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T cell immunity in glioma and potential implications for immunotherapy: A systematic review

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Abstract

Background. T cell-based immunotherapies have had limited success in glioma thus far. Here, we evaluate the literature on abundance, spatial distribution and phenotypical characteristics of T cells in the tumor micro-environment (TME) of IDH-mutant and IDH-wildtype glioma, with the aim to understand how these measures relate to immunotherapy resistance and to aid the development of immunotherapies for glioma.

Methods. Medline, Embase, Web of Science Core Collection, Google Scholar and the Cochrane Central Register of Controlled Trials were systematically searched up to May 6, 2025. Out of 4303 articles screened, 85 studies examining T cell immunity in human glioma were selected. We collected information about tumor subtype, grade, methods, T cell abundance, spatial distribution, phenotypes and prognostic significance.

Results. T cells are present in the glioma TME, but densities are heterogeneous and generally low, especially in IDH-mutant glioma. T cell abundance increases with higher WHO grade and upon recurrence. T cells cluster around blood vessels, especially in IDH-mutant glioma. Glioma-infiltrating T cells largely display a late-differentiated phenotype (CD45RA-CCR7-C62L-), expressing markers that signify sustained antigen activation and exhaustion (PD-1, CTLA-4, TIM-3, LAG-3, CD39, and TIGIT). This phenotype coincides with decreased anti-tumor cytotoxicity and is spatially enriched in the myeloid-rich, hypoxic tumor core. Prognostic significance remains controversial.

Conclusions. T cells in glioma are scarce, generally fully differentiated and functionally inert. Understanding and reinvigorating the deficient T cell response will be essential for successful immunotherapies. Future research should incorporate functional and spatial immune profiling to optimize and personalize immunotherapeutic strategies for glioma patients.

Key Points

- Glioma T cells are scarce, generally fully differentiated and functionally inert.
- Future research should include functional and spatial immune profiling to optimize immunotherapeutic strategies for individual glioma patients.

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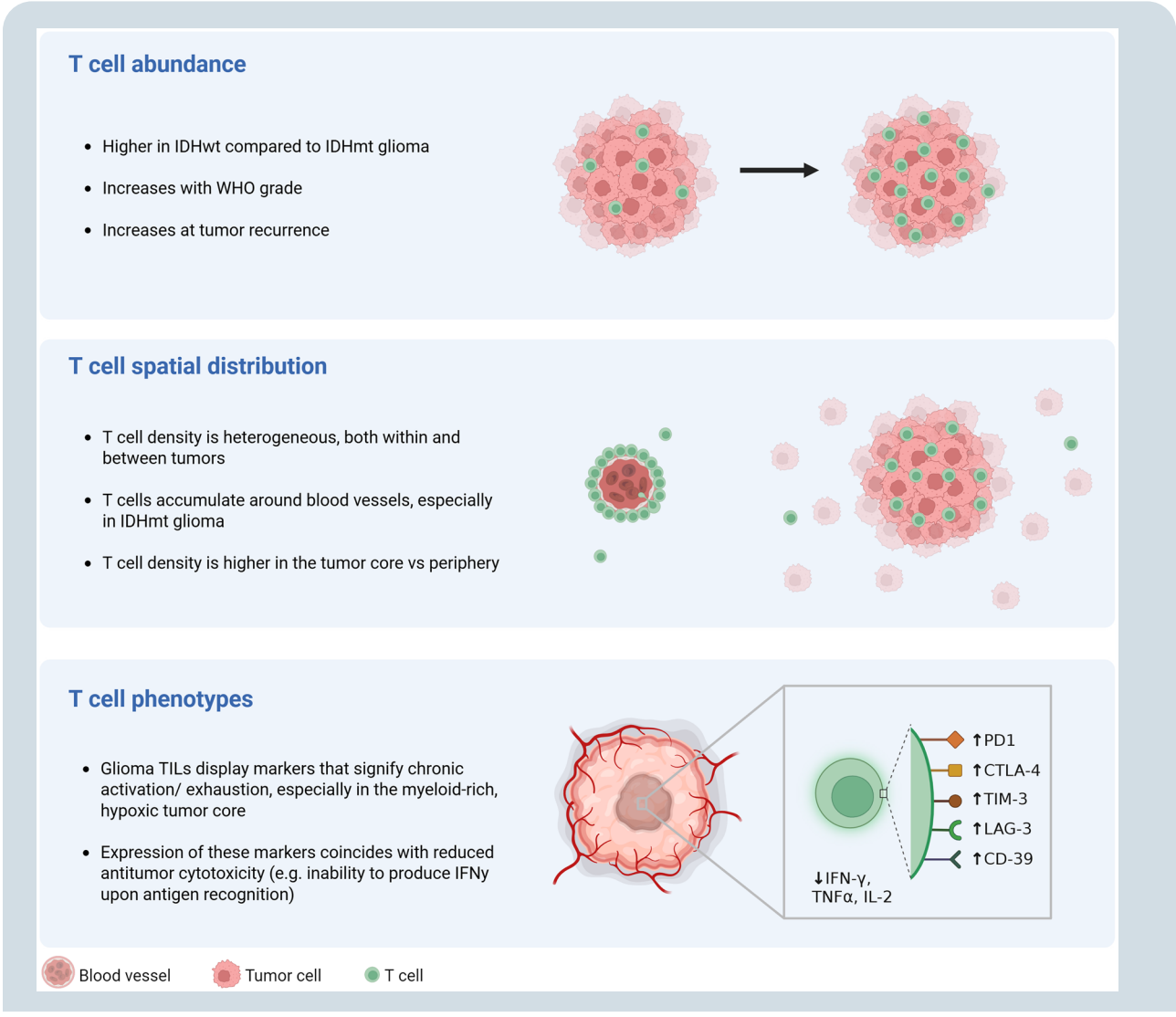
Graphical Abstract



Importance of the Study

Adult diffuse glioma remains notoriously difficult to treat. While immunotherapies represent a promising avenue of research, most T cell-based immunotherapies, such as immune checkpoint inhibitors (ICIs), have been unsuccessful in glioma thus far. Here, we systematically evaluate the literature on phenotypes, densities and spatial distribution of T cells in the tumor micro-environment (TME) of adult diffuse glioma, with the aim to understand current immunotherapy resistance and to guide future research. We find that T cells in glioma are scarce, spatially restricted, and display typical characteristics of exhaustion and reduced functional performance. We discuss underlying mechanisms, therapeutic implications and strategies to overcome these barriers, and highlight currently unanswered questions in the field. Moving forward, studies integrating transcriptomic, functional and spatial immune profiling, providing high-resolution insights into the composition and relevance of distinct immune-niches in glioma, will be essential to guide the optimization and personalization of immunotherapeutic strategies.

Adult diffuse gliomas are the most common primary malignant tumors of the central nervous system (CNS).[1] The 2021 WHO classification integrates histological and molecular parameters, with an important role for the isocitrate dehydrogenase (IDH) mutation, present in oligodendroglioma, IDH-mutant (mt) and 1p/19q co-deleted and astrocytoma,

IDHmt, but by definition absent in glioblastoma IDH-wildtype (wt).[2] Standard of care consists of maximal safe resection combined with radiotherapy and/or chemotherapy. While IDHmt glioma patients have a significantly better median survival time compared to those with IDHwt glioblastomas, all gliomas invariably recur, and there is an ardent need for new therapeutic approaches in both groups.[1]

Immunotherapies harness the capacity of the host's immune system to attack cancer cells, and have shifted the treatment standard for several advanced-stage solid and hematological malignancies.[3,4] T cells play an executive role in many immunotherapies because they are responsible for recognition and cytotoxic destruction of tumor cells.[3] The human T cell receptor (TCR) repertoire is enormously diverse, with 1×10^{15} distinct potential TCRs, allowing for the recognition of virtually any possible antigen including tumor neo-antigens.[5,6] T cells originate from hematopoietic stem cells in the bone marrow and mature in the thymus. To become activated, naive T cells require the presentation of their cognate antigen by antigen-presenting cells (APCs) along with costimulatory signals and pro-inflammatory cytokines, generally in the secondary lymphoid organs. Consequently, they undergo clonal expansion, traffic to affected target tissues and differentiate into effector cells upon encounter with their cognate antigen.[7] Immunotherapies that employ T cells to achieve tumor-directed immunogenicity include checkpoint inhibition, adoptive T cell transfer, bispecific T cell engagers, oncolytic viruses and cancer vaccines.[3] Especially monoclonal antibodies blocking the checkpoint molecules PD-1 and CTLA-4, as well as chimeric antigen receptor (CAR)-T cells, have generated dramatic and sometimes durable responses in several cancer types and are now widely embedded into clinical practice.

