Advance of immune checkpoint inhibitors in CNS disease

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Abstract: Immune checkpoint inhibitors, innovative immunotherapies that include programmed cell death 1, programmed cell death ligand 1, and cytotoxic T lymphocyte antigen 4 inhibitors, have achieved unprecedented benefits in a variety of malignancies. Activation of immune response in body organs may cause immune-related adverse reactions involving the central nervous system. There is growing evidence that immune checkpoint plays an important role in the central nervous system. Immune checkpoints play key roles in regulating the immune response of the central nervous system in a variety of situations, and immune checkpoint modulators are promising therapeutic agents for the treatment of central nervous system disorders such as brain tumors, Alzheimer's disease, ischemic stroke, multiple sclerosis and cognitive function. Further understanding of immune checkpoints signaling of cell types such as glial cells, neurons, and peripheral immune cells in the central nervous system will provide clues to immune regulation and barrier-breaking strategies for treating brain diseases. This article will discuss the application of common immune checkpoints in the treatment of central nervous system diseases, especially programmed cell death protein-1 and cytotoxic T lymphocyte-associated protein 4.
Keywords: Central nervous system; Immune checkpoints; Immunotherapy; PD-1; CTLA-4.

INTRODUCTION

The primary immune goal of T cells is to recognize microorganisms and antigens that may be associated with cancer and to kill microorganisms or cancer cells through various effector mechanisms. To maintain normal life, T cells can immunologically tolerate certain antigens through a variety of tolerance mechanisms. T-cell immune tolerance can be divided into central immune tolerance and peripheral immune tolerance according to the site of T-cell release, and one of the important mechanisms of peripheral tolerance is provided by coinhibitory receptors on the surface of T cells, which are collectively referred to as immune checkpoints (Schildberg, Klein, Freeman, & Sharpe, 2016). Immune checkpoints are expressed not only on T cells but also on various other types of immune cells and play important roles in maintaining self-tolerance and limiting the duration and intensity of immune responses by regulating the balance of costimulatory and coinhibitory signaling pathways.

Programmed cell death protein-1 (PDCD1; PD-1) is one of the most critical cosuppressor receptors that plays an important role in peripheral immune tolerance. PD-1 is widely expressed in various cell types,

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including T cells, macrophages, DCs and natural killer (NK) cells. The ligands of PD-1 are PD-L1 and PD-L2, and PD-L1 is widely expressed on antigen-presenting cells (APCs), macrophages, endothelial cells and cancer cells (Eppihimer *et al.,* 2002; Latchman *et al.,* 2001). During the activation of effector T cells in peripheral tissues, PD-1 binds to ligands and phosphorylates specific tyrosine residues in its cytoplasmic tail. This phosphorylation leads to the recruitment of protein tyrosine phosphatases that antagonize important signaling events associated with T-cell activation downstream of TCR and CD28.

Another cosuppressor receptor that plays an important role in immune tolerance is Cytotoxic T lymphocyte-associated protein 4 (CTLA-4; CD152). This cytokine is homologous to CD28 and is expressed mainly in dendritic cells (DCs), memory T cells and regulatory T cells (Tregs). During T-cell initiation in secondary lymphocyte organs, CTLA-4 levels on mature CD4$^+$ and CD8$^+$ T cells are temporarily upregulated, and high-affinity CTLA-4 steals costimulatory ligands from CD28, affecting T-cell initiation (Brunner *et al.,* 1999; Linsley *et al.,* 1994; van der Merwe, Bodian, Daenke, Linsley, & Davis, 1997). In addition, Tregs constitutively express CTLA-4 and indirectly inhibit T-cell activation through Tregs during the activation of peripheral tissues (Kerdiles *et al.,* 2010; Takahashi *et al.,* 2000; Wing *et al.,* 2008).

