CANCER IMMUNOLOGY

# The immunology of brain tumors

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**Brain tumors represent a unique challenge for cancer immunotherapies because of their location in an immune privileged site. However, the brain tumor immune microenvironment is dictated more by tumor type than the location within the brain per se. This feature is reflected by the higher immunogenicity and response to immunotherapies of metastatic brain tumors compared with primary brain tumors. Immunotherapies for brain tumors aim at inducing and boosting tumor T cell responses using vaccines, immune checkpoint inhibitors, or adoptive T cell therapies. A fundamental challenge in the field is how such brain tumor–targeting T cells gain access to brain tumors and maintain their function despite a hostile immunosuppressive microenvironment. Here, we review current knowledge of the cellular and molecular determinants of the antigenicity of brain tumors and the immunosuppressive brain tumor microenvironment. Expanding and exploiting this knowledge will provide the key for effective combinatorial therapies.**

## INTRODUCTION

Brain tumors pose a unique challenge for tumor immunology. They can form as intrinsic brain tumors originating from brain cells, a prominent example of which are gliomas originating from glial cells. These tumors usually do not metastasize. A second group of brain tumors, extrinsic brain tumors, originate outside the brain, which they then infiltrate or colonize. The most common types of extrinsic brain tumors are tumors metastatic to the brain, including melanoma, lung cancer, gastrointestinal cancer, or breast cancer, which typically metastasize through hematogenic seeding at a late disease stage. Brain metastases typically present as multiple lesions throughout the brain at the corticomedullary junction, whereas gliomas typically form solitary lesions in the deep white matter (Fig. 1). The distinct tumor immune microenvironment (TIME) in the central nervous system (CNS) is shaped by rich interactions between resident CNS cells such as neurons and glial cells, the CNS vasculature, and peripheral immune cells that patrol the CNS. Important interfaces at barriers such as the choroid plexus and meningeal layers are critical for the regulation of neuroimmune interactions, allowing for a bidirectional cross-talk between the CNS and peripheral immune system. Although CNS lesions involving breakdown of such barriers are associated with massive infiltration of peripheral immune cells, such infiltration may also occur through intact barriers, as exemplified in autoimmune neuroinflammation. Although the barrier function of the CNS is important for shaping the TIME, the distinct TIMEs of primary versus secondary brain tumors highlighted here provide evidence that the TIME is also critically shaped by the tumor itself.

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## Immunobiology of gliomas

The immunobiology of gliomas and brain metastases is quite different, because of their primary location, antigenicity, and clonal heterogeneity. Gliomas typically form over years, sometimes decades, and often originate from genetic driver mutations in neural or glial precursor cells. Typical driver mutations in gliomas are mutations in the genes for isocitrate dehydrogenase type 1 (IDH1) or histone H3 (Fig. 1). These mutations result in profound epigenetic changes, genomic instability, and subsequent acquisition of additional tumor-promoting genetic alterations such as amplifications and activating mutations of oncogenes or deletions or truncating mutations of tumor suppressor genes (*1*). The most malignant types of gliomas, glioblastomas, typically harbor several such tumor-promoting genetic alterations without a clear driver mutation (*2*). Because gliomas evolve slowly, there is high clonal heterogeneity. Adding to this complexity, single glioma cells transition between multiple cellular states in response to microenvironmental cues, which are associated with distinct transcriptional programs linked to mesenchymal, neural, or astrocytic cellular identities (*3*, *4*).

Most cells of glioma origin reside in the subventricular zone (*5*). During invasive progression, glioma cells preferentially colonize the perivascular niche, where they can long remain quiescent and maintain a stem-like, slow-cycling phenotype (*6*). This niche between the astrocytic end feet forming the glia limitans and endothelial cells of brain microvessels forming the blood-brain barrier (BBB) (Fig. 1) is similar to the neurogenic perivascular niche during development (*7*, *8*). Glioma cells typically retain or reactivate additional key features of neural or glial precursor cells (*9*). These features include a diffusely infiltrative growth pattern and the ability to form networks with other glioma cells and neurons. In addition, glioma cells form networks hijacking neuronal programs and connecting structures. Such networks and communicating activities may also shape the TIME, as has been recently reviewed (*10*).

Therefore, local therapies consisting of maximal safe resection and radiotherapy as standard-of-care modalities cannot cure gliomas (*11*). In particular, glioblastomas frequently recur within a year. Alkylating chemotherapy, also part of standard of care, may induce longer remissions in some types of gliomas but cannot prevent relapse, which is often associated with tumors that are more aggressive than the primary tumor (*11*). Despite decades of clinical trials, agents

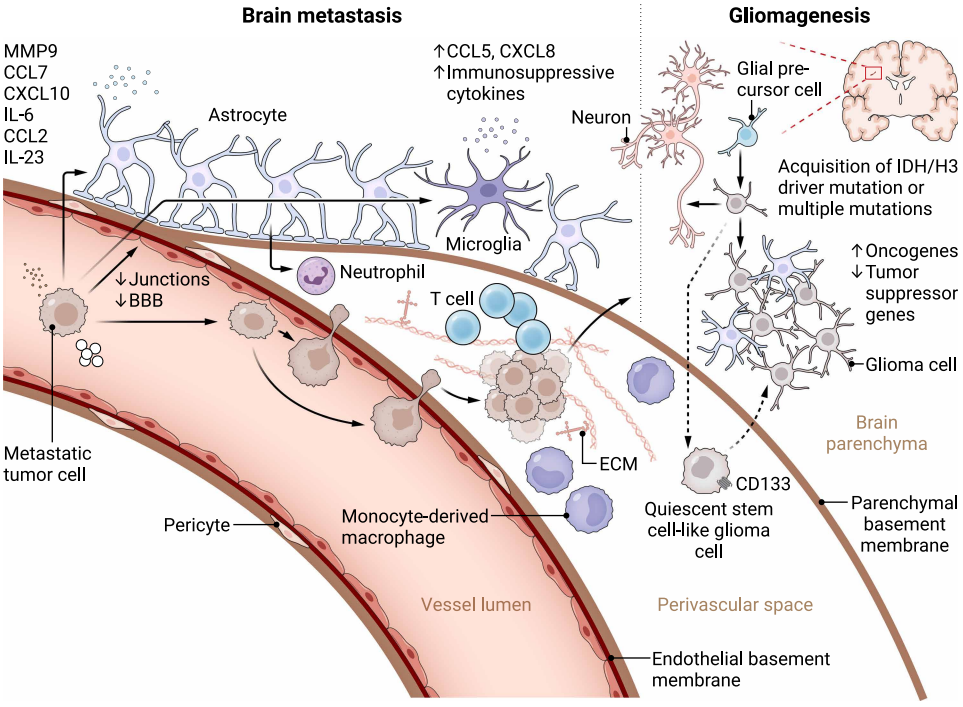


**Fig. 1. Development of brain metastases and gliomas.** Left: Brain metastatic cascade. Circulating tumor cells that have intravasated into the blood vessels secrete soluble factors like chemokines and microRNA-loaded extracellular vesicles, thereby affecting the local CNS microenvironment and inducing premetastatic niche formation. This involves loss of endothelial junctions and BBB integrity, microglial secretion of immunosuppressive cytokines and chemokines, and reprogramming of astrocytes, which secrete cytokines, chemokines, and other soluble factors to recruit tumor cells and facilitate BBB breakdown. Later, reprogrammed astrocytes and microglia promote metastatic niche formation and metastatic outgrowth. Via selectins, tumor cells adhere to the local CNS endothelium and extravasate via transcellular or paracellular routes into the perivascular space between endothelial and parenchymal basement membranes, where they, supported by the ECM, form micrometastases and exploit local blood supply by vessel co-option or angiogenesis before infiltrating the brain parenchyma. Right: Gliomagenesis. In contrast, gliomas originate from glial precursor cells in the brain parenchyma via IDH or H3 driver or multiple mutations and suppression of tumor suppressor and/or activation of oncogenes. They colonize the perivascular space during invasive progression, where they retain a quiescent stem cell–like phenotype before outgrowth. In the parenchyma, glioma cells form functional networks with neurons, astrocytes, and malignant cells to facilitate tumor growth and invasion. At the same time, they render their microenvironment immunosuppressive.