T cell-based immunotherapies have gained interest for glioma as well, but at least 2 important factors complicate the development of immunotherapies for glioma. Firstly, their location in the CNS, and secondly, the immunosuppressive properties of the glioma tumor micro-environment (TME). The CNS is structurally protected from pathogens and toxins by the blood-brain barrier (BBB), and was long thought to be an immunoprivileged site with very little to no infiltrating immune cells. However, it is now appreciated that the CNS is under tightly regulated immune surveillance.[6] There is drainage of CNS antigens through the cerebrospinal fluid (CSF) and a meningeal lymphatic network onto the deep cervical lymph nodes, where APCs can prime and activate T cells.[8] Activated T cells enter the CSF through leptomeningeal vessels in the subarachnoid space and the choroid plexus, and dynamic gradients of chemotactic cues including cytokines, chemoattractants, integrins and matrix metalloproteinases can induce and facilitate migration of effector lymphocytes across the BBB, allowing T cells to survey the brain and spinal cord.[9]

However, the glioma TME remains a T cell-poor environment compared to other solid tumors. Their location in the CNS is not the only cause, as brain metastases of other solid tumors do show marked T cell infiltration and can respond to treatment with checkpoint inhibitors.[10,11] In particular glioblastomas employ several tactics to drive their TME towards a highly immunosuppressive state.[11-14] Factors that influence immune evasion in glioma include a low mutational burden with limited neoantigens, the presence of immunosuppressive myeloid cells, the production of anti-inflammatory cytokines such as IL-10 and TGF-β, and the production of indolamine 2,3 dioxygenases and arginase, which influence tryptophan metabolism and negatively affect T cell activity.[15–17] Tumor-associated macrophages (TAMs), including bone marrow-derived macrophages and tissue-resident microglia, dominate the glioma TME and may comprise up to 50% of the tumor mass.[18,19] TAMs are generally considered pro-tumorigenic and suppress T cell responses in glioma.[19] Furthermore, T cell sequestration in the bone marrow,[20] therapy-induced systemic lymphopenia,[21] and the prescription of immunosuppressive medication such as dexamethasone to reduce cerebral edema[22] are factors that likely reduce T cell presence in glioma.

Clinical trials with checkpoint inhibitors have generally failed to show clinical benefit in glioma patients, as summarized elsewhere.[23,24] However, most of these trials have focused on the evaluation of classical efficacy endpoints and did not perform assessments of immunogenicity or appropriate target engagement in the TME.[17] Therefore, a better understanding of the immune micro-environment of gliomas is needed to fully understand why these trials failed. Furthermore, the first results of CAR-T cell therapies in glioma patients show signals of activity,[25–27] and future trials with CAR-T products are likely to benefit from a better understanding of what holds back T cell immunity in these tumors.

Aim and Research Question

In this study, we aim to systematically review the literature on abundance as well as spatial and phenotypical characteristics of T cells in the TME of adult diffuse glioma. A better understanding of T cell immunity in glioma might allow for the selection of patient subsets with a better response to certain immunotherapies, or might provide a rationale for combination therapies. Ultimately, this can aid decisions about whether and how to proceed with the development of immunotherapies for glioma.

Methods

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[28]

Literature Search

A systematic literature search was conducted in Medline, Embase, Web of Science Core Collection, Google Scholar and the Cochrane Central Register of Controlled Trials to retrieve all eligible articles published up to to May 6, 2025. The search terms *glioma* and *T lymphocytes* as well as synonyms were used. Complete search strings are reported in Supplement 1.

Eligibility Criteria

Only studies using human adult glioma samples were considered eligible, since extrapolating findings from preclinical

glioma models remains challenging due to significant differences in tumor biology and the immune micro-environment between preclinical models and human gliomas. Given their established role in clinical management, studies with patients who received standard therapies such as radiotherapy or chemotherapy were included; however, in order to focus on the endogenous T cell landscape, we excluded studies analysing samples obtained after specific immunotherapeutic interventions, which might reflect treatment-induced alterations rather than baseline immune architecture. Publications had to be available in the English language, and reviews and studies with less than 5 patients were excluded to reduce sampling error. Finally, bio-informatic analyses using public datasets like TCGA were excluded to prevent duplication.

Study Selection and Data Extraction

Eligibility assessment was performed by 3 independent reviewers (R.L., W.V., and L.D.) by sequentially screening titles, abstracts and full-text articles using Rayyan.ai. Doubts regarding in- or exclusion were resolved through discussion. Where available, the following information was extracted from each study: sample size, tumor subtypes, grade, methods, and most important findings on T cell abundance, phenotypes, spatial distribution, and prognostic significance. From articles combining human data with in vitro studies, animal studies or analyses of public datasets, only human data was extracted. All extracted data are summarized in **Table 1**, and full extracted data can be found in Supplement 2.

Risk of Bias

The risk of bias in individual studies was assessed by 1 reviewer (R.L.) according to the updated Cochrane Risk of Bias Tool[111] and was visualised using Robvis.[112] Risk of bias was deemed intermediate in most studies; eleven articles were deemed high risk, mostly due to bias in reporting of the results (Supplement 3 and 4).

Search Results

A total of 4303 records were screened on title and abstract after removing duplicates. Four thousand one hundred sixty-six articles were excluded at the title-abstract level. One hundred thirty-four articles were screened on full text, and finally 85 articles were selected for review (**Figure 1**).

Results

The 85 studies included in this review were published between 1999 and 2025. Due to changes in the WHO classification of CNS tumors during this period, patient populations might not be directly comparable among studies. Therefore, we have included subtype, WHO grade, WHO classification used, and IDH-mutation status (if reported) in **Table 1**. Out of 85 studies, only two articles focus solely on IDHmt glioma and 18 articles focus solely on IDHwt glioblastoma. The remaining articles include, and sometimes pool, both IDHwt and IDHmt gliomas in their analyses.

T Cells Are Present in the Glioma TME, but Abundance is Highly Variable and Lower than in Brain Metastases

Thirty-three included studies report quantitative data on T cell abundance in glioma. Despite different reporting methods, which impede direct comparisons, several observations can be made. First, T cells are present in the glioma TME. Four studies report a percentage of CD3+ T cells within the total amount of cells in the glioma TME, and find that CD3+ T cells comprise roughly 2% of all cells in 10 gliomas of different grades,[82] <2% of total cells in glioblastoma,[12] 2.1% of all DAPI+ cells in glioblastoma,[63] or "generally less than 5%" of total cells in glioblastoma.[73] Two studies report the percentage of T cells among all isolated tumor-infiltrating immune cells, and find that T cells comprise 11%[32] or 13%[11] of immune cells in heterogeneous groups of glioma patients. Mazzaschi and colleagues report a median of 37 total CD3+ tumor-infiltating lymphocytes (TILs)/mm^2 in glioblastoma,[77] and Weenink et al. report that nearly all WHO 2016 grade II glioma samples have T cell counts < 40 cells/mm^2.[103] Kim et al. find 262 696 T cells per gram for glioblastoma, 151 000 for WHO III astrocytoma, and 142 606 for WHO III oligodendroglioma.[57] Several studies mention that T cell numbers are increased in glioma when compared to adjacent normal-appearing brain tissue.[53,55,78,97,98] For instance, Tamura et al. report a mean of 10 CD8+ T cells and 15 CD4+ T cells per 3 high-powered fields (HPF) in the tumor core of IDHwt glioblastomas, compared to 0.5 and 0 in adjacent normal-appearing brain, respectively.[98]

Even though T cells are detected in the glioma TME, they are considerably less abundant in glioma compared to brain metastases of other cancers. Six studies report significantly lower quantities of T cells in both IDHmt and IDHwt glioma compared to brain metastases from non-small cell lung cancer, melanoma, breast cancer and renal cell carcinoma.[11–13,81,91,95] Friebel et al. observe that T cells comprise 13% of all intratumoral immune cells in glioma, compared to 50% in brain metastases of melanoma and NSCLC. Contrastingly, the glioma TME contains a much higher fraction of TAMs and monocytes (80%) compared to brain metastases (40%).[11] Furthermore, T cell abundance in glioma is highly heterogeneous, both between and within tumors, as mentioned in 9 of the included articles.[12,29,57,59,67,71,73,77,91,96] Mazzaschi et al. report a wide range of 9-360 CD3+cells/mm^2 of glioblastoma tissue,[77] and Soltani et al. find a range of 9-600 CD3+ T cells/mm2 of glioblastoma tissue.[96]

In short, T cells are present in the glioma TME, although numbers are low compared to brain metastases. T cell densities are heterogeneous, both between regions and between tumors.

