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# CD8 T Cell Exhaustion in Chronic Infection and Cancer: Opportunities for Interventions

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## Abstract

Antigen-specific CD8 T cells are central to the control of chronic infections and cancer, but persistent antigen stimulation results in T cell exhaustion. Exhausted CD8 T cells have decreased effector function and proliferative capacity, partly caused by overexpression of inhibitory receptors such as programmed cell death (PD)-1. Blockade of the PD-1 pathway has opened a new therapeutic avenue for reinvigorating T cell responses, with positive outcomes especially for patients with cancer. Other strategies to restore function in exhausted CD8 T cells are currently under evaluation—many in combination with PD-1-targeted therapy. Exhausted CD8 T cells comprise heterogeneous cell populations with unique differentiation and functional states. A subset of stem cell–like PD-1$^+$ CD8 T cells responsible for the proliferative burst after PD-1 therapy has been recently described. A greater understanding of T cell exhaustion is imperative to establish rational immunotherapeutic interventions.

## INTRODUCTION

CD8 T cells are central components of adaptive immunity. When naïve CD8 T cells recognize antigenic peptides presented via major histocompatibility complex class I (MHC-I) through their T cell receptors (TCRs), they are activated, undergo massive clonal expansion, and differentiate into potent effectors. Effector CD8 T cells (*a*) express cytotoxic molecules such as perforin and granzyme B, (*b*) produce effector cytokines like interferon (IFN)-γ and tumor necrosis factor (TNF)-α, and (*c*) express chemokine and homing receptors necessary for migration into peripheral tissues. Effector CD8 T cells kill target cells and secrete cytokines that help contain the spread of pathogens and cancer. During antigen clearance, most effector CD8 T cells die by apoptosis, but about 5–10% survive and differentiate into memory CD8 T cells. Memory CD8 T cells are maintained long-term in the absence of antigens and can exert rapid effector functions in response to previously encountered antigens (1, 2).

When host immune responses fail and antigens persist, antigen-specific CD8 T cells differentiate into a state called T cell exhaustion, in which they are distinct from naïve, effector, or memory CD8 T cells. T cell exhaustion was first described in the mouse model of chronic lymphocytic choriomeningitis virus (LCMV) infection, where virus-specific CD8 T cells exposed to continuous antigen stimulation show reduced effector function and poor proliferative capacity compared to functional memory CD8 T cells (1, 3–5). Exhausted CD8 T cells have unique transcriptional and epigenetic signatures that include overexpression of several inhibitory receptors, dysregulated cytokine signaling pathways, and altered metabolic fitness (1, 6–13).

Persistence of high levels of antigen is a major driver of T cell exhaustion (1, 14). Accordingly, T cell exhaustion occurs during human chronic infections and cancer (6, 15, 16). T cell exhaustion probably evolved to protect the host from immunopathology, but it results in limited control of pathogens and cancer. There is now great interest in establishing strategies to rescue exhausted CD8 T cells and reinvigorate immune responses. Understanding the basic mechanisms of T cell exhaustion is crucial for clinical applications. The importance of such research is demonstrated by the discovery of the role of programmed cell death (PD)-1 in CD8 T cell exhaustion during chronic viral infection in mice (13) and other early basic and preclinical studies (17–19) that drove clinical trials of PD-1-directed immunotherapy for cancer patients. Although CD4 T cell exhaustion also occurs during chronic infections (6), this review focuses on CD8 T cells. We describe several fundamental aspects of CD8 T cell exhaustion, potential strategies for restoring function in exhausted CD8 T cells to achieve therapeutic benefits in chronic infections and cancer, and novel findings regarding the existence of a stem cell–like PD-1$^+$ CD8 T cell subset (20).

## IMMUNE CHECKPOINT MOLECULES AND T CELL EXHAUSTION

A cardinal feature of exhausted CD8 T cells that persist during continuous antigen stimulation is sustained expression of inhibitory receptors (**Figure 1*a*,*b***) (6, 7, 13, 21–24). Most inhibitory receptors are induced by T cell activation, and thus are also expressed in effector T cells but at lower levels and in a transient manner. Inhibitory receptors vary in expression pattern, ligands, and signaling motifs, and their mechanism of suppression is not well understood. In exhausted CD8 T cells, most inhibitory receptors are coexpressed with PD-1 and provide a cumulative inhibitory effect (21–26). Our knowledge and therapeutic applications are more advanced for PD-1 and cytotoxic T lymphocyte antigen (CTLA)-4, but other inhibitory receptors also have blocking agents in clinical trials.

### PD-1

PD-1 is a transmembrane protein receptor of the CD28 family (27). The PD-1 cytoplasmic region contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an