specifically targeting dysregulated cell signaling pathways in glioma cells such as tyrosine kinase inhibitors have largely failed to show efficacy. This failure is due to the lack of selective vulnerability of glioma cells, high clonal heterogeneity and cellular plasticity, and the failure of many such agents to achieve sufficient drug levels in the tumor because of low blood-brain permeability. The recent approval of the selective inhibitor of mutant IDH vorasidenib marks the first approval of a targeted agent specifically developed for the treatment of gliomas (*12*). The mutant IDH enzyme produces the oncometabolite 2-hydroxyglutarate (2-HG), which blocks the normal differentiation of glial precursor cells, resulting in gliomagenesis. By blocking the production of 2-HG, IDH inhibitors induce redifferentiation of glioma stem cells and thereby slow tumor growth, as well as increase the number and activity of T cells infiltrating IDH-mutant gliomas (*13*) through multiple mechanisms (see below).

## Immunobiology of brain metastases

In contrast with gliomas, brain metastases develop from circulating tumor cells from a primary solid tumor outside the CNS. Ten to 40% of patients with solid tumors develop brain metastases, with increasing incidence (*14*), probably because of improved therapy of the primary tumor and improved diagnosis. Like primary brain tumors, brain metastases are associated with a poor prognosis of ~4 to 12 months (*15*, *16*). Metastases are initiated when tumor cells undergo epithelial-to-mesenchymal transition, migrate from the primary tissue site, and intravasate into the blood vessel. The formation of brain metastases requires extravasation through the BBB, a highly regulated barrier of complex functional and anatomical interactions between multiple cell types.

In addition to endothelial cells, the BBB is composed of pericytes, a type of mural cell covering microvessels, and astrocytes, which lie in close contact to neurons and microglia, which are the CNS-resident myeloid cells that derive from hematopoietic precursors and migrate from the yolk sac during development (*17*). The boundary between the endothelial layer and the brain parenchyma is composed of the endothelial basement membrane, in which pericytes form a layer around endothelial cells, and the parenchymal basement membrane called the glia limitans, which is formed by compacted astrocytic end feet and connected to microglial end feet. Mostly microscopically indistinguishable under physiologic conditions and at the capillary level, these two layers can be separated by the perivascular space, which is particularly prominent at the postcapillary level and during neuroinflammation (*18*–*20*). The perivascular space can be considered a regulatory checkpoint for CNS-infiltrating leukocytes, especially macrophages, because they congregate within these spaces to form perivascular cuffs before entering the brain parenchyma during neuroinflammation. Hence, leukocytes must cross a twofold border when extravasating from the blood stream into the parenchyma, as tumor cells must do when metastasizing to the brain parenchyma, albeit seemingly by completely different mechanisms once in the perivascular space.

Extravasation of tumor cells from blood vessels involves critical steps similar to those of leukocytes: (i) rolling and adhesion to endothelial cells, leading to arrest within the vessel lumen, which is mostly size-restricted and thus takes place at vascular branch points. These processes involve endothelial selectins and the cell adhesion molecules ICAM-1 (intercellular adhesion molecule–1) and VCAM-1 (vascular cell adhesion molecule–1). (ii) Active transmigration through the endothelial cell layer into the perivascular space, which may be paracellular or transcellular, is another critical step. The transmigration route depends on the primary tumor cell type, with melanoma cells more commonly using the paracellular route by matrix

metalloproteinase (MMP)–mediated degradation of junction proteins such as β-catenin and connexin 43 (*21*), whereas breast cancer cells seem to transmigrate transcellularly (*22*).

After successful transmigration, tumor cells reside within the perivascular space before onset of proliferation (Fig. 1). Here, tumor cells might acquire a dormant stem cell state, which could resemble tumor stem cells at the primary site, although they require direct contact with the vasculature for survival (*23*). Although cranial metastases are characterized by immunosuppressive pathway enrichment (*24*), overall gene expression is similar between extracranial and brain metastases, suggesting a limited tissue site–specific transcriptome of tumor cells. Longitudinal in vivo imaging in brain metastasis mouse models using intracardiac injection has demonstrated that brain-seeking circulating tumor cells prime the brain microenvironment for micrometastasis formation, a concept termed the formation of a premetastatic niche (*25*–*27*).

Cancer cells of different origin use distinct genetic programs to facilitate CNS metastasis formation, including transmigration through the endothelial lining, attachment to an extracellular matrix (ECM) within the perivascular niche, and exchanging signals with CNS-specific cells like astrocytes and microglia (Fig. 1). It is not surprising that tumor cell genetics equally define how the immune system combats, or fails to combat, brain metastases.

## ANTIGENIC PROPERTIES OF BRAIN TUMORS

### Antigens in brain tumors

A prerequisite for the immune control of tumors is the recognition of specific antigens expressed on or presented by tumor cells. Truly specific cell surface antigens in primary brain tumors are rare with very few exceptions (Table 1). A truncated variant of the epidermal growth factor receptor (EGFR), EGFRvIII, can be found in a subset of gliomas, however, typically only in a fraction of tumor cells within a tumor (*28*). This antigen subclonality predisposes gliomas to evasive resistance when EGFRvIII-targeting immunotherapies such as EGFRvIII chimeric antigen receptor (CAR) T cells are used (*29*). Antigen subclonality is also a key driver of resistance to immune checkpoint inhibitors (ICIs) in brain tumors (*30*, *31*). As in non-CNS cancers, ICIs act on brain tumors through nonspecific activation of circulating T cells (*32*), which home to brain tumors and become functionally active when binding glioma-associated or glioma-specific antigens presented on major histocompatibility complexes (MHC) (*33*). These antigens may stem from overexpressed self-antigens or mutated antigens.

In gliomas, several such overexpressed self-antigens have been identified using gene expression analyses, immunopeptidomics, and immunogenicity assays (*34*). More recently, neoantigens have been shown to stem from sources beyond single nucleotide variants, such as fusions, frame shifts, aberrant RNA splicing (*35*), or even intracellular bacteria (*36*). In pancreatic cancer, long noncoding RNAs, 5′ or 3′ untranslated regions, and alternative reading frames (internal open reading frames) generate epitopes that are also targets for T cell recognition (*37*). How relevant such antigens are for generating effective T cell responses against brain tumors remains to be demonstrated. Preclinical data suggest that response to ICIs is critically dependent on CD4 T cells (*38*). The MHC class II–mediated interaction of antigen-specific CD4 T cells with myeloid cells in the glioma microenvironment is critical for maintaining effector states of glioma-infiltrating CD8 T cells (*39*).

Compared with many other tumor types responsive to ICIs such as melanoma or non–small cell lung cancer (NSCLC), gliomas typically harbor few nonsynonymous mutations, giving rise to even fewer immunogenic neoepitopes. Hence, with very few exceptions, gliomas do not respond to ICIs (*40*–*42*). Even in cases where a hypermutator phenotype has evolved because of treatment with alkylating chemotherapy with subsequent inactivation of mismatch repair genes, there is little if any response to ICIs (*30*, *43*, *44*). This lack of response can be attributed to the subclonality of mutations that are induced by alkylating chemotherapy, underscoring the relevance of antigen subclonality as a key driver of evasive resistance to antigen-specific T cells (*30*). Compared with gliomas, brain metastases from lung cancer and melanoma show a higher mutational burden, albeit lower than in the primary tumor (*45*), suggesting that seeding in the brain occurs early during tumor evolution. This may explain why brain metastases, particularly from lung cancer and melanoma, display enhanced T cell infiltration and respond favorably to ICIs (*46*). The response of extracranial and intracranial metastases to ICIs is similar and dependent on the antigenicity of the primary tumor, indicating that the genetic makeup of the specific cancer, rather than its site, determines its immunogenicity and recognition by endogenous T cells. It is important to note that endogenous T cell responses are also, possibly to a much higher extent, directed against nonmutated tumor-associated antigens. In gliomas, spontaneous immune responses have been described against multiple nonmutated overexpressed antigens (*47*), which subsequently have been exploited for vaccination strategies (*48*).

