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Review

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Tumor infiltrating lymphocytes in glioblastoma: immunobiology and translational implications

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Glioblastoma (GBM) poses unique challenges to immunotherapy, owing to its low tumor mutational burden, profound antigenic heterogeneity, and highly immunosuppressive microenvironment. Observations of tumor-infiltrating lymphocytes (TILs) in human GBM differ from those in syngeneic orthotopic murine models: whereas TILs in several widely used murine glioma models display an abundance of exhausted T cells, patient tumors are enriched for clonally expanded granzyme K$^+$ T cells of uncertain function. We review the current understanding of the brain tumor immunity cycle in GBM, highlight the translational implications of TIL biology, and evaluate emerging TCR-based approaches, including adoptive TILs transfer, neoantigen vaccines, and engineered receptors. A refined focus on identifying and harnessing tumor-selective T cells may enable more rational, personalized immunotherapies for GBM.

For many cancers, immunotherapy has transformed both paradigms of treatment and the lives of patients, underscoring the extensive role of the immune system in cancer initiation and progression. However, immunotherapy has not shown the same benefit in glioblastoma (GBM), the most common primary malignant brain tumor in adults with a median survival of around 20 months[1–3]. Factors such as low mutational burden, intratumoral antigenic heterogeneity, and striking immunosuppression combine to make GBM an example of a severe cancer immunoediting process[4–7]. Moreover, the field is still trying to understand the unique features of the immune response in the central nervous system (CNS). In order to develop better therapies for GBM, it is therefore crucial to understand the features of this specialized immune response. This review will focus on tumor infiltrating lymphocytes (TILs) within GBM, first covering the brain tumor immunity cycle (BTI cycle) in GBM and then focusing on our current understanding of the TILs in the BTI cycle in malignant glioma, comparing observations in preclinical models and human glioma; finally, it discusses future directions for immunotherapy that harness T cells to treat the disease. This review explores how the limited presence of exhausted T cells in human GBM reflects a dearth of antigen stimulation and that this is critical for understanding immune checkpoint blockade (ICB) failure and designing future T cell–based therapies.

The brain tumor immunity cycle in GBM

The BTI cycle refers to the sequence of steps during which the immune system recognizes and generates responses to cancer in the brain[8,9]. While the general cancer immunity cycle (CI cycle) describes how the immune system targets tumors across tissues[8,9], the BTI cycle adapts this framework to the CNS and brain tumor specific context. The BTI cycle is impacted both by distinct immune features in the brain[5,10] and the unique characteristics of GBM.

Immune features of the brain

Historically, the CNS was considered immune privileged[10,11]. This belief stemmed from the finding that when engrafted with foreign tissue, the brain mounts a limited immune response in murine models[11]. Recent studies, however, have overturned the idea of CNS immune privilege by demonstrating the presence of immune cells in the brain and illuminating their role in brain development and disease[12]. For example, microglia appear to be involved in regulating several key cell types and processes in the brain, including the number of neural precursor cells in developing brains[13] and myelination[14]. Microglia have even been reported to prune synapses in a complement dependent manner[15]. In tumor models, conventional dendritic cells (cDC1) have been shown to be particularly important for anti CNS

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tumor responses, as mice deficient in cDC1 failed to mount a neoantigen specific response[16].

Beyond the brain parenchyma however, studies in humans and tumor models have highlighted the involvement of immune cells in other unique CNS tissues such as the meninges[17–22], the choroid plexus[23–25], and skull bone marrow[26–29]. The dural sinuses seem to serve as important sites of antigen sampling and presentation by APCs[19] and even development of lymphoid-like structures[22]. Also, studies have revealed the skull bone marrow is connected to the meninges through vascular channels[26,27] and may act as a hematopoietic source of both lymphoid and myeloid cells for CNS immunosurveillance[28,29].

In the setting of intraparenchymal tumors, such as GBM, these findings are particularly important in understanding how the immune system functions in homeostatic conditions and provides a foundation from which we can begin to understand how the immune system becomes dysfunctional, or even leveraged, by developing tumors.

BTI cycle

Indeed, in the setting of the CNS' distinct immune milieu, the BTI cycle likely occurs in the following manner: (1) release of tumor antigens from cancer cells, (2) uptake then presentation of MHC bound antigen by dendritic cells (DCs) to T cells, resulting in (3) activation of tumor antigen specific T cells; (4) trafficking of T cells across the blood brain barrier (BBB), which is more permeable in the setting of cancer; (5) T cell tumor infiltration, (6) T cell recognition of cancer cells displaying antigen on HLA molecules; finally, (7) T cell killing of cancer cells, thus, releasing more antigen and propagating the cycle[8–10]. This final step comprises the end effector arm of the cycle.

In addition to antigen uptake, DC maturation typically requires "licensing" by innate danger signals sensed through pattern-recognition receptors; in tumors, immunogenic cell death can release DAMPs such as extracellular ATP and HMGB1 and expose calreticulin, which promote DC activation and cross-presentation[30–32]. In parallel, tumor-derived DNA can likewise engage cGAS–STING signaling in DCs to induce type I interferons, a key pathway for effective T cell priming and antitumor immunity[33,34].

