of chronic infectious diseases is reviewed. The potential pitfalls and precautions, based on clinical experience from treating patients with cancer with PD-1/PD-L1 pathway inhibitors, are also described.
Conclusions: Anti-PD-1/PD-L1 therapy holds promise as adjunctive therapy for chronic infectious diseases such as tuberculosis and HIV, and must therefore be tested in randomized clinical trials.

Contents

Programmed death 1expression in cancer and infection: tissue versus periphery . . . 222
PD-1:PD-L1blockade as host-directed therapy . . . 224
Cancer . . . 224
Infectious diseases . . . 225
Anti-PD-1:PD-L1/2therapy: improved efficacy with acceptable toxicity . . . 225
Anti-PD-1 versus anti-PD-L1/2 therapy and opportunities for combination with other immunotherapies . . . 226
Conclusions . . . 226
Funding . . . 226
Conflict of interest . . . 226
References . . . 227

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Programmed death 1 expression in cancer and infection: tissue versus periphery

The chronicity of cancer and persistent infections provides constant antigen exposure to antigen-reactive T-cells, leading to cellular exhaustion and abrogation of effector functions.[1] The expression of cell surface-bound molecules such as programmed cell death protein 1 (PD-1/CD279) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4/CD152) on antigen-specific T-cells are markers of exposure to immunogenic stimuli.[2] PD-1 and CTLA-4 identify as immune checkpoints due to their crucial role in regulating the magnitude and quality of T-cell responses. Other immune checkpoint molecules of clinical relevance are the T-cell immunoglobulin and mucin-domain containing 3 (TIM-3), with its nominal ligand galectin 9, and to a lesser extent high-mobility group Box 1 (HMGB1)[3] and the lymphocyte-activation gene 3 (LAG-3), which binds to major histocompatibility complex (MHC) class II molecules with higher affinity than the CD4 receptor.[4]

This review focuses on PD-1 and its ligands. PD-1 expression on T-cells is rapidly induced after antigen exposure, following engagement between the T-cell receptor (TCR) and its cognate epitope-loaded MHC molecule in the draining lymph nodes.[5] PD-1 RNA is detectable in T-cells as rapidly as 2 h post TCR-specific stimulation.[6] In addition to TCR-dependence, the presence of interleukin 2 (IL-2), IL-7, IL-15, vascular endothelial growth factor (VEGF), IL-6, and transforming growth factor beta (TGF-β) in the local cytokine milieu in lymph nodes and diseased tissue additionally contribute to PD-1 up-regulation on T-cells.[7–10] PD-1 interacts with its ligands, namely PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273). The PD-1 ligands are expressed on transformed cells, professional antigen-presenting cells (pAPCs), and epithelial cells, as well as T-cells.[11] This interaction provides signals that tolerize T-cells to their antigenic targets, disarming their effector functions.[1,7] Type 1 interferons (IFN-α/β) and tumour necrosis factor alpha (TNF-α) can up-regulate PD-L1 expression on T-cells, B-cells, natural killer (NK) cells, myeloid cells, and epithelial cells,[5,7] while PD-L2 expression is inducible via interferon gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-4 signalling.[7] Figure 1 is a schematic representation of the factors driving PD-1 and PD-L1/2 expression by T-cells and transformed cells, respectively.

A summary of PD-1 expression by various human cell types is presented in Table 1.[88–99] Biologically, PD-1 expression may play a beneficial role in ameliorating and curbing the development of autoimmune disease – although this is not true for cancer and infections.[12] PD-1 expression on T-cells identifies immune cells that specifically recognize and react to transformed as well as to pathogen-derived antigens. More recent studies have shown that PD-1+ tumour-infiltrating T-cells (TIL) contain a distinct repertoire of tumour-reactive CD8 T-cells that express oligoclonal TCRs, specific for mutated tumour epitopes, termed neoantigens.[13] PD-1 + CD8 T-cells recognizing the patient's cancer cells are also found in peripheral blood mononuclear cells (PBMCs), albeit in significantly lower numbers compared to the tumour microenvironment.[13] PBMC-derived neoantigen-specific CD8 T-cells can also be expanded ex vivo for cellular therapy.[14] These PD-1+ CD8 TILs co-express additional surface markers associated with activation and potential tolerance, such as TIM-3, LAG-3, and the cytotoxic lymphocyte (CTL) activation marker 4-1BB.[13]

The biological significance of PD-1+ T-cells co-expressing other exhaustion/activation markers needs to be addressed, although analysis of PD1+ and 4-1BB+ T-cells, also associated with antigen-specificity, does not suggest that a distinct pattern of activation/exhaustion markers leads to a finer resolution of identifying antigen-specific immune cells.[13] Early results of phase 1 trials using activation/exhaustion marker-positive T-cells (e.g., 41-BB+ selected immune cells) showed potentiation of complete regression of the malignant disease (melanoma) in patients (Steven A. Rosenberg, personal communication). This offers clinical evidence that exhaustion/activation marker-selected immune cells are clinically relevant, warranting use for targeted adoptive cellular therapy (clinical trials identifier: NCT02111863).