### Presentation of brain tumor antigens

A pressing question in brain tumor immunology is whether such antigens are "seen" by the adaptive immune system and, if so, where (Fig. 2). Because of the challenges associated with obtaining brain tumor tissue, especially longitudinally, several other compartments have been assessed for brain tumor–reactive T cells as a surrogate for tumor-infiltrating T cells. The skull bone marrow adjacent to glioblastomas harbors glioblastoma-reactive T cell receptors (TCRs) at a frequency similar to glioblastoma tissue (*49*), although the routes of communication with the glioma microenvironment remain unclear (*50*). Antigen-specific CD4 T cells have also been identified in the cerebrospinal fluid (CSF) of patients with glioma after vaccination against the shared clonal driver mutation H3K27M (*51*). Whether draining cervical lymph nodes harbor brain tumor–reactive T cells remains controversial. In a preclinical glioma model, there was no enrichment of brain tumor–reactive TCRs in draining deep cervical lymph nodes (*52*). However, induction of meningeal lymphatic vessels by ectopic expression of vascular endothelial growth factor C (VEGF-C) in a glioblastoma model resulted in priming of therapeutic CD8 effector T cells in the cervical draining lymph nodes (*53*, *54*). CNS-derived antigens have been suggested to be continuously presented and drained to the cervical lymph nodes to maintain self-tolerance (*55*), but whether this is a relevant mechanism for brain tumors remains unclear.

## BRAIN TUMOR IMMUNE MICROENVIRONMENT

The TIME is a key determinant of brain tumor biology, response to therapy, and prognosis. Longitudinal transcriptomic profiling of gliomas has revealed that microenvironmental reorganization, rather than molecular evolution, of tumor cells determines the evolution of gliomas (*56*, *57*). The immune microenvironment is fundamentally

**Table 1. Clinical trials exploring vaccines and T cellular therapies targeting tumor-associated or tumor-specific antigens in primary CNS tumors.** BCAN, brevican; BIRC5, baculoviral IAP repeat containing 5 (survivin); CSPG4, chondroitin sulfate proteoglycan 4; EphA2, ephrin-receptor A2; FABP7, fatty acid binding protein 7; FOXM1, forkhead box protein M1; HER2, human epidermal growth factor receptor 2; IGF2BP3, insulin-like growth factor 2 mRNA-binding protein 3; NLGN4X, neuroligin-4, X-linked; NRCAM, neuronal cell adhesion molecule; TERT, telomerase reverse transcriptase; TMZ, temozolomide; VEGFR2, vascular endothelial growth factor receptor 2; WT1, Wilms tumor protein 1.

| Target antigen(s) | Description of target | Modality | Combination with | ClinicalTrials.gov | Phase | Primary outcome |
|---|---|---|---|---|---|---|
| B7-H3 | Immune checkpoint, promoting angiogenesis and invasion | CAR T | None | NCT04185038 | I | Not yet reported |
| | | CAR T | None | NCT04077866 | I | Not yet reported |
| EGFR | Conformational, tumor-specific subclonal epitope | CAR T | None | NCT03638167 | I | Closed prematurely |
| EGFR | Conformational, tumor-specific subclonal epitope | CAR T | None | NCT05768880 | I | Not yet reported |
| EGFRvIII | Subclonal in-frame deletion | Vaccine | TMZ | NCT01480479 | III | Negative |
| EGFRvIII | Subclonal in-frame deletion | Vaccine | TMZ | NCT00458601 | II | Positive |
| EGFRvIII | Subclonal in-frame deletion | Vaccine | Bevacizumab | NCT01498328 | II | Negative |
| EGFRvIII | Subclonal in-frame deletion | Vaccine | NY-ESO-1 antigen | NCT01967758 | I | Not yet reported |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT01454596 | I/II | Negative |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT02209376 | I | Positive |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT02664363 | I | Closed prematurely |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT03283631 | I | Not yet reported, suspended |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT05063682 | I | Not yet reported |
| EGFRvIII | Subclonal in-frame deletion | CAR T | None | NCT05802693 | I | Not yet reported |
| EGFRvIII | Subclonal in-frame deletion | CAR T | Bispecific engager | NCT05660369 | I | Not yet reported |
| EphA2 | Regulation of proliferation and differentiation | CAR T | None | NCT02575261 | I | Withdrawn |
| GD2 | Promoting survival, proliferation, and motility | CAR T | None | NCT04196413 | I | Positive |
| GD2 | Promoting survival, proliferation, and motility | CAR T | None | NCT05298995 | I | Not yet reported |
| GD2 | Promoting survival, proliferation, and motility | CAR T | None | NCT05544526 | I | Not yet reported |
| GD2 | Promoting survival, proliferation, and motility | CAR T | None | NCT04099797 | I | Not yet reported |
| GD2 | Promoting survival, proliferation, and motility | CAR T | None | NCT03170141 | I | Positive |
| H3 K27M | Clonal hallmark mutation defining diffuse midline glioma | Vaccine | None | NCT04749641 | I | Positive |

(*Continued*)

(*Continued*)

| Target antigen(s) | Description of target | Modality | Combination with | ClinicalTrials.gov | Phase | Primary outcome |
|---|---|---|---|---|---|---|
| H3 K27M | Clonal hallmark mutation defining diffuse midline glioma | Vaccine | Nivolumab | NCT02960230 | I | Not yet reported |
| H3 K27M | Clonal hallmark mutation defining diffuse midline glioma | Vaccine | Atezolizumab | NCT04808245 | I | Not yet reported |
| HER2 | Regulation of proliferation, migration, and invasion | CAR T | None | NCT03500991 | I | Not yet reported |
| HER2 | Regulation of proliferation, migration, and invasion | CAR T | None | NCT02442297 | I | Not yet reported |
| HER2 | Regulation of proliferation, migration, and invasion | CAR T | Ezabenlimab | NCT03383978 | I | Not yet reported |
| IDH1 R132H | Clonal hallmark mutation defining malignant astrocytoma and oligodendroglioma | Vaccine | Vorasidenib | NCT05609994 | I | Not yet reported |
| IDH1 R132H | Clonal hallmark mutation defining malignant astrocytoma and oligodendroglioma | Vaccine | Temozolomide | NCT02193347 | I | Positive |
| IDH1 R132H | Clonal hallmark mutation defining malignant astrocytoma and oligodendroglioma | Vaccine | Temozolomide | NCT02454634 | I | Positive |
| IDH1 R132H | Clonal hallmark mutation defining malignant astrocytoma and oligodendroglioma | Vaccine | Avelumab | NCT03893903 | I | Not yet reported |
| IL13Rα2 | Proinvasive | CAR T | None | NCT02208362 | I | Positive |
| IL13Rα2 | Proinvasive | CAR T | None | NCT04661384 | I | Not yet reported |
| IL13Rα2 | Proinvasive | CAR T | Without nivolumab, ipilimumab | NCT04003649 | I | Not yet reported |
| IL13Rα2, EGFR | Tumor-associated cell surface antigens up-regulated in glioblastoma | CAR T | None | NCT05168423 | I | Not yet reported |
| IL13Rα2, EphA2 | Proinvasive | CAR T | Anti-EGFRvIII SynNotch receptor | NCT06186401 | I | Not yet reported |
| Multipeptide vaccine | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | Nivolumab | NCT03422094 | I | Positive |
| Multipeptide vaccine | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | None | NCT02149225 | I | Positive |
| Multipeptide vaccine | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | None | NCT04842513 | II | Not yet reported |
| Multipeptide vaccine (BCAN, CSPG4, FABP7, IGF2BP3, NLGN4X, NRCAM, PTPRZ1, and TNC) | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | Pembrolizumab | NCT03665545 | II | Not yet reported |