Determinants of T Cell Abundance Include IDH-Status, WHO Grade and Tumor Recurrence

T cell infiltration is significantly lower in IDHmt gliomas compared to IDHwt glioblastomas, as mentioned in 10 articles.[11,13,33,36,40,56,57,71,99,109] Accumulation of the oncometabolite D-2-hydroxyglutarate (D-2HG) in IDHmt glioma drives TAMs

Table 1. Summary of data extracted from the 85 included studies

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Abdelfattah[29]	1 O2, 1 A2, 16 GBM	2021	scRNA seq	Majority of T cells are CD8+ in all samples; increased T cell infiltration at recurrence
Alban[30]	6 GBM (of which 1 IDHmt)	2016	CyTOF mass cytometry	More CD4+ T cells compared to CD8+ T cells across tumor grade; significantly increased amounts of double-positive and CD8+ T cells at recurrence
Asioli[31]	30 GBM	2021	IHC	More CD4+ than CD8+ T cells; of the 25 CD3 positive cases, 10 showed an exclusively perivascular distribution, 14 both perivascular and intratumoral, and 1 case had an exclusively intratumoral distribution
Bartkowiak[32]	32 GBM	2021	Mass cytometry	Lymphocytes in lateral ventricle-contracting GBM bear a hyperactivated/exhausted phenotype correlating with worse prognosis
Batchu[33]	1 A2, 7 GBM	2021	Spatial & scRNA seq	Prominent spatial overlap amongst TAM signatures and a T cell exhaustion gene signature
Belghali [34]	5 LGG (not specified), 19 GBM	2007	Immunohistochemistry (IHC)	Significantly more T cells in GBM compared to LGG; mostly localized in the perivascular space; CD3 inversely correlates with survival
Benotmane[35]	9 GBM	2021	Spatial TCR seq	Clonal T cell expansion was observed in 6 out of 9 samples
Berghoff[36]	43 LGG (15 A2, 7A3, 8 O2, 9 O3, 4 IDHwt LGG) & 131 GBM (of which 117 IDHwt)	2016	IHC	Significantly higher CD3+ T cell and PD1+ T cell infiltration in GBM compared to LGG; more prominent TIL infiltration and higher PD-L1 expression in IDHwt compared to IDHmt gliomas
Chen[37]	59 gr II or III, 84 gr IV gliomas (not specified)	2016	IHC, flow cytometry (FCM)	CCR5+CD38+HLA-DR+CD8+ T cells infiltrate the glioma TME
Davidson[38]	42 GBM, 10 LGG; 38 IDHwt, 13 IDHmt, 1 unknown	2016	FCM/FACS, mass cytometry, RNA seq	Tumor-infiltrating PD-1+ T cells represent a chronically activated, exhausted effector T cell population (CD45RO+ CD27- IL-7R-HLA-DR+ CD38+TIM-3+ LAG-3+ CTLA-4+)
Dejaegher[39]	10 LGG, 15 GBM (not further specified)	2016	FCM	PD1 is upregulated on TILS compared to peripheral T cells; no differences in PD1 expression between LGG vs GBM or primary vs recurrent tumors
Dejaegher[40]	93 GBM	2007	IHC, FCM, DNA methylation arrays	The mesenchymal subclass of GBM has the highest amount of infiltrating CD3+ T cells
Diaz[41]	4 LGG (1 O2, 2A2, 1 GA2), 11 GBM (IDH status not known)		Multiplex IF (mIF); EM	Highly proliferative gliomas show higher T cell infiltration into the tumor parenchyma; CD4+ T cells are abundant in perivascular areas
Dinevska[42]	98 LGG (22 A1, 42 A2, 34 A3), 28 GBM	2021	mIF	T cell infiltration increases with tumor grade. FOXP3 Tregs are rare, comprising <0.5% of T cells. Most T cells localize to the perivascular niche
Dobersalske[43]	39 GBM (30 IDHwt, 9 IDH status not known)	2007	FCM, mIF, scRNA seq	Tumor-reactive CD8+ clonotypes can be found in the cranial bone of glioblastoma patients
Friebel[11]	1A3, 1A4, 1O3, 19 GBM; 14 BrMs	2021	CyTOF mass cytometry	T cell infiltration is increased in brain mets compared to gliomas. No significant differences in frequencies of specific T cell subsets (CD4+, CD8+ and γδ-T cells) were found between IDHmt, IDHwt glioma and brain mets
Fu[44]	5 A, 10 O, all IDHmt, grade not mentioned	2016	CyTOF mass cytometry	The proportions of PD-1+ or TIM-3+ exhausted CD4+ and CD8+ T cells were substantially higher in TILs compared to peripheral T cells
Fu[45]	10 A (of which 1 IDHwt), 4 O (of which 1 IDHwt)	2016	CyTOF mass cytometry	The proportions of TIM-3+ CD4+ and PD1+ CD8+ T cells were substantially higher in TILs compared to peripheral T cells; CD8+ TILs show reduced ability to secrete IFNγ, TNFβ, T-bet and granzyme B

Continued

Table 1 Continued

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Fu[46]	16 GBM (of which 5 IDHmt)	2016	CyTOF mass cytometry	Both exhausted T cell (PD1, TIM-3 or LAG-3+) and regulatory T (Treg) cell percentages were significantly higher in TILs than in peripheral T cells
Gastearena[47]	76 GBM	2021	IHC	Perivascular lymphocyte cuffs were observed in 12 out of 76 samples (16%), while most slides showed areas of infiltrating lymphocytes. Higher CD8 values correlated with a worse OS
Gershon[48]	22 LGG (of which 3 IDHwt), 4 A4, 59 GBM, 10 BrMs	2016	FCM	A composite parameter combining the frequencies of 4 tumoral lymphocytes separates the survival curves of GBM patients with a median difference of 10 months
Gupta[49]	10 LGG (not further specified), 8 GBM	2021	scRNA seq, FCM	Increased T cell infiltration at recurrence, independent of IDH-status
Heimberger[50]	77 LGG (19 A3, 3A2, 21 O2, 16 O3, 13 OA3, 5 OA2, 6 gliosarcoma), 58 GBM	2007	IHC, FCM	CD4+ and CD8+ T cell infiltration increases with tumor grade; no significant difference in FOXP3+ Tregs between primary and recurrent tumors
Hijfte[51]	137 IDHmt astrocytoma	2021	mIF, bulk RNA seq, spatial RNA seq	Perivascular T cell confinement in IDHmt astrocytomas associates with gemistocytic tumor cell—TAM networks that mimic glial scarring mechanisms
Huff[52]	26 GBM	2021	FCM, RNA seq	There is a large population of CD8+CD28− T cells in GBM patients (blood + tumor) that is functionally and phenotypically distinct from exhausted T cells
Innocenti[53]	59 GBM (of which 56 IDHwt)	2016	IHC (on TMA's)	The number of CD4+ and CD8+ T cells is higher in GBM tissue compared to normal appearing brain tissue. CD4+ cells were inversely related to OS
Jacobs[54]	12 LGG (of which 6 gr II, 6 gr III), 31 GBM, 20 BrMs	2007	IHC, FCM	Treg accumulation positively correlates with WHO grade. Tregs are mainly localized in the perivascular space, in close proximity to other lymphocytes
Kaffes[55]	56 GBM	2007	IHC, IF	Mesenchymal tumors show markedly increased levels of TILs compared to other subtypes
Karimi[12]	120 GBM, 19 A4, 46 BrMs	2021	Imaging mass cytometry (IMC)	Certain spatial cellular neighborhoods with elevated numbers of CD4+ T cells were associated with improved survival
Kikuchi[56]	33 IDHwt, 22 IDHmt, grade not mentioned		RNA seq	Higher immune infiltration in IDHwt gliomas. Exhausted CD4+ and CD8+ T cell states were more abundant in IDHwt gliomas. Tumor-specific T cells are present, but do not correlate with clinical prognosis
Kim[57]	18 LGG (9 O3, 9 A3), 46 GBM	2016	FCM	Considerable heterogeneity between samples. T cells were more frequent in GBM compared to LGG. CD8+ T cells were more frequent than CD4+ T cells in all subgroups
Kim[58]	8 LGG (4 A3, 4 O3), 26 GBM	2016	FCM, RNA seq	The numbers of CD3+, CD4+, CD8+ T cells and FOXP3+ Tregs were significantly higher in the tumor core compared to the peripheral regions. A hypoxia signature positively correlated with the enrichment of severely exhausted CD8+ T cells, M2 TAMs and Treg cells in the tumor core