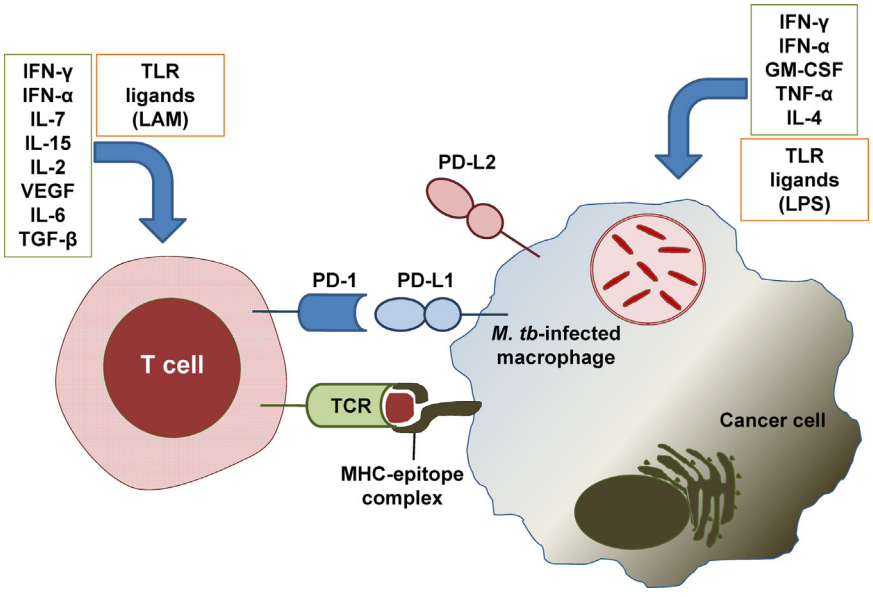

Figure 1. Factors that induce PD-1, PD-L1, and PD-L2 expression in host cells. Although the major trigger for PD-1 expression on T-cells is TCR engagement with the epitope-loaded MHC molecule on transformed cells (infected or cancerous cells), a cytokine milieu consisting of proinflammatory mediators such as IFN-γ, type 1 interferons, IL-6, VEGF, and IL-2 augment the activation of this immune checkpoint pathway. In addition, cytokines important for T-cell maintenance, i.e., IL-7 and IL-15, also contribute to PD-1 expression during chronic disease. TGF-β is another physiologically important cytokine that induces PD-1 expression and potentiates regulatory T-cell activity. Antigenic molecules such as *Mycobacterium tuberculosis* LAM can activate the PD-1 pathway in T-cells via TLR2 signalling. PD-L1/2 expression on transformed host cells is inducible by exposure to IFN-γ, type 1 interferons, TNF-α, and GM-CSF – thus indicative of intense inflammation. Bacterial LPS can also induce PD-L1 expression, via TLR4 involvement and NF-κB activation. The anti-inflammatory cytokine IL-4 can also trigger PD-L2 expression. Abbreviations: TCR, T-cell receptor; LAM, lipoarabinomannan; LPS, lipopolysaccharide; TLR, toll-like receptor; NF-κB, nuclear factor kappa B.

Table 1
Summary of PD-1, PD-L1, and PD-L2 in a variety of human cell types.