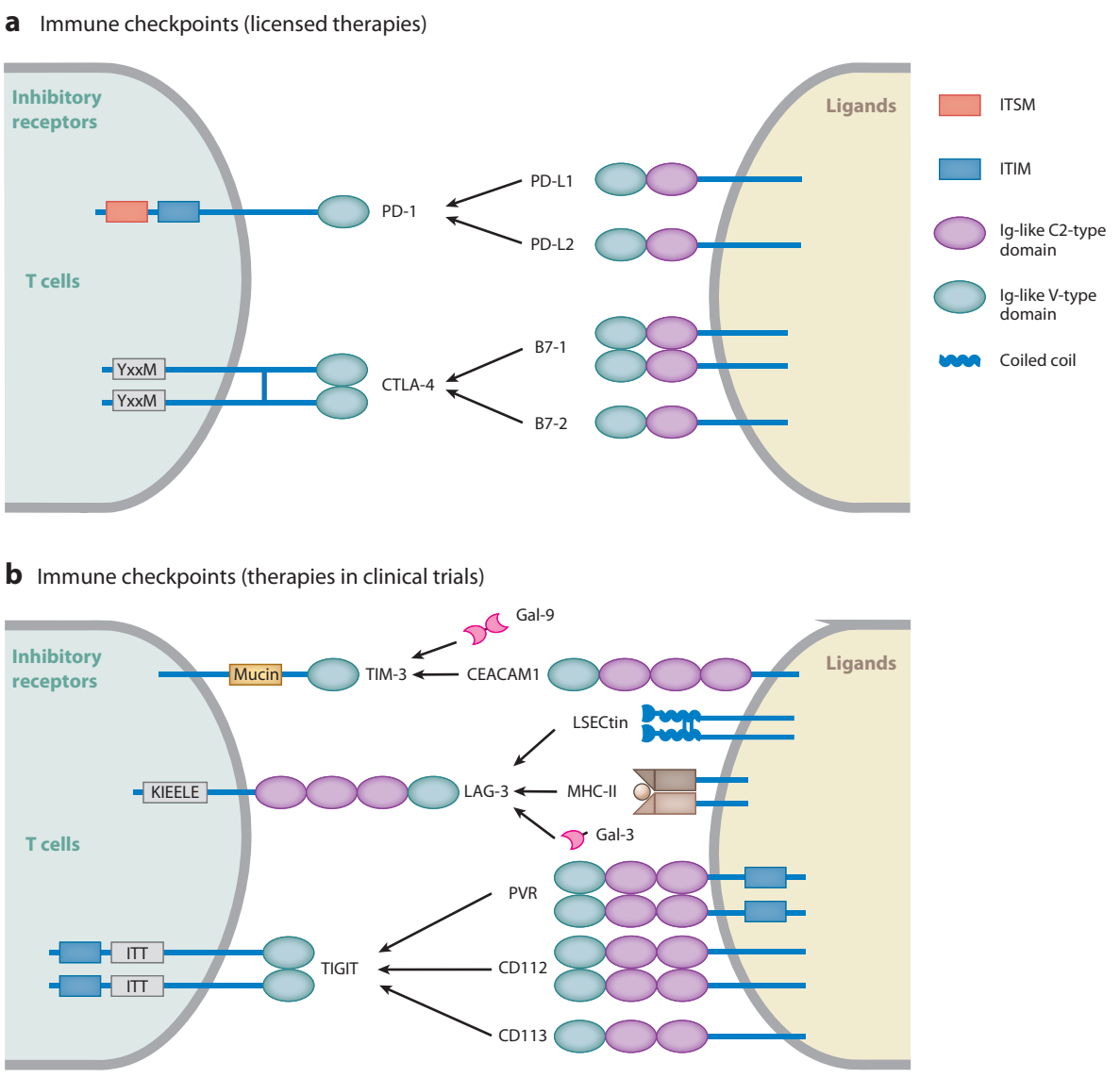



**Figure 1**

Immune checkpoints. T cells express inhibitory receptors that bind ligands present on antigen-presenting cells, infected cells, or tumor cells. (*a*) Immune checkpoints targeted by licensed therapies. (*b*) Immune checkpoints targeted by therapies in clinical trials. Abbreviations: CEACAM1, carcinoembryonic antigen cell adhesion molecule 1; CTLA-4, cytotoxic T lymphocyte antigen-4; Gal, galectin; Ig, immunoglobulin; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; ITT, Ig tail tyrosine motif; KIEELE, a conserved amino acid sequence motif that confers inhibitory function; LAG-3, lymphocyte activation gene-3; LSECtin, liver and lymph node sinusoidal endothelial cell C-type lectin; MHC-II, major histocompatibility complex class II; PD-1, programmed cell death 1; PVR, poliovirus receptor; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell Ig and mucin domain–containing-3.

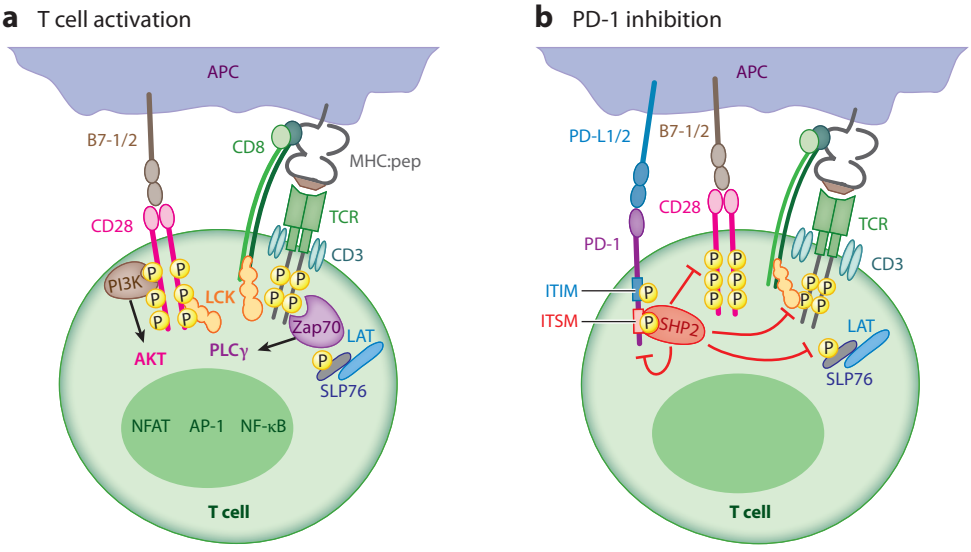



**Figure 2**

Regulation of T cell activation by PD-1. (*a*) TCR and CD28 ligations in the immunological synapse trigger a cascade of phosphorylation events initiated by LCK that ultimately result in T cell activation. (*b*) PD-1 inhibits T cell activation by SHP2-mediated dephosphorylation of CD28 and other molecules downstream of the TCR. Abbreviations: AP-1, activator protein 1; APC, antigen-presenting cell; LAT, linker for activation of T cells; LCK, lymphocyte-specific protein tyrosine kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; PD-1, programmed cell death 1; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase C gamma; SHP2, Src homology 2 (SH2) domain–containing tyrosine phosphatase 2; SLP76, SH2 domain-containing leukocyte protein of 76 kDa; TCR, T cell receptor; ZAP70, zeta-chain-associated protein kinase 70.