V domain-containing Ig suppressor of T-cell activation (VISTA), a member of the B7 family, is a unique immune checkpoint that, in addition to being a coinhibitory ligand expressed on APCs, is also widely expressed as a receptor in T cells, neutrophils, dendritic cells, monocytes and microglia. VISTA has two ligands, P-selectin glycoprotein ligand 1 (PSGL-1) and V-set and Ig domain containing 3 (VSIG3), the latter of which is expressed on only neurons and glial cells of the brain and Sertoli cells of the testis (Harada, Suzu, Hayashi, & Okada, 2005). Its interaction with VISTA inhibits the release of the cytokines IFN-γ, IL-2, and IL-17 (Wang *et al.,* 2019). Lymphocyte-activating gene 3 (LAG-3) is a member of the immunoglobulin superfamily and an important immune checkpoint. LAG-3 may inhibit the activation of host cells and enhance immune response suppression (Durham *et al.,* 2014). Although it is also expressed in NK cells, neurons, B cells, and plasmacytoid dendritic cells (pDCs), it has been studied mainly in regulatory T cells (Tregs) and conventional T cells (Shi *et al.,* 2021). The receptor for T-cell immunoglobulin and mucin-containing protein-3 (TIM-3) is expressed on DCs,

macrophages, T cells and NK cells. TIM-3 promotes the development and maintenance of immune tolerance by inducing T-cell apoptosis or innate immune cell suppression (G. Han, Chen, Shen, & Li, 2013).

Immune checkpoints are expressed in various resident cells of the central nervous system (CNS), including microglia, astrocytes, neurons and endothelial cells. In addition, these checkpoints are also expressed in peripherally infiltrating immune cells, such as T lymphocytes, NK cells, and macrophages (Table 1). PD-1 signals suppress the CNS immune response by resident microglia and infiltrating peripheral immune cells, and are also expressed in astrocytes and neurons (J. Zhao, Roberts, Wang, Savage, & Ji, 2021). PDL1 is highly expressed on astrocytes and microglia under inflammatory conditions (Yshii, Hohlfeld, & Liblau, 2017). In the CNS, CTLA-4 is expressed in CD4$^+$ T cells and CD8$^+$ cytotoxic T lymphocytes (CTLs), which inhibits T cell activation and thus participates in central immune regulation(Liu & Zheng, 2020). VISTA, LAG3 and TIM-3 are all mainly expressed in microglia, and they are differentially expressed in CNS pathologies (Borggrewe *et al.,* 2018; Z.-Q. Chen *et al.,* 2019; Morisaki, Ohshima, Suzuki, & Misawa, 2023).

IMMUNE CHECKPOINT INHIBITORS AS CANCER THERAPIES AND irAEs

Cancer immunotherapy is a type of immunotherapy in which the host immune system is used to recognize and destroy cancer cells. To evade immune surveillance, cancer cells have developed several mechanisms to induce immune tolerance states and evade immune destruction, one of which is the use of immune checkpoints. Immune checkpoint inhibitors (ICIs) are mainly inhibitory antibodies that target PD-1 and CTLA-4; these agents prevent tumor-related antigens from triggering activation of signaling pathways and have inhibitory effects on a variety of tumors. To date, the US Food and Drug Administration (FDA) has approved seven types of ICIs including Ipilimumab, Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab, Avelumab and Cemiplimab, which are now used to treat various types of cancer, including bladder cancer, head and neck cancer, and different types of advanced non-small cell lung cancer. Specific immune-related adverse effects (irAEs) caused by ICIs include colitis, dermatitis, pneumonia, and, less commonly, neurotoxicity and myocarditis. (Johnson *et al.,* 2019; S. Khan & Gerber, 2020; Yshii *et al.,* 2017).