Continued

Table 1 Continued

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Klemm[13]	FCM: 5 LGG, 2 GBM, 16BrMs. RNAseq: 7 LGG, 17 GBM, 17 BrMs	2016	FCM/FACS, RNA seq, IF, ELISA	Gliomas contain an abundance of TAMs, whereas T cells were much fewer, particularly in IDHmt tumors. Tregs were rare in gliomas
Kmiecik[59]	65 GBM	2007	IHC, FCM	Great heterogeneity in abundance and spatial localization. Increased CD3+T cell infiltration was associated with prolonged survival
Knudsen[60]	23 GBM		IF	No significant differences in the fraction of CD3+T-lymphocytes nor the distribution of CD3+/CD8+ cytotoxic- and CD3+/FOXP3+-regulatory T-lymphocytes in primary vs recurrent tumours
Kollis[61]	30 GBM		IF, FCM, scRNA seq	T-cell subsets expressing CCR2, CCR5, CXCR3, CXCR4, CXCR6, CD49a, and CD49d are enriched in glioblastoma tumours compared to matched peripheral blood samples
Koshkaki[62]	9 GBM (of which 1 IDHmt)	2016	IHC	Expression of PD-1, PD-L1, TIGIT, IDO, and FOXP3 was higher in the tumor core compared to infiltration zone, along with an increase in CD163+TAMs
Kumar[63]	22 PXA, 29 LGG (all IDHmt astrocytoma grade II or III), 30 GBM (all IDHwt)	2016	mIF, spatial RNA seq	PXAs harbored a significantly higher mean percentage of CD3+T cells as compared with IDHmt astrocytoma and IDHwt GBM
La Manna[64]	20 GBM	2016	FACS/FCM, IF	The CD8+CD103+ CD69+TRM subset was significantly more abundant in GBM samples than in control (meningioma) samples
Lee[65]	32 GBM	2021	FCM	An increased frequency of precursor exhausted T cells (CD8+KLRG1–PD-1+CXCR5+TCF1+TIM3–) was found in male vs female tumor samples
Liu[66]	22 LGG, 31 HGG (unspecified)	2007	IHC, FCM	Tim-3 expression was increased ~4-fold on the CD4+TILs and ~2-fold on the CD8+TILs as compared to PBMCs from glioma patients
Lohr[67]	28 LGG (13 WHO II, 15 WHO III), 65 WHO IV GBM	2007	mIF, FCM	T cell infiltration increases with WHO grade; no difference between primary and recurrent tumors; effector T cell infiltration positively impacts survival
Luce[68]	32 LGG (A2 or A3), 60 GBM	2007	IHC (on TMA's)	Increased expression of PD-L1 in GBM compared to LGG
Maddison[69]	13 GBM	2021	IHC	Low T cell density. Perivascular T cells are common in both primary and recurrent tumors. CD8+TILs increased significantly in recurrent tumors
Magri[70]	63 GBM (57 IDHwt, 6 IDH unknown)		FCM, mIF	Increased frequency of CD8+T cells both in central and marginal areas of recurrent tumors. Steady high frequency of PD-1 expression among TILs
Makarevic[71]	130 IDHmt LGG (59 gr II, 71 gr III), 13 IDHwt GBM	2016	mIF	Higher T cell infiltration in IDHwt compared to IDHmt tumors. Tregs are rare and comparable across glioma groups. Increased CD3+ infiltration in recurrent tumors, especially after treatment with radiotherapy alone
Marinari[72]	RNAseq: 19 (16 gr IV, 3 gr III; 2 IDHmt, 17 IDHwt) FCM: 39 (14 gr II, 8 gr III, 17 gr IV; 20 IDHmt, 15 IDHwt, 4 unknown)	2016	RNA seq, FCM	Significant enrichment of CD8+ and CD4+T cells and NK cells in patients with a poor prognosis
Martinez-Lage[73]	98 GBM (96 IDHwt, 2 IDHmt)	2016	IHC	Great heterogeneity among and within samples. Highest lymphocyte infiltration in mesenchymal GBMs compared to other subgroups. The percentage of CD3+ and CD8+T cells did not correlate with patient survival
Marx[74]	35 GBM (29 IDHwt, 6 IDH status unknown)		FCM, IHC	TEM comprised the dominant T cell subpopulation both in intratumoral CD4+ and CD8+T cells

Continued

Table 1 Continued

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Mathewson[75]	15 LGG (3 O2, 2 O3, 4A3, 5 A4, 16 GBM (all IDHwt)	2016	scRNA seq	TCR repertoire analysis revealed the presence of highly expanded clonotypes in each patient. Clonal CD8 T cells had higher expression of cytotoxicity genes (GZMB and NKG7) and NK receptors (KLRB1 and KLRD1) than non-clonal cells in GBM. A large fraction (>90%) of GBM-infiltrating CD8 T cells and most CD4 T cells were CD161-positive
Mauldin[76]	77 GBM	2021	mIF	Median numbers of CD8, CD4, and CD20 infiltrates were 26.9, 6.0, and 10.6 cells/mm^2 of tissue, respectively. No correlation between total densities of infiltrating CD4, CD8, and CD20 and survival
Mazzaschi[77]	GBM; 53 IDHwt, 3 IDHmt, 1 unknown	2016	IHC	IDHmt tumors were characterized by a significantly higher density of perivascular TILs compared to wildtype tumors
Mitsdoerffer[78]	RNAseg: 9 GBM FCM: 4 GBM	2021	RNA seq, FCM	While CD4+ T cells were found in association with the wall of brain vessels and in necrotic areas, CD8+ T cells were largely located either perivascularly or scattered in the tumor bed. GBM-associated CD8+ T cells showed a partially exhausted phenotype with TIM3 (but not PD1) differentially expressed in GBM. Th17 cell signatures are markedly enriched in GBM TILs
Mohme[79]	40 GBM (all IDHwt)	2016	FCM, cytokine assays, TCR sequencing	TILs were largely comprised of differentiated CD4+ and CD8+ T cells, a mixture of TTM and TEM in the CD8+ compartment and a majority of TTM in the CD4+ compartment. High expression of PD-1, and CD39, and HLA-DR was found in rGBM (vs primary GBM). PD-1 was the most prominently expressed marker on TILs, both for CD8+ and CD4+ cells
Mu[80]	15 DA, 14 DA recurred as GBM, 15 GBM. 22/44 were IDHwt (not mentioned which)	2007	RNA seq, IHC, mIF	More perivascular than intratumoral TILs in primary and recurrent tumors; in primary tumors, two-thirds of the TILs were in the perivascular niche. Perivascular T cells were enriched at recurrence
Musca[81]	19 GBM, 20 BrMs, IDH status unknown		FCM	Significantly more tumor-infiltrating T cells in brain mets compared to glioma; high levels of exhaustion markers, irrespective of the tumor's origin
Najem[82]	2 LGG (1 A2 IDHmt, 1 A2 IDHwt), 8 GBM IDHwt, 10 BrMs		scRNA seq, mIF	T cells were much less frequent across tumors relative to myeloid-derived lineages. In GBM, T cells were mostly found in perivascular regions and showed strong nuclear p-STAT3 expression
Noorani[83]	59 GBM	2021	IHC	There are functional interactions between anti-inflammatory TAMs and CD4+/CD8+ T cells in all regions. Suppression of T cells via PD-L1 may be present in the leading edge and may be driven by anti-inflammatory TAMs
Ou[84]	75 LGG, 50 GBM (mix of IDHwt and IDHmt, not further specified)		IHC	Increased infiltration of CD8+ T cells in recurrent gliomas, especially in GBM
Perrin[85]	16 grade 3 or 4 astrocytoma (no IDH status known)		RNA seq (CDR-3 analysis & sequencing of amplified TCR transcripts)	CDR-3 analysis revealed multiple T cell oligoclonal expansions in all astrocytomas. Unique T cell clones were present in different adjacent areas of a given tumor, but never detected in the blood. Expanded clones were almost exclusively confined to the CD8+ subset