Many elements of the BTI cycle in GBM remain to be discovered, including the process and location of antigen presentation, or the exact contributions of specialized structures in the brain like the meninges, choroid plexus or skull bone marrow. Regardless, GBM develops in part from the failure of the cycle in eradicating it, and this failure can arise from any of the steps in the cycle.

Characteristics of glioblastoma that influence the BTI cycle

GBM has many features that could contribute to the malfunctioning of its BTI cycle. The disease is often referred to as "cold" in part due to its baseline low tumor mutational burden (TMB), a term that quantifies the number of somatic mutations in the exome[35], and its low endogenous T cell infiltration compared to other solid malignancies. A low TMB presumably confers an even lower number of neoantigens, or mutated antigens, which can be presented on MHC molecules and elicit T cell activation, so step 1 of GBM's BTI cycle is likely compromised. In addition, somatic variants do not always result in neoantigen presentation, whether due to antigen processing, antigen downregulation, or other causes of immunoediting. However, other tumor antigens (TAs) can also be released to initiate the cycle; these include cancer testis antigens (CTAs), tumor associated antigens (TAAs), and alternative sources of tumor specific antigens (TSAs). Antigens from all three of these classes have been observed in GBM and reported as being able to elicit T cell reactions[5].

Next, steps 2-5 are likely constrained by the immunosuppressive environment[36] derived from the tumor itself, the immune compartment, or treatment with corticosteroids, radiotherapy and chemotherapy. Moreover, GBM is highly heterogeneous[37] with sub-clonal populations of discrete mutations[38,39], limiting the efficacy of T cells within the tumor microenvironment (TME). The majority of GBM mutations are passenger mutations–that is, unrelated to the tumor's oncogenicity–which may allow for further clonal evolution of tumor cells in response to T cell killing. In this way, steps 6 and 7 of the BTI cycle are likely limited as well. Lastly, another way that the BTI cycle can fail occurs when the previously activated T cells lose their function and cease to be able to engage in either steps 6 or 7. While some have suggested this occurs in GBM due to the presence of exhausted TILs, the full impact of these exhausted T cells, and other dysfunctional T cells, requires further study.

BTI cycle applications for therapy

Understanding the CI cycle for a particular cancer is key to the development of immunotherapy[40,41]. Indeed, immunotherapy endeavors either to 1) revitalize or augment a patient's endogenous CI cycle, as in the case of ICB, antigen-targeted vaccination, adoptive cell therapy with TIL (ACT-TIL) or oncolytic viruses, or 2) confer the end effector arm, as in the case of Chimeric Antigen Receptor (CAR) T cells or engineered T Cell Receptor (TCR) based therapies (Fig. 1). Thus, elucidating the source of dysfunction in GBM's BTI cycle will lead to insights that will inform the development of future therapies.

Preclinical observations of T cells in GBM models

Preclinical research in syngeneic orthotopic mouse models has elucidated elements of the BTI cycle, offering a window into how the cycle might transpire in humans, and thus, revealing how it may be possible to leverage it for therapy.

Tumor antigens

Studies from our lab have identified endogenous tumor-specific neoantigens in the GL261 and SMA-560 models of glioma[42]. These include the H-2D^b–restricted Imp3$_{D81N}$ (GL261), H-2D^b–restricted Odc1$_{Q129L}$ (SMA-560) and H-2K^b–restricted E2f8$_{K272R}$ (SMA-560); all of which were confirmed to be immunogenic via IFN-γ ELISPOT[42]. Plus, T cells from intracranial tumors and cervical draining lymph nodes were confirmed via tetramer staining to be neoantigen specific to Imp3$_{D81N}$ and Odc1$_{Q129L}$[42]. Notably, in mice with GL261 brain tumors that were treated with an infusion of T cells with TCRs specific for Imp3$_{D81N}$, the majority of the mice experienced long-term cures[43]. This represents the potential of ACT-TIL as an immunotherapy for treating patients with GBM. However, developing such treatments has been limited by a scarcity of tumor-specific antigens shared across a tumor and between patients. This topic is reviewed in detail elsewhere[5].

TILs

The foundational evidence for immunoediting and immune surveillance in preclinical oncology was initially presented by Shankaran, van den Broek, et al.[4,44–46]. Their studies showed recombinase-activating gene 2 (RAG2)-deficient mice models lacking B and T cells, or perforin-deficient mice lacking cytotoxic T cell capabilities were highly susceptible to tumorigenesis[4,46–48]. Further studies have since expanded on the concept of cancer immunoediting, shedding light on its role, particularly on how it contributes to understanding the fatality of GBM or immunotherapies to prolong survival. Crucially, these data established that the presence of lymphocytes was protective in cancer.

T cell exhaustion

T cell exhaustion is one key outcome of the cancer immunoediting process. Although initially identified in chronic viral infection in murine models as loss of effector function, T cell exhaustion is often cited as a major contributor to the lack of efficacy of immunotherapies in GBM. T cell exhaustion is thought to be catalyzed by progressive loss of effector function, persistent expression of inhibitory receptors and immunomodulators, and extensive transcriptional, epigenetic, and metabolic reprogramming[49–53].

