Norollahi *et al.*
Journal of the Egyptian National Cancer Institute (2024) 36:33
https://doi.org/10.1186/s43046-024-00240-4

Journal of the Egyptian National Cancer Institute

REVIEW

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Practical immunomodulatory landscape of glioblastoma multiforme (GBM) therapy

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Abstract

Glioblastoma multiforme (GBM) is the most common harmful high-grade brain tumor with high mortality and low survival rate. Importantly, besides routine diagnostic and therapeutic methods, modern and useful practical techniques are urgently needed for this serious malignancy. Correspondingly, the translational medicine focusing on genetic and epigenetic profiles of glioblastoma, as well as the immune framework and brain microenvironment, based on these challenging findings, indicates that key clinical interventions include immunotherapy, such as immunoassay, oncolytic viral therapy, and chimeric antigen receptor T (CAR T) cell therapy, which are of great importance in both diagnosis and therapy. Relatively, vaccine therapy reflects the untapped confidence to enhance GBM outcomes. Ongoing advances in immunotherapy, which utilizes different methods to regenerate or modify the resistant body for cancer therapy, have revealed serious results with many different problems and difficulties for patients. Safe checkpoint inhibitors, adoptive cellular treatment, cellular and peptide antibodies, and other innovations give researchers an endless cluster of instruments to plan profoundly in personalized medicine and the potential for combination techniques. In this way, antibodies that block immune checkpoints, particularly those that target the program death 1 (PD-1)/PD-1 (PD-L1) ligand pathway, have improved prognosis in a wide range of diseases. However, its use in combination with chemotherapy, radiation therapy, or monotherapy is ineffective in treating GBM. The purpose of this review is to provide an up-to-date overview of the translational elements concentrating on the immunotherapeutic field of GBM alongside describing the molecular mechanism involved in GBM and related signaling pathways, presenting both historical perspectives and future directions underlying basic and clinical practice.

Keywords Glioblastoma multiforme, Signaling pathways, Immunotherapy, Translational element

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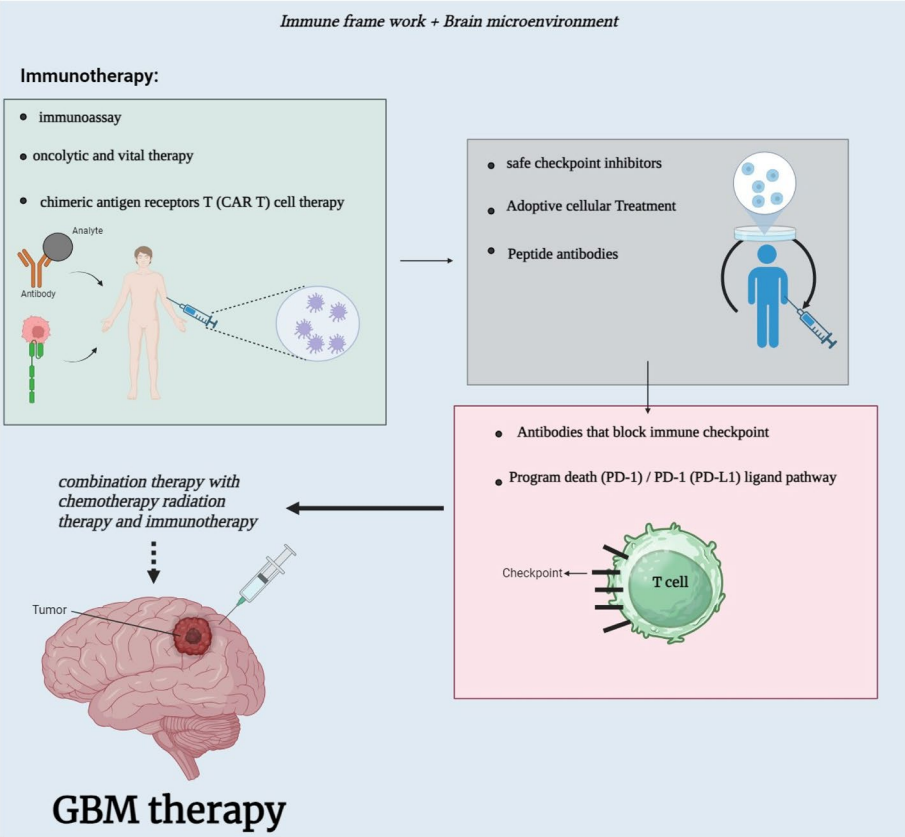
Graphical Abstract



Introduction

The most common and deadly primary brain tumor among adult patients is glioblastoma (GBM) [1]. With notable variations between patients, GBM is a physiologically diverse tumor that demonstrates all of the traditional characteristics of malignancy [2]. Notably, the gold standard of treatment is combined radio-chemo- and tumor-treating field therapy [3, 4], which increases the mean overall survival of patients to 21 months [4]. Although prognostically significant, GBM subtypes have been identified by genetic [5] and epigenetic [6] approaches and personalized treatments that target certain pathogenic processes or molecular targets that have not yet been developed. Specifically, designed cells are expected to coordinate invasion along pre-existing central nervous system (CNS) structures such as blood arteries, subarachnoid space, and white matter tracts [7], which may result in a collective invasion [8, 9]. GBM cells can infiltrate as single cells [10] or as a group [11, 12] by modifying their cellular skeleton and extracellular matrix [7]. Local and distant bulky relapses may be brought on by distant GBM cells' reinvasion into the main tumor position and the invasion of distant tissues. Many early tumor mutations are shared by recurring tumors and their offspring [13], recommending that phylogenetic progenitor clones of primary tumors live in habitats where they can emerge from their latent state and proliferate locally [14]. Numerous invasion patterns have been identified [15, 16], and they all rely on interactions with the microenvironment [8] and genetic programs [17]. GBM brain tumor-initiating cells (BTIC) and differentiated cells can be used to imitate invasion [18–20]. Anyway, the involved cellular and molecular elements in GBM are of great importance and can help researchers strongly to find a better diagnostic and therapeutic method.

Immune structure of GBM

Various considerations in the use of quality expression from the Omnibus database Quality Expression and The

Cancer Genome Atlas (TCGA) have shown that qualitatively rich expression is associated with immune responses, particularly of the tumor-associated macrophage (TAM) genes, in the mesenchymal (MES) subtype of GBM compared with other subtypes [21], most likely indicating that TAM has a specific sub-role in terms of GBM. Indeed, TAMs have a major role in tumor development. However, several correlated studies suggest that TAMs may perform different functions in GBM subtypes. In contrast, despite increasing evidence from animal models and TCGA analyses of human glioblastoma (hGBM) [22], the clinical value of these data remains unclear, as neurofibromatosis type 1 (NF1) deficiency increases TAM infiltration. Although a growing body of preclinical data proposes that disease-specific therapies may be of preferred patient benefit, these subtypes have not been clinically proven to be biomarkers to predict survival rates [5]. However, it remains unclear what governs the variations in the immunological composition of the GBM subtypes. One possibility may be that genetically modified tumor-associated or tumor-specific antigens exist in distinct subtypes that influence different molecular immune responses and underlie differences [5, 23]. The inflammatory and proangiogenic microenvironment that is produced by glioblastoma increases adhesion molecule expression and decreases tight junctions in endothelial cells, which in turn increases blood–brain barrier (BBB) permeability. These changes allow leukocytes to exit the bloodstream through extravasation through the brain's endothelial wall and infiltration of tumor masses. In addition to TAMs, many additional immune cells could be identified in GBM parenchyma, albeit at a much lower frequency. T cells certainly make up the majority of lymphocytes in GBM, but according to flow cytometric analysis, their frequency is less than 0.35% of cells isolated from hGBM tumor tissue biopsy samples. Despite being an important cytoprotective agent in tumor cell elimination, CD8 + cytotoxic T cells are only sporadic present in GBM parenchyma and account for less than 35% of total CD3 + T cells [24]. The sensitivity of T cells isolated from GBM patients is lower than T cells from healthy controls sensitive to direct in vitro anti-CD3 activation, which indicates an immunosuppressed condition [24]. Recent research has demonstrated an association between immune-inhibitory receptor indoleamine 2,3-dioxygenase 1 (IDO1) levels, which is expressed more frequently by T cells that have infiltrated a GBM, with poor prognosis of the disease [25, 26]. Regulatory T cells (Tregs) could also be identified in GBM parenchyma and are thought to have immunosuppressive functions and inhibit antitumor immunity in different solid tumors like breast, ovarian, and pancreas cancers [27]. A phase I clinical trial investigating the utility and safety of an IDO1 inhibitor combined with temozolomide (TMZ) in children with primary malignant brain tumors is currently underway [28, 29]. In vitro, T cell activity in GBM patients is restored to levels comparable to healthy controls after Treg depletion removes the T cell proliferative defect [30]. Therefore, targeting Tregs may reverse tumor immune evasion and contribute to conventional or tumor immunotherapy. An in silico investigation of 22 human NP immune cell types confirmed and indicated collective increases in multiple cell types, including memory T cells (CD4 +), neutrophils, and polarized type 2 macrophages in cell MES tumors compared with non-MES tumors and classic (CL) and MES samples [22]. Theoretically, the immunosuppressive properties of TAM could block the effector T cell infiltration at higher concentrations. However, the etiology of the direct invasion of TAMs and T cells specific to this subtype is not immediately clear. This may be because T cells leave the blood stream passively following TAMs secondary to BBB damage during GBM development. However, this is unlikely because the T cell-to-TAM ratio in the tumor differs from that in the blood, and the number of lymphocytes is higher than monocytes. One possible explanation is that the chemokines chemokines C–X–C motif ligand (CXCL) and C–C motif ligand (CCL) secreted by MES tumors attract T or TAM cells, respectively, in tandem with other subtypes of GBM. Transgenic mouse models (GEMMs) can have enhanced, stable summary hGBM subtypes, providing an important tool for studying subtype-specific and related immunopathology development of effective therapies [31, 32]. These individual GEMMs make an unprecedented opportunity to identify the molecular signaling and immune cells that contribute to glioma formation and their continued proliferation driven by the microenvironment subtype-specific glioma. GEMM with different GBM subtypes is a better choice than other models for specific questions about interactions between the tumor and its microenvironment. Mouse orthologous allograft employing murine GBM cell lines which have been cultured for many years in serum or species-incompatible hGBM xenografts, especially those that are incompatible with chemokine and receptor them, indicated its significance. One of the desirable features of these biological models is the use of immunocompetent mice, where immune and tumor cells belong to the same species. This may eliminate species-specific interactions between cytokines, chemokines, and their receptors, which are essential for differential immune mobilization and cell-type incompatibility. The GEMM model of GBM can be used to answer critical biological questions on the relevance of differential immune cell infiltration in different subtypes of hGBM. Several additional studies later