Continued

Table 1 Continued

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Pimenta[86]	51 GBM (if which 50 IDHwt)	2016	FCM	No significant differences in the different immune cell subsets were observed between primary and recurrent tumors
Ran[87]	9 GBM	2021	scRNA seq	T cell subpopulations in glioma tissues exhibited higher exhaustion signature scores compared to PBMCs
Ravi[88]	8 GBM	2021	scRNA seq, spatial RNA seq	CD8+T cells revealed a stronger probability for tumor-associated exhaustion compared to the CD4+ population. Tumor regions enriched for mesenchymal-like (MES-like), and astrocytic-like (AC-like) transcriptional signatures were colocalized with CD8+T exhausted clusters and CD4 Th17-like clusters. HMOX1+ myeloid cells were spatially correlated with T cell exhaustion and the mesenchymal state of glioblastoma
Romagnoli[89]	45 GBM	2021	FCM	CD8+CD103+ TRM cells abundantly infiltrate GBM, and low frequency of these cells expressing PD1, TIM3, or both correlates with better OS
Sayour[90]	39 GBM		IHC	No significant difference in absolute cell counts of CD4+, CD8+, and FOXP3+ cells between primary and recurrent GBMs. Recurrent tumors showed a significant decrease in proportion of CD4+T cells. Individual lymphocyte populations did not correlate with clinical outcomes
Schaettler[91]	1 O3, 18 GBM (of which 3 IDHmt), 11 BrMs		Whole exome, RNA and TCR seq	The T cell fraction among all cells was significantly higher within the BrMs compared to glioma, whereas the TCR repertoires within GBM had a higher degree of clonality. There is increased spatial heterogeneity of neoantigens within GBM compared to BrMs. The intratumoral TCR repertoire was significantly more similar between spatially distinct regions of BrMs than gliomas, which often harbored locally expanded T cell clones
Schmassman[92]	3 GBM, 2 A4	2021	scRNA seq, FCM	Tumor CD8+T cells displayed features of tissue-resident memory T cells and were characterized by an exhaustion phenotype
Scholl[93]	7 IDHmt LGG (grade/subtype not mentioned), 9 A4, 12 GBM	2021	FCM, RNA seq	Increase in all ectoenzymes (CD38, CD39, CD73) in each lymphoid subset analyzed in the TME when compared to peripheral blood
Shen[94]	7 LGG (5 A3, 2 O3), 13 GBM	2016	FCM	LAG3, BTLA, VISTA, TIM-3 and CTLA-4 were detected in various degrees on CD3+T cells in the HGG microenvironment
Simonds[95]	19 GBM		CyTOF mass cytometry	Nearly all subsets of T cells, plus NK cells, were decreased in the GBM TIME compared to RCC and sarcoma primary tumors
Soltani[96]	5 GBM, all IDHwt	2016	IHC, IF, qPCR	Highly variable numbers of CD3+ cells in GBM (ranging from 9 to 600 cells/mm^2)
Tamma[97]	30 GBM	2021	IHC	Both CD4+ and CD8+ cells in the tumor zone significantly increased compared to the surrounding normal appearing brain area; the increase of CD4+ cells was about ten times greater than that observed in CD8+ cells
Tamura[98]	10 GBM (all IDHwt)	2016	IHC	Most T cells (both CD4 and CD8) were observed in the perivascular area. FOXP3+ cells were mainly observed in the perivascular area. The tumor core had more PD-1+CD8+T cells compared to the infiltration zone
Tang[99]	41 IDHmt, 46 IDHwt (grade and subtype not mentioned)		IHC	CD3+T cells, CD4+T cells, CD8+T cells, neutrophils and macrophages were significantly less abundant in IDHmt glioma compared to IDHwt glioma
Wang[100]	21 GBM	2021	mIF, FCM	PD-1 was expressed in about 46% of CD4+T and 38% of CD8+T cells in GBM tissue. CD8+T cells were more abundant in patients with a better prognosis (survival > 14 months)

Continued

Table 1 Continued

Author	Glioma subtype, grade & IDH status (if known)	Used WHO classification (if known)	Methods	Main findings on T cell abundance, spatial distribution, phenotypes & prognosis
Wang[101]	15 HGG (unspecified; either A4 or GBM)	2021	scRNA seq, V(D)J seq, spatial RNA seq	CD8+GZMK+ TILs comprised the most clonally expanded T cells, and localize near vasculature and CD163+ MHCII+ myeloid cells
Wang[102]	26 GBM	2021	Histopathology, IF, scRNA seq	Within the peritumoral brain zone, the proportion of T cells in the high cerebral blood flow interface was higher than in the lower cerebral blood flow interface (10.08% vs. 3.30%)
Weenink[103]	FCM: 12 LGG (gr II), 8 HGG IF: 28 LGG (gr II), 28 HGG	2016	FCM, IF, WES & RNA seq, qPCR	LGG have lower numbers of intra-tumoral CD8 T cells compared to HGG. In LGG, T cells were predominantly located perivascularly. LGG and HGG harbor an equally diverse TCR-Vβ repertoire
Wischniewski[104]	26 GBM (of which 2 IDHmt). 4 O, all IDHmt. 6 A, of which 1 IDHwt (grade not mentioned). 48 BrM		Bulk and scRNA seq, FCM, mIF, TCR profiling	>90% of TILs were CD45RO+, indicating a conserved broad activation and differentiation program in brain TILs independent of the underlying disease. CD8+ TILs in gliomas had a high abundance of C1 which contained early active and effector memory CD8+ T cells
Woroniecka[105]	FCM: 21 GBM TCR analysis: 5 GBM		FCM & TCR analysis	PD-1, LAG-3, TIGIT, and CD39 were highly expressed on patient CD8+ TILs. PD-1 was especially prominent, being found on up to 96% of tumor infiltrating CD8+ T cells
Xu[106]	FCM: 2 A3, 7 GBM IHC: 3 A3, 16 GBM		FACS/FCM, IHC, functional assays	Robust CD39 expression was observed in CD4+ TILs, with a prevalence of 61.8+19.3% relative to the 8.0+5.7% from peripheral CD4+ T cells
Yang[107]	108 GBM		IHC	Tumors from long-term survivors were more likely than those from short-term survivors to have intermediate or extensive T cell infiltrates
Yang[108]	28 pilocytic astrocytoma, 63 GBM	2007	IHC	Significantly more glioblastomas had intermediate or extensive perivascular CD8+ T cells compared to intratumoral CD8+ T cells
Zhang[109]	23 A3, 14 A4, 35 GBM		IHC	The IDH mutation was significantly associated with reduced numbers of tumor-infiltrating CD4+, CD8+ and FOXP3+ T cells. D-2HG significantly inhibited the migration of both CD4+ and CD8+ T cells
Zhao li[110]	24 GBM		FACS/FCM	Compared to healthy controls, GBM patients had significantly more CD4+ FOXP3- IL-10+ Tr1 cells in peripheral blood. The Tr1 frequency was further elevated in the tumor. By surface marker expression, the Tr1 cells were enriched in the antigen-experienced effector/memory cell compartment

This table includes a summary of information on glioma subtype, grade, IDH status, used WHO classification, methods and main findings on T cell abundance, spatial distribution, phenotypes and prognosis for each study included in the review. A more detailed overview of all extracted data can be found in Supplement 2.

Abbreviations: A2, grade 2 astrocytoma; A3, grade 3 astrocytoma; A4, grade 4 astrocytoma; BrMs, brain metastases; CyTOF, cytometry by time of flight; FACS, fluorescence-activated cell sorting; FCM, flow cytometry; GBM, glioblastoma; HGG, high grade glioma; IDH, isocitrate dehydrogenase; IF, immunofluorescence; IHC, immunohistochemistry; LGG, low grade glioma; mIF, multiplex immunofluorescence; O2, grade 2 oligodendroglioma; O3, grade 3 oligodendroglioma; PBMCs, peripheral blood mononuclear cells; qPCR, quantitative polymerase chain reaction; scRNAseq, single-cell RNA sequencing; TAM, tumor-associated macrophage; TCR, T cell receptor; TILs, tumor-infiltrating lymphocytes; TMA, tissue micro-array; WES, whole exome sequencing.

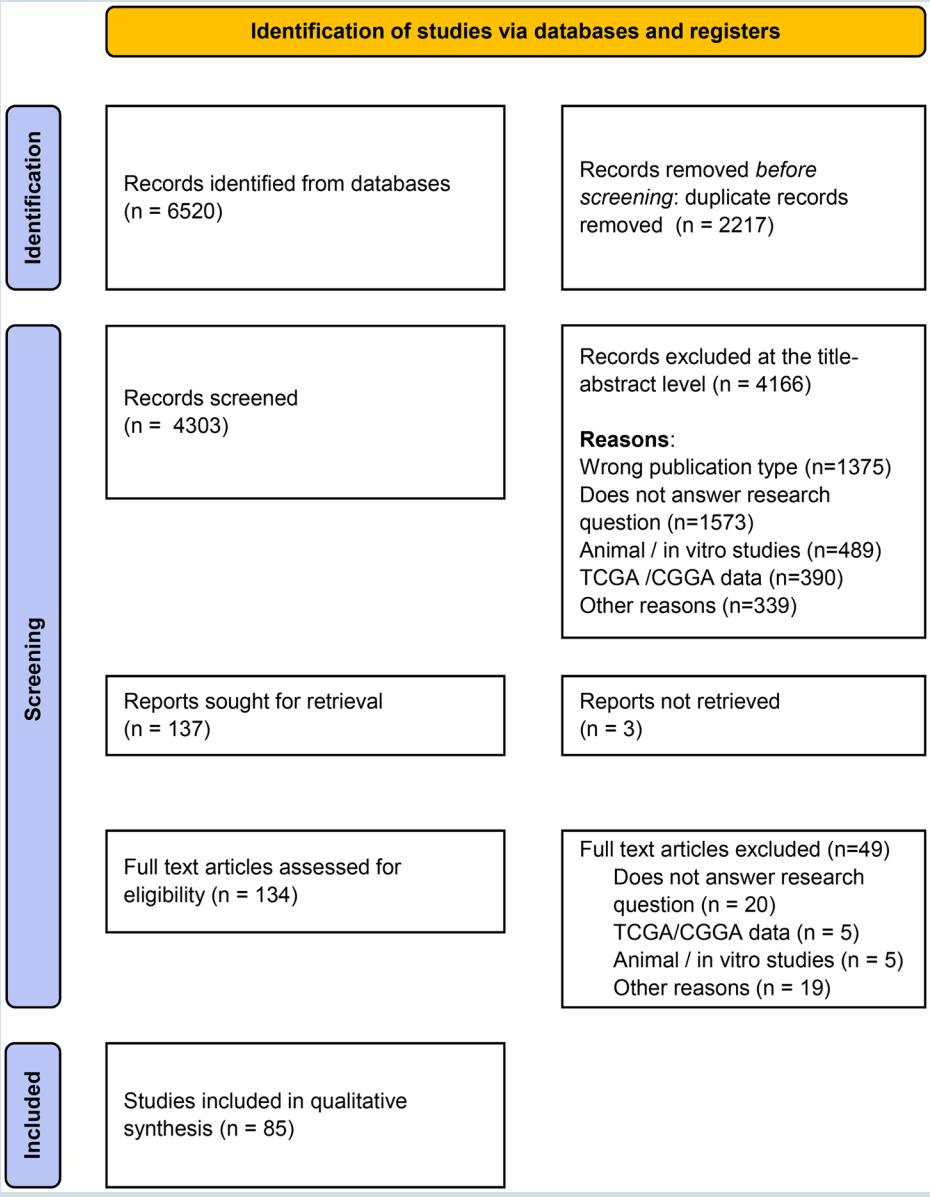


Figure 1. PRISMA flowchart describing the systematic approach to identification, screening and inclusion of studies in this review.