immunoreceptor tyrosine-based switch motif (ITSM) (**Figure 1*a***). When PD-1 binds its ligands and is recruited to the immunological synapse, the tyrosines within ITIM and ITSM motifs are phosphorylated (17, 27). Phosphorylated PD-1 recruits SHP2 [Src homology 2 (SH2) domain–containing tyrosine phosphatase 2], which then dephosphorylates several mediators of T cell activation (28). Recent work with elegant cell-free biochemical analysis demonstrated that SHP2 associated to PD-1 dephosphorylated CD28 with higher efficiency than TCRs and other downstream components such as CD3-ζ, ZAP70 (zeta-chain-associated protein kinase 70), and LAT/SLP76 (linker for activation of T cells/SH2 domain-containing leukocyte protein of 76 kDa) (**Figure 2**) (29). The differential effect of PD-1 signaling on CD28 phosphorylation compared to CD3-ζ was due to a higher rate of rephosphorylation of CD3-ζ by LCK (lymphocyte-specific protein tyrosine kinase). Higher net dephosphorylation of CD28 by PD-1 was further confirmed by in vitro cellular assays. Nevertheless, when immunological synapses do not involve CD28/B7 interactions, PD-1 should still attenuate T cell activation since SHP2 associated to PD-1 also targets TCRs and downstream molecules—for example, during the interactions of cytotoxic CD8 T cells with $B7^-$ target cells (28, 29).

PD-1 has two ligands, both members of the B7 family: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (B7-DC, CD273) (27). PD-L1 is constitutively expressed by many different cells of hematopoietic and nonhematopoietic origin. PD-L1 expression can be induced by type

I and II IFN and other cytokines (27), and upregulation of PD-L1 in response to IFN-γ has been described as adaptive resistance—a mechanism to dampen ongoing immune responses (30). In several studies, PD-L1 expression in the tumor microenvironment, which is usually strongly associated with CD8 T cell infiltration, increases the likelihood of clinical response to PD-1-targeted immunotherapy (31). In contrast to PD-L1, PD-L2 expression is inducible and more restricted. PD-L2 can be expressed by dendritic cells (DCs), macrophages, and B-1 cells, given specific environmental cues, such as IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (27).

In T cells, PD-1 expression is induced and maintained by TCR stimulation, but can also be modulated by cytokines and other signals (27). Furthermore, unlike memory cells, in exhausted T cells the PD-1 promoter remains demethylated and poised, even in the absence of antigen stimulation (32, 33).

The PD-1 pathway affects T cell function in many aspects, including metabolism. PD-1 signaling reduces Akt (protein kinase B) activation and thus inhibits mTOR (mammalian target of rapamycin) activity, switching T cell metabolism from glycolysis to fatty acid oxidation (34–36). An earlier study based on molecular signatures found that exhausted CD8 T cells were defective in their metabolic fitness (7). Mechanistically, it was shown that PD-1 regulated early glycolytic and mitochondrial alterations and repressed the transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α). Overexpression of PGC-1α improved metabolism and function in exhausted CD8 T cells during chronic viral infection (12). Therefore, the metabolic switch in exhausted T cells may play a core role in T cell dysfunction. Decreased mTOR activation in PD-1$^+$ CD8 T cells ensures increased activity of the transcription factor FoxO1 (forkhead box O1), which sustains PD-1 expression and survival of exhausted CD8 T cells (35).

Therapeutic blockade of the PD-1 pathway reinvigorates exhausted CD8 T cell responses by reprogramming the metabolism and promoting proliferation and increased expression of effector molecules (perforin, granzymes and cytokines) (6). In numerous mouse models of cancer and chronic infection, PD-1-targeted therapy suppresses tumor growth (18, 19) and reduces viral load (6, 13). Similar results have been confirmed for chronic infections in nonhuman primates. Blockade of PD-1 during chronic simian immunodeficiency virus (SIV) infection in macaques enhanced SIV-specific CD8 T and B cell responses, improving viral control and survival (37). In chimpanzees chronically infected with hepatitis C virus (HCV), one of three animals receiving PD-1-targeted therapy demonstrated reinvigoration of HCV-specific CD8 and CD4 T cell responses followed by 100-fold suppression of viremia during treatment (38).

In clinical trials, some advanced cancer patients (up to 30% depending on the cancer type) experienced reduced tumor burden and improved survival following PD-1-targeted therapy (39, 40). In September 2014, the first anti-PD-1 blocking antibody was approved by the US Food and Drug Administration (FDA) for second- and third-line treatment of metastatic melanoma, and as of August 2017, several drugs targeting the PD-1 pathway have been approved for different cancer types and for solid cancers sharing genetic abnormalities that confer higher numbers of mutations, regardless of tissue of origin (**Table 1**). Cancer mutations generally increase the number of neoantigens that can function as T cell epitopes to sustain antitumor T cell responses. Indeed, in many studies the number of neoantigens has a positive association with clinical responses to PD-1-targeted therapy (41).