showed that blood-derived myeloid progenitor cells in mice did not contribute to postnatal adult microglia significantly. Thus, the major number of adult microglia arise from the yolk sac, maintained by longevity, and have low self-renewal capacity [33–35]. By monitoring the lifespan of microglia with long-term imaging from a single cell in mice model, it has been demonstrated that resident neural microglia have an average lifespan of 15 months, which is roughly equal to the lifespan of other microglia. Although the naive parenchyma of CNS is dominated by resident microglia, different condition was identified in the tumor-bearing CNS. In tumor-bearing brains, the BBB is damaged and mononuclear chemoattractant protein (MCP) family expression is increased. This allows monocytes to enter the tumor from the peripheral border and then differentiate into macrophages. Hematopoietic stem cell-derived DC macrophage progenitor cells are the progeny that form monocytes. These progenitor cells differentiate into monocytes in the bone marrow, then released into the bloodstream to invade peripheral organs [36]. Mouse monocytes could be divided into two main cellular populations, CX3CR1int, Ly6C+, CCR2+inflammatory monocytes, and Ly6C−, CX3CR1hi as well as circulating monocytes CCR2− [37]. It is well known that Ly6C+, CX3CR1int, and CCR2+inflammatory monocytes leave the bloodstream and migrate to inflamed tissues. Once these cells reach the inflamed tissue, they differentiate into macrophages as they gradually upregulate CCR2 and simultaneously upregulate CX3CR1 [38]. Interestingly, TAMs express multiple levels of CCR2 and CX3CR1 in a reciprocal new model, suggesting that these cells are continuously transformed from infiltrated monocytes to mature macrophages [39]. This dynamic surface molecule switching suggests that myeloid-derived monocytes have high plasticity and that they mature after localization in the tumor [40]. It has been found that bone marrow-derived microglia and macrophages respond differently to different types of CNS injury and may have different functions [41, 42]. A recent example applying the complex parabiosis model shows that peripheral mononuclear cells infiltrate the inflamed CNS in an experimental autoimmune encephalomyelitis model and play an important role in the process of progression to paralysis [43]. Bone marrow-derived cells were found in the perivascular region, while resident glial cells were more strongly expressed in the peritumoral region (Fig. 1). RNA-seq analysis revealed that bone marrow-derived TAMs and microglia-derived TAMs mainly shared this gene involved in "cell migration," while genes

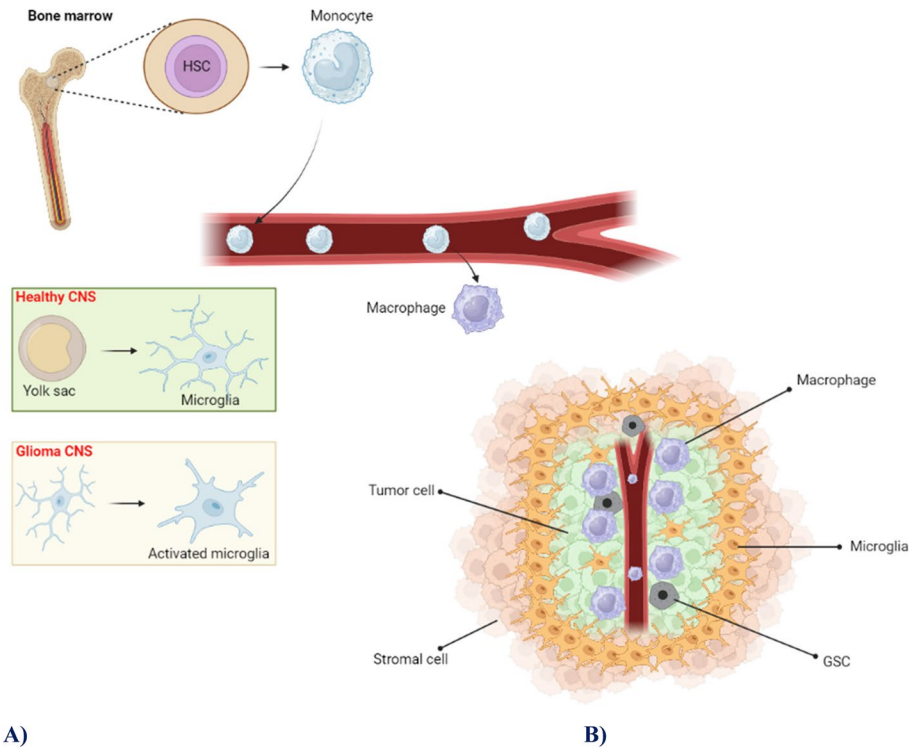


Fig. 1 Tumor-related macrophages (TAM) in GBM. **A** TAMs originated from two eclectic originations. The brain is full of bone microglia or marrow-derived monocytes. **B** In the proneural GBM cellular population, the major number of TAMs are BMDMs, which are chiefly, located in the perivascular niches which are also a niche for glioma stem cells (GSCs). Most microglia occur in the peritumoral area

involved in "inflammatory cytokines" and "transformation" are related. It became clear that there is an adjusted upward [39]. These differences can be partly explained by the difference between progenitor cells that these two cell populations are derived from and differences in transcription factors that they selectively use for gene regulation [44]. These results indicate that Cx3cr1 deletion indirectly promotes the transport of inflammatory monocytes to the central nervous system and leads to increased accumulation in perivascular regions [45]. However, there was no direct effect on microglial accumulation in the peritumoral region. Bone marrow-derived monocytes promote glioma stem cell proliferation through IL-1β production [45]. These novel findings recommend that bone marrow compartment-derived TAMs promote glioma formation, whereas microglia have not a central role in tumor growth and they appear to be mainly involved in tumor cell invasion.

Role of immunosuppression in GBM

Macrophages that reside in tumors are mainly considered important promoters of tumor growth due to their pro-angiogenic and immunosuppressive effects. These cells include myeloid-derived suppressor cells (MDSCs). Interestingly, murine MDSCs are the main cells that can express both Gr1 and CD11b markers and can differentiate into granulocyte subsets and monocytes. Importantly, in GBM, a few granulocytic MDSCs can occur within the tumor microenvironment [39]. Monocytic MDSCs can yield many different mechanisms to suppress cell-mediated immune action, including upregulating Arg1 product, promoting the expansion of Treg populations, and/or T cell apoptosis [46]. Considerably, most of these features are permanent in the macrophages with M2 phenotype. Previous in vitro studies suggested that myeloid monocytes may dichotomize into a standard active pro-inflammatory phenotype and also a main anti-inflammatory phenotype [47]. Remarkably, M1 cells generate many inflammatory cytokines and oxidative metabolites that are necessary for host defense, but they can hurt all different tissues [48]. Menacingly, M2 cells improve the healing of the wound and suppress the responses of the unwanted immune system [49]. However, according to these impressive findings from practical experiments in cell culture, the absolute dichotomous dispensation of M1 and M2 is not commonplace in vivo environment [50]. Indeed, bone marrow-derived macrophage and tumor-associated microglia transcriptional investigation indicated mixed structures of M1 with M2 phenotypes in all TAM colonies. For instance, the canonical M2 marker arginase 1 had an upregulation level in both microglia and bone marrow-derived macrophages, and notably, the special M1 cytokine IL-1β had an upregulation level in all cells. Anyway, it is not evident whether these M1 and M2 features belong to diverse colonies or whether single cells can be able to express two molecular subsets at diverse levels. Transparently, TAMs have high plasticity and have been indicated to switch between two M1 and M2 phenotypes responding to foreign environmental stimulators [51]. There are several endeavors to polarize the TAMs to the fate of M1. Anyhow, there are major challenges as soluble parameters yielded by tumor cells may revert TAMs to the M2 phenotype despite translational medicine.