toward an immunosuppressive state through a complex re-orchestration of tryptophan metabolism,[16] decreasing pro-inflammatory cytokines and inhibiting proliferation and migration of both CD4+ and CD8+ T cells. Furthermore, D-2HG suppresses T cell activity by modulating the transcription factor NFAT and the complement system.[109] Besides IDH-status, 5 studies have investigated the association between WHO grade and T cell density, and find that T cell density significantly increases with higher WHO grade.[42,50,57,67,103] Grade 4 gliomas have a 5-fold higher T cell density/mm^2 than grade 2 gliomas.[103] Within IDHwt glioblastomas, 3 different transcriptome-based subtypes have been described; proneural, classical and mesenchymal, of which the mesenchymal subtype is considered most aggressive, most dedifferentiated and most inflammatory.[113] Accordingly, 3 studies find that the mesenchymal subtype is associated with increased amounts of both myeloid cells and T cells.[40,55,73] Whereas non-CNS tumors with a mismatch-repair deficiency (MMRd) often show increased immune infiltration, likely due to increased neo-antigen burden,[114] a landmark study by Touat et al. showed no significant difference in T cell infiltration between MMR-deficient and MMR-proficient gliomas.[115] Differences in T cell abundance between primary and recurrent glioma are reported in 9 studies.[29,46,49,69–71,79,80,86] Three studies report increased CD3+ T cell presence at recurrence, both in IDHmt and IDHwt glioma.[29,49,71] In a dataset of 78 primary and 66 recurrent IDHmt gliomas, Makarevic et al. report a 2-fold increase in CD3+ T cells at recurrence. In their study, treatment with radiotherapy alone was associated with increased T cell infiltration compared to chemotherapy alone or combined chemoradiotherapy.[71] Others specifically report an increase in CD8+,

but not CD4+ T cells in recurrent glioblastoma,[69,70,79] or an increase of perivascular T cells in recurrent IDHmt and IDHwt glioma.[80] The final 2 studies do not find significant differences between primary and recurrent tumors.[46,86] The effect of corticosteroid treatment on T cell density is mentioned in 3 studies. Whereas 2 studies report no correlation between CD3+ TIL density and dexamethasone treatment,[36,76] in the third study, where 18/31 patients did not receive dexamethasone before surgery, dexamethasone use was associated with a 4-fold reduction in CD3+ TIL density.[75]

In short, T cell abundance is higher in IDHwt glioblastomas compared to IDHmt gliomas, and increases with WHO grade and recurrence, whereas dexamethasone therapy likely reduces T cell presence. At present, there is no compelling evidence for increased T cell abundance in MMRd gliomas.

Tumor-Specific TILs Are Present in the Glioma TME, but Are Spatially Restricted

Several immunotherapies, including checkpoint inhibitors, require the presence of tumor-specific T cells for active tumor targeting. Seven articles report on such potentially tumor-reactive T cells (pTRTs) in glioma. Already in 1999, Perrin et al. looked into TCR-clonality in astrocytoma by determining the length of the TCR-β chain complementarity-determining region (CDR-)3 as a proxy for TCR variability.[85] In all 16 samples, they detected several oligoclonal enrichments among CD8+ TILs in different tumor areas, but not in autologous PBMCs, suggesting local expansion of T cells expressing the same TCR-β chain.[85] Reconstructing TCR sequences from single-cell RNA-reads confirms the presence of clonally expanded T cells in the majority of IDHmt and IDHwt gliomas.[75] Interestingly, clonally expanded CD8+ T cells had higher expression of cytotoxicity genes and NK-receptors,[75] pointing to the expansion of tumor-specific effector CD8+ T cells. Clonal diversity in the TCR-Vβ repertoire does not differ substantially between low-grade and high-grade glioma.[103] Schaettler et al. find that in 6 out of 22 glioblastoma patients, a dominant T cell clone was present that comprised >12% of the total T cell repertoire in a certain region. The most highly expanded intratumoral T cell clones could also be detected in the peripheral blood, but at substantially reduced frequencies (<1%).[91] Furthermore, T cell clones accounting for >5% of the clonal space were enriched in recurrent compared to primary glioblastoma.[79] Stable and homogeneously expressed neo-antigens as potential targets for T cell responses are not well established in glioma.[78] Accordingly, in contrast to brain metastases, where the TCR repertoire was relatively similar between spatially distinct regions, clonal expansion in glioblastoma is highly local and highly heterogeneous, corresponding to the genomic and transcriptional heterogeneity in glioblastoma.[35,91]

In short, there is evidence for heterogeneous and locally confined oligoclonal T-cell expansion in the glioma TME, suggesting the presence of potentially tumor-reactive effector cells.

T Cell Subsets Are Not Evenly Present: Tregs Are Enriched in the Tumor Core

The ratio between CD4+ and CD8+ T cells among glioma-infiltrating T cells is debated. Four studies report a predominance of CD8+ T cells,[29,42,57,69] whereas four others report a predominance of CD4+ T cells,[30,32,78,97] and 2 describe no significant differences.[11,74] This may partly be due to different study designs; some studies include both primary and recurrent tumors,[69] both IDHwt and IDHmt gliomas,[29,42,57] and both low- and high-grade gliomas,[29,42] whereas these factors might all influence CD4/CD8 ratio.[103] Furthermore, CD4/CD8-ratio can be confounded by age.[116] Tregs can promote tolerance and suppress anti-tumor T cell responses through upregulated expression of T cell inhibitory receptors, such as CTLA4 and TIGIT, and secretion of anti-inflammatory cytokines such as TGF-β and IL10, which can inhibit T cell activation, proliferation and migration.[67,117] Treg frequencies in glioma are relatively low; Tregs make up only a few percent of all lymphocytes in all of the reviewed articles.[12,13,37,42,59,67,86] However, whereas one group finds that the proportion of Tregs does not differ between circulating T cells and glioblastoma-infiltrating T cells,[79] three other groups find a more prominent population of Tregs in the TME compared to peripheral blood.[11,45,110] Spatially, multiple groups report that Tregs are enriched in hypoxic core regions in mostly IDHwt glioblastomas.[57,58,98] Regarding other specific CD4+ T cell subsets (Th1, Th2, Th17, Th17.1) not much is known yet, although a preferential commitment to the Th17-lineage has been suggested, which might contribute to the suppression of a sufficient Th1- and cytotoxic T cell antitumor response.[78] Combining single-cell RNA sequencing and multicolor flow cytometry, Kollis et al. have examined profiles of chemokine receptor expression within both CD8+ and CD4+ T cells in glioblastoma. Within CD8+ TILs, expression of CCR2, CCR5, CXCR3, CXCR4, and CXCR6 was enriched compared to peripheral blood T cells; within CD4+ TILs, these were CCR2, CCR5, CXCR3, and CXCR6, both highlighting a potential role for these chemokine receptor axes in T cell-homing to the glioma TME.[61] Elevated CCR5-expression on glioma TILs was confirmed by Chen et al.[37]

Recently, more innate-like T cell subsets have been described in glioma as well, such as γδ-T cells, natural killer-T cells and Malt-like T cells.[49] Of these, especially γδ-T cells have gained interest within the area of cancer immunotherapy.[118] In contrast to αβ-T cells, γδ-T cells are able to exert antitumor cytotoxicity independent of neoantigen burden and conventional MHC-dependent antigen presentation, making them theoretically suitable for the treatment of MHC-deficient tumors such as gliomas. Whereas their role in antitumor immunity has not been fully elucidated yet, γδ-T cells have been associated with favourable outcomes in most tumor types.[118] Six articles out of the 85 articles included in our review mention γδ-T cells in glioma.[11,34,48,49,64,78] Although they can be detected in most samples, frequencies of γδ-T cells are generally at least 10-fold lower than those of CD8+ or CD4+ T cells.[11,48] La Manna et al report an average frequency of γδ-T cells of 1.7% of the CD45+ leukocyte population,[64] and Belghali et al report heterogeneous immunohistochemical staining patterns for γδ1-T cells with a median of 3.5 positive cells per HPF, and report that does not correlate with age, glioma subtype or prognosis.[34] The functional significance of such innate-like T cell subsets in human glioma has not been investigated to date, and remains incompletely understood.