(*Continued*)

(*Continued*)

| Target antigen(s) | Description of target | Modality | Combination with | ClinicalTrials.gov | Phase | Primary outcome |
|---|---|---|---|---|---|---|
| Multipeptide vaccine (IL13Rα2, BIRC5, and FOXM1) | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | Nivolumab | NCT04116658 | Ib/IIa | Positive |
| Multipeptide vaccine (IL13Rα2, EphA2, and Survivin) | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | None | NCT02078648 | II | Positive |
| Multipeptide vaccine (IL13Rα2, EphA2, and Survivin) | Tumor-associated antigens up-regulated in glioblastoma | Vaccine | None | NCT02358187 | II | Not yet reported |
| Multitarget (EGFRvIII, IL13Rα2, Her-2, CD133, EphA2, and GD2) | Tumor-associated cell surface antigens up-regulated in glioblastoma | CAR T | None | NCT03423992 | I | Status unknown |
| Survivin | Key regulator of apoptosis and mitosis | Vaccine | None | NCT01250470 | I | Positive |
| Survivin | Key regulator of apoptosis and mitosis | Vaccine | None | NCT02455557 | II | Positive |
| Survivin | Key regulator of apoptosis and mitosis | Vaccine | None | NCT04978727 | I | Not yet reported |
| Survivin | Key regulator of apoptosis and mitosis | Vaccine | None | NCT05163080 | II | Not yet reported |
| Survivin | Key regulator of apoptosis and mitosis | Vaccine | Pembrolizumab | NCT04013672 | II | Not yet reported |
| TERT promoter | Counteracts telomere shortening | Vaccine | None | NCT04280848 | II | Positive |
| VEGFR2 | Neoangiogenesis | Vaccine | Avelumab | NCT03750071 | I/II | Positive |
| WT1 | Regulation of cell proliferation, differentiation, and apoptosis | Vaccine | None | NCT01621542 | I | Positive |
| WT1 | Regulation of cell proliferation, differentiation, and apoptosis | Vaccine | None | NCT02498665 | I | Positive |
| WT1 | Regulation of cell proliferation, differentiation, and apoptosis | Vaccine | Bevacizumab | NCT03149003 | III | Negative |



different when comparing brain metastases with gliomas (Fig. 3). By mere abundance, brain metastases are generally infiltrated by more immune cells than gliomas, which is independent of glioma IDH status and primary tumor type of brain metastases (*58*). Interestingly, this reduced immune cell sequestration in glioma tissues mostly reflects that of adjacent normal CNS tissue, highlighting the importance of the CNS as a tissue of origin of primary brain tumors in this regard. Within the immune cell population, brain metastases are skewed toward lymphocytes, mostly T cells, whereas gliomas harbor more myeloid cells (*59*). Both glioma and brain metastases rely on chemokines to shape the TIME, but the specific molecules involved and the surrounding microenvironment differ substantially, leading to distinct immune microenvironments (Fig. 1). Furthermore, the ECM composition, particularly tenascin-C (TNC), plays a role in trapping T cells in gliomas, whereas brain metastasis ECM might have a different impact on immune cell infiltration (*60*). Targeting these chemoattractant pathways, particularly the CXCL12/CXCR4 axis, and ECM molecules is a promising approach for both glioma treatment and prevention of metastasis.

Brain tumor–associated astrocytes secrete chemokines like CCL2 and CSF1, which recruit and reprogram immune cells, particularly macrophages, to adopt a protumorigenic phenotype (*61*). A recent study found that interleukin-11 (IL-11)–driven signal transducers and activators of transcription 3 (STAT3) activation in tumor-associated astrocytes up-regulates TRAIL (tumor necrosis factor–related apoptosis-inducing ligand) expression, which, in turn, suppresses local $CD8^+$ T cell responses (*62*). Another study revealed that metastatic cells co-opt reactive astrocytes to support tumor growth by maintaining an immunosuppressive niche, again via STAT3 activation (*63*). STAT3-activated astrocyte-derived TIMP1 (tissue inhibitor of metalloproteinases 1) was identified as a critical mediator that enhances metastatic colonization and modulates immune cell infiltration. Together, these studies position astrocytes as active participants in tumor progression and immune modulation.

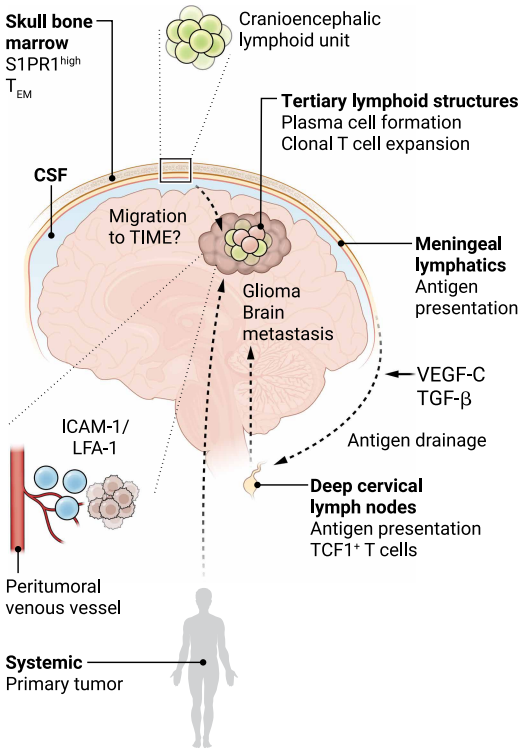


**Fig. 2. Routes of T cell priming to brain tumor–derived antigens.** Recognition of tumor-derived antigens is a prerequisite for effective T cell–driven antitumor immune responses. Several sites of brain tumor–associated antigen presentation and tumor-reactive T cell activation have been described: (i) The deep cervical lymph nodes are a reservoir for TCF1$^+$ tumor-reactive T cells and are an active site of antigen presentation. (ii) Antigen-specific T cells can be found in the CSF of patients receiving tumor-specific vaccination. (iii) Tumor-derived antigens can drain through meningeal lymphatics to the deep cervical lymph nodes. Antigen drainage can be induced by expression of VEGF-C and TGF-β. (iv) TLS are found in ~15% of patients with glioma and are potential hubs for clonal T and B cell expansion. (v) The skull bone marrow is infiltrated by tumor-reactive T cells defined by S1PR1 expression and effector memory ($T_{EM}$) state. (vi) Peritumoral venous vessels are extravasation points for tumor-reactive T cells in brain metastasis, facilitated via ICAM-1/LFA-1 (lymphocyte function–associated antigen 1) interaction. (vii) Antigens derived from primary tumors can prime tumor-reactive T cells in distant lymph nodes that subsequently migrate to the brain metastases.

## Myeloid cells

### *Macrophages in gliomas*

Gliomas are infiltrated by many tumor-associated myeloid cells, which may account for up to 50% of the nonmalignant cell population. In contrast, brain metastases are signified by a higher immune cell infiltration and T cell–to–macrophage ratio (*58*, *59*, *64*). The reduced T cell sequestration in gliomas may in part be explained by low antigenic properties but is in large part driven by immunosuppressive factors released by tumor-associated macrophages (TAMs). The brain tumor microenvironment is distinct, given that it harbors microglia, an additional class of myeloid cells that resemble macrophages but are distinct in origin. Distinct from monocyte-derived macrophages (MDMs) (*39*, *65*), microglia are resident myeloid cells of the brain, originating from hematopoietic precursor cells that migrate from the yolk sac to the CNS during embryonic development (*17*). Like MDMs, microglia display a continuum of immunosuppressive and immunostimulatory phenotypes within the tumor microenvironment and exert similar functions.