Cell type	PD-1 expression level		PD-L1 expression level		PD-L2 expression level		Ref.
	Constitutive	Cytokine-induced	Constitutive	Cytokine-induced	Constitutive	Cytokine-induced	
Naive T-cells	Nil	Yes	Nil	Yes	Nil	Yes	5,7,18,31, 61,88–99
Activated T-cells	Yes	Increased	Yes	Increased	Nil	Yes	
Exhausted T-cells	Yes	Increased	Yes	Increased	Nil	Yes	
Regulatory T-cells	Yes	Increased	Yes	Increased	Nil	Yes	
NK cells	Nil	Yes	Nil	Yes	Nil	Yes	
Naive B-cells	Yes	Increased	Yes	Increased	Yes	Increased	
Plasma cells	Yes	Increased	Yes	Increased	Yes	Increased	
Plasmacytoid DCs	Nil	Nil	Yes	Increased	Yes	Increased	
Myeloid DCs	Nil	Yes	Yes	Increased	Yes	Increased	
Macrophages	Nil	Yes	Yes	Increased	Yes	Increased	
Monocytes	Nil	Yes	Yes	Increased	Nil	Yes	
Airway and lung epithelia	Nil	Nil	Nil	Yes	Nil	Yes	
Liver non-parenchymal cells	Nil	Yes	Yes	Increased	Yes	Increased	
Mesenchymal stromal cells	Nil	Nil	Yes	Increased	Yes	Increased	
Pancreatic islets	Nil	Nil	Yes	Increased	Yes	Increased	
Fibroblasts	Nil	Nil	Yes	Increased	Yes	Increased	
HUVEC	Nil	Nil	Nil	Yes	Yes	Increased	

PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; PD-L2, programmed cell death 1 ligand 2; NK, natural killer; DC, dendritic cell; HUVEC, Human umbilical vein endothelial cells.

Similar to long exposure to mutant neoantigens in cancer, the immune system in patients with tuberculosis (TB) is exposed to new *Mycobacterium tuberculosis* antigens for an extended period. Elevated PD-1 expression has been reported on CD4 and CD8 T-cells,[15–19] neutrophils,[20] NK T-cells,[21,22] and regulatory T-cells (Tregs)[23,24] isolated from patients with drug-susceptible as well as drug-resistant TB. Furthermore, *M. tuberculosis* infection of PBMCs can up-regulate PD-1, PD-L1, and PD-L2 expression on T-cells,[15,19] NK cells,[25] and monocytes[19] therein. Individuals with latent TB infection (LTBI) also have high levels of PD-1-expressing circulating CD4 T-cells, which can produce IFN-γ (mean frequency of $29.0 \pm 2.6\%$), although T-cells negative for PD-1 expression are twice as likely to respond to antigenic stimulation via IFN-γ, IL-2, and TNF-α production.[26] This observation highlights the need to identify which PD1+ T-cell subset would indicate 'exhausted' and the antigen-experienced T-cells that can be rescued from activation-induced cell death or 'anergy', and PD1+ T-cells that cannot be reverted to functional immune cells[27] for target identification in infectious diseases.

Thus, the identification of PD1+ T-cells and their nominal targets does not reflect merely an academic exercise, but rather a clinical reality to use these T-cells for targeted cellular therapy. The analysis of T-cells from lung granulomas of *M. tuberculosis*-infected cynomolgus macaques, which very closely mimic the dynamics of human TB, revealed that approximately 8% of all granuloma-derived CD3 cells (CD4 and CD8 T subsets) were found to express PD-1.[28] The functional impact of these T-cells (exhausted and non-functional, or exhausted and antigen-experienced defining clinically relevant target epitopes) is, however, yet to be addressed.

CD8 T-cell exhaustion, mediated by PD-1 expression in HIV infection, is an important cause of weakened immune clearance of virus-infected host cells.[29,30] Increased PD-1+ CD8 T-cell numbers in PBMCs of patients with HIV infection correlate positively with impaired immune effector functions, disease progression, and declining CD4 T-cell counts.[29,31] Similar to TB, Tregs from patients with HIV infection also express high levels of PD-1.[32] Mucosal-associated invariant T-cells (MAITs) highly expressing CD161 have also been shown to up-regulate their surface expression of PD-1 protein in HIV-positive as well as HIV–TB co-infected patients; this is reversible with antiretroviral therapy.[33]

Effector memory CD8 T-cells isolated from Kenyan children with persistent exposure to *Plasmodium falciparum* were also found to strongly express PD-1 on their surface.[34] Although PD-1 expression on CD8 T-cells is implicated in the chronicity of malaria,[35] PD-1 expression on T follicular helper cells (T_{FH}) seems to correlate with protection in children.[36] T_{FH} cells are a subset of antigen-experienced (thus, antigen-specific) CD4 T-cells resident in the B-cell follicle of germinal centres within secondary lymphoid organs such as the tonsils and spleen, and play an important role in promoting B-cell activation and antibody production.[37] Another important attribute of T_{FH} cells is their ability to abundantly produce IL-21 (important for T-cell differentiation) and CXCL13, an important B-cell chemoattractant.[37] As such, the functionality of T_{FH} cells can activate antigen-specific effector T-cell responses while priming B-cells to strengthen pathogen-directed humoral immune responses.