Preclinically, transcriptomic analyses have revealed similarities between exhausted TILs and exhausted T cells in chronic viral infections, suggesting shared mechanisms of exhaustion driven by persistent antigen exposure in the TME[53,54]. Specifically, it has been shown that exhausted TILs

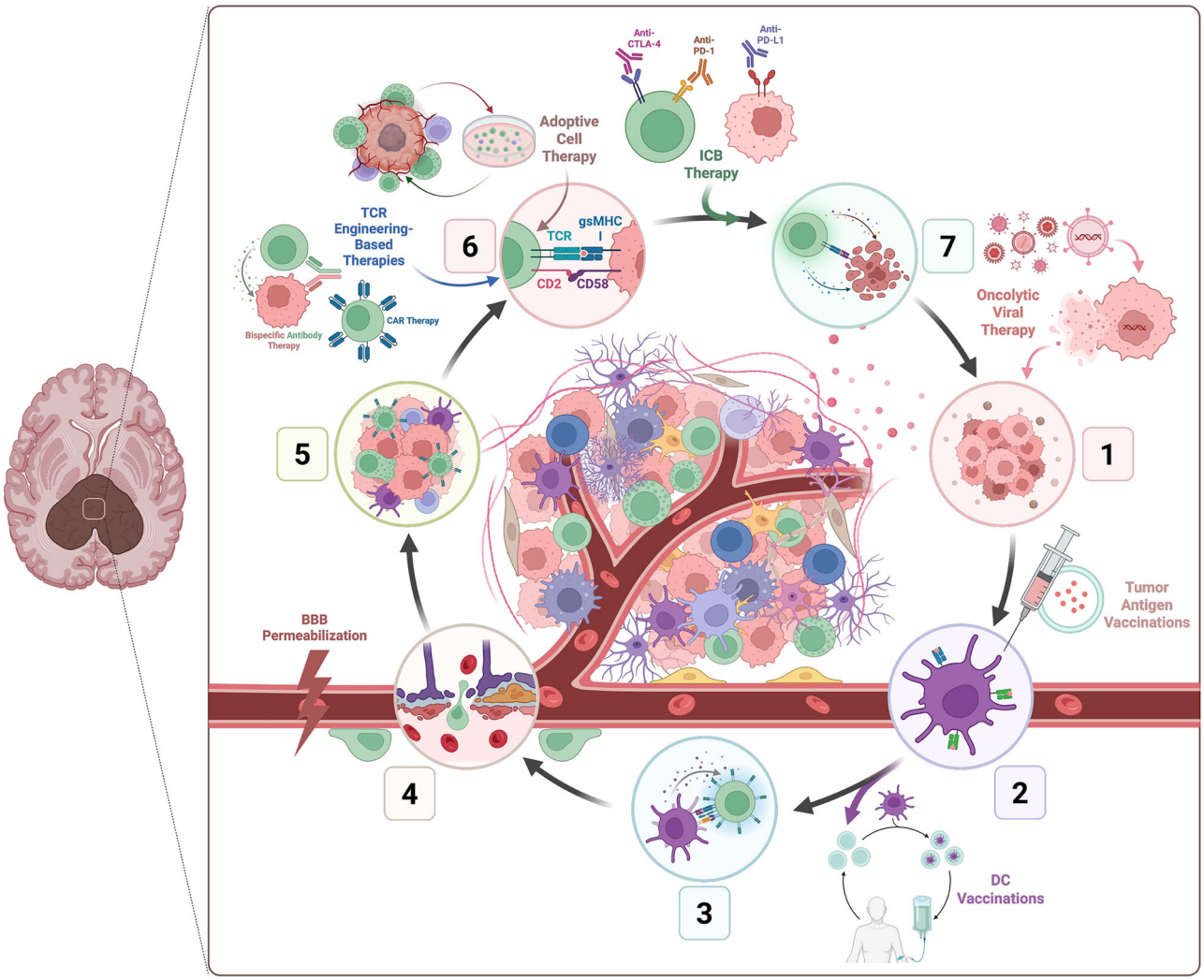

Fig. 1 | Harnessing the cancer immunity cycle with immunotherapy. The cancer immunity cycle begins with the release of tumor-associated antigens from dying tumor cells (1), followed by their uptake and MHC presentation on antigen-presenting cells (APCs) (2), and the priming and activation of local and peripheral T cells (DC -mediated T cell activation) (3). Activated T cells must then traffic across the BBB into the TME (4) before infiltrating the tumor niche (5). Once within the TME, cytotoxic T lymphocytes (CTLs) recognize tumor cells via TCR–MHC interactions (6), resulting in T cell mediated tumor cell lysis (7) and contributing to tumor antigen release. Multiple immunotherapeutic strategies target, activate, or amplify these steps, including tumor antigen (2) and DC vaccinations (3), BBB permeabilization (4), immune checkpoint blockade (ICB) (6 and 7), adoptive cell transfer (6), TCR engineering (step 6), and oncolytic viral therapies (step 1).

in CT2A mice glioma model demonstrate elevated Eomes, decreased T-bet levels, and highly expressed Blimp-1, a transcription factor associated with T cell exhaustion in chronic viral infections[49,54,55]. PD-1 was also shown to be highly expressed and demethylated in said exhausted state[54,55], as has been shown in terminal states of T cell exhaustion in viral infection[56–59].