Some cancers have a viral origin, and viral antigens provide immunogenic epitopes in addition to neoantigens generated by mutations. In the study that established a survival benefit of PD-1 blockade over standard of care for platinum-refractory recurrent head and neck cancer,

**Table 1 FDA-approved clinical indications for checkpoint inhibitors**

| PD-1 targeted therapy FDA approved between 2014 and August 2017 for the following indications |
|---|
| Melanoma[a,b] |
| Non–small cell lung cancer[a,b,c] |
| Head and neck squamous cell cancer[a,b] |
| Renal cell carcinoma (kidney cancer)[a] |
| Urothelial carcinoma (bladder cancer)[a,b,c,d,e] |
| Classical Hodgkin's lymphoma[a,b] |
| Merkel cell carcinoma[d] |
| Colorectal cancer with microsatellite instability (MSI-high) or defects in DNA mismatch repair (MMR)[a,b] |
| Solid tumors MSI-high or MMR deficient[b] |
| **CTLA-4 targeted therapy FDA approved in 2011** |
| Melanoma[f] |

[a]Nivolumab (Opdivo®), hIgG4 anti-PD-1.
[b]Pembrolizumab (Keytruda®), hIgG4 anti-PD-1.
[c]Atezolizumab (Tecentriq®), hIgG1κ anti-PD-L1.
[d]Avelumab (Bavencio®), hIgG1 λ anti-PD-L1.
[e]Durvalumab (Imfizi®), hIgG1κ anti-PD-L1.
[f]Ipilimumab (Yervoy®), hIgG1 κ anti-CTLA-4.
Abbreviation: hIg, human immunoglobulin.

the survival benefit of PD-1-targeted therapy was higher in patients with human papillomavirus (HPV)-positive tumors (42). Interestingly, the rates of response to PD-1-targeted therapy for Merkel cell carcinoma—a skin cancer typically associated with Merkel cell polyomavirus—were similar between viral antigen-positive and -negative tumors (43, 44). However, virus-negative Merkel cell tumors harbor a higher number of neoantigens than virus-positive Merkel cell tumors or even melanoma (45). These data so far suggest that either neoantigens or viral epitopes (both potential targets for T cells) increase the response rate to immunotherapies.

The clinical advances of immunotherapy in the cancer field have not been matched for chronic infections. Powerful antiviral drugs now manage chronic infections with relatively few adverse events. However, treatment can be life-long for patients with HIV; HCV can rebound after treatment; and treatment for chronic hepatitis B virus (HBV) infection is still not optimal. Since clinical trials enrolling chronically infected patients need to be cautious about potential toxicities of PD-1-targeted therapy in otherwise healthy individuals, trials have so far only assessed single-dose regimens. Although there was only a modest response rate for chronic HCV, among 20 patients receiving the highest anti-PD-1 dose (10 mg/kg), three patients displayed significant reductions in HCV RNA (>10,000-fold drop), and in one patient, HCV was undetectable for at least one year. Mild to moderate immune-related adverse events occurred in 6 of 54 patients but were resolved without specific intervention (46). Single-dose PD-1-targeted therapy was also evaluated in HIV-infected individuals on clinically effective combination antiretroviral therapy (47). Increases in HIV-specific CD8 T cell responses were observed in the blood in 2 of 6 participants, but without effects on viral load, possibly because the dosage of anti-PD-L1 antibodies was tenfold lower than dosages selected for activity in patients with cancer. These clinical trials show that there is potential to use PD-1-targeted therapy for management of chronic infections and that combination treatments should be further evaluated.

## CTLA-4

Blockade of CTLA-4 (ipilimumab) was approved by the FDA in 2011 for the treatment of advanced melanoma based on clinical trials that demonstrated increased overall survival (48, 49) (**Table 1**). However subsequent studies have shown higher efficacy with PD-1 therapies than with ipilimumab (50, 51). Nevertheless, combined CTLA-4 and PD-1 blockade achieved higher numerical response rates than either monotherapy, and the combination was approved to treat metastatic melanoma in 2015 (50). Recent data on 3-year overall survival confirms the superiority of anti-PD-1 over anti-CTLA-4, but whether combination therapy provides enough benefit over anti-PD-1 monotherapy still remains to be fully resolved (52).

CTLA-4 is expressed constitutively in regulatory T cells (Tregs) and induced upon activation in other T cells. CTLA-4 is mostly found in intracellular vesicles but is recruited to the immunological synapse when CTLA-4 binds to B7–1 (CD80) or B7–2 (CD86) (**Figure 1*a***). CTLA-4 competes with the positive T cell costimulatory molecule CD28, and the cytoplasmic domain of CTLA-4 may recruit phosphatases that attenuate TCR/CD28 signaling (53). Direct inhibitory effects of CTLA-4 on exhausted CD8 T cells have been proposed. For example, in vitro blockade of PD-1 did not restore function in hepatic PD-1$^+$ CTLA-4$^+$ HCV-specific CD8 T cells, but combined blockade of CTLA-4 and PD-1 reinvigorated HCV-specific CD8 T cells in a CD4 T cell–independent manner (54). However, given the suggested role of Treg depletion for the effectiveness of anti-CTLA-4 therapy in tumor models, an indirect effect on CD8 T cells may play a major role (55–57). It is important to point out that in vivo blockade of CTLA-4 during chronic viral infections (LCMV, SIV, and HIV) has failed to reduce viral load or increase CD8 T cell function (13, 58). Hence, further mechanistic studies are required to fully understand the potential applications of CTLA-4 blockade.

# POTENTIAL THERAPEUTIC TARGETS FOR MODULATING T CELL EXHAUSTION

The efficacy of PD-1 and CTLA-4 blockade for cancer treatment has reinvigorated interest in immunotherapies. Increased understanding of altered pathways and the molecular signature of exhausted T cells has uncovered many opportunities for interventions. Due to the favorable safety profile and broad action of therapies that block the PD-1 pathway, most clinical trials now focus on combinations with PD-1-targeted therapy. Below we highlight a few of the promising strategies.

## Other Immune Checkpoint Molecules

Exhausted CD8 T cells can express many inhibitory receptors that function as checkpoint molecules. The success of immune checkpoint inhibitors targeting PD-1 and CTLA-4 has prompted testing co-blockade with other inhibitory receptors. Drugs targeting at least three other immune checkpoints are now in clinical trials (**Figure 1*b***): LAG-3 (lymphocyte activation gene-3), TIM-3 (T cell immunoglobulin and mucin domain–containing-3), and TIGIT (T cell immunoreceptor with immunoglobulin and ITIM domains). Despite minimal effects of single therapy, co-blockade of LAG-3, TIM-3, or TIGIT synergized with PD-1 pathway blockade in chronic viral infection and tumor models (21–24, 59). However, signaling transduction of these receptors on T cells remains largely undefined. It is still unclear whether the positive biological outcome of blockade of these inhibitory pathways is due to direct effects on CD8 T cells, and further mechanistic studies are warranted to provide a better understanding of the mechanism of action for these new drugs.