Main signaling pathways in GBM

Notch signaling in GBM

One of the main signaling pathways in GBM is Notch signaling. Noteworthy, the Notch signaling pathway has a crucial responsibility in the regulation of many eclectic molecular, developmental, and cellular functions comprising number determination apoptosis, differentiation, neurogenesis, self-renewal, homeostasis, cell migration, and stem cell maintenance [52–54]. These main elements are strongly and widely spread in the brain cells, and Notch 1 is also expressed in astrocytes, neurons, epithelial and endothelial cells, and progenitor cells [55–57]. Interestingly, Notch 2 and 3 molecules are expressed strongly in progenitor cells [55–58], and in this way, the Notch 4 molecule is expressed in the endothelial cells [59]. DII1 is shown to be expressed in all parts of intermediate neuron progenitors and neuron cells [57, 58, 60, 61]. DII3 is expressed also in approximately all sections of intermediate neuron precursors [60]. In addition, DII4 is also expressed in the endothelial cells [62]. It is remarkable to say that many cells express Jagged 1 including neurons, progenitors, and intermediate neural progenitors [56, 57, 60, 63–65], and alongside this, the only cell that could express Jagged 2 is a neuron [57, 59]. The Notch pathway is operated by many different factors comprising metabolic interactions of Notch receptors and neighboring cellular ligands and suppressed by cis interactions through binding to the cell [66–68]. Activation of the Notch signaling pathway results in the expression of proteins included in the diagnosis of lineage. Dysregulation in the Notch signaling pathway is related to several types of cancers including colon, pancreatic, brain tumors, skin, breast, cervical, and blood [69–72]. Several translational researches about GBM confirmed that Notch receptors including Notch 1, 3, and 4, or their molecular components such as Hey1 and DII1 have irregular and unusual expression in brain tumors [73–75]. The high expression of Notch receptors 1 and 4 or other elements of Notch signaling including DII1, DII4, Hey1, Hey2, Jagged 1, and Hes1 have been confirmed and reported in many different studies [73, 76, 77]. Notch receptor 4 is also related to primary GBM with high grades. Anyhow, many studies

have indicated low expression levels of Notch proteins including Notch 1 and Notch 2 in GBM [78]. The association between GBM and expression of Notch molecule has been evaluated in different cells like mesenchymal [79], classical [5], and nervous [5, 80]. So far, there are not sufficient studies examining the epigenetic performance of the Notch signaling pathway in GBM [81, 82]. Hey1 methylation status is thought to mediate the pathogenesis of GBM, and past research suggested it as a predictive marker for GBM patients [83]. Moreover, in the xenograft cell lines 4910 and 5310, treatment with inhibitors of histone deacetylase (HDAC), comprising sodium butyrate, mediated apoptosis of GBM cells, downregulating Hey1 expression and reducing DNA (cytosine-5) expression. In this regard, cerebellar growth and neurodevelopmental interaction between glial cells and Bergmann cells are regulated by the delta/Notch-like epidermal growth factor receptor (DNER), which results in the expression of Purkinje cells in a delta-dependent manner [84]. Also, in GBM-derived neurons, deletion of HDAC can activate the DNER/Deltex signaling, resulting in the inhibition of cell differentiation and neuronal growth [84]. However, more studies are needed to investigate the epigenetic role of Notch signaling in GBM and find potential therapeutic targets.

Hedgehog signaling in GBM

Hedgehog (HH) signaling has an important role in embryogenesis as well as tumorigenesis [85, 86]. Regulation of cellular proliferation and differentiation can be an important pathway for embryonic patterning [87, 88]. Regulation of tissue repair, stem cell maintenance, and regeneration can play an important role in HH signaling after puberty [86]. Congenital malformations such as holoprosencephaly and microcephaly are the result of dysregulation of the HH signaling pathway [85, 89]. Cancer susceptibility syndromes such as Guerlain syndrome [90, 91] and various cancers such as glioma are associated with upregulation of HH [92, 93]. Signaling pathways are activated by three of the HH ligands, namely, desert hedgehog (DHH), Indian hedgehog (IHH), and sonic hedgehog (SHH) [94]. HH ligands activate signaling pathways by binding to patch receptors (PTCH) that override smoothing (SMO) receptors as shown in Fig. 2 [85]. The CDO mediates the binding of PTCH to HH, a binding-reducing cell adhesion molecule that is regulated by oncogenes, GAS1, and BOC [95, 96]. Activated SMO inhibits the suppressor of fused (SUFU) gene action, thus preventing degradation of the zinc finger (GLI) of the glioma-associated oncogene family (GLI) [86]. The GLI family includes three transcription factors (GLI1, 2, and 3) [97]. Activated GLI1 upregulates several genes like PTCH1GLI1, VEGF, Bcl-2, and cyclin D2 (CCND2). In this way, the HH signaling pathway

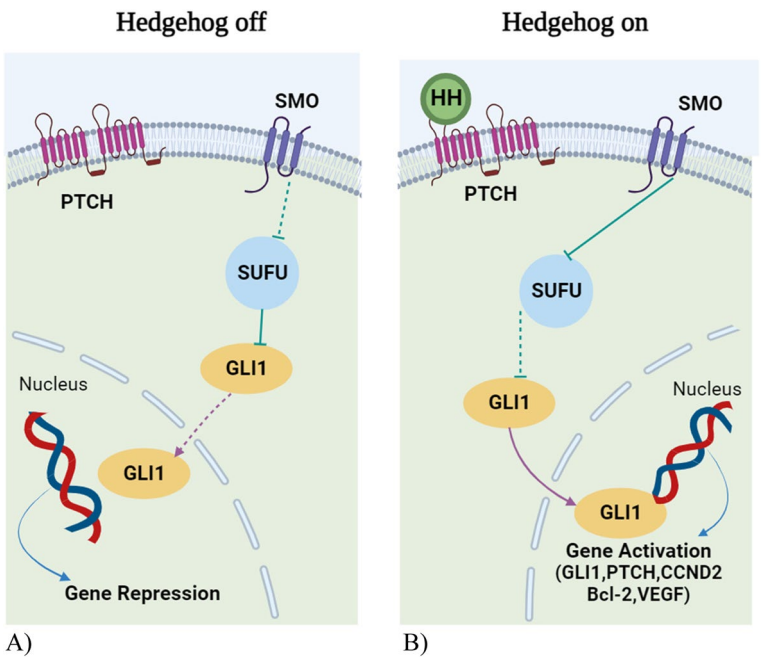


Fig. 2 Hedgehog signaling pathway. **A** Hedgehog signaling pathway inactivation in the absence of a Hedgehog ligand. **B** The binding of the Hedgehog to the patched receptor (PTCH) revitalizes the hedgehog signaling pathway. SMO, smoothing receptor; SUFU, suppressor

upregulation is related to glioma. Expression of the HH signaling pathway is associated with glioma growth and progression through cancer stem cell augmentation [98, 99]. SHH ligands are highly expressed in both gliomas and surrounding tissues [98, 100]. GBM aggressiveness is thought to correlate well with truncated tGLI1 isoforms [101, 102]. GBM cells in contrast to normal healthy cells express tGLI1 [101]. Migration and invasion of glioma cells are reduced by inhibiting the HH signaling pathway [103, 104]. Bromodomain-containing protein 4 regulates GLI1 transcription by directly binding to gene promoters [105, 106]. Furthermore, lysine acetyltransferase 2B levels correlate with the expression of HH target gene [107]. The HH signaling through cancer stem cell maintenance has also been suggested to play an important role in GBM development and progression [98, 99]. Therefore, in GBM treatment, the signaling cascade is considered a promising target. In this regard, epigenetic regulators may have a crucial role in cancer improvement by deregulating the HH signaling cascade [107, 108]. Consequently, HH signaling could be a useful therapeutic target for GBM.

Wingless signaling pathways in GBM

The wingless (WNT) signaling plays a critical role in the development, proliferation, migration, and final fate of embryonic cells [109, 110]. It also regulates the adult stem cells' differentiation, regeneration, and maintenance [111]. Deregulation of WNT signaling causes various CNS pathologies [112, 113] and various tumors, including GBM [75, 114–116]. Importantly, Fig. 3 shows the WNT signaling pathway. The binding of WNT ligands to the cell membrane frizzled receptors (FZDs) activates this signaling pathway [117]. WNT signaling activation inhibits glycogen synthase-3 (GSK-3) and leads to cytosolic β-catenin stabilization [81, 118]. GSK-3 plays a role in promoting WNT-FZD complex formation and phosphorylation and degradation of the β-catenin [81]. During activation of the WNT signaling pathway, a high proportion of cytoplasmic β-catenin translocates to the nucleus, making multimeric complexes through binding to the transcription factors like T cell factor/lymphocyte-enhancing factor (TCF/LEF), which inhibits target genes transcription As c-Myc, CCND1, CD44 SOX9, and COX2 [119–121]. Aberrant WNT signaling cascades regulate various pathways involved in the maintenance of stem cells [122] and therapeutic resistance [123]. Unlike some other cancers, changes in the WNT signaling pathway leading to constitutively active signalings are rare in gliomas. Nevertheless, it is also approved that WNTs play a key role in the dysregulation cascade of glioma stem cells [124]. Furthermore, WNT signaling pathway alterations distinguish between healthy and

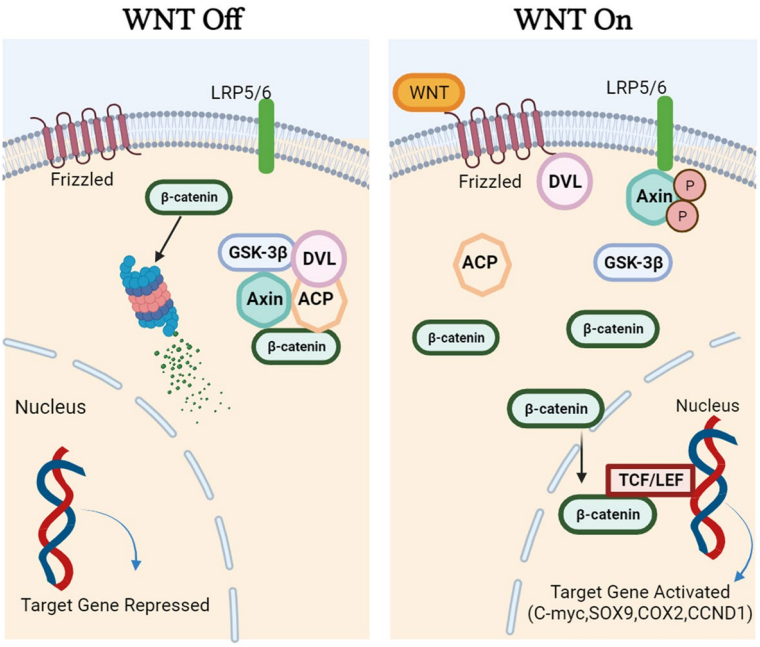


Fig. 3 Schematic illustration of the WNT signaling pathway. Left: Inactivation of the WNT signaling pathway. Right: The binding of a WNT ligand to the FZD receptor activates the WNT signaling pathway. FZD, frizzled receptor; LRP5/6, low-density lipoprotein receptor-related proteins; APC, adenomatous polyposis colon. GSK3β, glycogen synthase kinase 3β. DVL, disturbed. CCND1: Cyclin D1; TCF/LEF: T cell agent/lymphoid enhancer agent