Recent studies in chronic infection and tumor models have also identified distinct subsets of exhausted T cells with varying potential for reinvigoration and expression of PD-1, Eomes, amongst other T cell exhaustion markers, spelling a potential window of opportunity[53]. For instance, progenitor-exhausted T cells expressing TCF1 retain some plasticity and can respond to PD-1 blockade, whereas terminally exhausted T cells, characterized by high Eomes expression, are less responsive[53,55,60,61]. The balance between these subsets is regulated by transcription factors such as T-bet and TOX, which coordinate transitions between progenitor, intermediate, and terminally exhausted states[53,55,61]. Specifically, T-bet tended to foster transition to cell states that were reminiscent of effector T cell function in tumor cells, while preventing TOX-mediated terminal exhaustion[53]. Therapeutic strategies that preserve or expand progenitor-exhausted T cells while limiting terminal exhaustion could improve responses to ICB.

Congruently, exhausted T cells in the CT2A glioma model have been stratified into progenitor-exhausted T (Tex_prog) cells and terminally exhausted T (Tex_term) cells with the former having PD-1 and TCF expression and maintaining effector functionality, while the latter has been characterized by high TOX, PD-1, and TIM-3 expression, with a tendency to be tolerized[62–66]. ICB tends to be more efficacious when the Tex_prog state is more prominent[62]. Moreover, a similar state had been shown in human GBM with low expression of several immune inhibitory receptor genes that are less reflective of the finality of the anergic terminal T cell exhausted state with more regenerative capabilities[67]. Nonetheless, the T cell states in the above studies were still noted to be mostly hypofunctional with higher tolerization-like features and non-proliferative immunomodulatory cells when compared to other tumor types such as melanoma, NSCLC[54,62,67].

Contributions of the immune microenvironment to T cell exhaustion

T cell states may further be shaped by the distinct characteristics of the hostile GBM microenvironment, particularly the prevalence of tumor-associated macrophages (TAMs)[68,69]. TAMs can occupy up to 30% of the TME and their abundance has been associated with poor prognosis[68,70].

Recently, TAMs have been shown to propagate the transition from Tex_prog to Tex_term state in syngeneic CT2A glioma mice, suggesting a therapeutic opportunity for these immunomodulators[62]. Particularly, TAMs were shown to be the primary antigen-presenting cells driving said transition to Tex_term cells via MHC I, as against microglia or DCs. Although TAM depletion in combination with ICB in CT2A glioma mice, canonically ICB non-responsive mice, showed no improvement in survival, there was a preponderance of Tex_prog cells[62]. Of note, in an ICB-responsive melanoma model, survival did improve, corroborating the importance of a predominantly Tex_prog TME[62].

Similarly, it has been proposed that TAMs dampen $CD4^+$ helper T cell function via a PD-L1/PD-1/CD80 axis[71–73]. In ICB resistant syngeneic mice, $PD\text{-}L1^+$ TAMs are able to bind PD-1 on T cells, notably imparting immune checkpoint resistance and induction of regulatory T cells[69,73]. TAMs via SPP1 also highly express osteopontin which coupled with CD29 integrin, induce Akt signaling and T cell activation. However, when this interaction is prolonged, this same axis further activates NFAT2 signaling pathway, which in turn propagates TOX-mediated $CD8^+$ T cell dysfunction[71–75]. $CD4^+$ T cells via MHCII are able to counteract this interaction by releasing IFN-γ which suppresses TAM osteopontin expression, hence fostering non-exhaustive cytotoxic T cell states[72].

The TME bolsters a hypoxic environment which then exacerbates T cell exhaustion via angiogenesis, cell migration, and metabolic reprogramming[76–79]. Hypoxia-inducible factor 1-alpha (HIF-1α), a key regulator under hypoxic conditions, modulates epigenetic changes in TILs[77,80]. The hypoxic TME also favors glycolysis over oxidative phosphorylation, in which less energy is produced for TIL activity, and lactate levels accumulate, in turn catalyzing progression to an exhaustive state[49,81–84]. Additionally, the depletion of key amino acids, such as tryptophan and arginine, as a result of dysfunctional expression of enzymes like IDO1, creates metabolic stress that drives T cell dysfunction[85–89]. Gliomas particularly harbor high IDO1 expression, which correlates with poor patient prognosis, underscoring the role of metabolism in shaping the exhausted T cell phenotype[85–89].

Together, these findings suggest that T cell dysfunction in glioma is shaped less by a single dominant suppressive pathway and more by the combination of antigen presentation dynamics and microenvironmental constraints. In syngeneic models, tumor-associated macrophages emerge as key regulators of T cell fate by both presenting antigen and promoting transition toward terminal exhaustion, while hypoxia and metabolic stress further limit T cell effector capacity. Enzymatic depletion of critical nutrients, including tryptophan and arginine via IDO1 and related pathways, adds an additional layer of suppression by imposing metabolic bottlenecks on infiltrating T cells.

Thus, accumulating evidence demonstrates that glioma TILs in syngeneic orthotopic mouse models harbor abundant, classically exhausted TILs; however, patient tumors tell a different story. The next section contrasts these model findings with human data.