## Costimulatory Molecules

In a therapeutic setting, increasing positive costimulation has been a focus for improving immune responses (60). However, while the relationship between inhibitory receptors and T cell exhaustion has been a major topic of investigation, the role of positive costimulatory molecules in the differentiation or rescue of exhausted T cells remains mostly unexplored. Positive costimulatory molecules vary in signaling motifs and intracellular adaptors, as well as in cellular expression pattern and kinetics of the receptors and ligands. It is interesting that both PD-1 and CTLA-4 interfere with CD28-mediated T cell activation (29, 61).

CD28 is a major costimulatory molecule for T cell priming, yet the requirements for CD28 costimulation vary according to antigenic strength and T cell differentiation status (62). The intracellular tail of CD28 contains tyrosine-based signaling motifs (**Figure 2**) and recruits adaptors, such as the lipid kinase PI3K, growth factor receptor-bound protein 2 (GRB2), and LCK, that ultimately result in the activation of NFAT, activator protein (AP)-1, and NF-κB (62). CD28 signaling amplifies TCR signaling in addition to having effects independent of TCR, which may include expression of IL-2 and B cell lymphoma (Bcl)-2, modulation of metabolism (63), and epigenetic changes (62).

We recently demonstrated that during chronic viral infection, CD28 signals are necessary for reinvigoration of virus-specific CD8 T cells that follows blockade of the PD-1 pathway (64). Conditional deletion of the CD28 gene after establishment of chronic infection, but before PD-1-targeted therapy, demonstrated that loss of CD28 precluded expansion of virus-specific CD8 T cells in a cell-intrinsic manner. Likewise, the efficacy of PD-1-targeted therapy to control CT-26 tumor growth was also dependent on the CD28/B7 pathway (64). Based on these findings, we propose that the CD28/B7 pathway may also determine success of PD-1-targeted therapy in cancer patients. In support of our hypothesis, we found heterogeneity in CD8 T cells infiltrating human non–small cell lung cancer: 10–80% of CD8 T cells were $CD28^-$. Yet, CD8 T cells that proliferated in the peripheral blood of lung cancer patients after PD-1-targeted therapy were mostly $CD28^+$ (64). These data imply a role for the CD28/B7 pathway in the efficacy of PD-1-targeted therapy. In addition, our data and other studies suggest that T cell reinvigoration may rely on T cell interactions with $B7^+$ hematopoietic cells, such as DCs (65, 66).

Despite the expression of other positive costimulatory molecules in $PD\text{-}1^+$ CD8 T cells (7, 20), our data suggest that CD28 signaling plays a major and nonredundant role in T cell reinvigoration by PD-1-targeted therapy (64). Directly targeting CD28 to improve immunotherapy may not be feasible, given the broad expression profile of CD28 and severe immunotoxicity in a clinical trial with CD28 superagonist (67).

A number of drugs targeting costimulatory molecules are in cancer clinical trials, including members of the TNF receptor superfamily OX40 (CD134), CD27, and 4-1BB (CD137). However, the basic research to understand the function of this new class of drugs still needs to address signaling pathways as well as the target cell population (68, 69). Aside from immunotherapies that directly target T cell activation, there is also interest in therapies that target antigen-presenting cells (APCs) and thereby increase the availability of ligands to engage costimulatory molecules in T cells (60, 70). A deeper understanding of costimulatory molecule function is necessary to optimize therapeutic interventions.

## Cytokines

Exhausted CD8 T cells have unique cytokine signaling pathways and cytokine receptor expression profiles (1, 6, 7). For instance, exhausted CD8 T cells express lower levels of IL-7Rα (CD127) and IL-2Rβ (also known as IL-15Rβ or CD122) than functional memory CD8 T cells. Therefore,

unlike memory CD8 T cells, which rely on signals from IL-7 and IL-15 for homeostatic proliferation and survival, exhausted CD8 T cells rely on antigenic stimulation for cell division and maintenance (71, 72). Likewise, during chronic infection, virus-specific CD8 T cells downregulate IL-18Rα (CD218a) and have reduced responsiveness to certain combinations of inflammatory cytokines such as IL-12, IL-18, and IL-21 (73). Furthermore, in contrast to acute infection settings, IL-21 is vital for sustaining CD8 T cell responses during chronic viral infection, and loss of IL-21R in CD8 T cells exacerbates exhaustion (74–76).

From the perspective of immunotherapy, modulating the actions of cytokines on exhausted CD8 T cells is a promising approach to improve biological outcome during chronic infections and cancer. For example, IL-2 administration was approved by the FDA for treating metastatic renal carcinoma in 1992 and for metastatic melanoma in 1998 after IL-2 therapy demonstrated some efficacy in these diseases (77). However, our immunological understanding of the mechanism of action of each cytokine in exhausted CD8 T cells is limited.

Earlier studies found that IL-2 or IL-7 administration improves virus-specific CD8 T cell responses and accelerates viral clearance during chronic viral infection (78–80). IL-2 or IL-7 treatment also increased T cell numbers during SIV or HIV infection, but did not reduce viral load (81–84). IL-2 and IL-7 increase the number of CD4 T cells, which are the primary targets for SIV/HIV; thus, an increase in target cells might hamper the positive effects of IL-2 and IL-7 on the immune responses directed to these viruses. Similarly, IL-15 administration during SIV infection increased SIV-specific CD8 T cell and natural killer (NK) cell numbers. However, the treatment did not improve viral control; in fact, the viral set point increased (85, 86).