In patients with chronic hepatitis B virus (HBV) infection, PD-1+ CD8 T-cells have been found to be impaired in their antiviral effector functions, and these cells are likely to be targeted for apoptotic depletion in vivo[38,39] – a scenario also true for NK T-cells in TB.[17,22] High levels of PD-1+ CD4 and CD8 T-cells have been observed among liver-infiltrating T-cells in tissue biopsies as well as explants from patients with hepatitis C virus (HCV) infection.[40] Cytomegalovirus (CMV) viraemia in renal transplant recipients also appears to up-regulate PD-1 expression on CD4 T-cells.[41] PD-1 + CD8 T-cells with antigen-specific TCRs may also reside in the bone marrow. IFN-γ-producing T-cells derived from bone marrow of patients with pancreatic cancer have been reported to exhibit pronounced reactivity to pancreatic tumour cell lysate in an MHC-I-dependent manner.[42] PD-1+ CD8 TILs have been found in brain metastases in patients with renal cell carcinoma,[43] while increased numbers of HIV-specific PD-1+ CD8 T-cells in cerebrospinal fluid have been reported for patients with HIV infection.[44]

The expression of PD-1 on T-cells circulating in PBMCs is gaining momentum as a marker of disease progression. In patients with non-small cell lung cancer (NSCLC), a dramatic decrease in circulation of CD8 T-cells expressing PD-1 appeared to be concomitant with overall survival after vaccination with a peptide vaccine candidate.[45] In contrast, an increase in circulating PD-1+ CD4 T-cells also correlated with overall survival, suggesting a greater degree of epitope spreading recognized by CD4 T-cells. Circulating CD8 T-cells from patients with RCC who underwent surgical resection displayed a higher degree of activation, concomitant with reduced PD-1 expression and increased CD69 expression on the cell surface.[46] The prognostic value of PD-1 expression on immune cells may also apply to malaria,[34,47]

TB,[17,20,22] and chronic viral infections.[29,39,48] These studies indicate that the dynamics of T-cell populations in peripheral blood of patients with cancer and infectious disease are, to some extent, representative of the changes occurring in the microenvironment of affected tissues.

PD-1:PD-L1 blockade as host-directed therapy

Cancer

Immune checkpoint blockade using monoclonal antibodies (mAbs) now constitutes an integral component of host-directed therapy (HDT). HDTs exploit aberrant host biological pathways that can be corrected by interfering with the respective ligands. The most clinically successful HDT has been the immunological blockade of PD-1 in cancer. The United States Food and Drug Administration (FDA)-approved anti-PD-1 mAbs, e.g. nivolumab and pembrolizumab, successfully disrupt contact with PD-L1/2 to reverse T-cell exhaustion (CD8 and CD4) and restore their anti-tumour potential, combining minimal off-target toxicity with high efficacy (Figure 2).[49] The induction of this specific tumouricidal activity by anti-PD-1 therapy correlates with increased proportions of neoantigen-specific CD8 TCRs in the peripheral blood of patients.[50] Thus, anti-PD-1 therapy is able to mobilize and to activate a subpopulation of specialized CD8 TILs to improve patient survival.

Tregs express exceptionally high levels of PD-1 on their surface; this is necessary for the maintenance of immunological balance to avoid overt inflammation and tissue destruction.[5] By targeting PD-1, Treg populations are as likely to be blocked from dampening host-protective effector T-cell responses, in addition to the direct effect on the latter. PD-1 blockade with pembrolizumab has also shown superiority over monotherapy with ipilimumab (anti-CTLA4) in patients with advanced melanoma.[51] In a prospective study, 23 of 53 advanced patients with melanoma treated with a combination of ipilimumab and nivolumab displayed an objective response, some with over 80% tumour regression.[52] Thus, compared to CTLA-4, PD-1 appears to a have a greater impact on establishing T-cell tolerance due to the magnitude of the clinical effect of its blockade.

At least two other anti-PD-1 mAbs (PDR001 and REGN2810) are in clinical trials for advanced cancers. The combinatorial effect of anti-CTLA4 and anti-PD-1 treatment imparts an additive effect of a two-pronged tumour reduction, should patients not respond to anti-CTLA-4 monotherapy. For patients with tumours expressing more PD-L1 and PD-L2 molecules than B7.1 (CD80) and B7.2 (CD86), the ligands for CTLA-4 may not respond as effectively to anti-CTLA-4 therapy. The same is true for the number of PD-1+ and CTLA-4+ tumour-specific TILs and circulating T-cells in the patient. A useful selection criterion prior to initiation of anti-PD-1 therapy would be to perform cellular analysis of the proportion of T-cells and tumour cells expressing PD-1/PD-L1/2 as opposed to CTLA-4/B7.1/2 in the tumour biopsy (microenvironment) as well as PBMCs of the patient. Several biopsies from the same patient, to account for tumour heterogeneity in the same individual, will further facilitate clinical decision-making to initiate combination therapy with immune checkpoint inhibitors.