Observations of TILs in clinical GBM

T cell phenotypes

Studies in animals do not always recapitulate what we see in humans. Despite the preclinical evidence that the majority of infiltrative T cells in GBM are "exhausted", studies of human samples have illuminated a more nuanced perspective. We recently showed that the majority of $CD8^+$ T cells in GBM do not express a transcriptional profile similar to that of exhausted T cells[90], which supports findings of other studies[67,91]. Rather, most $CD8^+$ T cells express markers including *GZMK* rather than classic cytotoxic markers like *GZMB, GNLY*, and *PRF1*. Furthermore, we found that the most clonally expanded TCRs in the tumor were associated with $GZMK^+$ T cells and were selectively expanded in the tumor (Fig. 2). Overall, these results suggested that $GZMK^+$ T cells were tumor enriched and may play an important role in the TME. $GZMK^+$ T cells have been observed in several different cancer types[92–97], though the specific function of GZMK is still relatively unknown. Outside the context of cancer, $GZMK^+$ $CD8^+$ T cells have been implicated in inflammatory diseases such as recurrent airway inflammation, rheumatoid arthritis, and dermatitis[98,99]. Mechanistically, recent studies (not in the context of GBM) have shown that GZMK induces the complement cascade by either cleaving C2 and C4, generating the C3 convertase, or cleaving C3 directly[98,99]. This ultimately results in anaphylatoxins C3a and C5a, the opsonins C4b and C3b, and the membrane attack complex (MAC)[99].

Whether $GZMK^+$ T cells mediate complement activation within the GBM TME remains unknown. However, if operative, such a mechanism could plausibly influence both tumor cells and infiltrating immune populations. Intriguingly, both anti-tumorigenic and pro-tumorigenic effects of complement activation have been reported in cancers[100]. On one hand, complement-mediated cytotoxicity is leveraged therapeutically by antibody-based immunotherapies such as rituximab in B cell lymphomas. Conversely, chronic complement activation, particularly through anaphylatoxins such as C3a and C5a, can promote tumor-supportive inflammation, recruitment of myeloid-derived suppressor cells, angiogenesis, and immune suppression[100,101]. Consistent with this, inhibition of the C5a receptor has been shown to slow tumor growth in preclinical lung cancer models[102]. Thus, should $GZMK^+$ T cells activate complement in GBM, the downstream consequences are likely to be highly context dependent. Defining whether GZMK-driven complement activation occurs in GBM and determining its net impact on tumor progression and immune control represents an important area for future investigation.

Reconciling the differences in T cell phenotypes observed in preclinical compared to clinical GBM

One potential reason for the discrepancy between preclinical and patient observations is that GBM preclinical models have much higher levels of TMB than clinical GBM (Table 1). For instance, CT2A and GL261 murine tumor models have been reported to have 79 or 179 mutations/megabase (MB), respectively[103]. These levels are greater than the FDA's definition of a "high-TMB" of 10 mutations/MB[104] and even greater than TMB levels found in GBM, which has a median of 1-2 mutations/MB[105,106]. Similarly, the GBM model SMA-560 has been observed to have 2,171 nonsynonymous (SNV) mutations per tumor[42], while GBM has been reported to have 30-50 SNV mutations per tumor[107]. Alternatively, the model SB28 has been reported to have 108 SNV mutations per tumor[108]. While still higher than GBM's, SB28's lower TMB compared to other murine GBM models, combined with other features signifying its low immunogenicity, suggest its value as a model in researching GBM[108]. Beyond TMB, growth kinetics and intact antigen-presentation efficiency differ across models and can impact exhausted T cell formation even at similar antigen densities.

Stochastically, a higher TMB will lead to a higher tumor neoantigen burden (TNB). Since chronic antigen stimulation has been observed to cause T cell exhaustion[109], it is likely that in cancers,or preclinical models,-with high TMB, the large number of presented neoantigens in these rapidly-growing tumors results in fast and persistent antigen stimulation of the TILs, causing exhaustion. Conversely, perhaps the dearth of exhausted T cells in human GBM reflects limited antigen stimulation in the tumor, secondary to a low number of neoantigens and the immunosuppressive milieu as well as more prolonged growth kinetics. As such, GBM models with high TMB and rapid growth may not be ideal tools to most faithfully recapitulate GBM patient TIL phenotypes.

Understanding the failure of immune checkpoint blockade in clinical trials for GBM

The results of clinical trials with ICB in GBM are consistent with the observation that GBM has a paucity of exhausted T cells. ICB operates by blocking canonical exhaustion markers PD-1 or CTLA-4, proteins that attenuate T cell activation. In this way, ICB improves T cell functionality by either revitalizing or preventing further exhaustion in pre-existing exhausted T cells, or limiting exhaustion in T cells newly recruited to the TME via clonal replacement[110]. Thus, ICB's efficacy seems to depend on the