In short, published results show distinct presence of CD4 and CD8 T cells as well as Tregs in glioma. While Treg frequencies are low, they are enriched in hypoxic regions in the tumor

core. More inconventional, innate-like T cell subsets such as γδ-T cells are present, but remain underexplored in glioma.

Glioma TILs Display a Late-Differentiated, Tissue-Resident Memory Phenotype

T cells in the parenchyma of post-mortem brain donors display a late-differentiated phenotype compared to circulating T cells.[119] Smolders et al. show that these T cells largely match a transcriptional phenotype of tissue-resident memory T cells (TRM s), expressing CD103 and CD69 along with inhibitory receptors CTLA-4 and PD-1.[120] TRM cells reside within peripheral tissues after previous antigen exposure, patrolling the local environment to perform a 'sensing-and-alarm' function upon re-exposure.[121] Similarly, glioma-infiltrating T cells are more often antigen-experienced and display a further differentiated phenotype compared to circulating T cells.[92, 104] Four studies find that effector memory T cells (TEM, defined either as CD45RO+CD62L-, CD45RA-CD62L-, or CD45RA-CCR7-CD28-) and/or transitional memory T cells (TTM, CD45RA-CCR7-CD28+) make up the dominant T cell subpopulations in the glioma TME, both within CD4+ and CD8+ subsets.[74,78,79,105] Moreover, glioma-infiltrating T cells predominantly have a phenotypic and transcriptomic profile consistent with TRM cells, including expression of CD49a, CD103, CD69, CXCR3, CXCR6, CCR5, HOBIT, and BLIMP1 and reduced expression of TBET and EOMES.[64,89,92]

In short, T cells in the glioma TME largely display a late-differentiated, tissue-resident phenotype, which may reflect their earlier encounter with antigen and their capacity to enter the brain parenchyma upon chemotactic cues.

Different Spatial Distribution Patterns: glioma TILS Accumulate in the Perivascular Space, Especially in IDHmt Glioma

Sixteen studies mention spatial distribution patterns of glioma TILS. Of these, 3 studies investigate regional differences between the tumor core and infiltration zone, and find that T cells densities are consistently higher in the tumor core compared to the infiltration zone.[36,58,62] Eleven articles describe that T cells can be found both in the tumor parenchyma and in the perivascular space.[34,41,42,47,62,63,69,77,78,103,108] Five studies report a preference for the perivascular space compared to the parenchyma, both for CD4+ and CD8+ T cells.[34,42,78,80,98] In a cohort of primary astrocytomas and glioblastomas, two-thirds of the TILs were found in the perivascular space and only one-third infiltrated the tumor parenchyma.[80] Two other studies find that specifically CD4+ T cells accumulate in the perivascular space, whereas CD8+ T cells tend to infiltrate further.[41,80] Accordingly, only a small fraction of FOXP3+ regulatory T cells (Tregs) infiltrates deep into the tumor parenchyma, and most Tregs are retained in the perivascular space.[54] Looking at differences between tumor types, 2 studies find that perivascular T cell-containment occurs more frequently in IDHmt glioma compared to IDHwt glioblastoma.[77,103] T cell density in IDHmt astrocytomas is highest near blood vessels, and declines with increasing distance from vasculature.[42] Van Hijfte et al. recently elucidated a cellular network between gemistocytic tumor cells and reactive TAMs which co-occurs with perivascular retainment of T cells in IDHmt astrocytoma. They observe a process reminiscent of glial scarring, mediated by tumor-TAM interactions through the expression of receptor-ligand pairs such as SPP1/CD44 and IL-1β/IL1R1, which might contribute to immune evasion in IDHmt glioma.[51]

Thus, perivascular clustering of T cells is a recurring observation, especially in IDHmt glioma. While it is tempting to speculate that this phenomenon may represent early attempts at tertiary lymphoid structure (TLS) formation, the perivascular lymphocyte aggregates observed in IDHmt glioma cannot be formally classified as TLS's due to the lack of typical CD4+ T cell and B cell organization.[51] However, this hypothesis merits further study, as TLSs have been associated with improved immunotherapy response in other cancers.[122] Interestingly, Hotchkiss et al suggest that perivascular accumulation of T cells might support antigen presentation and T cell activation in glioblastoma; in their study, glioblastoma samples that were more permissive to successful TIL expansion seemed to contain more structured perivascular T cell aggregates.[123] This is a hypothesis that will need further testing.

In short, spatial T cell phenotypic patterns are observed in glioma, and while certain T cells do infiltrate the tumor parenchyma, others are retained in the perivascular space. In IDHmt glioma, perivascular T cell retainment seems to be mediated through tumor-TAM interactions in a process reminiscent of glial scarring.

Glioma TILs Display Typical Characteristics of Exhaustion, Especially in the Hypoxic Tumor Core

Woroniecka et al. extensively profiled glioblastoma TILs, and were among the first to describe a tumor-imposed T cell-exhaustion phenotype characterized by the sequential co-expression of immune checkpoints molecules including PD-1, TIM-3, LAG-3, CTLA-4, TIGIT, and CD39 along with transcriptional programs that imply decreased cytotoxic functionality and closely resemble classical virus-induced exhaustion.[105] PD-1 expression alone is not enough to characterize T cells as "bona fide" exhausted, as PD-1 single-positive TILs were able to produce similar amounts of IFNγ and TNFα compared to PD-1-negative TILs, whereas those expressing multiple immune checkpoints (PD-1, TIM-3, and LAG-3) lost their ability to produce IFNγ, TNFα, and IL-2 upon TCR triggering, signifying a more profound state of exhaustion.[105] Furthermore, glioma TILs often lack expression of Ki-67 and Granzyme B, suggesting limited proliferative capacity and effector function.[32] Seven studies confirm that PD-1 is expressed on the majority of glioma TILs;[38,39,45,70,74,79,98] 8 separate studies find that a substantial proportion of glioma TILS co-express multiple immune checkpoints, consistent with T cell exhaustion.[11,32,38,45,46,66,79,94] For example, Davidson et al. show that PD1+TILs often co-express CTLA-4, TIM-3, LAG-3, CD38, and HLA-DR.[38] Spatially, the tumor core seems more immunosuppresive than the infiltration zone. The relative number of CD8+ of T-cells expressing PD-1, CTLA-4, TIGIT, 4–1BB, and OX40, a phenotype that makes T cells functionally inert, is significantly higher in the tumor core compared to infiltration zone.[58] Hypoxia is often proposed as a driving factor,[62] along with increased TAM density contributing to T cell exhaustion.[11,62,88] Spatial transcriptomic analyses show a prominent overlap between neighborhoods

with higher expression of suppressive TAM-signatures and T cell exhaustion signatures.[33] Ravi et al. find that cellular neighbourhoods enriched for mesenchymal-like transcriptional signatures are enriched in CD163+ HMOX1+TAM subsets that correlate with exhausted T cell clusters in glioblastoma. They show that both astrocytes and TAMs contribute to an immunosuppressive micro-environment through STAT-3-driven release of IL-10, which is crucial in the shift from T cell activation to exhaustion.[88] Several factors involved in driving T cell exhaustion in glioma, including hypoxia, nutrient deprivation, tumor-TAM interactions, and the release of immunosuppressive cytokines including IL-10 and TGF-β are also elegantly reviewed elsewhere.[124]

Interestingly, Dobersalske et al. recently discovered accumulations of T cells in the cranial bone adjacent to the tumor site in glioblastoma patients.[43] These aggregates contain mainly activated effector-type T cells that share clonotypes with intratumoral T cells, suggesting that they possess the same tumor reactivity. Notably, cranial bone-derived T cells retained proliferative capacity, show increased MHC-dependent tumor reactivity and are markedly less exhausted than tumor-infiltrating T cells, supporting the notion that tumor-derived cues drive T cell exhaustion.[43]

In short, T cells in the glioma TME often bear an exhausted phenotype, characterized by the expression of multiple immune checkpoint molecules and decreased cytotoxicity, especially in the hypoxic and TAM-high tumor core.