Cell type	Immune checkpoints
Microglia	PD-1 (J. Zhao *et al.,* 2021), PD-L1 (Yshii *et al.,* 2017), VISTA (Borggrewe *et al.,* 2018), LAG3 (Morisaki *et al.,* 2023), TIM-3 (Z.-Q. Chen *et al.,* 2019)
Astrocytes	PD-1 (J. Zhao *et al.,* 2021), PD-L1 (Yshii *et al.,* 2017)
Neurons	PD-1 (J. Zhao *et al.,* 2021), LAG-3 (Angelopoulou, Paudel, Villa, Shaikh, & Piperi, 2020)
Endothelial cells	PD-1 (Tobias, Steinberger, Drinić, & Wiedermann, 2021), PD-L1 (Eppihimer *et al.,* 2002), VISTA (X. Huang *et al.,* 2020), LAG3 (Shi *et al.,* 2021), TIM-3 (L. Zhao, Cheng, Fan, Zhang, & Xu, 2021)
Macrophages	PD-1 (W. Li *et al.,* 2022), PD-L1 (W. Li *et al.,* 2022), VISTA (X. Huang *et al.,* 2020), LAG3 (Shi *et al.,* 2021), TIM-3 (G. Han *et al.,* 2013)
Natural killer cells	PD-1 (Tobias *et al.,* 2021), LAG-3 (Shi *et al.,* 2021), TIM-3 (G. Han *et al.,* 2013)
CD4+ and CD8+ T cells	PD-1 (Tobias *et al.,* 2021), CTLA-4 (Liu & Zheng, 2020), VISTA (X. Huang *et al.,* 2020), LAG-3 (Shi *et al.,* 2021), TIM-3 (G. Han *et al.,* 2013)
Dendritic cells	PD-1 (Tobias *et al.,* 2021), PD-L1 (Eppihimer *et al.,* 2002), CTLA-4 (Ghorbaninezhad *et al.,* 2022), VISTA (X. Huang *et al.,* 2020), LAG-3 (Shi *et al.,* 2021), TIM-3 (G. Han *et al.,* 2013)

Table 1. Expression of immune checkpoints in various cell types of central nervous system.

IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED NEUROLOGICAL irAEs

ICIs can cause neurological irAEs involving the central, peripheral, or autonomic nervous system, with an incidence of 1%-5% (Kao, Brickshawana, & Liewluck, 2018; Larkin *et al.,* 2017), and 80% of neurological irAEs have been reported to occur within the first four months of ICI therapy (Spain *et al.,* 2017). CNS complications associated with ICIs include aseptic meningitis, necrotizing encephalitis, brainstem encephalitis, transverse myelitis, etc. (Haugh, Probasco, & Johnson, 2020). Recently, Cristina Valencia-Sanchez *et al.* retrospectively analyzed 31 patients with CNS autoimmunity caused by ICI treatment of malignant tumors. The clinical characteristics of spontaneous paraneoplastic syndromes (PNSs) were compared with those of spontaneous paraneoplastic syndromes (PNSs). The results showed that 62% of patients using ICIs had MRI abnormalities, 70% had inflammation in the cerebrospinal fluid, and 47% had neuroautoantibodies detected in the serum, which is more common than in patients with neuroendocrine tumors. Ninety-seven percent of ICI-related CNS autoimmune patients were forced to stop using ICIs, and 39% of patients had unfavorable outcomes (Valencia-Sanchez *et al.,* 2023). In irAEs, involvement of the peripheral nervous system has been shown to be more common than that of the CNS; this involvement includes peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP), acute inflammatory demyelinating polyneuropathy (AIDP), myasthenic syndrome, etc. (Cuzzubbo *et al.,* 2017; E. Khan *et al.,* 2022).

IMMUNE CHECKPOINT MODULATORS IN NERVOUS SYSTEM DISEASE

Glioma

Several common immune checkpoints are beginning to be used to treat CNS diseases such as glioma, neurodegenerative diseases, and stroke (Table 2). Gliomas are the most common primary tumors of the CNS. Although relatively rare, they can cause significant morbidity and mortality. High-grade glioma or glioblastoma (GBM) is the most aggressive and deadly form of glioma, with a survival rate of only 12-15 months, even with current treatments, such as surgical resection, chemotherapy and radiation (Ostrom *et al.,* 2014). In the past decade, there have been no major advances in the treatment of glioma (Yeo & Charest, 2017).