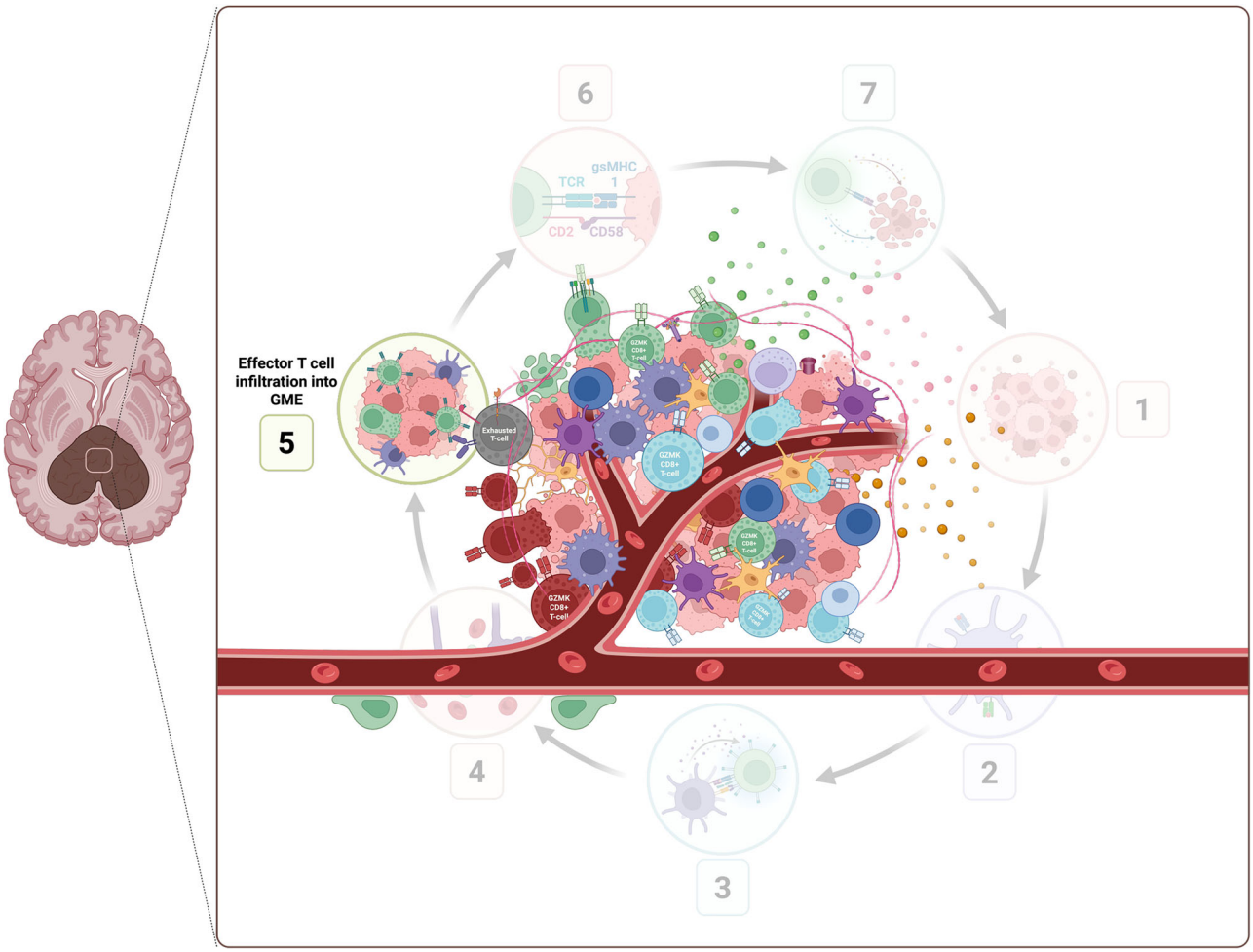

Fig. 2 | Clonal expansion of granzyme K$^+$ T cells in the GBM microenvironment. Mounting a central immune response to glioblastoma depends on the release of tumor antigens and the balance between functional effectors and dysfunctional immune populations. At step 5 of the GBM immunity cycle, effector T cells infiltrate the GBM microenvironment. The TME in GBM is characterized by clonally expanded *GZMK*$^+$ CD8$^+$ T cells (light blue, green, and red with cytotoxic granules), activated microglia, macrophages (dark purple), DCs (magenta), and peripheral myeloid infiltrates (dark and pale blue), with few exhausted T cells (grey). The role of *GZMK*$^+$ T cells in GBM remains incompletely defined but has been reported peripherally to independently induce complement activation and modulate local immunoinflammatory milieu.

Table 1 | Key immunologic contrasts across mouse models, melanoma, and human GBM

	Preclinical orthotopic mouse syngeneic GBM Models	Melanoma	Clinical GBM
Tumor Mutational Burden	High[a]	High	Low
Presumed Tumor Neoantigen Burden	High	High	Low
Prevalence of Exhausted T Cells	High	High	Lower fraction among TILs (assay and cohort dependent) compared to other cancers
ICB Responsiveness	Variable depending on model[149]	Confers survival benefit	No survival benefit in phase III trials

[a]GL261, CT2A and SMA-560 GBM models have high TMB.

presence of tumor-reactive T cells encountering sufficient antigen to drive exhaustion.