glioma tissue. The expression intensity of beta-catenin and TCF4 (its transcription factor) is higher in glioma cells than in normal brain cells [125]. In high-grade gliomas, some WNT signaling activators, such as TCF4 and SOX, are increased [75, 126]. In addition, oncogenic phenomena such as cell proliferation, apoptosis, and inhibition of invasion are thought to be associated with the Wnt/β-catenin signaling pathway in GBM [127]. Other WNT signaling factors like FZD1, DKK1, and LEF1 are expressed at more than normal levels in glioma and are associated with poor disease consequences [128]. Active oncogenic activity in glioma cellular populations is thought to be related to changes in the WNT/β-catenin signaling pathway [128]. These results are supported by the fact that chemoresistance and radiation are associated with changes in standard WNT signaling [129]. Several studies have shown that advanced GBM invasion and poor prognosis are associated with WNT expression [130]. High-grade gliomas are associated with both WNT signaling pathway factors, such as LEF1 and HOXA13, which promote glioma growth and cell migration [131]. This is in line with studies showing that WIF-1 loss increases tumor invasion through mediating metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) activity [132].

Epigenetic alterations in GBM

DNA methylation

DNA methylation is believed to be one of the most important discoveries of epigenetic modification processes. There are four possible positions, including the C-5 position of cytosine, the N-4 position of cytosine, the N-6 position of adenine, and the N-7 position of guanine [133]. Also, 5'-CpG-3' cytosines to generate 5-methylcytosine (5mC) take place in DNA methylation mainly in the mammalian cells. The methylation reaction is also performed by a DNA methyltransferase (DNMT) that uses a methyl donor group called the *S*-adenosylmethionine cofactor [134]. DNA methylation patterns of glioma cells and normal cells have been shown in many studies [135] to be different. It is noteworthy that the simultaneous presence of general

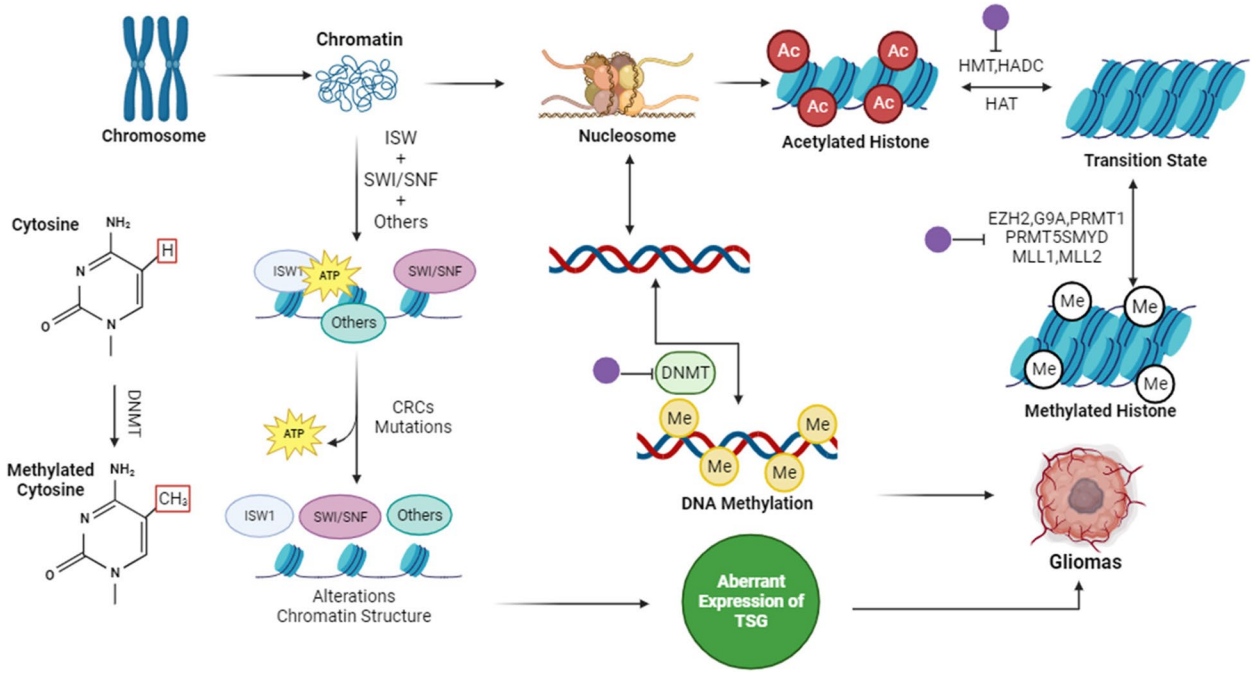


Fig. 4 Performance of epigenetic-based therapeutic landscape in the treatment of glioma. Nucleosomes are formed and organized by chromatin as a result of DNA strands coiled near histone octamers. In general, reactions in the presence of DNMTs lead to DNA methylation at the 5-carbon position of cytosine residues (CpG sites), ultimately leading to glioma development. Epigenetic-based therapeutic targets inhibit DNMT, but DNMT also plays an important role in reactivating TSG and related genes to treat glioma. In addition, acetylation, methylation, and phosphorylation reactions lead to histone modifications (HM) at multiple sites. Epigenetic-based therapeutic strategies inhibit HMT and HDAC function, freeing up more sites on histone ends for acetylation, a process that reverses aberrant HM and ultimately tumor cell proliferation may inhibit and cause apoptosis. In addition, chromatin remodeling combinations comprising ISW I and SWI/SNF rely on ATP hydrolysis to provide energy to complete changes in chromatin structure. Mutations in the CRC protein led to an aberrant expression of TSG or other genes included in cell cycle control, leading to the development of glioma. ATP, adenosine triphosphate; DNA, deoxyribonucleic acid. DNMT, DNA methyltransferase; HM, histone modifications. TSG, tumor suppressor gene; HMT, histone methyltransferase; HDAC, histone deacetylase

hyper- and hypomethylation levels of CpG islands is another characteristic of cancer cells. Therefore, in glioma cells, the status of DNA methylation in various related genes is the standard biomarker for the diagnosis of GBM (Fig. 4) [136]. In the promoter region of the gene, DNA gain is the most frequent epigenetic alteration in tumor cells. Also, the regulation of gene expression in the human genome is dependent on the methylation status of the promoter regions. In addition, promoter regions control approximately 50% of tissue-specific genes and all constitutive genes. Nearly all CpG islands in normal physiological conditions are hypomethylated, but some constituent genes, such as DNA repair genes, and tumor suppressor genes (TSGs) are commonly hypermethylated in tumor tissue. This abnormal status of methylation plays an important function in repressing gene transcription and gene's biological function loss. Also, 5-hydroxymethylcytosine is an epigenetic marker of 5mC oxidation and plays a role in glioma development. In this regard, it has been confirmed that tumor grade has a negative correlation with low levels of 5hmC DNA [137]. Furthermore, in patients of all ages, the CpG island methylator phenotype (G-CIMP) is suggested to be a prognostic marker of glioma [138, 139]. In particular, Jha et al. indicated distinct methylomas in pediatric GBM compared with geriatric GBM. This study indicates that the G-CIMP prognosis marker of glioma in senile GBM cannot be readily extrapolated to GBM in pediatric patients and that there is an urgent need to identify clear prognostic indicators. Also, aberrant DNA methylation is an important marker of TSG inactivation. Several TSGs have also been recognized in gliomas comprising p14ARF, p16INK4a [140], MLH1, and NDRG2 [141]. In addition, the p16INK4a gene maintains the dephosphorylated activated state of the retinoblastoma tumor suppressor protein in the normal cyclin D-Rb cascade and regulates cell cycle progression. Over 50% of homozygous deletions of the p16INK4a gene were also detected in GBM tissue, and p16INK4a is altered in 80% of glioma cells. Thus, restoration of p16INK4a inhibits cell proliferation and induces cell cycle arrest [140]. In addition, MGMT (O-6-MethylGuanine DNA Methyltransferase) is a key DNA damage repair gene that can repair alkyl damage induced by BCNU (bischloroethylnitrosourea). Approximately 40% of glioma tissues have been observed to have hypermethylation of MGMT promoter [142]. The degree of methylation has a strong association with tumor prognosis and incidence. Therefore, its importance could be more than prognosis according to tumor grade or age. Several studies have shown that the degree of MGMT promoter methylation is the most important marker for assessing sensitivity to temozolomide (TMZ) in glioma treatment. Conversely, downregulated MGMT could significantly restore TMZ chemosensitivity in vivo and in vitro [143]. In addition to the above genes, CpG islands methylation in the promoter regions of LATS1, LATS2 [144], and p73 [145], and the genes described in Table 1 are also strongly related to GBM progression.

