# Vaccine-Based Immunotherapeutics for the Treatment of Glioblastoma: Advances, Challenges, and Future Perspectives

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**Key words**
- Brain neoplasm
- Clinical trials
- Glioblastoma
- Immunotherapeutic
- Immunotherapy
- Vaccine

**Abbreviations and Acronyms**
APC: Antigen-presenting cell
APVAC: Actively personalized vaccination
BBB: Blood–brain barrier
CMV: Cytomegalovirus
CNS: Central nervous system
CSF: Cerebrospinal fluid
DC: Dendritic cell
EGFRvIII: Epidermal growth factor receptor variant type III
GAA: Glioma-associated antigen
GBM: Glioblastoma
GSC: Glioblastoma stemlike cells
HLA-I: Human leukocyte antigen class I
HSP: Heat-shock protein
HSPPC-96: Heat-shock protein peptide complex-96
IDH1R132H: Isocitrate dehydrogenase 1 Arg132His
IL-13Rα2: Interleukin-13 receptor subunit-α2
iRANO: Immunotherapy Response Assessment in Neuro-Oncology
MHC: Major histocompatibility complex
MRI: Magnetic resonance imaging
Neovax: Neoantigen-based vaccine
OS: Overall survival
PFS: Progression-free survival
pp65: Phosphoprotein 65
RANO: Response Assessment in Neuro-Oncology
TAA: Tumor-associated antigen
TGF-β: Transforming growth factor-β
TMZ: Temozolomide
T-regs: T-regulatory cells
TSA: Tumor-specific antigen
WT-1: Wilm's tumor protein-1

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*Citation: World Neurosurg. (2018) 120:302-315. https://doi.org/10.1016/j.wneu.2018.08.202*

*Journal homepage: www.WORLDNEUROSURGERY.org*

*Available online: www.sciencedirect.com*



**Glioblastoma is a highly aggressive neoplasm with an extremely poor prognosis. Despite maximal gross resection and chemoradiotherapy, these grade IV astrocytomas consistently recur. Glioblastoma cells exhibit numerous pathogenic mechanisms to decrease tumor immunogenicity while promoting gliomagenesis, which manifests clinically as a median survival of less than 2 years and few long-term survivors. Recent clinical trials of vaccine-based immunotherapeutics against glioblastoma have demonstrated encouraging results in prolonging progression-free survival and overall survival. Several vaccine-based treatments have been trialed, such as peptide and heat-shock proteins, dendritic cell-based vaccines, and viral-based immunotherapy. In this literature review, we discuss the immunobiology of glioblastoma, significant current and completed vaccine-based immunotherapy clinical trials, and broad clinical challenges and future directions of glioblastoma vaccine-based immunotherapeutics.**

## INTRODUCTION

Glioblastoma (GBM) is an aggressive brain tumor with a dismal prognosis. The current standard of care for GBM, consisting of maximal safe resection, radiotherapy, and temozolomide (TMZ), translates into a median survival of 14.6 months and 2-year medial survival of 27%.[1] The unfortunate survival rate of GBM has been attributed in part to intratumoral heterogeneity, GBM stem cells, and various mechanisms of glioblastoma-induced immunosuppression. As such, clinical trials of vaccine-based immunotherapeutics have emerged as a potential novel treatment modality intended to overcome GBM pathogenesis and improve patient outcomes. Contrary to the standard of care, immunotherapy is unique in being highly specific to both the patient and the tumor. Recently, clinical trials of vaccine-based immunotherapies against GBM have demonstrated promising results in prolonging progression-free survival (PFS) and overall survival (OS) and by exhibiting a favorable safety profile. Several types of vaccine-based immunotherapies have been trialed, including peptides, heat-shock proteins, (DC)-based vaccines, and viral-based immunotherapies. Here, we provide a detailed discussion of GBM immunobiology, and we review the significant current and completed GBM vaccine immunotherapy trials. Furthermore, clinical challenges and future directions of vaccine-based immunotherapies are considered.

## CENTRAL NERVOUS SYSTEM "IMMUNE PRIVILEGE"

Historically, the central nervous system (CNS) was considered an immunoprivileged organ because of the presence of the blood–brain barrier (BBB) and lack of classic lymphatic vessels. However, it has been known for decades that brain tumors can provoke an immune response against tumor antigens. Although the CNS is an immunologically unique organ, the immune system is still capable of surveying CNS antigens. This process initially entails antigens acquiring entrance into the cerebrospinal fluid (CSF) by one of several mechanisms such as disruption of the BBB, direct extension of the tumor into CSF spaces, or via glymphatic clearance.[2] Subsequently, CSF antigens can be transported to cervical lymph nodes by 1 of 3 anatomic routes. First, CSF can enter recently described glymphatic vessels (i.e., meningothelial lymphatics of the dura) that drain into cervical lymph nodes.[3-6] Second, CSF can traverse the