Blocking the actions of inhibitory cytokines, such as IL-10, is another promising approach. Increased levels of IL-10 were found during chronic LCMV infection, and blockade of IL-10/IL-10R improved virus-specific CD8 T cell responses and promoted viral clearance (87, 88). Given that elevated IL-10 levels are also detected during human chronic viral infections (6), targeting the IL-10/IL-10R axis may be a promising clinical approach. Conversely, the role of IL-10 in antigen-specific CD8 T cells in cancer immunology is still controversial, and both immunosuppressive and immunostimulatory activities are reported (89, 90). These observations might be context dependent, and further studies are necessary.

Targeting the actions of cytokines is an attractive strategy to modulate exhausted CD8 T cells, and establishing the effective combination of cytokine-targeted therapy and immune checkpoint inhibitors is of great interest. IL-2 administration during chronic viral infection has striking synergistic effects with PD-1 blockade, enhancing virus-specific CD8 T cells and reducing viral load (91). The synergy of exogenous IL-2 and PD-1 blockade combination therapy was also confirmed in a cancer model in conjunction with tumor-antigen-targeting antibody and T cell vaccine (92). Blockade of IL-10R also improved the efficacy of anti-PD-L1 treatment during chronic viral infection, enhancing immune response and viral clearance (93). Given this variety of possible combination therapies, it is critical to establish the immunological rationale to obtain synergistic effects in modulating T cell exhaustion and improving biological outcomes.

## EPIGENETICS OF T CELL EXHAUSTION

T cell exhaustion is regulated at an epigenetic level. Comprehensive whole-genome analysis of chromatin accessibility has shown that exhausted CD8 T cells have a distinct epigenetic signature (8–11, 94, 95). For instance, exhausted CD8 T cells lack several open chromatin regions present at the *Ifng* locus in effector and memory cells (11). In contrast, open chromatin regions specific to exhausted CD8 T cells were identified for immune checkpoint molecules such as PD-1 (8, 11). The unique epigenetic landscape for exhausted CD8 T cells was similar in virus-specific CD8 T

cells in mice and in humans during chronic viral infections, suggesting that epigenetic changes associated with exhaustion are conserved (8).

The demonstrations that CD8 T cell exhaustion is heritable and associated with epigenetic changes have important implications for therapies aiming to restore T cell function. Even though treatment with anti-PD-L1 antibody restores some functions in exhausted CD8 T cells, the epigenetic signature of reinvigorated CD8 T cells presents only minimal changes (11, 95). Consequently, after cessation of PD-1 therapy, the gene expression profile in reinvigorated CD8 T cells returns to the original exhausted state (11, 95). These data suggest that for long-term results it is insufficient to simply block immune checkpoint molecules such as PD-1, and that exhausted CD8 T cells may need to be epigenetically reprogrammed to achieve long-lasting reinvigoration. A similar phenomenon of resilience due to epigenetic stability was previously reported for tolerant T cells (96).

More recently, it was reported that DNA methyltransferase 3A (DNMT3A), which is responsible for de novo genomic DNA methylation (97), is required to establish the exhausted epigenetic program (98). This study also found that exhausted CD8 T cells within tumors underwent significantly greater proliferation when anti-PD-L1 antibody was combined to DNA methylation inhibitor decitabine (98). These data are the first to suggest that combining immune checkpoint inhibitors with an epigenetic-targeted therapy may have a synergistic effect on T cell responses. Currently, several drugs that target molecules with chromatin- or DNA-modifying activity are undergoing clinical trials in different cancers (99). Whether and how these epigenetic modifying compounds affect immune cells will be of great interest.

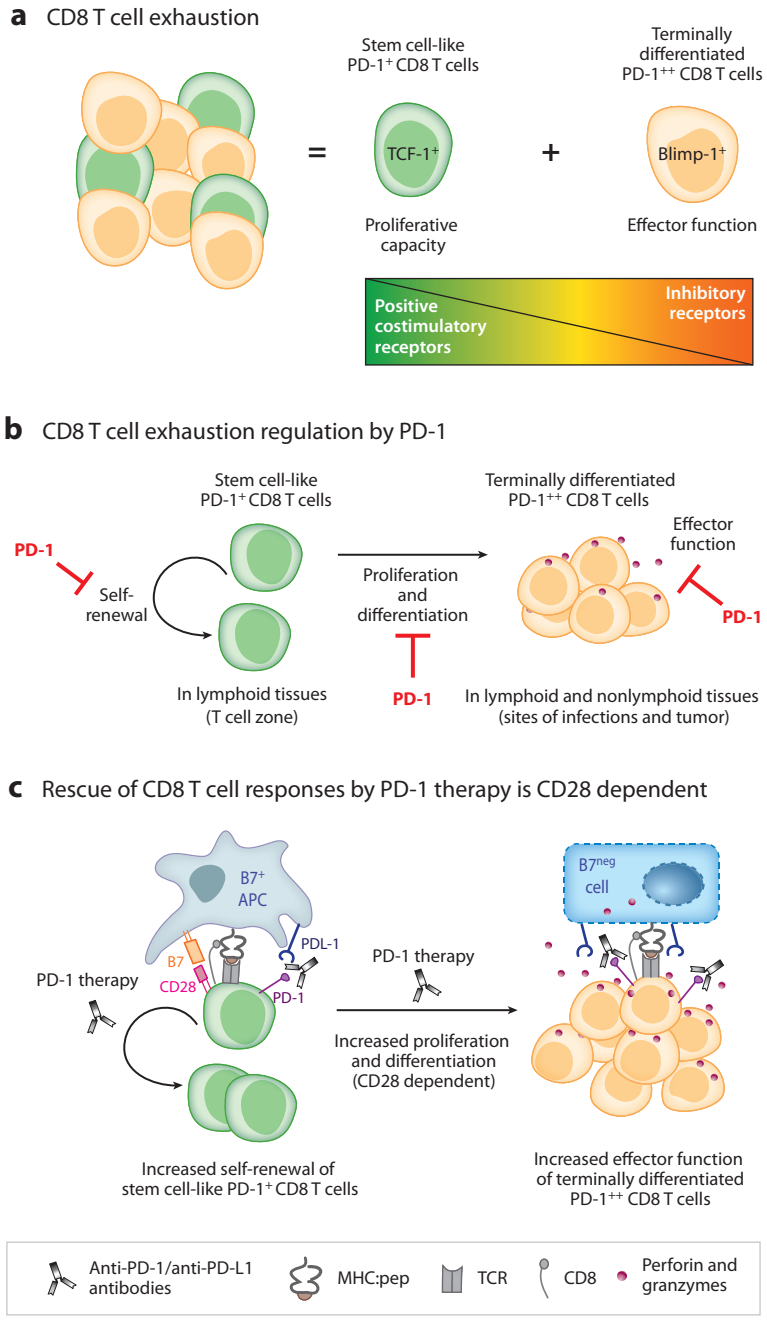
## DISCOVERY OF A STEM CELL–LIKE CD8 T CELL SUBSET THAT PROLIFERATES IN RESPONSE TO PD-1 THERAPY