To the best of the authors' knowledge, four monoclonal antibodies targeting PD-L1 have entered clinical trials for cancer.[53] The first of these to be tested was BMS-936559; treatment resulted in disease stabilization up to 24 weeks in patients with metastatic melanoma (27%), NSCLC (12%), ovarian cancer (18%), and RCC

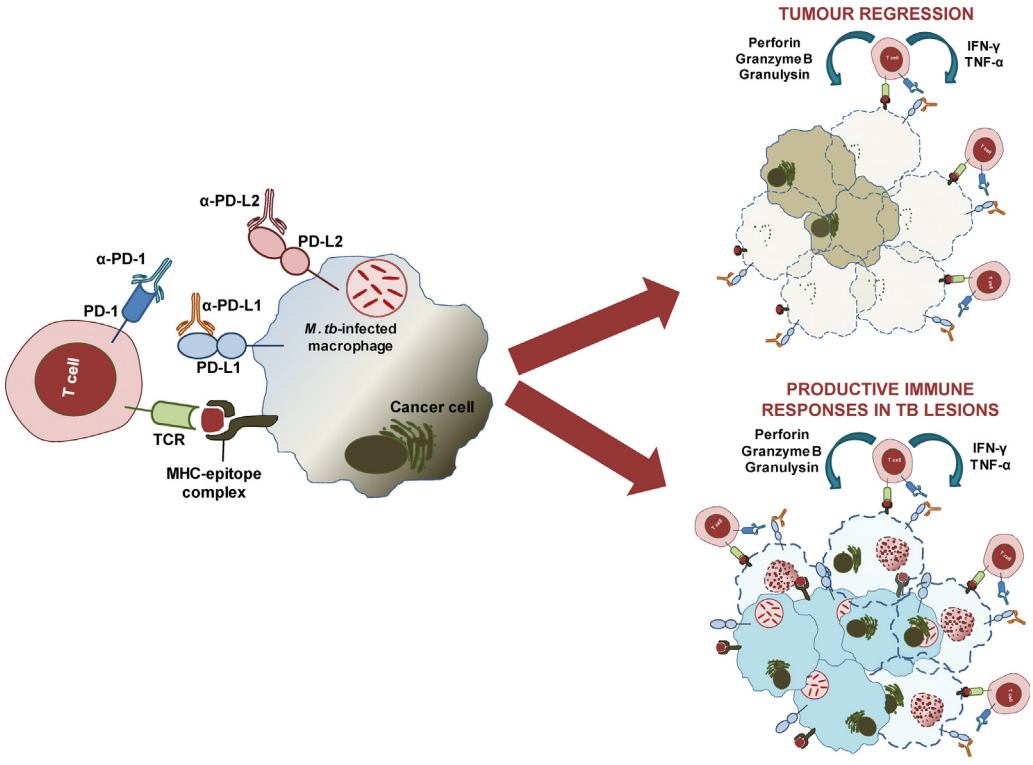

Figure 2. Blockade of the PD-1/PD-L1/2 pathway potentiates productive immune responses in cancer and infectious diseases. Immune checkpoint inhibition of the PD-1-mediated signalling pathway (initiated upon interaction between PD-1 and PD-L1/2) using monoclonal antibodies leads to activation of exhausted antigen-specific T-cells in cancer. The same is envisaged for pathogen-specific T-cells in infectious diseases – as seen in TB and chronic viral infections. Activation of these antigen-specific T-cells unleashes their ability to produce proinflammatory cytokines, i.e., IFN-γ and TNF-α, as well as cytolytic molecules such as perforin, granzyme B, and granulysin – which can lyse and kill transformed cells in the host to control the spread and burden of disease.

(41%).[54] Similar if not better responses were observed with MPDL3280A treatment,[55,56] while trials for two other anti-PD-L1 antibodies, namely MEDI4736 and MSB0010718C, are underway.