It is now widely accepted that the CNS has an innate immune system and is connected to the peripheral immune system. Glioma cells secrete different types of chemokines, cytokines and growth factors, inducing the infiltration of a series of immune cells. In addition to central resident immune cells, namely, microglia, there are also various peripheral immune components in the glioma microenvironment. Macrophages, myeloid-derived suppressor cells (MDSCs), natural killer cells, and tumor-infiltrating lymphocytes (TILs) all play important roles in the glioma microenvironment. Immune cells are highly inhibited in the glioma microenvironment of the CNS through various mechanisms, which makes glioma cells prone to immune escape and subsequent growth and rapid development. First, the unique immune state of the brain (to prevent an inflammation-mediated intracranial hypertension crisis, the brain encourages only limited immunity) limits the activity and number of TILs in the brain, and Treg cells secrete the anti-inflammatory cytokines IL-10 and TGF-β, which play important inhibitory roles in the inflammatory immune response in tumors (Gong *et al.*, 2012; Vitkovic, Maeda, & Sternberg, 2001). Second, the expression of certain genes in glioma itself also leads to the inhibition of TILs. For example, tumor-derived Fas ligands promote the apoptosis of activated T cells by inhibiting the maturation of DCs and T cells, leading to immune escape of tumor cells (Jansen *et al.*, 2010). Overexpression of PD-L1 in glioma cells prevents T-cell activation and induces T-cell apoptosis by binding to PD-1 (Butte, Keir, Phamduy, Sharpe, & Freeman, 2007). In addition, due to the lack of CD80/86 costimulatory molecules in glioma cells, CTLA-4 is overexpressed in CD4^{+} T cells and CD8^{+} CTLs, which inhibit T-cell activation (Wintterle *et al.*, 2003). Microglia are antigen-presenting cells of the CNS that participate in the immune response and are crucial for maintaining the stability of the CNS environment. Microglia can mediate tumor microenvironment immunosuppression by regulating mammalian target of rapamycin (mTOR), hindering the infiltration, proliferation and immune response of effector T cells, thus contributing to tumor immune escape and promoting the growth of glioma (Dumas *et al.*, 2020).

Given that immune cells are strongly inhibited in the glioma microenvironment through multiple mechanisms, the role of immune checkpoint inhibitors in the treatment of gliomas is also of concern. The application of immune checkpoint inhibitors has improved the survival rate of patients with

different types of cancer. In glioma cases, different immune checkpoint molecules, such as CTLA-4, PD-1, TIM-3, and LAG-3, have been described, and these receptors have corresponding ligands (Roesch, Rapp, Dettling, & Herold-Mende, 2018; Tomaszewski, Sanchez-Perez, Gajewski, & Sampson, 2019). Many drugs tested in clinical trials for patients with GBM fail due to their inability to be delivered across the blood-brain barrier (T. Li *et al.*, 2022), thus compromising their therapeutic effectiveness against intracranial tumors and making it critical to design unique immunotherapy strategies for the CNS. Studies have shown that anti-PD-1 agents can irreversibly bind to PD-1 or CTLA-4 on peripheral lymphocytes and then penetrate the blood-brain barrier, and once they successfully pass through the blood-brain barrier, they can bind to tumor-infiltrating lymphocytes and exert therapeutic effects (van Bussel, Beijnen, & Brandsma, 2019). To address the inability of CTLA-4 antibodies to cross the blood-brain barrier, Galstyan *et al.* attempted to combine immunotherapy with nanotechnology by delivering nanoscale immunocoupling (NIC) drugs across the blood-brain barrier to treat GBM (Galstyan *et al.*, 2019). They used a multifunctional drug carrier and poly (β-L-malic acid) (PMLA), a natural polymer, to deliver covalently coupled CTLA-4 and PD-1 antibodies to brain tumor cells. This led to local immune system activation and prolonged survival in glioblastoma mice. Currently, clinical trials of anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) agents are being conducted in glioma patients to test their safety, toxicity and efficacy (J. Huang *et al.*, 2017).

Of course, recent studies have shown that the use of ICIs in combination with multiple treatments can enhance the efficacy of glioblastoma immunotherapy. For example, targeting microglial metabolic recombination can synergically enhance the effect of immune checkpoint therapy on glioblastoma. Specifically, in the immune microenvironment of glioblastoma, targeted inhibition of NR4A2 reduces oxidative stress in microglia, which can enhance the antigen-presenting function of microglia against CD8^{+} T cells, thus improving the therapeutic effect of immune checkpoint blockade in vivo (Ye *et al.*, 2023).