We recently found that during chronic viral infection, the pool of virus-specific CD8 T cells comprises two distinct PD-1-expressing subsets: one stem cell–like and the other terminally differentiated/exhausted (**Figure 3*a***) (20). The stem cell–like CD8 T cells have a transcriptional program driven by TCF-1 (T cell factor 1)/Bcl-6 with a gene expression signature similar to those of CD8 memory precursor cells, CD4 follicular helper T ($T_{FH}$) cells, and hematopoietic stem cells. In contrast, the more differentiated virus-specific CD8 T cell subset has the characteristic gene signature of exhausted T cells. In addition to striking differences in transcription factors, effector molecules, and chemokines, the two subsets also differ in the expression of inhibitory receptors and costimulatory molecules. Although both subsets express PD-1, the stem cell–like CD8 T cells

**Figure 3**

Regulation and maintenance of CD8 T cell exhaustion. (*a*) Two distinct subsets of PD-1-expressing CD8 T cells make up the pool of exhausted CD8 T cells. A few of the important differences between stem cell–like and terminally differentiated PD-1-expressing CD8 T cells are highlighted. (*b*) Lineage relationship and regulation by PD-1 of stem cell–like PD-$1^{+}$ and terminally differentiated PD-$1^{++}$ CD8 T cell subsets. Stem cell–like PD-$1^{+}$ CD8 T cells undergo self-renewal and also generate terminally differentiated PD-$1^{++}$ CD8 T cells. The PD-1 pathway regulates proliferation/differentiation of stem cell–like PD-$1^{+}$ CD8 T cells. Terminally differentiated PD-$1^{++}$ CD8 T cells migrate to sites of infection or tumor. The PD-1 pathway dampens effector function of terminally differentiated PD-$1^{++}$ CD8 T cells, hampering control of infections and tumors. (*c*) PD-1 and CD28 regulation of exhausted CD8 T cell reinvigoration. TCR and CD28 signaling are required for proliferation/differentiation of stem cell-like PD-$1^{+}$ CD8 T cells during PD-1 therapy. However, blockade of PD-1 inhibitory signals may improve the effector function of terminally differentiated PD-$1^{++}$ CD8 T cells to eliminate target cells (infected/tumor) in a CD28-independent manner. Abbreviations: APC, antigen-presenting cell; Blimp-1, B lymphocyte-induced maturation protein 1; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; TCF-1, T cell factor 1; TCR, T cell receptor.

have low or no expression of the inhibitory receptors such as 2B4 and TIM-3, and they have higher levels of costimulatory molecules like CD28 and ICOS (inducible T cell costimulator) compared to the terminally differentiated CD8 T cells. The divergent transcriptional profiles of these two virus-specific CD8 T cell subsets raise interesting questions about their epigenetic regulation. Future studies are necessary to delineate how these two subsets are regulated at the epigenetic level.



The stem cell–like CD8 T cells are critical for maintaining the pool of virus-specific CD8 T cells during chronic infection. Not only do these cells undergo a slow self-renewal but they also proliferate and differentiate into the more terminally differentiated CD8 T cells. Most importantly, proliferative capacity is preserved predominantly in the stem cell–like CD8 T cells, and it is this population that almost exclusively provides the proliferative burst seen after PD-1 blockade (**Figure 3*a*,*b***). Our studies in chronic LCMV infection show that PD-1 therapy substantially enhanced the proliferation and differentiation of stem cell–like CD8 T cells into terminally differentiated CD8 T cells and also slightly increased the number of stem cell–like CD8 T cells (**Figure 3*b*,*c***) (20).

There are also interesting differences regarding the tissue distribution and localization of the two CD8 T cell subsets. Stem cell–like CD8 T cells are present predominantly in the T cell zones of lymphoid tissues and these cells are rarely detected in nonlymphoid tissues, whereas terminally differentiated CD8 T cells localize at major sites of infection (and possibly in tumor) in both lymphoid and nonlymphoid tissues. We hypothesize that stem cell–like CD8 T cells continuously interact with DCs in the T cell zones, which may provide niches for maintaining this stem cell–like subset by protecting it from exposure to the high levels of antigen present on infected or tumor cells. The localization of stem cell–like CD8 T cells (in proximity to $B7^+$ DCs in the T cell zone) is consistent with the requirement of CD28 signals for T cell reinvigoration by PD-1-targeted immunotherapy (64). Terminally differentiated CD8 T cells preferentially interact with infected or tumor cells that lack B7 ligands and thus do not provide CD28 signals. Accordingly, stem cell–like CD8 T cells have higher levels of CD28 expression than terminally differentiated CD8 T cells (20). However, given that PD-L1 expression in infected nonhematopoietic cells was shown to suppress viral clearance and prevent immunopathology during chronic viral infection (103), PD-1/PD-L1 interactions may play a CD28/B7-independent role by inhibiting the cytotoxic activity of the terminally differentiated CD8 T cells in the infected tissues or tumor (**Figure 3*c***).