Relying on a single therapeutic agent to obtain long-term and stable disease remission may not be viable. Topalian et al. recently published the long-term survival of patients with metastatic melanoma who received nivolumab at different doses: the mean overall survival reported was a little over 2 years (approximately 25 months).[57] Given that anti-PD-1 therapy mobilizes the repertoire of neoantigen-specific TILs into circulation,[50] adoptive T-cell therapy covering the epitopes recognized by the patient's immune system would serve as an excellent adjunct to chemotherapy, as well as immune checkpoint inhibition.[58] The same applies to the chronic infectious diseases such as TB, HIV, hepatitis, and malaria discussed in this review.

Infectious diseases

The phenomenon of T-cell exhaustion in chronic infections and its similarity to cancer has been reviewed elsewhere.[1] In light of its success in cancer therapy, anti-PD-1/PD-L1 therapy has adequate precedent to be trialled in the treatment of severe chronic infectious diseases. Immune checkpoint inhibition in infectious disease therapy may be administered adjunctively to standard drug treatment – akin to cancer chemotherapy.

Exaggerated proinflammatory cytokine responses and suboptimal antigen-specific T-cell activity are equally responsible for severe tissue damage in human TB and poor clearance of *M. tuberculosis* infection.[21,22,26,59] There is some evidence in the literature to support the potential benefit of anti-PD-1 therapy in TB. In vitro blockade of PD-1 with monoclonal antibodies has been shown to restore TB antigen responsiveness, cytokine secretion, and proliferation and cytolytic activity of circulating T-cells and NK cells from TB patients. More recently, anti-TB therapy was reported to promote reduced expression of PD-1 and its ligands,[18,60] as well as increased expression of the anti-apoptotic protein B-cell lymphoma 2 in T-cells.[17] The same has been observed for PD-L1 expressed on neutrophils. Using anti-PD-1 therapy in adjunct to standard anti-TB treatment may, therefore, potentiate a two-pronged (antibiotics and T-cell-driven) antimycobacterial effect, offering an overall clinical benefit to patients.

Preclinical evaluation of anti-PD-1 therapy for HIV infection has been evaluated using the non-human primate model of simian immunodeficiency virus infection.[61] PD-1 blockade reversed T-cell exhaustion while reducing viral load as well as various opportunistic infections in non-human primates. More recently, ex vivo blockade of PD-L1 on PB Tregs from HIV-positive individuals independent of viraemia was shown to trigger expansion and potentiate effector functions of virus-specific CD8 T-cells.[32] In addition to this effect, the proliferative capacity of Tregs, although increased by PD-L1 inhibition, did not influence the ability of the cells to induce immunosuppression. PD-1 blockade appears to augment reduced hyper-activation of type 1 IFN responses, which in part translates to a lower incidence of systemic cytokine storms and immune dysregulation in HIV infection,[61] which can reduce tissue pathology in patients. In pulmonary TB, an exaggerated type 1 interferon response in the lung markedly promotes *M. tuberculosis* replication and extensive tissue destruction.[62] Accordingly, pre-antiretroviral therapy IFN-α levels were shown to be up-regulated by at least 10-fold in HIV–TB patients who went on to develop immune reconstitution inflammatory syndrome (IRIS), as well as those who died within 6 months of antiretroviral therapy initiation.[63]

In chronic human HBV infection, in vitro blockade of PD-L1 on CD8 and CD4 T-cells from PB and liver biopsies of patients resulted in enhanced IFN-γ and IL-2 production.[64] Patients with chronic HCV infection treated with nivolumab were reported to respond well, some achieving more than a 4-log reduction in viral load after treatment.[65] The analysis of sera from healthy individuals as well as haematopoietic stem cell transplantation (HSCT) recipients with transient or persistent CMV infection revealed that the latter group had the highest amount of IL-6 produced by CD33 + PD-L1+ adherent cells, which in turn induced PD-1 expression on virus-specific CD8 T-cells.[10] In vitro blockade of PD-L1 in PBMCs isolated from these patients led to significant expansion of tetramer-positive virus-specific CD8 T-cells, indicating that PD-L1 blockade may be clinically favourable in managing chronic CMV disease.[10] Blockade of PD-L1 in a murine model of cerebral malaria exacerbated inflammation and haemorrhage in the brain compared to untreated controls.[66] However, blocking PD-1 as well as LAG-3 in the blood stage of murine malaria (which resembles the erythrocyte stage in human malaria) proved beneficial, manifesting in dramatic parasitaemia reduction and improved antibody as well as CD4 (also T_{FH}) and CD8 T-cell responses, associated with elimination of chronic infection as well as extended survival.[67]