Since the amount of exhausted T cells likely reflects the levels of antigen presentation in the tumor, cancers with low levels of exhausted T cells would presumably not respond as robustly to ICB while those with high levels would. This paradigm is supported by clinical trial data: ICB did not improve clinical outcomes for unselected GBM patients in phase III clinical trials[1,111,112], but did improve outcomes in cancers like melanoma[113] or non-small cell lung cancer (NSCLC)[114], which are both notable for significant levels of exhausted T cells and high TMB[115,116]. Consequently, TMB is now used as a biomarker for pembrolizumab (an ICB that targets the PD-1/PD-L1 pathway) eligibility with a threshold of 10 mutations/MB guiding treatment decisions[104]. In pancreatic ductal adenocarcinoma (PDAC), patients with high-TMB tumors were found to have markedly better overall survival following ICB (25.7 vs. 5.2 months)[117], and high-TMB was observed to correspond to a higher frequencies of exhausted CD8$^+$ T cells[118]. Also, the frequency of dysfunctional CD8$^+$ T cells with high PD-1 expression was reported to be predictive for response and survival during treatment with

anti-PD-1 therapy in non-small-cell lung cancer patients[119]. Together, these data suggest that the presence of exhausted T cells, driven by sufficient tumor antigen, is a critical marker of ICB responsiveness, and their scarcity in GBM may underlie its resistance.

While ICB has failed in unselected cohorts of GBM patients, some have reported benefits for select GBM patients, including those with a hypermutation phenomenon, and thus an increased TMB, due to genetic causes[120,121], but not due to temozolomide induction, which may induce mutations without a corresponding induction of antigenicity[122]. These findings suggest that ICB may provide benefit for a select group of GBM patients. Furthermore, they complicate the proposed paradigm of a high TMB tumor leading stepwise to exhausted T cells that can be treated with ICB. While multiple preclinical studies demonstrated that immunosuppression alone does not lead to exhausted T cells in cancer, and that T cell exhaustion requires antigen recognition[123–125], immunosuppressive mechanisms very likely impact exhaustion by tuning antigen presentation or inhibiting T cell activation. Perhaps antigen presentation inefficiency acts synergistically with low TMB (and the immunosuppressive effects of treatment) to further limit the number of exhausted T cells in GBM, and thus, the amount of exhausted T cells may be more of a reflection of tumor immunogenicity than simply its TMB. Ultimately, the minimal levels of exhaustion in T cells in the majority of human GBM tumors is important to recognize because our understanding of the BTI cycle in GBM informs the immunotherapies that we trial in the disease.

TCR–based immunotherapies in GBM: from biology to therapy

Successful recognition of tumor antigens by T cells underpins the mechanisms of many immunotherapies that have proven successful in other cancers, like ACT, antigen vaccines, and engineered TCR therapies. While CAR-T therapies are an important and rapidly evolving modality in GBM, they are beyond the scope of this review, which centers on TIL immunobiology and TCR/HLA-restricted antigen recognition and its translational extensions. We therefore refer readers to dedicated reviews of CAR-T strategies in high-grade glioma[126].

Adoptive cell transfer of autologous tumor-infiltrating lymphocytes

ACT-TIL, adoptive transfer of autologous TILs that are expanded ex vivo and then infused back to the patient, is one promising immunotherapy for cancer. In a phase III clinical trial of ACT-TIL for melanoma, the median overall survival for the ACT-TIL treated group was 25.8 months compared to 18.9 months for the control group which received ipilimumab[127]. Lifileucel(Amtagvi), a type of ACT-TIL, received FDA approval for melanoma[128,129]. Notably, ACT-TIL is being explored as a treatment in GBM: a phase I clinical trial demonstrated the safety and feasibility of administering TILs engineered to secrete anti PD-1 antibodies[130], and a separate trial of ACT-TIL with pembrolizumab is currently underway (NCT06640582).

Antigen vaccines

Personal neoantigen vaccines, where mutant antigens are administered to a patient with the aim of inducing clonal expansion of antigen-reactive T cells, have shown promise in clinical trials for some cancers[131–136], including melanoma, in which some patients were reported to experience complete tumor regression after vaccination and subsequent anti–PD-1 therapy[131]. Notably, personal neoantigen vaccines have been reported to elicit neoantigen reactive TILs in GBM[5], and may provide clinical benefits. One clinical trial of a personal neoantigen vaccine showed that patients with T cell responses to more than one peptide had a median overall survival of 53 months compared to 27 months in those with response to only one or zero[137]. A H3K27M neoantigen vaccine resulted in complete remission for one patient with diffuse midline glioma, and the patient was observed to have T cells reactive to the vaccine neoantigen[138]. Many clinical trials for vaccines administering tumor antigens in GBM are in progress[5].

Engineered TCRs

Other FDA approved therapies that harness the CI cycle include tecelra, a TCR-based therapy that targets the MAGE-A4 CTA[139] for metastatic synovial sarcoma, and tebentafusp, an engineered T cell engager bispecific for CD3 and a glycoprotein 100 TAA that treats metastatic uveal melanoma[140]. A treatment with a design similar to tebentafusp is potentially on the horizon for GBM, as a clinical trial for a T cell engager bispecific for CD3 and EGFRvIII has entered early-phase clinical testing (NCT04903795). A downside of these TCR-based therapies is their limited therapeutic application: tecelra and tebentafusp target tumor antigens that are HLA bound and thus are only approved for patients with particular HLA molecules. That is, tecelra is only approved for people with certain HLA-A*02 types[141], and tebentafusp is only approved for HLA-A*02:01 positive patients[142]. Contrastingly, other therapies that treat patients with their own autologous cells, like ACT, do not have such a limitation and thus are more broadly applicable to all patients, independent of genetic background, since the treatment uses an individual patient's own cells.