We propose that PD-1 blockade modulates the T cell response in two distinct ways. First, PD-1 blockade induces the proliferation and differentiation of stem cell–like CD8 T cells in a CD28-dependent manner into terminally differentiated CD8 T cells that will go to sites of infection or tumor. This will result in a substantial increase in the number of antigen-specific CD8 T cells in the infected tissues/tumor. Then blockade of the PD-1 pathway at the target site will unleash effector functions of the antigen-specific CD8 T cells to efficiently kill virally infected cells or cancer cells (**Figure 3*c***). This increased effector function at the site of infection/tumor after PD-1 blockade would not be CD28 dependent, since virally infected nonhematopoietic cells and tumor cells do not express B7-1/B7-2.

The $PD\text{-}1^+$ stem cell–like CD8 T cells that we have defined during chronic LCMV infection of mice may represent a specific adaptation of the CD8 T cell response to chronic antigenic stimulation. In fact, several recent studies have described similar virus-specific CD8 T cells with a $T_{FH}$-like program in other chronic viral infection models of mice and nonhuman primates, and also in human chronic viral infections (100–102, 104–108). It will be of considerable interest to determine if similar stem cell–like CD8 T cells are present in cancer patients and, if so, whether such cells are located in the tumor itself or only in draining lymph nodes and other lymphoid tissues.

## CONCLUSION

During the past few years, our understanding of T cell exhaustion and the role of PD-1 has shown great progress at the molecular and epigenetic levels. Now, the distinction between stem cell–like and terminally differentiated cell subsets of exhausted CD8 T cells has been uncovered. Given that these two cell subsets have distinct expression patterns of inhibitory receptors and costimulatory molecules, delineating how each immunotherapeutic intervention influences these

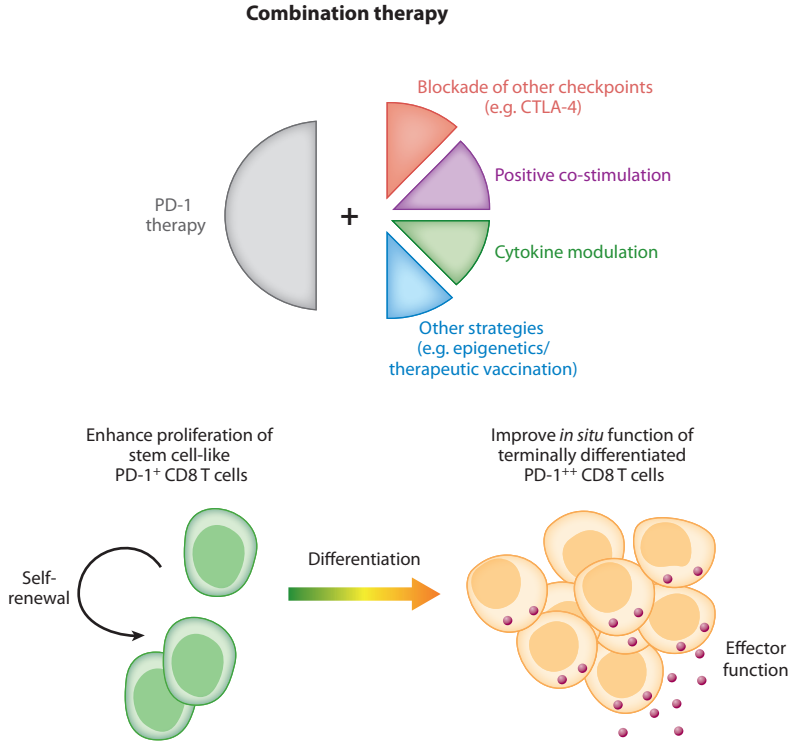



**Figure 4**

The future of immunotherapy. There are numerous opportunities to modulate immune response. Optimal strategies will emerge when the specific biological effects of each intervention are understood at the cellular and molecular levels. How each intervention modulates the newly described subsets of PD-1-expressing CD8 T cells needs to be taken into account. Abbreviations: PD-1, programmed cell death 1; CTLA-4, cytotoxic T lymphocyte antigen-4.

two subsets is important for understanding the mechanistic basis of the efficacy of current and future immunotherapies that target exhausted CD8 T cells (**Figure 4**). Moreover, insights obtained by basic research are fundamental to the development of novel CD8 T cell immunotherapeutic strategies for a broad range of diseases.

## SUMMARY POINTS

1. T cell exhaustion is a unique differentiation status of antigen-specific CD8 T cells during chronic infections and cancer that is characterized by distinct molecular/epigenetic signatures from naïve, effector, or memory CD8 T cells.
2. Overexpression of inhibitory receptors such as PD-1 dampens function in exhausted CD8 T cells, which can be partially restored by blocking this pathway using immune checkpoint inhibitors.
3. A stem cell–like CD8 T cell subset is present among exhausted CD8 T cells during chronic viral infection, and this population expands in response to PD-1-targeted immunotherapy.

4. Combining immune checkpoint inhibitors with agents targeting other coinhibitory/costimulatory molecules, cytokines, or epigenetic programs is a promising approach to improve immunotherapies.
5. More detailed understanding of T cell exhaustion, especially of the stem cell–like CD8 T cell subset, is essential to develop effective combination immunotherapies.

### FUTURE ISSUES

1. How can we improve and prolong the reinvigoration of T cell responses?
2. Does a population of stem cell–like CD8 T cells also exist in cancer? Where are these cells located (tumor, tertiary lymphoid structures in the tumor, lymph nodes, etc.)?
3. How are the different subpopulations of PD-1-expressing CD8 T cells (stem cell–like and terminally differentiated exhausted cell subsets) generated and maintained?
4. How do stem cell–like and terminally differentiated CD8 T cells respond to different immunotherapies?
5. What are the underlying reasons for PD-1-targeted therapy failure in cancer patients?
6. Why do most patients who respond to therapy not achieve complete responses?

## DISCLOSURE STATEMENT

R.A. holds patents and receives patent royalties related to the PD-1 inhibitory pathway. The remaining authors declare no competing financial interests.

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