The myeloid compartment is highly dynamic, with coevolving reciprocal interactions with tumor cells, T cells, neurons, glial cells, and vascular cells (Fig. 3) (*66*). Brain tumors, particularly high-grade gliomas, recruit large numbers of monocytes from the peripheral circulation via extremely robust and redundant chemoattractant cues. This redundancy is likely responsible for the failure of therapeutic approaches aiming to block monocyte chemokine signaling. In preclinical models, neutrophils compensate for the loss of monocytes after depletion and similarly skew tumor cells to a mesenchymal phenotype (*67*). Once recruited, monocytes rapidly differentiate to TAMs.

Attempts to classify TAMs according to phenotypic criteria determined by morphology, developmental programs, or in vitro culture conditions, such as M1-/M2-like macrophages or myeloid-derived suppressor cells, fall short in encompassing the dynamics and breadth of functional states found in gliomas (*68*). In general, in both human and clinical samples, TAMs accumulate over time of progression (*65*) and undergo a transition to immunosuppressive phenotypes (*69*). Several signaling pathways orchestrating this transition to immunosuppressive TAMs have been described, notably triggering receptor expressed on myeloid cells 2 (TREM2) (*69*), tryptophan metabolism (*65*, *70*), glucose metabolism, and lactate signaling (*71*, *72*). Monocytes recruited to gliomas receive signals from glioma cells to differentiate into glioma-associated myeloid cells, which, in turn, affect glioma cell states and function (*73*, *74*). TAM-derived oncostatin M (*75*), EGFR/FGFR (fibroblast growth factor receptor) ligands (*76*, *77*), and TAM-derived myelin-derived lipids (*78*) have been shown to induce an unfavorable mesenchymal phenotype in glioma cells. This phenotype is sustained by TAM-induced epigenetic programs activated in glioma cells (*74*).

### *Macrophages in brain metastases*

In contrast with TAMs in glioma, TAMs in brain metastases are characterized by more prominent classical monocyte and dendritic cell (DC) states (*31*, *58*, *79*). Using approaches such as RNA sequencing (RNA-seq) (*64*), mass cytometry [cytometry by time-of-flight (CyTOF)] (*59*), imaging mass cytometry (*58*), and multisector immunogenomic profiling (*31*), several recent studies identified striking differences in the myeloid composition between glioma and brain metastases. IDH-mutant low-grade gliomas were characterized by a high frequency of microglia that was similar to that in nontumor brain tissue (*65*), whereas brain metastases were more infiltrated by MDMs (*31*, *64*). Interestingly, IDH–wild-type high-grade gliomas were more similar to brain metastases in this regard. Hence, microglia were enriched in IDH-mutant glioma, MDMs in IDH–wild-type glioma, and MDMs and T cells in brain metastases. Melanoma-originating brain metastases were the most infiltrated with T cells, reflecting findings in primary tumors (*64*). These differences highlight once more how tumor cell genetics and origin shape distinct microenvironments in the CNS.

A more recent analysis based on imaging mass cytometry including nonimmune cells did not confirm a clear higher abundance of microglia in gliomas compared to brain metastases (*58*). This might be due to differences in annotation and identification of microglia versus MDMs, which has remained a particular challenge in the field because of phenotypical and functional similarities especially in tumors (*64*, *80*). Although gene expression profiles of microglia and MDMs seem to be largely defined by tumor type (glioma versus

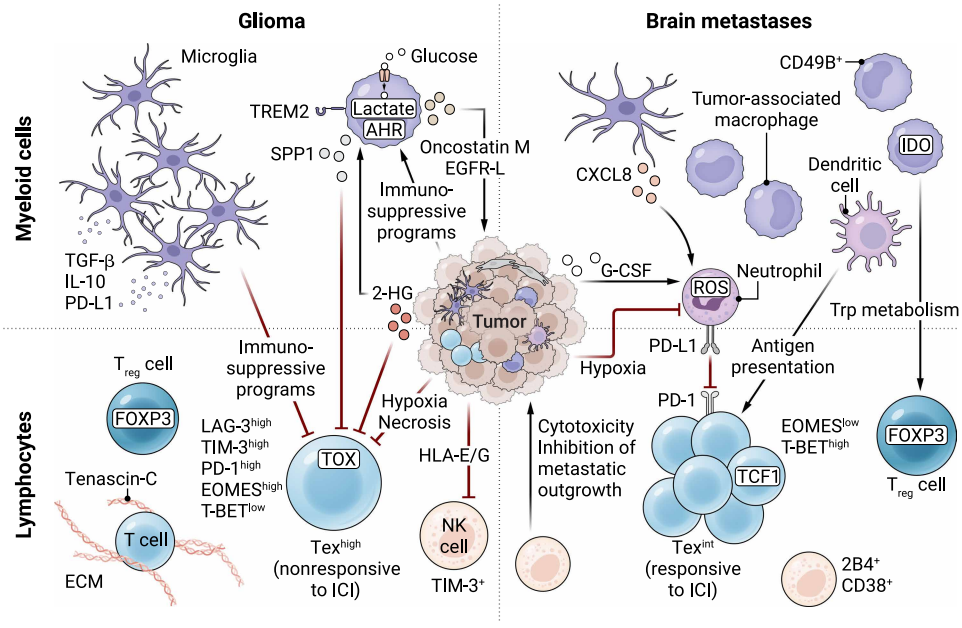


**Fig. 3. Immune microenvironmental differences between brain metastases and gliomas.** The immune microenvironment of brain tumors is defined by tumor cell genetics and origin. Primary brain tumors like gliomas are characterized by a high microglial content and limited T cell infiltration. Tumor cells as well as myeloid cells in the glioma TIME express immunosuppressive surface markers as well as chemokines that inhibit T cell and NK cell effector functions. Thus, T cells predominantly exhibit an exhausted and dysfunctional phenotype. Brain metastases are defined by a higher T cell, NK cell, and myeloid cell infiltration, leading to increased antigen presentation by macrophages and dendritic cells and increased T cell function, ultimately resulting in elevated responsiveness to ICIs. AHR, aryl hydrocarbon receptor; IDO, indoleamine-2,3-dioxygenase; Trp, tryptophan; SPP1, secreted phosphoprotein 1 (osteopontin); TOX, thymocyte selection–associated high mobility group box.

brain metastasis), in glioma, MDMs displayed a greater potential for antigen processing and presentation than microglia (*64*).

Macrophages reside in perivascular spaces in both glioma and brain metastases. In contrast, microglia seem to be more restricted to the tumor margin and excluded from the tumor core in brain metastases than in glioma, where they are diffusely distributed throughout the tumor (*58*, *59*). This is particularly important, because myeloid cells play important oxidative and metabolic roles in the tumor core to fuel glioma stem cells that have a different metabolic state than the tumor periphery (*81*).

### *Neutrophils and dendritic cells*

CXCL8 expression by brain metastasis–associated microglia seems to recruit neutrophils, which is why brain metastases harbor significantly more neutrophils than glioma (*64*). In glioblastoma, neutrophils have also been shown to be recruited from the skull bone marrow (*82*). During early metastasis formation, astrocyte-mediated inflammation, which is induced by the primary tumor, recruits neutrophils into the metastatic niche (*83*). Later, neutrophils can also be attracted by granulocyte colony-stimulating factor (G-CSF) secreted by brain metastatic tumor cells (*84*) and drive metastasis progression.