Histone modifications in GBM

Histone modifications (HM) occur in different ways in the epigenome of mammalian species. The histone building block, the nucleosome, is an octamer consisting of 147 compositionally coiled base pairs with two H2A, two H2B, two H3, and two H4s. Correspondingly, nuclear histones have both N-terminal and C-terminal binding sites. Relatively, because the N-terminal half of lysine extends outside the nucleosome, the N terminus has a significance situation and is critical to modifications comprising acetylation, methylation, ADP-ribosylation, ubiquitination, and phosphorylation. For gene expression changes without alterations of base pairs, these modifications and differences play an important role. Transcription errors permanently take place in the expression of various genes that have an important function in the progress and development of glioma, and these transcription errors may be due to misplaced HMs. Among the various HM proteins, histone methyltransferases, histone deacetylation, and two proteins that cause methylation at multiple sites on histones have received more consideration than other HDACs. Interestingly, HDAC, HDAC1, HDAC2, HDAC3, HDAC5, and HDAC9 enzymes from diverse classifications in glioma cell lines have main alterations and differences, as well as the HDAC5 and HDAC9 expression in high-grade medulloblastoma, the level of H3 acetylation in astrocytoma of high-grade compared to low-grade medulloblastoma and considerably the normal tissue of the brain increases. Notably, type II mRNA levels and class IV HDAC levels had downregulation results in GBM in comparison with low-grade astrocytoma and healthy and normal brain tissue. In this way, the application of HDAC inhibitors (HDACIs) has become an active and practical research category for curing some different cancers. In addition, HDACIs have been employed combined with radiotherapy and chemotherapy to cure and control GBM. Meanwhile, the mechanisms of antitumor activity of HDACIs, comprising promoting cell differentiation, inducing apoptosis, inhibiting angiogenesis, and blocking the cell cycle, may eventually prevent and control the proliferation and programmed cell death of various tumor cells [147]. Several recent studies have indicated and confirmed that the degree of histone lysine methylation is regulated by histone methyltransferases

Table 1 DNA methylation of genes or proteins in glioma cells

Genes/Proteins	Location on chromosome	Functions	Symbol	References
ARF tumor suppressor	9	Controls cell cycle	P14ARF	[145, 146]
Cyclin-dependent kinase inhibitor 2A	9	Inhibits cell proliferation and triggers cell growth arrest	CDKN2A/p16INK4a	[147]
Tumor protein P73	1	Reduces cell proliferation and enhanced apoptosis	P73	[148, 149]
Mitogen-activated protein kinase	8	Inhibits cell growth	MKP-2	[150]
Nuclear receptor binding SET domain protein 1	5	Suppresses cell growth	NSD1	[151]
MicroRNA 129–2	11	Suppresses cell growth, and triggers apoptosis	miR129-2	[152, 153]
HIV-1 tat interactive protein 2	11	Suppress cell growth and proliferation	HTATIP2	[154]
Solute carrier family22 member 18	11	Inhibits cell growth and induces apoptosis	SLC22A18	[155–157]
TNF receptor superfamily member 11a	18	Elevates cell apoptosis	RANK(TNFRSF11A)	[158]
Neogenin	15	Induces cell apoptosis	NEO1	[159, 160]
Nonsteroidal anti-inflammatory drug-activated gene-1	19	Induces cell apoptosis and Inhibits cell growth	NAG-1	[161]
Glioma pathogenesis-related protein 1	12	Promotes cell apoptosis	GLIPR1	[162]
Testin	7	Triggers cell apoptosis	TES	[163]
Brain expressed X-linked1	X	Increases sensitivity to chemotherapy-induced apoptosis	BEX1	[164]
Brain expressed X-linked2	X	Enhances apoptosis, as well as inhibits migration and invasion	BEX2	[164, 165]
N-Myc downstream-regulated gene 2	14	Decreases cell proliferation	NDRG2	[166, 167]
Human mutl homolog 1	3	Repairs damage DNA	hMLH1	[168]
O^6-alkylguanine DNA alkyl transferase	10	Repairs damage DNA	MGMT	[169–171]
Epithelial membrane protein 3	19	Reduces cell proliferation	EMP3	[172, 173]
Kruppel-like factor 4	9	Decreases cell proliferation	KLF4	[173–175]
WNK lysine deficient protein kinase 2	9	Suppresses cell invasion	WNK 2	[176]
Slit guidance ligand 2	4	Inhibits cell migration and invasion	SLIT 2	[177]
Micro RNA 124–1	8	Inhibits cell proliferation, invasion, and migration	miR-124a	[178, 179]
Tissue factor pathway inhibitor 2	7	Suppresses cell proliferation, invasion, and migration	TFPI-2	[180–182]
Protocadherin 10	4	Triggers the cell growth arrest and apoptosis	PCDH10	[183]
RUNX family transcription factor 3	1	Suppresses cell migration and invasion	RUNX3	[184]

containing EZH2, MLL1, MLL2, and G9a in all types of glioma cells. These modifications are closely correlated to genome integrity and gene transcription regulation [166]. The protein arginine methyltransferase 5 (PRMT5) gene is used in the diagnosis and treatment of GBM, whose nuclear expression is associated with poor survival in glioma patients. GBM cell treatment with a PRMT5 inhibitor mirrored the action of PRMT5 knockdown and played and led to the apoptosis of differentiated GBM cells [168]. It is acknowledged that glioma cell proliferation inhibition and apoptosis activation may be achieved through suppressing the function of histone methyltransferases or HDACs [185], recommending that these proteins It has been suggested that the suppressor could be used as a potential drug to treat glioma. A recent study showed that the G9a histone methyltransferase, which regulates the demethylation of H3K9, is also associated with glioma progression [186]. Therefore, its suppressor is considered a promising candidate for the treatment of glioma [187]. Recent studies [188] have shown overexpression of EZH2 in many tumor tissues, such as tumor tissue. It is a glioma and is closely associated with cancer cell development, metastasis, and invasion. Advances in clinical research suggest that the use of EZH2 gene silencing techniques or EZH2 suppressors could prevent glioma cell proliferation [189]. Therefore, EZH2 has been suggested as a new target that could open new avenues for the treatment of glioma [190]. In addition, H3F3A

contains two changes in the histone tail, a glycine (G) amino acid to arginine (A)/valine (V) amino acid change at codon 34 (G34R/V) and a methylation at lysine (K) 27 (K27). It is one of the key regulators of post-transcriptional modifications in pediatric GBM [146].

Chromatin remodeling in GBM

The chromatin remodeling complex (CR) has an adenosine triphosphates (ATPase) function and relies on ATP hydrolysis to provide the energy to fulfill changes in the structure of chromatin [167]. The complexes could be classified as ISW I, SWI/SNF, and others based on the different subunits capable of hydrolyzing ATP. Also, these proteins and complexes that are related to cell cycle inhibition and activation, DNA repair, DNA methylation, and DNA transcription have a significant role. Mutations in the CR protein are associated with many diseases in humans. Additionally, these mutations are responsible for CR failure, which ultimately leads to chromosome misalignment, blocking the transcription machinery and making DNA inaccessible to complexes capable of repairing the damage. This can lead to abnormal gene expression. If these mutations make abnormalities in TSGs or proteins controlling the cell cycle. They may finally contribute to cancer incidence [169]. A recent study found that CR controlled drug resistance in the GBM [170]. Targeting the GBM stem cells with kinase inhibitors could reversibly induce these cells into a slow, cyclical steady state. In addition, this status activates the Notch signaling and significantly upregulates the histone demethylases KDM6A/B. This has a key role in the removal of H3K27 trimethylation in cis-regulatory regions of the genome, subsequently contributing to increased H3K27Ac levels. CR has an important function in these types of cell shifts, and this study provided new targets for future beneficial therapeutic advances. Furthermore, by targeting developmental and epigenetic cascades, it could be possible to destroy drug-resistant tumor cells and prevent recurrence of disease. Research approved that upregulated CR factors, including lymphocyte-specific helicase (LSH), accelerate glioma progression [171]. In addition, this study shows that glycogen synthase kinase-3β (GSK3β) and the regulated transcription factor E2F1 in astrocytoma and GBM are involved in the development of glioma and expression of LSH [171]. Also, reduction of E2F1 decreases the expression of LSH and cell proliferation, while deletion of GSK3β increases its accumulation in E2F1 in the LSH promoter and ultimately increases LSH expression. In this regard, lipoprotein receptor-related protein 6 (LRP6), which serves as an upstream regulator for GSK3β signaling, is often overexpressed in glioma cells. Degradation of LRP6 reduces the recruitment of E2F1 to the promoter of LSH, thus reducing LSH the expression levels. LSH ultimately plays an important role in suppression of cell growth. Overall, there is a mechanistic relationship between expression of LSH in glioma cells and induction of the LPR6/GSK3β/E2F1 axis, suggesting a novel role for LSH in both malignant astrocytoma and GBM. So, understanding the contribution of LSH in the development of gliomas will therefore improve our understanding of gliomas and suggest LSH as a promising therapeutic target in patients with these types of brain tumors.

Noncoding RNA in GBM

Role of microrna in GBM

A group of small RNA sequences known as microRNAs (miRNAs) exhibit post-transcriptional regulation during mRNA degradation [148, 172–175]. Considerably, miRNAs are currently being used to control cellular metabolism in GBM. Moreover, as shown in Fig. 5, miRNAs direct the expression of metabolic genes either directly or through the regulation of signaling cascades of cancer [149], metabolic oncogenes, and tumor inhibitors [150]. Some miRNAs can target mRNA enzymes that promote metabolic processes such as lipid, glutamine, and glucose metabolism, oxidative phosphorylation, and glycolysis. Also, this miR-106a gene affects GLUT3 (SLC2A3) and decreases glucose influx during glycolysis [151]. In GBM stem cells, the miR-143 gene affects HKII and induces differentiation [152]. Also, let-7a and miR-326 regulate GBM metabolism through inhibiting PKM2 expression [153, 154]. In addition to glucose metabolism, miRNA-153 also targets glutaminase and downregulates the metabolism of glutamine in glioma cells [155]. miR-100, miR-16, miR-101, and miR-23 target the mitochondrial ATP synthase ATP5B or ATP5A1 to control mitochondrial energy metabolism in GBM cells [156]. Many anticancer factors in its downstream cascade play key roles as controllers in metabolism in GBM cells. Therefore, miRNAs targeting these factors may also indirectly affect the GBM cell metabolism. EGFR is also expressed in high levels in approximately 50% of GBM cells and is also a GBM pathological target with EGFR amplification or mutation. EGFR activates PKCε monoubiquitination, which leads to the induction of NF-κB and an increase in the expression of PKM2 to promote glycolysis and tumorigenesis in GBM [157]. Of note, expression of EGFR is decreased in GBM compared with gliomas of low-grade malignant potential by various miRNAs like miR-7, miR-219-5p, and miR-128 [158–160]. Oncogenic K-Ras induces the growth of cancer cells through dissociating glutamine and glucose metabolism [161]. MiRNAs, including let-7a and miR-134, that target KRAS that are downregulated in GBM are associated with disease-poor prognosis [162]. Similarly, miR-9 indirectly affects K-Ras