Challenges in identifying antigen targets for vaccines and TCR-based immunotherapies

Despite their promise, the development of antigen targeting vaccines or TCR therapies in GBM is often limited by the ability to identify targets. This is due to GBM's low TMB, high levels of intra-tumoral and inter-tumoral heterogeneity, and the limitations of the strategies commonly employed for antigen identification. An antigen derived from the EGFRvIII mutant protein is one of the only shared neoantigens identified in GBM[5]. Plus, many neoantigen vaccines in GBM identify a patient's personal neoantigens for inclusion in vaccination using computational analysis of a patient's exome to look for variant epitopes that are predicted to bind to a patient's HLA molecules. Cancer immunogenomics approaches do not incorporate a proteomics step to validate that a tumor expresses the predicted "neoantigen" is in its immunopeptidome, which is important to confirm since not every mutation will lead to neoepitopes. In one personal vaccine trial for GBM, zero of the over 600 algorithm-predicted neoantigens from 15 patients were identified by mass spectrometry, but neoantigens were identified by mass spectrometry in one patient not in the trial who had a hypermutated variant of GBM[107]. Moreover, very few predicted neoantigens appear to elicit immune responses; one study observed that less than 2% of mutations in gastrointestinal cancer had T cells reactive to the matched mutant antigen[143]. Thus, identifying which antigens to target, whether via vaccine or TCR therapies can be challenging.

Leveraging markers of tumor-selectivity to improve T cell based immunotherapies

The ability to identify and selectively expand the most therapeutically potent T cells may be a crucial step in ACT-TIL for low TMB cancers like GBM. The understanding that antigen stimulation leads to exhaustion may be a concept that can be leveraged to identify tumor specific T cells ideal for therapy. That is, since chronic antigen activation leads to exhaustion, the limited number of tumor-infiltrating T cells that are exhausted in GBM might, in fact, contain TCRs reactive to tumor antigen. Indeed, in a neoantigen vaccination study for GBM, patient T cells specific for vaccine antigens from pre- and post- vaccination time points exhibited increased expression of exhaustion marker PD-1 after vaccination[107]. In a separate study that vaccinated GBM patients with neoantigen vaccines, some neoantigen specific T cells were identified as expressing canonical exhaustion markers[144]. Some have identified neoantigen-reactive T cell signatures in other cancers, some of which include exhaustion markers[145–148]. One study in melanoma demonstrated that patients' $CD8^+$ T cells reactive to tumor were enriched in an exhaustion phenotype compared to non tumor reactive T cells[147]. More research should be done to explore the relationship between exhaustion, T cell phenotype, and antigen specificity of T cells, as exhaustion markers can possibly serve as tools for isolating tumor-selective T cells or individual TCRs for ACT-TIL and TCR-engineered therapies, respectively.

Similarly, since *GZMK*$^{+}$ CD8^{+} T cells are clonally expanded in GBM TILs and selectively expanded in tumor compared to peripheral blood, it is possible that GZMK could also help flag therapeutically valuable TCRs. Defining whether tumor-specific T cells exhibit exhaustion markers, GZMK expression, or other distinguishing features will be critical to optimizing ACT-TIL and TCR-based therapies.

The recent results of antigen vaccines in other cancers and GBM, as well as the FDA approval of lifileucel, tecelra, and tebentafusp, and current clinical trials exploring similar treatments in GBM all highlight the potential for immunotherapies that leverage T cell biology for GBM. As we better define tumor-reactive T cell phenotypes in GBM, we can design more rational, personalized immunotherapies that overcome the unique challenges of this disease.

Conclusion

Elucidating the BTI cycle in GBM, and in particular, identifying the features that distinguish GBM from other cancers, is imperative for the design of immunotherapies for the disease. The low TMB levels, concomitant low TNB, and subsequent limited presence of exhausted T cells distinguish GBM from cancers like melanoma or lung cancer, and these qualities suggest that GBM requires different strategies for therapy. Recognizing that the majority of T cells in GBM are not exhausted, likely due to minimal antigen stimulation, and that the majority express GZMK are important steps in re-directing efforts to investigate arms of the BTI cycle for treatment. Namely, emphasis should be placed on identifying appropriate antigen targets and TCRs that are reactive to tumor cells, since TCR-based therapies are powerful tools that have shown success in other cancers. Ultimately, success in GBM immunotherapy may require prioritizing T cell therapies tailored to the unique antigenic and immune landscape of each patient.

Data availability

No datasets were generated or analysed during the current study.

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Author contributions

C.M.H. and G.P.D. conceived the manuscript. C.M.H., C.D.N., and A.Z.W. wrote the first draft of the manuscript. C.M.H., A.Z.W., and G.P.D. revised and edited the manuscript. C.M.H. and G.P.D. conceived the figures and table. A.O.O made the figures. B.H. provided intellectual input and critically reviewed the manuscript. All authors have read and agree with the final version of this manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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