Once recruited, neutrophils are functionally reprogrammed to an immunosuppressive phenotype in the brain tumor microenvironment, such as by hypoxia (*85*). Neutrophils seem to exert their immunosuppressive phenotypes through limited reactive oxygen species (ROS) production and expression of PD-L1 (programmed death ligand 1) in proximity to CD8 T cells (*86*), contributing to T cell exhaustion. These immunosuppressive functions make neutrophils an attractive potential target for combinatorial immunotherapeutic approaches for brain metastases. Conversely, tumor-associated neutrophils may exhibit a distinct inflammatory signature, which is more pronounced in brain metastasis than glioma (*86*). Therefore, when targeting neutrophils, similar to monocytes and T cells, their plasticity, different subtypes, and wide range of stimuli affecting phenotypes and functions need to be considered.

Although DCs are generally of low abundance in brain tumors, imaging mass cytometry recently found less infiltration of DCs in brain metastasis compared with glioma samples (*59*). Among DCs, both plasmacytoid (pDC) and conventional DC (cDC1 and cDC2) types were present in both glioma and brain metastases, although DCs in the latter seemed to exhibit a more classical phenotype. Whereas pDCs have been shown to also derive from lymphoid progenitors (*87*) and are important links between innate and adaptive immunity via their large production of type I interferons (IFNs) upon infection, cDCs mainly initiate an adaptive immune response by antigen presentation and costimulation—cDC1s primarily by cross-presentation to cytotoxic CD8 T cells and cDC2s by presentation to CD4 T cells. Given the dual and contradictory role of type I IFNs, which, depending on the dynamics, might inhibit or stimulate antitumor immune responses (*88*, *89*), the more classical cDC phenotype in brain metastases may in part account for the stronger T cell infiltration of these tumors compared with gliomas. Conversely, in gliomas, IDH mutations may render DCs dysfunctional by paracrine reprogramming, limiting T cell responses (*90*). Evidence for the functional relevance of intrabrain metastatic DCs is limited because it mainly stems from RNA-seq data (*90*–*92*), so further studies are warranted.

### *Cross-talk of TAMs with brain tumor cells*

Overall, the signals responsible for the recruitment and differentiation of TAMs seem to be tumor-specific rather than brain-specific. Whereas tumor-infiltrating myeloid cells in brain metastases recapitulate the primary tumor, glioma-infiltrating myeloid cells display distinct microglial inflammatory and scavenger immunosuppressive programs that are not strongly present in brain metastases (*68*). Such microglial programs can be acquired by glioma-infiltrating monocytes recruited from the periphery, which up-regulate canonical microglial markers such as TREM119 and P2RY12. The presence of such myeloid cell states or programs is determined by the malignant phenotype of gliomas, particularly in high-grade gliomas (*68*). In addition, myeloid cell states are affected by glioma genotypes. IDH1-mutant gliomas are characterized by an enrichment of microglial inflammatory programs and a depletion of immunosuppressive programs, which is driven by the oncometabolite 2-HG (*65*, *77*).

Last, myeloid cell states are critically determined by specific niches in which they reside. The perivascular hypoxic niche appears to not only preferentially attract peripheral monocytes but also provide an environment that favors conversion into TAMs with specific programs of anabolism and response to hypoxia and cell stress. Such perivascular TAMs in the vicinity of pseudopalisading necrosis, a hallmark of glioblastomas, interact with stem-like tumor cells, which produce chemokines and cytokines responsible for both myeloid cell recruitment and differentiation (*77*). Endothelial cells of the glioma vasculature provide signals to the perivascular niche, such as oncostatin M, to induce differentiation of recruited monocytes into TAMs (*93*). Given that TAMs, in principle, retain their phagocytic function, glioma cells evade phagocytosis by up-regulating "don't eat me" cell surface receptors that suppress phagocytosis such as CD47, which engages SIRPs (signal regulatory proteins) on macrophages. In preclinical models, TAMs, if appropriately activated, may induce regression of experimental gliomas (*94*, *95*). A CD47 blocking antibody targeting both antiphagocytic pathways in TAMs and resident microglial cells (*96*) was effective in preclinical brain tumor models in enhancing phagocytosis and tumor control (*97*), but clinical trials were halted because of lack of efficacy and side effects.

The symbiotic cross-talk of TAMs with stem-like tumor cells in the perivascular niche is specific to gliomas and a key driver of tumor growth, resistance to therapy, and an immunosuppressive microenvironment, resulting in reduced T cell infiltration. Ultimately, it is the environment of the niche with hypoxia, limited glucose, and accumulation of lactate that is the key driver of adaptive programs in both tumor cells and TAMs. Conversely, T cells are less well equipped in adapting to these conditions while maintaining effector function. TAMs create an immunosuppressive microenvironment by down-regulating mechanisms (re)activating brain tumor–specific T cells while also up-regulating mediators that suppress T cell function.

## Lymphoid cells

### *Tertiary lymphoid structures*

Tertiary lymphoid structures (TLS) are important hubs not only for B cell maturation and isotype switching but also for mounting antigen-specific T cell responses in cancer and chronic diseases (*98*–*100*). In the CNS, TLS can be found in infectious diseases, autoimmunity, and presumably brain tumors (*101*). In preclinical glioma models, TLS are induced in an otherwise dysfunctional vasculature by exogenous expression of tumor necrosis factor superfamily member 14 (TNFSF14, LIGHT), which promotes efficient antitumor immune responses (*102*). One study, which analyzed TLS in human brain tumors, found that 15% of human gliomas harbor TLS, which were associated with the perivascular niche and were hubs of clonal B and T cell expansion (*103*). The frequency of TLS in brain metastases seems to be considerably higher than that in gliomas, reflecting higher immunogenicity (*104*). To the best of our knowledge, well-defined and consensus-based characterization of TLS is lacking, hindering robust interstudy and entity comparisons.

### *T cell recruitment*

In physiological conditions, T cells actively survey the CNS and continuously control viral latencies, highlighting the role and potency of antigen-specific T cells for CNS homeostasis. Activated T cells can access the CNS through three distinct routes (*105*). One pathway involves migration from blood vessels into the stroma of the choroid plexus, followed by passage across the blood-CSF barrier. This barrier is formed by epithelial cells of the choroid plexus, which are joined by tight junctions. Alternatively, activated T cells can enter the subarachnoid space by extravasating through meningeal venules. These vessels are composed of endothelial cells also connected by tight junctions. A third route allows activated T cells to cross the BBB at postcapillary venules that penetrate the brain parenchyma. These venules, like the others, consist of tightly connected endothelial cells. In brain metastases, distinct venous vessels have been identified as the key structures for T cell recruitment (*106*).

To access the brain parenchyma or tumor site, T cells must first enter these perivascular spaces and subsequently traverse the glia limitans, a dense layer of astrocytic end feet that forms the final barrier between the perivascular compartment and the neural tissue. To our knowledge, it has not yet been investigated whether direct T cell transmigration through the ventricle-lining ependymal layer occurs in proximity to the brain tumor site.

### *T cell phenotype and function*

T cells make up ~1 to 10% of glioblastoma immune infiltrates and only 0.1 to 1% of astrocytoma and oligodendroglioma immune infiltrates (*107*, *108*). Brain-tumor infiltrating CD8 cytotoxic T lymphocytes are generally considered to be the main effector cells in antitumor immunity. However, in glioblastomas, these T cells are often found in a state of functional exhaustion, marked by high expression of inhibitory receptors, such as PD-1 (programmed death 1), and less pronounced LAG-3 (lymphocyte-activation gene 3), and TIM-3 (T cell immunoglobulin and mucin domain-containing 3) (*109*, *110*). This exhausted phenotype reflects chronic antigen exposure and a progressive decline in effector function. T cell apoptosis has also been described (*111*, *112*). The majority of CD8 T cells are memory T cells, with no significant differences in their relative proportions between brain metastases and gliomas (*59*). In patients with glioblastoma, higher frequencies of central memory CD4 and CD8 T cells showed a positive correlation with overall survival or length of follow-up, suggesting a potential prognostic relevance (*59*). Nonetheless, in glioblastomas, PD-$1^+$ T cells have also been characterized as being more terminally exhausted T ($T_{EX}$) cells, as reflected by an $EOMES^{high}T\text{-}BET^{low}$ phenotype, which is inversely correlated with T cell proliferation (*109*). In contrast, in brain metastases, a notable proportion of stem-like progenitor exhausted T cell factor–1–positive ($TCF1^+$) T cells has been reported (*113*).