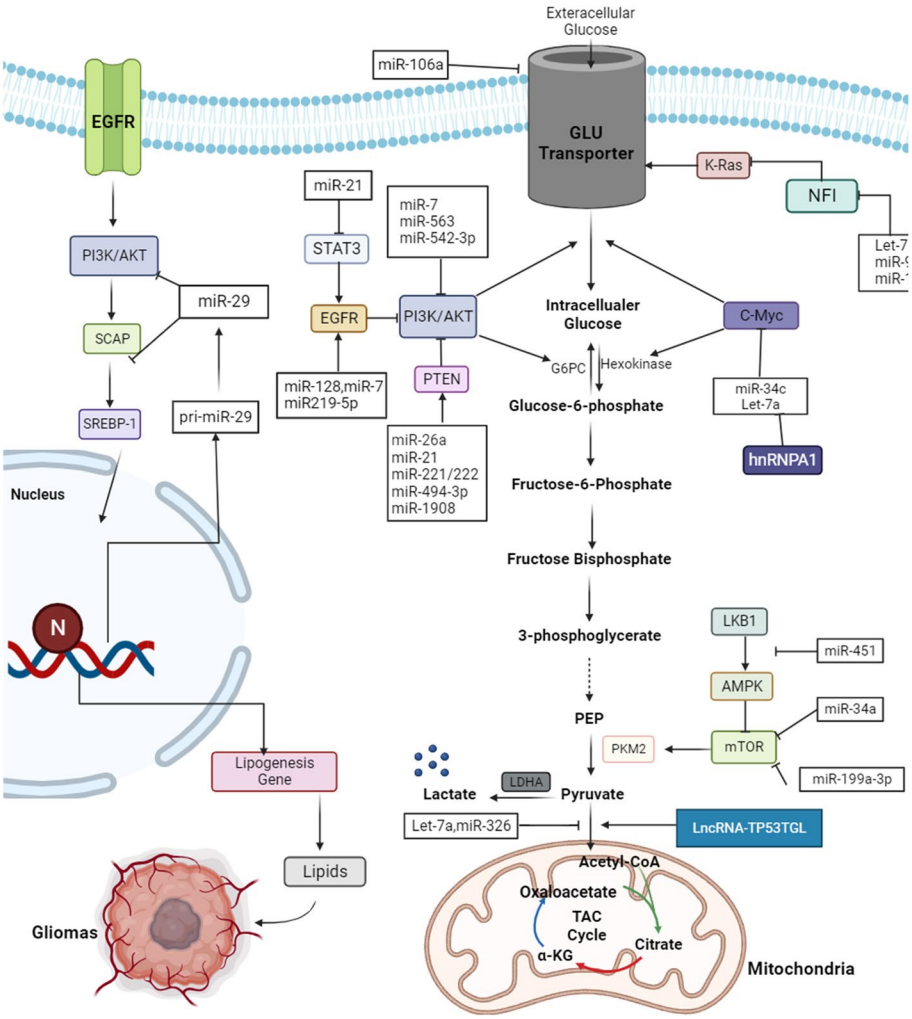


Fig. 5 Role of miRNAs in regulating lipid metabolism and glycolysis in glioma. Glycolysis takes place in the cytosol when D-glucose enters the cell through membrane transporters of glucose. After a series of enzymatic reactions, D-glucose is converted to pyruvate and then is entered into the TCA cycle. Conversely, in conditions with limited oxygen levels, pyruvate is converted to lactate. Moreover, miRNAs and long noncoding RNAs control glycolysis in oncogenic conditions. Glucose transporter family expression is regulated by noncoding RNAs, thereby altering glucose internalization rates. It is important to say that the miRNAs also inhibit the F PI3k/AKT signaling, which plays an important role in the metabolism of GBM cells. In addition, miRNAs inhibit c-Myc and mTORC2, which regulate GBM glycolytic metabolism. On the other hand, miRNAs also have a central role in the lipid metabolism of GBM cells. The transcription factor SREBP-1 triggers cholesterol synthesis and is highly upregulated in various tumors, including GBM [187]. Furthermore, SCAP/SREBP-1 signaling mediated by EGFR upregulates miR-29 by binding to precise sites within the promoter. Interaction between miR-29, SREBP-1, and the 3'-UTR of SCAP subsequently represses their expression. Thus, the miR-29-SCAP/SREBP-1 feedback loop regulates EGFR signaling-mediated GBM proliferation through the regulation of cholesterol synthesis: G6PC, glucose-6-phosphatase; G6PD, glucose-6-phosphate dehydrogenase, PEP, phosphoenolpyruvate; PKM2, pyruvate kinase M2; LDHA, lactate dehydrogenase A; α-KG, α-ketoglutarate; EGFR, epidermal growth factor receptor; STAT3, signal converter and transcriptional activator 3; PTEN, phosphatase, and tensin homologs; NF1, neurofibromin 1; AMPK, AMP-activated protein kinase; LKB1, liver kinase B1; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; cMYC, c-myelocytoma virus oncogene homolog; SREBP-1, sterol regulatory element binding protein 1; and SCAP, SREBP cleavage-activating protein

by targeting neurofibromin 1[163]. In addition, miR-9 is expressed more than normal levels in GBM and this expression is also associated with poor disease prognosis [164]. In this regard, C-Myc is one of the main regulators of metabolism in tumoral cells by increasing the target gene expression like LDH-A, Glut1, glycolytic enolase, and serine hydroxymethyltransferase which increases C1 metabolism [157]. Various studies have shown that Let-7a and miR-34 directly affect c-Myc in GBM [154, 165]. In addition, the PI3K/Akt signaling pathway is also an important controller that has a key role in regulating the Warburg effect in cancer and GBM cell metabolism

[176]. miR-542-3p [177] and miR-7 [178] target Akt and PI3K in GBM, respectively. miR-503 [179] also could inhibit the PI3K/Akt signaling. PTEN (a PI3K antagonist) is a major tumor inhibitor of GBM regulated by miR-26a, miR-221/222, miR-21, miR-10a/10b, miR-1908, and miR-494-3p [180–182]. Also, the Warburg effect regulation has a key role in the LKB1-AMPK cascade [183]. On the other hand, mTORC2 affects glycolytic metabolism through acetylation of FOXO and upregulation of c-Myc in GBM [165]. Considerably, miR-199a-3p has been observed to target mTORC2, which is downregulated in GBM compared with normal brains [184]. In addition to the aforementioned mechanisms, regulation of miRNA may be a more promising approach. For example, c-Myc targets let-7a downregulate hnRNPA and subsequently inhibit expression of let-7a by binding to and inhibiting the progression of pri-let-7a [154]. Additionally, let-7a directly targets PKM2. Consequently, the let-7a/c-Myc/hnRNPA1/PKM2 feedback loop upregulates PKM2 in GBMs and induces glycolysis. Interestingly, miRNAs also have a crucial role in GBM lipid metabolism. Indeed, malignant tumors are fundamentally characterized by this metabolic dysregulation. Also, the regulatory element of sterol transcription factor binding protein 1 (SREBP-1) causes cholesterol synthesis and has increased expression in various tumors such as B. GBM [191]. For instance, SCAP/SREBP-1 upregulates miR-29 through EGFR signaling and by binding to precise sites within the promoter. The interaction between miR-29, SREBP-1, and three major untranslated regions (3′-UTR) of SCAP subsequently represses their expression. Thus, the miR-29-SCAP/SREBP-1 feedback loop regulates glioma cell proliferation through the regulation of EGFR signaling and cholesterol synthesis [192]. Approximately 50% of miRNA genes are thought to reside in cells of glioma or their susceptibility sites, and these genes may regulate approximately 3% of all tumor genes of glioma and 30% of coding genes there is. Similarly, one miRNA could affect 100 GBM mRNAs simultaneously [193], whereas one mRNA in glioma can relax one or multiple miRNAs [194]. This study suggested that many miRNAs are altered in GBM, ultimately affecting the regulation of mRNAs associated with gene expression [195]. Correspondingly, miRNAs have several important functions in glioma proliferation. In particular, miRNAs have a significant role in modulating the expression of cancer genes and genes involved in tumorigenesis and regulating different signaling pathways. These are viruses that also regulate glioma stem cell differentiation, are encoded as oncolytic, and play an important role in the growth of tumors [196]. In comparison, the knockdown of miR-221/222 decreased cell invasion by altering TIMP3 levels of the tissue inhibitor of metalloproteinase-2. Degradation of miR-221/222 further enhanced the expression of TIMP3 and significantly shortened the development of tumors in xenograft models. Another study found that overexpression of miR-221/222 decreased p27kipl-level staging [197]. P27kipl prevents cell cycle transition from the G1 phase to the S phase through binding to the cyclin E complex and CDK2. Therefore, downregulated miR-221/222 may have an up regulatory effect on p27kipl to suppress tumor growth [194]. In cancer metabolism, circular RNAs and long non-coding RNAs (lncRNAs) play a very important role [198] and play a central role in gene regulation [199–202]. It is confirmed that 198 lncRNAs were downregulated and 27 lncRNAs were upregulated in GBM, indicating the critical role of these nucleic acids in GBM. Recently, there has been some evidence suggesting that lncRNAs are also involved in the cellular metabolism of GBM. For example, TP53TG1 lncRNA supports cell proliferation and migration by expressing genes like IDH1 and PKM2 in glioma cultures with reduced levels of glucose [203]. The lncRNA LEF1-AS1 increases GBM cell proliferation and prevents apoptosis through the Akt/mTOR signaling, which controls glycolysis [22]. However, further investigations are needed to confirm the association of lncRNAs and GBM regulation. In contrast, circular RNAs have an important role in the GBM cellular metabolism. For example, Fbxw7circRNA translates a novel 21 kDa protein called FBXW7-185aa, which reduces USP28-mediated stabilization of c-Myc [204]. This may be related to the Warburg effect. However, little is known about the role of circRNAs in the regulation of cell metabolism [205].