Neurodegenerative disease

Almost all neurodegenerative diseases are accompanied by chronic neuroinflammation, and anti-inflammatory and immunosuppressive therapies have

shown some efficacy in treating neurodegenerative disease models, the most typical example being the treatment of multiple sclerosis (MS). MS is a chronic inflammatory disease of the brain and spinal cord characterized by focal lymphocyte infiltration leading to myelin and axon damage. The expression of PD-1 on T cells and PD-L1 on APCs is significantly increased in MS patients. In MS model mice, deletion of the Pdcd1 gene or blockade of PD-1 via drugs can enhance the activation and expansion of T cells and aggravate neuroinflammation and pathological changes (Ortler *et al.*, 2008). Enhancing PD-L1/PD-1 inhibition signaling is expected to be a therapeutic strategy for MS patients (J. Zhao *et al.*, 2021).

There are also arguments that systemic immunity should be enhanced in chronic neurodegenerative diseases to drive the immune-dependent cascade needed for brain repair. Alzheimer's disease (AD) is an age-related neurodegenerative disease and the most common cause of dementia. In AD model mice, systematic blockade of the immune checkpoint PD-1 can induce an IFN-γ-dependent systemic immune response and subsequently recruit monocyte-derived macrophages to the CNS to promote the clearance of amyloid beta plaques, thereby improving cognitive function. This approach aims to enhance the immune system's overall ability to clear brain pathology, suggesting immune checkpoint blockade as a novel strategy for treating Alzheimer's disease and possibly other neurodegenerative diseases (Baruch *et al.*, 2016). Other studies in AD model mice have also shown that PD-1 blockade can reduce the Aβ plaque load in the brain, and repeated anti-PD-1 therapy can produce lasting beneficial effects on AD pathology (Rosenzweig *et al.*, 2019). However, other studies have shown that the expression of PD-L1 and PD-1 in astrocytes and microglia around amyloid plaques is upregulated in AD patients and in APP/PS1 AD model mice and that the synergistic effect of soluble PD-L1 secreted by astrocytes and PD-1 in microglia promotes the uptake of Aβ by microglia. Neuroinflammation is inhibited, and the pathology of AD is improved (Kummer *et al.*, 2021). In addition, most hippocampal CA1/CA3 neurons in mice and humans also express PD-1, and PD-1 inhibits neuronal activity. Blocking the PD-L1/PD-1 axis in hippocampal excitatory neurons can enhance the excitability and synaptic plasticity of mouse neurons, thereby improving their learning and memory ability (J. Zhao *et al.*, 2023).

In addition to common immune checkpoints, genome-wide association analyses revealed a number of key microglial immune-related genes, such as CD33 (an immune receptor that inhibits microglial phagocytosis), TREM2 (a trigger receptor expressed on myeloid cells) and SHIP1 (inosito-5-phosphase 1 with a selective SH2 domain, a microglial immunocytocytosis receptor). All of them are genetic risk factors for AD (Efthymiou & Goate, 2017). Take CD33 as an example. CD33 is a transmembrane glycoprotein expressed on the surface of microglia. Increased expression of CD33 leads to inhibition of microglial activity and a decrease in phagocytic function. In the J20 amyloid mouse model, CD33 expression resulted in decreased microglia-mediated Aβ phagocytosis and increased amyloid pathology (Bradshaw *et al.*, 2013). CD33 gene knockout resulted in a pathological reduction in amyloid protein levels and cognitive improvement in 5xFAD mice (Griciuc *et al.*, 2019). In addition to traditional immune checkpoint-related therapies, the exploration of other immune target-related therapies is equally critical for treating AD, and new immune checkpoints involved in the neuropathological mechanism of AD are expected to develop.