At recurrence or progression, intratumoral T cell abundance is increased and exhaustion is enhanced relative to the initial tumor (*114*). Memory T cells typically localize to "hypoxic" and "vasculature" niches (*4*). CD4 T cells are also present in gliomas but are often skewed toward regulatory phenotypes rather than proinflammatory helper subsets. Regulatory T ($T_{reg}$) cells, identified by FOXP3 (forkhead box P3) expression, are enriched in the brain metastasis microenvironment but less abundant in gliomas (*64*, *115*). This may be because of counterregulatory mechanisms induced by activated TAMs reprogramming T cells to a regulatory phenotype, for instance, through tryptophan metabolism (*115*). $T_{reg}$ cells contribute to the suppression of effective antitumor immune responses by secreting immunosuppressive cytokines such as IL-10 and transforming growth factor–β (TGF-β) and inhibiting the activation and proliferation of other T cell subsets (*116*, *117*). In postvaccination inflammatory glioma lesions, vaccine-induced antigen-specific follicular T helper cell–like $CXCL13^+$ CD4 T cells have also been found (*108*).

In contrast with gliomas, brain metastases often exhibit higher levels of T cell infiltration, although this varies substantially depending on the tissue of origin. Brain metastases from melanoma and

NSCLC, for instance, display higher densities of $T_{reg}$ cells and $CD8^+$ T cells, increased expression of IFN-stimulated genes, and greater responsiveness to immune checkpoint blockade. Interestingly, this greater ICI responsiveness in the brain was found to be associated with peripheral expansion of homing-competent effector CD8 T cells (*118*). Nevertheless, even in these cases, the immune infiltrate can be spatially restricted and mostly found concentrated around blood vessels or at the tumor margin.

***Therapeutic T cells***

Adoptive T cell therapies, including TCR-engineered cells and CAR T cells, are under investigation for gliomas. Several CAR T cell targets have been explored, including the disialoganglioside GD2 (*119*), IL13Rα2 (interleukin 13 receptor alpha 2) (*120*), and EGFRvIII (*29*), and early studies have demonstrated strong tumor regression after intracerebroventricular delivery of CAR T cells (Table 1) (*120*), but challenges remain. These include heterogeneity of antigen expression, limited T cell persistence, and ongoing immunosuppression in the tumor. Hence, current advances focus on improving efficacy and specificity and overcoming tumor resistance. For example, glioblastoma cells deficient in IFN-γ signaling are resistant to killing by CAR T cells because of insufficient IFN-γ–induced ICAM-1 expression. Killing by CAR T cells can be restored by exogenous expression of ICAM-1 in glioblastoma cells (*121*). Enhanced killing and prevention of exhaustion through tonic signaling can also be prevented by introducing a synthetic receptor (SynNotch) recognizing a specific priming antigen (*122*). Genetic screens have identified genes that can be targeted to prevent CAR T cell dysfunction (*123*). Additional measures to increase CAR T cell resilience include the production of proinflammatory cytokines or metabolic or epigenetic modifiers.

Different routes of T cell administration, including intravenous, intracerebroventricular, and intra-arterial delivery, have been investigated for treating primary brain tumors (*124*). As a monotherapy, intracerebroventricular T cell delivery has shown superior feasibility and early efficacy (*125*). Recent clinical trials have explored the intracerebroventricular delivery of bivalent CAR T cells targeting multiple glioblastoma antigens, such as EGFR and IL13Rα2, aiming to overcome antigen heterogeneity and reduce tumor escape (*119*). These approaches are being refined by optimizing CAR costimulatory domains and delivery methods to enhance efficacy and safety in brain tumors. Another attempt to target glioblastoma heterogeneity is the development of CARv3-TEAM-E T cells, which are CAR T cells engineered to target the subclonal EGFRvIII tumor-specific anigen and the wild-type EGFR protein through site-specific secretion of a T cell–engaging antibody molecule (TEAM) (*126*). Whereas cellular therapies for primary brain tumors are evaluated in early clinical trials and conceptual advancements are currently prioritized, a large body of evidence supports that intravenous CAR T cells targeting lymphoma metastatic to the brain are efficacious (*127*). This suggests that the brain tumor entity, rather than its localization in the CNS, dictates susceptibility to tumor-targeting therapeutic T cells. In distinct immunological contexts, intravenous cellular therapies for brain tumors can be efficacious.

Broadening the targetable antigen repertoire in glioblastoma, we have developed TCR-engineered T cell therapies that target glioblastoma-specific antigens such as protein tyrosine phosphatase receptor type Z1 (PTPRZ1), a molecule highly expressed in glioblastoma cells with stem cell–like properties (*128*). By isolating TCRs from vaccinated patients and engineering them into donor T cells, we have produced transgenic T cells capable of recognizing and killing PTPRZ1-expressing glioblastoma cells both in vitro and in animal models. Similarly, the recurrent H3K27M mutation (*129*) was exploited for neoantigen-specific TCR-engineered T cell therapy in diffuse midline gliomas (*130*), although the relevance of endogenous presentation on human lymphocyte antigen A2 (HLA-A2) remains controversial (*131*, *132*).

Overall, these approaches are dependent on sufficient immunologically visible peptide-MHC complexes and, in general, limited to patients with certain HLA types. Efforts are ongoing to expand the range of targetable HLA types and antigens to make the therapy accessible to more patients. T cells are indispensable players in antitumor immunity, yet their function is severely constrained in primary and secondary brain tumors. Gliomas, particularly glioblastomas, present an exceptionally hostile environment for T cells, characterized by low infiltration, high exhaustion, and multiple layers of suppression. Brain metastases, although more permissive to T cell activity, still exhibit immune evasion mechanisms. The growing understanding of the immunobiology of CNS tumors is opening previously unrecognized avenues for targeted therapies aimed at restoring intratumoral T cell responses. Progress will likely depend on integrated strategies that combine different immunotherapeutic modalities as well as medical devices such as focused ultrasound.

***NK cells***

Belonging to the lymphoid lineage and the innate immune system, natural killer (NK) cells are the major class of innate lymphoid cells. NK cells are rapidly activated during infections, acquiring high cytolytic activity without the need for antigen-specific activation. NK cells have also been shown to play an important role in the antitumor immune response, where they can directly detect and kill transformed cells. However, their activation is tightly controlled by activating and inhibitory ligands, whose signals are integrated to mount a respective response (*133*). For example, the presence of an activating ligand such as MHC class I–related proteins as well as the loss of inhibitory molecules on a target cell results in rapid cytolysis (*134*). Adding complexity, activated T cells expressing B7H6 can also be NK cell cytotoxicity targets via binding to the NK cell receptor NKp30 (*135*), marking a type of NK cell–dependent checkpoint to reduce T cell activity. Thus, albeit at a low frequency compared with T cells in tumors, NK cells can limit cell therapy or ICI efficacy by reducing T cell persistence and activity (*135*).