Therapeutic approaches for GBM therapy

Mesenchymal stem cells could suppress the development, invasion, and metastasis of hard tumors. They are therefore considered to be excellent therapeutic modalities to treat tumors, but their exact role in tumorigenesis is currently unknown [206]. The response of GBM tumors to surgery, chemotherapy, and radiation is not completely clear, so new treatments are greatly needed [207]. Because miRNAs affect the expression of various genes, they are potential candidates for GBM therapy. For example, miR-873 downregulates IGF2BP1 expression which results in decreased carcinogenesis and metastasis of GBM [208]. Instead, miR610 reduces cell proliferation and GBM proliferation by inhibiting the expression of CCND2 and AKT3 at translational as well as transcriptional levels [209]. In this account, lncRNAs such as ASLNC20819 and ASLNC22381 that target IGF-1 play several crucial roles in GBM progression. Targeted therapy of lncRNAs is therefore likely to be an effective therapeutic approach [210]. Compared to normal cells,

altered epigenetic changes occur in tumor cells, which can be inhibited by using inhibitors that alter the activity of epigenetic enzymes (EEs). In this regard, for example, a potential epigenetic therapeutic agent such as 5-aza-2'-deoxycytidine (5-aza-CdR) increases GBM cell apoptosis through the caspase-8 pathway [211].

Epigenetic drugs for GBM therapy

Epigenetic therapies have been investigated in clinical trials, but only a few have received approval from both the European Medicines Agency and the Food and Drug Administration for use in treating cancer [212]. Epigenetic regulators such as BMI1 and EZH2 are useful in vitro and in vivo. The application of EZH2 inhibitors aids control of the progression of GBM [210]. Agents that inhibit DNMT1 are said to reduce DNA methylation and possibly activate tumor suppressor genes. For example, decitabine and azacitidine, which belong to the DNMT inhibitor known as 5-aza-CdR, are a type of FDA-approved epigenetic drugs for the treatment of medulloblastoma, myelodysplastic syndrome, and acute myeloid leukemia [213, 214]. HDACI, a histone deacetylase inhibitor, blocks the glioma gene transcription and further influences the cell cycle. They work by blocking cell division in the G1 and G2 phases, which in turn promote apoptosis and cell differentiation [215]. HDACIs can reduce glioma growth and development by further degrading a combination of heat shock and matrix proteins and inhibiting angiogenesis in tumors [216]. DNMT and HDACI inhibitors may be used individually or synergistically in combination with other agents to treat various tumors [217]. Therefore, HDACIs offer new opportunities as therapeutic agents for GBM. Studies of HDACI are ongoing and include phase I and II trials [218, 219]. A combination of temozolomide and vorinostat in a clinical phase I study which was conducted by the Children's Oncology Group, suggested that the combination of vorinostat and TMZ is recommended for refractory or relapsed primary tumors of CNS [220]. Vorinostat and TMZ combination was well tolerated for 5 days in pediatrics with recurrent malignancies of CNS, and the dose-limiting toxicity was myelosuppression. Vorinostat causes acetylated H3 accumulation in peripheral blood mononuclear cells. A phase II trial investigating vorinostat application for recurrent cases of GBM was conducted by the North Central Cancer Treatment Group [221]. This study demonstrated that vorinostat monotherapy was beneficial in recurrent GBM. After treatment, the acetylation rates of H2B, H3, and Hand4 were significantly increased. RNA microarray analysis reveals changes in vorinuler-regulated genes with E-cadherin upregulation [194].

Role of CAR T cell therapy in GBM

CAR T cells (chimeric antigen receptor T cell) are allogeneic and autologous modified T cells obtained from a patient's peripheral blood and expanded in vitro. They are genetically engineered using electroporation or viral vectors to express CAR cell membrane molecules. Their extracellular domains may identify tumor-associated antigens, and their intracellular domains contain signals that activate T cells. The modified T cells then are injected into the patient's body, where they will identify cells bearing the corresponding tumor antigens [222]. The TCR-CD3 complex has six independent genetic products. CD3 g, d, ϵ, z, and TCR a, b chains. TCR a and b chains may bind to HLA-peptide complexes. The g, d, ϵ, and z chains of CD3 could activate T cells [223]. The intracellular signaling domains of the activated T cells typically contain signaling domains that are recognized as first-generation CARs even in the absence of other signaling domains. The addition of costimulatory signaling domains (usually 41BB or CD28) creates second-generation CARs. The third-generation CARs arise from the combination of many different co-stimulatory proteins and several co-stimulatory domains [224]. This is thought to stimulate T cell production, leading to cancer cell killing by cytotoxic cells [225, 226]. In a phase 2 trial of patients with refractory or relapsed B cell acute lymphoblastic leukemia, nearly 81% of the patients achieved remission 3 months after CAR-T cell therapy. The survival rate after 6 months was 73% and the event-free rate was 90%. Furthermore, after 12-month the survival rate and event-free rate were around 50% and 76%, respectively [227]. Another phase 1–2 trail involving 22 centers reached similar conclusions [228]. In addition to the successful clinical experiences in hematological malignancies mentioned above, many of these CAR-T therapies have also been demonstrated in some other solid malignancies such as GBM [229], pancreatic [230], colorectal [231], and renal cell disease. Clinical trials are underway [232], for ovarian cancer [233], and breast cancer [234]. Although CAR-T therapy has not yet been clinically implemented in solid tumors, it offers hope for patients with other types of cancer who have few therapeutic options.

Clinical application of AUTO-T cells for GBM

CAR-T cell application in GBM patients is still limited because GBM does not express tumor-specific antigens [235]. However, with the advent of this CAR in the second and third generations overcoming the low heterogeneity of GBM tumors has been made possible which resulted in improved clinical efficacy. This includes studies on various CAR-T cell targets and combined therapeutic strategies like combination immune checkpoint

blockade and chemotherapy. Nevertheless, only three of these trials reported clinical responses for CAR-T cell targets. Importantly, interleukin 13 receptor alpha 2 (IL13-Ra2) [236], epidermal growth factor receptor variant III (EGFRvIII) [237], and human epidermal growth factor receptor 2 (HER2) [238], are clinically shown to be an effective and safe target for the efficacy of CAR-T cell therapy in GBM disease.

Allogeneic CAR-T cells for GBM therapy

Although allogeneic T cells have many superiorities over autologous T cells, they pose unique challenges that must be addressed to achieve clinical success. These challenges include (A) proper selection of T cell sources, as well as (B) avoidance of GVHD and (C), and host immune rejection to achieve potent activation and proliferation in vivo [239].

Source of T cells for GBM therapy

Non-mobilized peripheral blood leukapheresis derived from the patient is the main and most commonly used material to start the production of autologous CAR-T cells. In contrast, in healthy adult volunteers apheresis is conducted in an allogeneic setting [240] (Fig. 6). Recruiting healthy donors yields a large number of donated cells from every single subject. Peripheral blood mononuclear cells are preferred because donors, unlike patients with cancer, do not undergo radiotherapy or chemotherapy [239]. Other cell sources, such as umbilical cord blood (UCB)-derived T cells, could also be considered for the development of allogeneic CAR-T cells. Application of UCB-derived T cells reduces the activation of the NF-κB pathway resulting in decreased responsiveness and consequent reduced generation of many pro-inflammatory cytokines, thus decreasing the frequency and intensity of GVHD. [241]. In the hematopoietic stem cell transplantation (SCT) as a therapy for hematological malignancies, transplantation of UCB has a better outcome than the corresponding unrelated donor in terms of incidence of GVHD, late effects, and overall survival, showing similar outcomes compared to matched related-donor transplants [242–244]. UCB-derived CAR-T cells have already been used, demonstrating the feasibility and efficacy of this approach, as UCB-derived CAR-T cells can identify and destroy target cells [245]. Another promising choice is induced pluripotent stem cells (iPSCs). This makes it possible to generate pluripotent stem cells using adult somatic cells by introducing specific transcription factors [246]. In this way, iPSC-derived T cells pose longer telomeres which results in higher proliferation capacity in comparison with mature T cells. So far, one study has shown that anti-CD19 CARs are derived from iPSC-derived T cells and that these CAR T cells can specifically recognize and kill target cells [247]. However, no major progress in