Prognostic Impact of Glioma TILs Remains Controversial

Abundance of CD8+ TILs is a recognized prognostic factor in several cancers,[125] yet the clinical relevance of T cell abundance in glioma is strongly debated. Prognostic significance of T cell abundance in glioma is mentioned in 24 studies. Some find that higher CD3+ or CD8+ T cell numbers are associated with a better prognosis;[59,64,67–107] conversely, others find that CD3+ T cell abundance correlates with a worse prognosis,[34,40,57–72] or that CD4+ or CD8+ T cell abundance correlates with a worse prognosis.[47–53] In some studies, the presence of CD3+ T cells does not significantly influence prognosis,[73,76,77–80] nor does infiltration by Tregs.[50,58,67] Others find that specific subsets relate to outcome; for instance, the presence of central memory T cells correlates with better overall survival,[11] PD-1+CTLA-4+CD8+ T cells in core regions are associated with decreased progression-free survival,[58] CD8+ TRM cells positively correlate with survival,[64] or Ki-67-high CD8+ T cells positively correlate with overall survival.[76] Perivascularly localized CD4+ T cells and FOXP3+ Tregs were associated with shorter progression-free survival in one study, although there was no association with overall survival,[80] while an increased proportion of CD4+ Tregs within total TILs was associated with reduced survival in another study.[90] Furthermore, recent mass cytometry and spatial transcriptomics efforts have revealed that spatial relationships between immune cell subsets might be more important than absolute numbers. Karimi et al. find that neighbourhoods enriched in MPO+ or M1 macrophages and neighbourhoods enriched in CD4+ T cells correlated with improved survival in glioblastoma.[12]

Thus, while there is some suggestion that antigen-experienced T cell subsets may associate with outcome, there is little consensus. Contributing factors to these discrepancies across studies include differences in patient demographics (including tumor subtype, grade, and prior treatments), significant inter- and intratumoral heterogeneity, different methods of TIL analysis and inconsistent definitions of T cell subsets between studies. Furthermore, most studies are retrospective with small sample sizes, and often do not take the spatial context into account. Without carefully stratifying for these factors, comparison across studies remains difficult.

Discussion

In this review, we systematically evaluated the abundance, phenotypic characteristics and spatial organization of T cells in glioma, integrating recent advances from spatial and single-cell profiling studies. We confirm and refine the understanding that gliomas are generally T cell-poor tumors, and that T cell abundance is higher in IDHwt gliomas compared to IDHmt gliomas, increases with higher WHO grade, and increases at recurrence. Paradoxically, increased T cell abundance does not directly correspond with improved outcomes, highlighting the importance of T cell functionality and spatial localization for an effective antitumor immune response. Indeed, glioma TILs largely display a late-differentiated phenotype (CD45RA-CCR7-C62L-), with markers for tissue residency (CD103, CD96) and co-expression of markers that signify profound T cell exhaustion (PD-1, CTLA-4, TIM-3, LAG-3, CD39, and TIGIT), coinciding with transcriptional programs pointing to decreased cytotoxic functionality. Furthermore, spatial constraints to T cell immunity in glioma are increasingly recognized. Clonally expanded T cell populations can be detected in glioma, but are more spatially restricted compared to brain metastases, corresponding to glioma-intrinsic genetic and transcriptional heterogeneity. Additionally, the cellular interactions within immunosuppressive niches in the glioma TME are beginning to be unraveled. For instance, tumor-TAM interactions contribute to T cell dysfunction in the hypoxic tumor core of glioblastomas through secretion of IL-10 and TGF-β, shifting the balance from T cell activation towards T cell exhaustion,[88] and by promoting T cell exclusion in the perivascular space in IDHmt gliomas in a process reminiscent of glial scarring.[51] A more granular understanding of such mechanisms will pave the way for better patient selection and rational combination therapies.

A major limitation for this review is the inclusion of retrospective studies with highly variable study designs. Differences in selected patient populations, tumor regions, methods of analysis and reporting methods hamper drawing fair comparisons between studies. The relatively low incidence of glioma and high costs of advanced techniques lead to small sample sizes, increasing the risk of selection bias, especially considering the heterogeneity of glioma samples.

Present barriers to successful immunotherapies for glioma patients include tumor-intrinsic heterogeneity, low neo-antigen burden, MHC class 1 downregulation, T cell scarcity, spatial restrictions, functional T cell deficits and strong tumor-driven immunosuppression. Several combinatorial immunotherapy strategies are now under investigation to overcome these barriers. Since T cell abundance positively correlates with response to checkpoint inhibitors in advanced melanoma and NSCLC,[126] as well as in brain

metastases of solid tumors,[127] strategies that combine checkpoint inhibitors with treatments that increase T cell recruitment to the TME are of interest. Besides minimizing corticosteroid use, radiotherapy, laser interstitial thermal therapy, oncolytic virotherapy or vaccination therapies might promote T cell recruitment to the TME by increasing available neo-antigens, disrupting the BBB and/or stimulating the inflammatory cascade;[128,129] however, concrete evidence for efficacy in glioma is currently lacking. Combination therapies that simultaneously target Tregs or the immunosuppressive TAMs are also of interest to enhance numbers and/or activity of glioma TILs.[130,131] In order to mobilize potentially tumor-reactive T cells that are confined to the perivascular space[51] or the cranial bone of glioblastoma patients,[43] therapies that enhance T cell chemotaxis and entry into the brain parenchyma might be employed and are being tested preclinically. [132,133] Furthermore, since D-2HG accumulation likely plays a role in T cell exclusion in IDHmt tumors, treatment with the recently approved IDH-inhibitor vorasidenib might sensitize IDHmt tumors to immunotherapies, and the combination of vorasidenib and pembrolizumab is now under investigation in NCT05484622.

Whether the exhausted phenotype of glioma TILS reflects irreversible dysfunction or a reversible adaptation to the immunosuppressive TME currently remains an open question. In an attempt to reverse T cell exhaustion profiles, therapies that simultaneously target multiple checkpoints are being tested in clinical trials, such anti-PD1 combined with anti-CTLA4, anti-LAG3, anti-TIM3, or anti-TIGIT,[134] and may be used in addition to those that enhance numbers of TILs. Locoregional administration of immunotherapies is another valuable strategy to bypass some of the present barriers. Intratumoral administration of checkpoint inhibitors[135] as well as local administration of oncolytic viruses[136] are being evaluated clinically. Furthermore, adoptive T cell therapies based on the ex-vivo expansion and locoregional infusion of naturally occurring or genetically engineered autologous tumor-specific T cells are rapidly gaining international momentum, and could potentially circumvent both tumor-driven T cell exclusion and T cell exhaustion. The first clinical trials using CAR-T cell products targeting EGFRvIII or IL13Rα2 describe rapid but transient radiological responses in glioma.[25–27] In the largest CAR-T cell trial to date, tumors with higher CD3+ T cell infiltrates showed a better response to CAR-T therapy, suggesting that CAR-T cell therapies would benefit from co-treatments or additional engineering of T cells to counteract the immunosuppressive microenvironment.[26] Major efforts are being made to enhance the persistence and potency of CAR-T cell products,[137] and will be critical to realizing their full potential as the field progresses.

Conclusions and Future Perspectives

Despite disappointing trial outcomes to date, recent advances in spatial and single-cell techniques provide high-resolution insights into the glioma TME and are now rapidly expanding our understanding of the composition of distinct immune niches and their therapeutic relevance, offering new perspectives on immunotherapy for glioma patients. In order to move the field of glioma immunotherapy forward, we strongly support a framework that integrates transcriptomic, functional and spatial immune profiling into the design of early-phase immunotherapy trials. This includes prospective tissue acquisition, preferentially from both pre- and post-treatment tissue samples, in order to assess factors such as T cell abundance, clonality, activation/exhaustion states and spatial organization within the TME and relate these factors to clinical outcomes. This approach will be essential to verify that the intended therapeutic effect of a treatment is achieved in the TME and to correlate immune changes with therapeutic response, and will improve our understanding of the factors that currently hold back efficient antitumor immune responses in glioma. The value of study designs that incorporate serial tissue sampling and multimodal functional and/or spatial profiling is increasingly recognized,[138] and similar approaches have been successfully embedded into more recent immunotherapy studies.[139–141] For instance, Skadborg and colleagues showed that systemically administered nivolumab can reach and bind to glioblastoma TILS and causes significant changes in markers for T cell activation and proliferation, but also causes a strong upregulation of negative checkpoint inhibition molecules TIGIT, LAG-3, TIM3, and CTLA-4 that might mitigate its clinical efficacy.[140] Furthermore, Hotchkiss et al. show that it is feasible to adapt a spatially resolved, multimodal profiling approach into a prospective clinical trial platform, which they employ to identify genomic and spatial factors that determine TIL expandability in glioblastoma.[123] Such studies, where prospective tissue acquisition and functional and spatial immune profiling are incorporated into a clinical trial setting in a standardized way, may provide answers to some of the burning questions that currently remain unanswered and will guide the optimization of rational, biomarker-informed combination therapies, ultimately paving the way toward more effective, individualized immunotherapies for glioma patients.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (https://academic.oup.com/neuro-oncology).

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Glioma | TME | T cells | phenotype | immunotherapy

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Data Availability

No new data were generated or analyzed in support of this research.

Ethics Statements

This study is a systematic review of previously published literature and does not involve any original data collection involving human or animal participants. Therefore, ethical approval and informed consent were not required.

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