In esophageal cancer, a higher NK cell/T cell ratio was associated with nonresponse to ICIs and reduced progression-free survival. In brain tumors, little is known about NK cell contributions to tumor control or limiting T cell function. NK cell frequencies in these tumors are low, about 2.5% of leukocytes in glioma and brain metastases (1 to 5%) (*59*). Therefore, NK cells are more frequent than DCs and, hence, might be more relevant in the orchestration of the TIME, and their abundance among leukocytes does not seem to differ between primary and metastatic brain tumors. However, within the entire tumor mass, NK cell frequency is higher in brain metastases (*58*), probably because of a stronger T cell infiltration compared with gliomas. Interestingly, immature $CD16^-$ NK cells were enriched in IDH1–wild-type gliomas, whereas IDH1-mutated gliomas and brain metastases harbor more cytolytic $CD16^+$ NK cells (*59*). A more immunosuppressive phenotype of these cells in gliomas, especially IDH1–wild-type gliomas, compared with brain metastases, is signified by reduced expression of the activating receptors 2B4 and CD38, increased proliferation, and increased Tim-3 expression (*59*), which renders NK cells nonfunctional when activated (*136*). This

observation is in line with a generally more immunosuppressive microenvironment in gliomas compared with brain metastases, making the latter more prone to effective immunotherapies.

Functionally, NK cell cytotoxicity seems to be mostly evaded by brain tumor cells, best described for glioblastoma and IDH-mutant glioma, via a variety of immunosuppressive mechanisms. These include reducing the expression of activating ligands (like NKG2D ligands), primarily in response TGF-β, and shedding of these ligands (*137*–*139*). TGF-β, secreted by astrocytes and immunosuppressive brain tumor–associated myeloid cells, also impairs NK cell metabolism and function by inhibiting mammalian target of rapamycin signaling and promoting production of immunosuppressive factors like galectins (*140*). Glioblastoma cells further resist NK cells by expressing high levels of inhibitory molecules such as MHC class I variants (HLA-E and HLA-G) (*141*). In lung and breast carcinoma brain metastasis models, NK cells exerted cytolytic activity against extravasated proliferating micrometastatic clusters, limiting metastatic outgrowth and thereby forcing such metastatic cells into a quiescent cell state to evade NK cell–mediated immune surveillance (*142*). Together, NK cells in the brain tumor microenvironment have mostly been rendered immunosuppressive or inactive by tumor cells to circumvent cytotoxicity. Nevertheless, NK cells have increasingly been exploited for cell therapy.

## IMPLICATIONS FOR COMBINATORIAL THERAPIES

To mount an effective, therapeutic immune response to brain tumors, particularly gliomas, several challenges need to be addressed: The transmigration of T cells through the BBB needs to be facilitated. Then, the number of antigens "seen" by T cells needs to be increased. Proper activation and restoration of effector functions compromised by the immunosuppressive microenvironment need to be ensured. Last, key signaling pathways in tumor cells need to be modified such that they present antigens and are susceptible to T cell–mediated killing.

To facilitate the transmigration of T cells through the BBB, local measures such as focused ultrasound, introduction of T cell–attracting chemokines, or genetic engineering of T cells with brain tumor–homing receptors are currently being explored. To increase T cell antigen recognition, the induction of antigen or epitope spreading holds great promise. This process is well described in autoimmune disease to sustain and broaden tissue-specific immune responses (*143*). In tumors, this phenomenon has been much less described and only recently substantiated (*144*, *145*). In many types of gliomas, particularly IDH-mutated gliomas, there is abundant genome-wide DNA hypermethylation (*146*) silencing the expression of potential antigens (*147*) but also antigen processing and presentation machinery (*110*). Consequently, demethylating agents such as 5-aza-2′-deoxycytidine can improve antigen presentation and increase stimulation of tumor-specific T cells in patients with recurrent glioblastoma (*148*). In addition, the recently approved inhibitor of the oncogenic IDH mutation in gliomas, vorasidenib, blocks 2-HG production, stimulates T cells in gliomas, and improves progression-free survival (*12*, *13*). 2-HG is released into the microenvironment, where it is taken up by immune cells and suppresses their function (*65*, *110*, *149*, *150*). Blunting 2-HG production by IDH inhibitors has a direct immune stimulatory effect by derepressing the cGAS-STING (cyclic GMP-AMP synthase–stimulator of interferon genes) pathway (*151*, *152*). On the basis of preclinical studies rationalizing the combination of IDH inhibitors with ICIs (*110*, *153*, *154*) or IDH-directed vaccines (*108*), clinical trials are being conceptualized or underway.

Another means to stimulate antigenicity is the brute-force approach of inducing virus-mediated tumor cell death. This approach is probably necessary given that there is little if any evidence in brain tumors that immunogenic cell death can be induced by chemotherapy. In contrast, local injection of oncolytic viruses (OVs) induces massive cell death, resulting in the release and presentation of viral and tumor-derived antigens. The resulting immune activation is a critical determinant of clinical outcome of patients with glioblastoma treated with OVs (*155*). Consequently, recent approaches have combined OV therapy with T cell–activating therapies such as ICIs. A combination of intratumoral injection of the OV DNX-2401 followed by pembrolizumab was safe with notable survival benefit in select patients (*156*). To amplify the immune response, OVs can be equipped with proinflammatory cytokines such as IL-12, a cytokine that has been shown to reprogram the immunosuppressive glioma microenvironment and induce regression of established gliomas in preclinical models (*157*, *158*) and is safe when administered locally in patients with glioblastoma (*159*). Treatment with IL-12–encoding OV results in up-regulation of MHC class II and stimulation of polyfunctional CD4 T cells in the glioma microenvironment in preclinical models (*160*) and was safe and immunogenic in patients (*161*). Next-generation OVs coexpress multiple immunomodulatory molecules (e.g., IL-12, anti-PD-1, and anti-TREM2) to simultaneously target several mechanisms of immunosuppression operating in the TIME. Alternatively, to stimulate antigen-specific T cell responses, tumor-targeting viruses can be equipped with a model antigen. In a recent phase 1 clinical trial, treatment with measles viruses expressing carcinoembryonic antigen was safe and induced CD8 T cell infiltration in posttreatment samples (*162*).

To (re)activate effector functions of T cells compromised by the immunosuppressive microenvironment, transgenic T cells can be engineered to express immunostimulatory cytokines, intracellular signaling molecules, or metabolic switches enhancing T cell function and resilience. It is also clear that many other highly dynamic factors important for evasive resistance, such as T cell anergy, antigen down-regulation, intertumoral heterogeneity, and reprogramming of the immune microenvironment to sustain a meaningful antitumor immune response, need to be addressed along the way.

## CONCLUSIONS

Our understanding of the immunology of brain tumors has advanced substantially in recent years. Immunotherapeutic concepts such as adoptive cell therapies, vaccines, virotherapies, ICIs, and immune modulators have shown encouraging results. At the same time, because of the enormous interindividual and intratumoral heterogeneity both at the tumor cell and the immune level, numerous negative randomized phase 2 and phase 3 trials highlight that a "one fits all" approach is unlikely to be viable. To create meaningful subgroups of patients benefitting from immunotherapy beyond the presence of a specific target antigen is difficult, because robust immune biomarkers are largely lacking and immunotherapies for brain tumors are only beginning to show efficacy in early clinical trials.

The complex, heterogeneous, and dynamic immune microenvironment requires decoding samples from individual patients to identify subgroups responding to immunotherapy. To retrieve meaningful biomarkers of response and resistance and to rationalize an

informed combinatorial approach stimulating specific tumor-targeting T cells and counteracting the immunosuppressive brain tumor microenvironment, innovative preclinical models and reverse translation approaches from smart, biology-driven, window-of-opportunity trials (*163*) are necessary (*164*). The data provided from such models and trials, including genetic, immune monitoring, imaging, and clinical data, in combination with novel methods to identify mechanisms of immunosuppression operating in the TIME (*165*), need to be shared and used to train new algorithms for hypothesis generation (*166*) to ensure an efficient iterative cycle of immunotherapy development for brain tumors.

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