Anti-PD-1:PD-L1/2 therapy: improved efficacy with acceptable toxicity

Generally mild to moderate immune-related adverse effects (irAEs) have been reported in patients with anti-PD-1:PD-L1 therapy.[68] Three out of 296 patients with advanced solid tumours (two patients with NSCLC, one patient with CRC (colorectal cancer)) who received nivolumab treatment in the first anti-PD-1 clinical study were reported to have died due to treatment-induced pneumonitis (inflammation of the alveolar wall, grade 3 or 4 irAE), nevertheless accounting for only 1% of total mortality (21%) in the cohort.[69] A clinical trial of nivolumab therapy in patients with advanced NSCLC ($n = 129$) also reported three deaths (2% of patients) due to treatment-induced pneumonitis, although objective responses were seen in 22 patients (17%).[70] A more recent phase 2 evaluation of nivolumab in patients with metastatic RCC reported that only eight patients who received treatment subsequently developed pneumonitis, with no treatment-related deaths occurring over the duration of the trial.[71] Thus, the fatality rate due to anti-PD-1:PD-L1 therapy is significantly low compared to the clinical benefits gained by the patients with advanced cancer undergoing immune checkpoint inhibition treatment.

Sporadic cases of the development of anti-PD-1 therapy-associated type 1 diabetes mellitus (autoimmune insulin-dependent diabetes mellitus, T1DM) in patients have also been reported.[72,73] In general, high titres of autoantibodies against glutamate decarboxylase (GAD), and in some patients with an HLA-A2+ background, CD8 T-cells recognizing epitopes from insulin alpha and beta chains, preproinsulin, GAD65, and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) (0.2% to 2% tetramer-specific cells of total PBMCs) were detected in peripheral blood. Autoimmune myocarditis, concomitant with increased CD8 T-cells and reduced Tregs (in a myocardial biopsy) has been reported in one patient with metastatic melanoma who received pembrolizumab.[74] These case reports contribute to a better understanding of irAEs that may be valuable for the clinical immune-monitoring of patients undergoing anti-PD-1:PD-L1 therapy, rather than to discourage their use altogether.

Essentially, the adverse effects reported for patients with metastatic cancer receiving anti PD-1 therapy may be far less harmful than the off-target toxicities of standard chemotherapy, which also impairs the patient's quality of life.[53,75] In addition, the inflammatory side effects associated with anti-PD-1:PD-L1 therapy, i.e., pneumonitis and gastrointestinal inflammation, are clinically manageable with immunosuppressive corticosteroids.[53,69,70] Weber et al. reported 38% objective responses in

patients with advanced melanoma treated with nivolumab ($n = 268$) versus a meagre 5% of patients who underwent the investigators' choice of chemotherapy or ICC ($n = 102$).[76] Furthermore, more patients who were treated with ICC (32 patients, 32%) exhibited grade 3 or 4 AEs as compared to patients treated with nivolumab (24 patients, 9%). In a more recent retrospective report of a European multicenter clinical trial assessing the efficacy and safety of nivolumab or pembrolizumab therapy for advanced melanoma, 138 of 496 treated patients exhibited grade 1 or 2 irAEs, conditions that are either treatable with steroids or even negligible.[68] Thus, the proven clinical efficacy of anti-PD-1:PD-L1 therapy against melanoma and other solids tumours compensates for acceptable and manageable side effects observed in a small fraction of patients.

Anti-PD-1 versus anti-PD-L1/2 therapy and opportunities for combination with other immunotherapies

Due to its robustness, blocking PD-1 delivers the probable risk of activating subpopulations of autoreactive and pathogenic T-cell subsets in the patient, as seen with the onset of inflammatory conditions in individuals receiving anti-PD-1 therapy without a previous history of the disease. In contrast, blockade of PD-L1 alone would elicit less prominent T-cell responses compared to blocking PD-1, since it can still interact with PD-L2, although expression of the latter is more restricted than PD-L1.[5] This, however, comes with the advantage of reduced toxicity.[77] Nevertheless, the relative efficacy and toxicity of anti-PD-1 and anti-PD-L1 agents at present appear to be quite similar, and further work is necessary to delineate the potential differences in efficacy and toxicities of these agents.