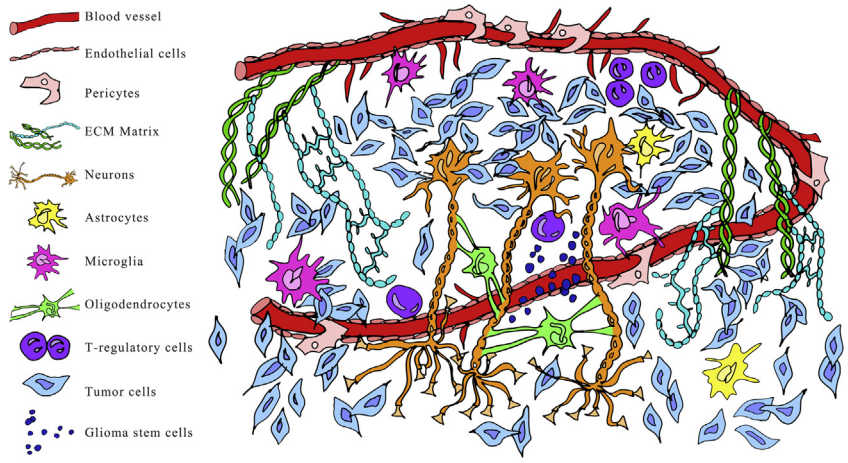


**Figure 1.** Schematic diagram of the glioblastoma microenvironment; Glioblastoma tumors create a heterogeneous microenvironment rich in neoplastic glial cells, glioblastoma stem-like cells, non-neoplastic parenchymal cells (e.g., astrocytes), neurons, immunologic cells (e.g., microglia, macrophages, T-lymphocytes), and perivascular cells (e.g., endothelial, pericytes).

cribiform plate and enter the lymphatic vessels of the nasal mucosa, which also drain into cervical lymph nodes.[7,8] The prior anatomic routes of CSF drainage are acquiescent to both antigens and immune cells. The last route to cervical lymph nodes, amendable only to CSF antigens, entails interstitial fluid transport through the basement membranes of arteries and capillaries within the brain parenchyma.[9]

Following antigen presentation and activation in cervical lymph nodes, T-cells may enter the CNS parenchyma through any of several routes. First, T-cells may pass through or between the BBB endothelial cell layer of postcapillary venules into perivascular spaces followed by infiltration of the glia limitans into the brain parenchyma.[10-12] Second, T-cells may enter the CSF from subarachnoid leptomeningeal venules followed by possible entrance into the parenchyma after passing the glia limitans.[13] Third, although not yet confirmed, experimental evidence suggests that T-cells within blood vessels may travel into the stroma of the choroid plexus and infiltrate its epithelium.[14] In the choroid plexus, T-cells may enter the ventricular system, gain access to the subarachnoid space, and, ultimately, penetrate the parenchyma.[14,15] Upon arrival at the site of the tumor (e.g., GBM), immune cells will encounter an extraordinary immunosuppressive microenvironment distinct from physiologic brain parenchyma.

## THE MICROENVIRONMENT OF GLIOBLASTOMA

Tumors are unable to thrive independently; rather, they are closely intertwined and are reliant on their proximate cellular environment. Tumor cells demonstrate the ability to adjust to their surrounding environment and alter it to their own advantage. Indeed, such a task requires complex interactions between the tumor progeny with various supporting cells. This microenvironment is a focused nook composed of neoplastic cells, immune cells, blood vessels, and stroma intertwined through an extracellular matrix (ECM).[16] The brain microenvironment is unique from peripheral environments such that the brain is largely secluded by the BBB.[16,17] Even though the CNS is not as immunologically privileged as once alleged, GBMs prefer to proliferate within its local microenvironment as even highly aggressive CNS tumors rarely metastasize outside of the brain parenchyma.[18]

To create efficacious immunotherapeutics against GBM, it is imperative to understand the molecular basis of the tumor microenvironment and how this niche supports gliomagenesis. Glioblastoma creates a heterogeneous microenvironment rich in neoplastic glial cells, glioblastoma stemlike cells (GSCs), nonneoplastic parenchymal cells (e.g., astrocytes), neurons, immunologic cells (e.g., microglia, macrophages, T-lymphocytes), and perivascular cells (e.g., endothelial, pericytes) (Figure 1).[19-21] Each cell type serves a purpose in supporting the tumor microenvironment and promoting gliomagenesis. Tumor initiating cells, known as GSCs, demonstrate the capability to maintain the malignant features of GBM, such as tumor cell inception, angioneogenesis, and proliferation as well as acquiring resistance to chemotherapeutics.[20-24] Interestingly, GSCs have recently been shown to secrete extracellular vesicles containing vascular endothelial growth factor from ex vivo cultured patient-derived GSCs, which elicited an angiogenic reaction within the microenvironment.[20] Nonneoplastic astrocytes are the most prevalent glial cell within the GBM perivascular niche. Upon activation, astrocytes can encompass and shield tumors from the gliotoxic effects of chemotherapeutics.[25] Furthermore, histologic analysis has demonstrated that infiltrating microglia composes 5% to 20% of GBM tumor mass.[26] Microglial cells actively recruit peripheral macrophages and increase neoplastic migration as well as tumor evasion.[27,28]

To meet their metabolic requirements, these tumors use numerous mechanisms of neovascularization such as angioneogenesis,[29] vasculogenic mimicry[30] (tumor synthesis of vessel-like entities), or vessel co-option[31] (tumor sequestering of physiologic vessels). Each mechanism is dependent on pericyte and endothelial recruitment, which can occur through GBM secretion of vascular endothelial growth factor.[32,33] Remarkably, GBM cells have been shown to transdifferentiate into vascular endothelial cells[34] and to generate vascular pericytes[35] in vivo to further angioneogenesis. The extracellular matrix, consisting of fibrous proteins and several other biomolecules (e.g., hyaluronic acid, glycosaminoglycans, proteoglycans), interweaves the heterogeneous microenvironment together and also relays biochemical and mechanical signals to tumor cells.[36,37] Last, neoplastic glial cells refine their microenvironment and drive pathogenesis through a multitude of immunologic mechanisms.

## MECHANISMS OF IMMUNOSUPPRESSION IN GLIOBLASTOMA

Patients with GBM can become significantly immunosuppressed. Translational research