Stroke

In many cerebrovascular diseases, neuroinflammation is closely related to neurological impairment and poor prognosis. The expression of immune checkpoints was upregulated in patients with traumatic brain injury, stroke, cerebrovascular inflammation and hemorrhagic vasospasm. There is growing evidence that immune checkpoint regulation is a promising strategy for the treatment of cerebrovascular diseases by reducing immune cell recruitment, cytokine secretion, brain edema, and neurodegeneration. For example, stroke can be an acute cerebrovascular disease caused by brain tissue damage due to sudden rupture of blood vessels in the brain or blockage of blood vessels that prevent blood from flowing to the brain. Stroke is associated with intense and persistent neuroinflammation. After stroke, there is a large increase in inflammatory factor levels in the damaged area of the brain, local microglia are activated, and damage to the blood-brain barrier leads to a large infiltration of peripheral immune cells, including macrophages, T cells, and B cells. PD-1 signaling on T cells, B cells, microglia, and macrophages is involved in poststroke neuroinflammation (Bodhankar, Chen,

Vandenbark, Murphy, & Offner, 2013a; Ren, Aki-yoshi, Dziennis, *et al.,* 2011). In an animal model of stroke, the expression of PD-1 in both microglia and macrophages was significantly upregulated, and a lack of PD-1 led to greater cerebral infarction and more severe neurological dysfunction (Qin *et al.,* 2019; Ren, Akiyoshi, Vandenbark, Hurn, & Offner, 2011). Thus, activating the PD-1 inhibitory pathway in microglia and macrophages may provide neuroprotective effects after stroke. B cells produce IL-10 and increase the expression of PD-1 on T cells, thereby providing neuroprotection against stroke. However, the specific role of PD-L1 in stroke remains controversial. Studies have shown that PD-L1 can aggravate the inflammation of stroke patients and thus aggravate their disease. In contrast, other studies have shown that PD-L1 can significantly reduce neurological deficits and provide neuroprotection in stroke patients (Bodhankar *et al.,* 2015; Bodhankar, Chen, Vandenbark, Murphy, & Offner, 2013b; R. Han, Luo, Shi, Yao, & Hao, 2017). These opposite results suggest that PD-L1/PD-1 signaling has dual effects on CNS inflammatory conditions. PD-L1/PD-1 signaling in stroke can lead to positive or negative results, depending on the different stages of stroke. The release of catecholamines after stroke can inhibit the upregulation of CTLA-4 in activated T cells, resulting in the secretion of large amounts of proinflammatory cytokines (Vogelgesang *et al.,* 2010). In addition, CTLA-4 is needed for the immunosuppressive function of Tregs, and considering the key neuroprotective effect of Tregs (S. Chen *et al.,* 2013), CTLA-4 may also be a particularly interesting therapeutic target for poststroke inflammation (Kim, Patel, & Jackson, 2021).

CNS Disease	Immune checkpoints
Glioma	PD-1, CTLA-4, TIM-3, LAG-3
Neurodegenerative disease	PD-1
Stroke	PD-1, CTLA-4

Table 2. The applications of immune checkpoints in CNS disease including glioma, neurodegenerative disease, and stroke

CONCLUSION

There is growing evidence that immune checkpoints play important roles in the CNS. Immune checkpoints have been linked to brain tumors, Alzheimer's disease, ischemic stroke, multiple sclerosis, cognitive function and other CNS diseases. Immune checkpoint signals suppress the CNS immune response through resident microglia and infiltrating peripheral immune cells. Immune checkpoint blockade is the most promising approach for cancer immunotherapy, including for glioma treatment. Immune checkpoint blockade to enhance the immune response is a novel strategy for treating neurodegenerative diseases such as Alzheimer's disease. In multiple sclerosis, enabling immune cells (such as T cells, B cells, and DCs) to overexpress immune checkpoints to prevent excessive autoreactive immunity can significantly reduce disease severity. In many cerebrovascular diseases, immune checkpoint regulation is a promising therapeutic strategy for reducing immune cell recruitment, cytokine secretion, brain edema, and neurodegeneration. The development of new immune checkpoints is highly important for the treatment of CNS diseases.

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Conflict of Interest

The authors declare no conflict of interest.

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