In addition to using monoclonal antibodies targeting the PD-1/PD-L1 pathway, cytokine therapy, antibodies targeting cytokines and other cell-surface markers, as well as cellular therapy are potentially powerful interventional tools. Given that the local and systemic cytokine milieu in patients with chronic infection and cancer contributes to T-cell exhaustion and tolerance, timely delivery of cytokines that can reverse exhaustion could augment the therapeutic activity of anti-PD-1/PD-L1 antibodies. Alternatively, antibodies that neutralize TNF-α, IL-6, TGF-β, and/or VEGF in combination with anti-PD-1/PD-L1 antibodies could be as clinically beneficial, in terms of reducing unwanted and destructive inflammation. Using the example of 3/15 patients with advanced melanoma who had disease progression after at least 6 months of remission, Zaretsky et al. recently reported that the outgrowth of metastatic tumours with mutations in the Janus kinase 1 or 2 genes (*JAK1 or 2*), which disrupts IFN-γ-induced cell growth arrest as well as mutations in the β2-microglobulin gene (which is involved in MHC-1 antigen presentation), might promote late acquired resistance to anti-PD-1 therapy.[78] *JAK1* mutations could, therefore, serve as a biomarker for the efficacy of anti-PD-1:PD-L1 therapy, and could be tested for prior to initiating treatment.

Another interesting host target is CD70, a surface receptor expressed on lymphocytes and myeloid cells associated with T-cell activation and memory formation, albeit with a poor prognosis in cancer and infection.[79–82] CD70 expression inhibits the functionality of effector memory TILs in RCC and melanoma,[79,80] while viraemic and lymphopenic HIV-infected individuals have high frequencies of CD70-expressing PB CD4 T-cells.[81] Anti-CD70 treatment has been shown to reduce Treg proliferation, activate TILs, potentiate antibody-mediated cellular cytotoxicity to kill human tumour cells, and also to extend the survival of Burkitt's lymphoma-bearing mice.[83] There are at least two anti-CD70 mAbs in clinical trials for RCC and non-Hodgkin's lymphoma at present (ARGX-110 and MDX-1203).

The clinical effect of anti-PD-1/PD-L1 therapy is very pronounced in tumour-reactive TILs since PD-1 expression is significantly higher on TILs than circulating T-cells from cancer patients.[13] It has also been learned that most anti-cancer T-cell responses potentiated by PD-1 therapy target mutated epitopes.[50] This may also resonate in infectious diseases, especially those perpetrated by drug-resistant pathogens. While subdued CD8 T-cell responses to viral escape neoantigens has been shown to facilitate viral persistence and disease progression in the host,[84,85] host-protective T-cell responses to *M. tuberculosis* mutated antigens is not known but warrants formal testing using clinical material from patients with drug-sensitive and drug-resistant TB.[86,87]

Conclusions

The therapeutic efficacy of monoclonal antibodies against PD-1 and PD-L1 in patients with cancer prompts extensive clinical evaluation in patients with chronic infectious diseases, especially in light of increasing drug resistance. Regardless of the shortcomings reported in patients receiving anti-PD-1/PD-L1 therapy, the proven clinical success of blocking this immune checkpoint pathway certainly encourages its further use – particularly if clinically meaningful inclusion criteria and markers of responses can be identified (thus, precision medicine). Taking TB as a paradigm, the high expression of PD-1 in patients with drug-refractory disease as well as those with HIV-related IRIS is targetable for anti-PD-1 therapy. Clinical studies involving metastatic melanoma patients have revealed that doses up to 3 mg/kg are well tolerated by patients (in terms of acceptable irAEs), and produce highly encouraging treatment outcomes.[52,68,69] Designing clinical trials to test the efficacy of PD-l blockade in TB should therefore consider involving patients with extensive chest radiographic findings and blood inflammatory markers (including cytokines such as IL-6, IFN-γ, TNF-α, IFN-α/β etc.), as well as poor responsiveness to drug therapy. Patients with multidrug-resistant or extensively drug-resistant (MDR/XDR) TB would represent an excellent cohort for intervention. The adjunctive administration of anti-PD-1 antibodies to TB patients undergoing anti-TB drug therapy – for drug-susceptible or drug-resistant TB disease – could potentially eradicate *M. tuberculosis* reservoirs in organs inaccessible to chemotherapeutic agents but not immune cells. Anti-PD-1 treatment, after the intensive phase of anti-TB therapy, may activate anergic and exhausted *M. tuberculosis*-specific T-cells in the patient. In addition to offering an improved point-of-care intervention for otherwise helpless patients, immune checkpoint inhibition has also shed new light on the pathobiology of disease. This opens up new and exciting avenues of basic and clinical research that may materialize in hitherto unknown therapeutic strategies. Borrowing this knowledge should lead to revised regimens of combination therapy incorporating immune checkpoint inhibitors and antibiotics, as well as repurposed drugs for the effective management of chronic diseases.

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Conflict of interest

The authors declare no conflicts of interest.

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