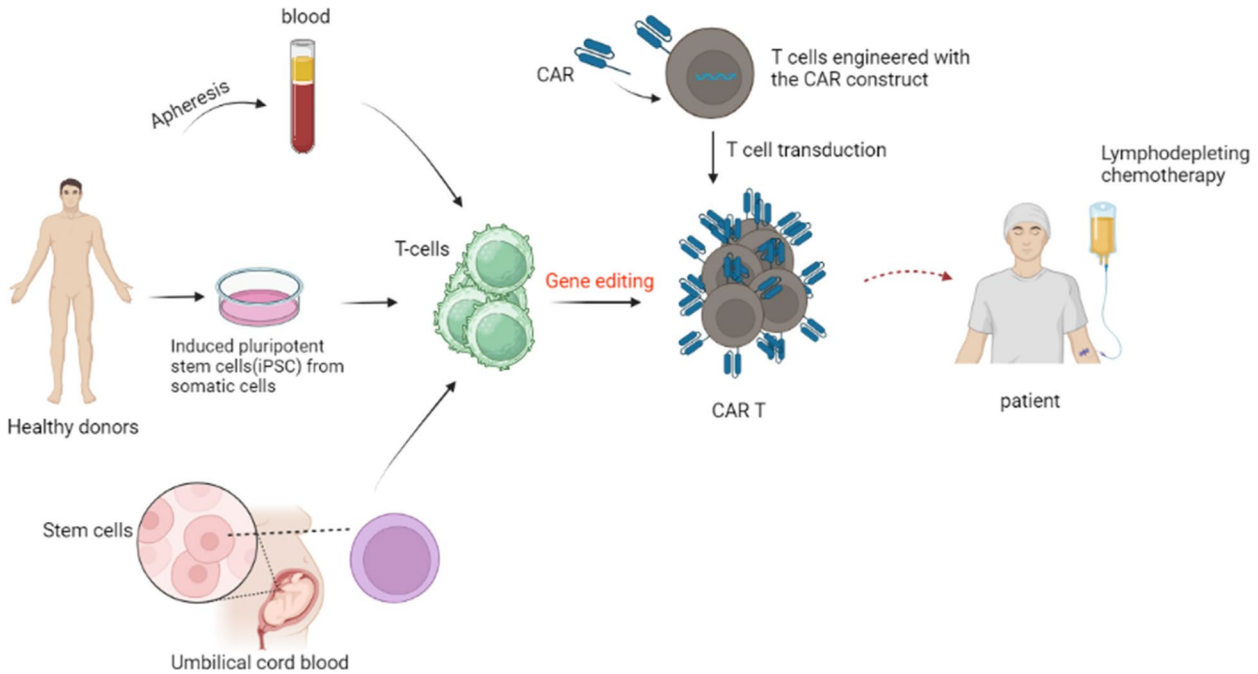


Fig. 6 Generation of allogeneic ("standard") CAR T cells and T cell sources. Allogeneic T cells could be obtained from healthy donor peripheral blood mononuclear cells, cord blood, or induced pluripotent stem cells (iPSCs). CAR-T cells are produced by viral transduction and in vitro expansion

generating CAR-T cells using iPSCs has been achieved recently. Given that GVHD is a leading cause of death post allogeneic SCT, the main focus has been placed on generating allogeneic CAR-T cells to avoid GVHD [248]. In recent years, many groups have worked to improve the classification and diagnosis of GVHD. Currently, there is a consensus on defining two primary GVHD categories, acute and chronic [249]. Nevertheless, the investigations on CAR-T cells, in particular allogeneic CAR-T cells, do not mention the differential effect of these approaches on each category of GVHD, especially chronic GVHD. With the advent of allogeneic CAR-T cells in the clinical setting, further studies are needed to clarify their effect on both categories of GVHD. Many groups hypothesize that the primary cause of GVHD is ab T cells, the most commonly used type of T cells to develop CAR T cells [250]. Two primary strategies have been proposed to reduce the risk of GVHD, based on either virus-specific T cell selection or genetic TCR locus ablation. Because the alloreactivity risk increases with the diversity of the donor's TCR repertoire and the number of transferred T cells [239], there are reasons to use purified T cells having low diversity of TCR repertoire. Indeed, the application of virus-specific memory T cells in hematopoietic SCT may control viral infection without inducing GVHD [251, 252]. Repeated stimulation of donor T cells may reduce the GVHD risk by increasing the frequency of virus-specific memory cells, but predicting the extent of alloreactivity of these cells in advance remains challenging [253]. A small clinical study applying allogeneic virus-specific T cells that express anti-CD19 CAR constructs showed that they were safe and could exert antitumor activity without clinically developing GVHD [254]. A new clinical trial is underway with anti-CD30 and anti-CD19 CAR T cells modulated with Epstein-Barr virus-specific allogeneic T cells [255]. Using virus-specific T cells as a source for allogeneic CAR T cells remains a promising approach that needs to be investigated in next-generation clinical studies. In recent years, the robust development of technologies for gene editing has made available the main tools needed to block endogenous TCR expression and minimize the GVHD risk (Fig. 2). Various groups have reported that by genetically knocking out the exons of the TCRa constant (TRAC) and/or TCRb constant 1 or 2 (TRBC1 or 2) loci using small interfering RNAs, T cell surface eliminates expression of the ab TCR [256], ZFNs [257], TALENs [258], MegaTAL nucleases [259], artificial homing endonucleases, or CRISPR/Cas9 [260]. A direct comparison between TALENs, megaTAL nucleases, and CRISPR/Cas9 showed that the latter two were the best at disruption of TCR [259]. As there is only one α-chain constant region gene, this is considered the most efficient and direct approach to disrupt the ab-TCR and is, therefore, the most commonly used [261]. Additionally, further modifying CAR-T cells is possible by multiplexing. Indeed, the CRISPR/Cas9 technique has been applied to produce MHC class I- and TCR-deficient allogeneic CAR T cells supplemented with PD1 [262], PD1/CTLA4, or Fas, knockout. Multiple gene editing may help reduce the alloreactivity of CAR-T cells while enhancing their resistance to immunosuppression and apoptosis. However, it also improves the off-target cleavage risk, which may lead to hyperproliferation of CAR-T cells because of tumor suppressor gene disruption [263]. One of the most intriguing alternatives to avoid GVHD and achieve functional advantage in a more controlled manner is the direct introduction of CAR transgene into the TRAC locus. Indeed, in addition to the reduction in GVHD, this manipulation allows regulated and homogeneous CAR expression under the control of the TCR promoter. This is a trait that has been shown to lead to reduced differentiation and depletion of CAR T cells [261, 264]. This mutant has similar advantages, and it has also been studied in the context of TCR-manipulated T cells [265]. Other strategies that have been proposed to reduce the risk of GVHD include the application of non-Ab T cells [266] or T cells derived from hematopoietic SCT donors. The first involves populations of innate lymphocytes like NK [267], gd-T cells [268], or invariant NKT cells (iNKT) [269]. In the case of gd-T cells, these rare cells (5% of T lymphocytes) can proliferate in vitro, exhibit potent anti-tumor cytotoxic activity, and identify targets independently of restriction of MHC, possible and unlikely to cause GVHD [270]. Preclinical experiments with CAR-gd T cells have shown some promising results, including glioma-associated targets such as disialoganglioside GD2 [271]. NK cells and iNKT cells will be explained in detail later. The use of T cells from SCT donors is limited to cases who relapse after allogeneic hematopoietic SCT. Here, the same donated CAR-T cells could be used in case of relapse, and this procedure showed GVHD in only 6.9% of cases in a meta-analysis conducted on seven studies [272].

Another important challenge in therapies using allogeneic CAR-T cells is that these cells must be kept and expanded in vivo. This property has been demonstrated in studies of autologous CAR-T cells in neuroblastoma and hematologic malignancies [273]. It is associated with response to therapy [274]. As previously mentioned, allogeneic CAR-T cells do not have the same limitations as autologous cells. A major concern in enhancing T cell function and thus persistence in vivo is to decrease its immunogenicity. Repeated administration is possible because allogeneic CAR-T cells can be produced in higher numbers compared to autologous CAR-T cells (Fig. 7). Some early findings using this approach as an attempt to

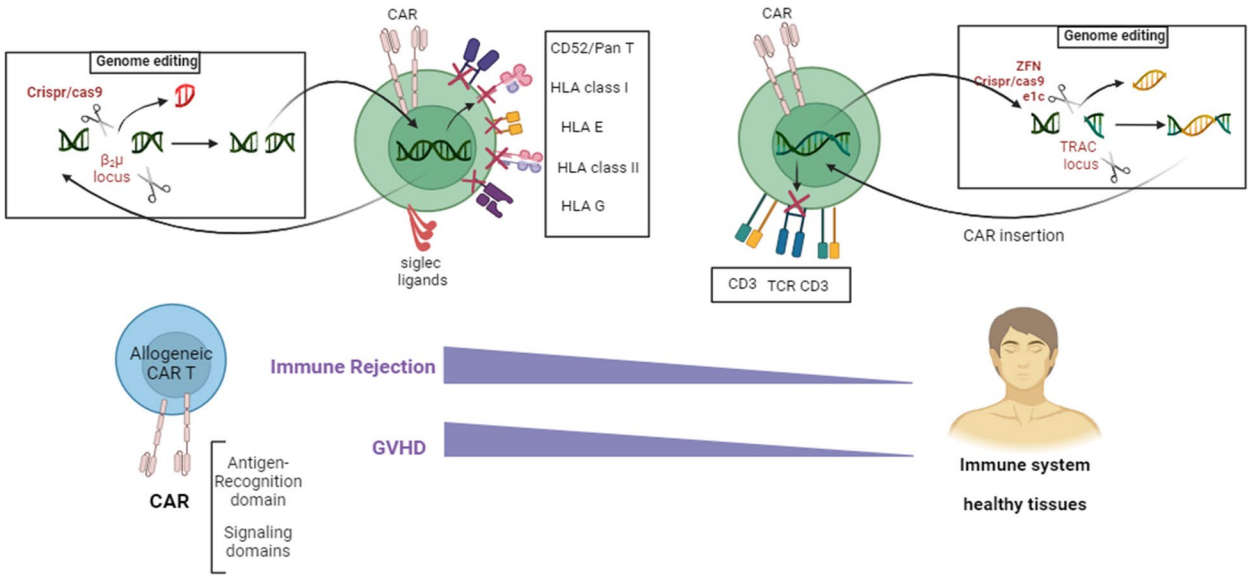


Fig. 7 Allogeneic CAR-T cells must evade host immune rejection and GVHD. Allogeneic CAR-T cells evade the patient's immune response by genetically disrupting HLA class I and II molecules, resist anti-CD52 antibody lymphocyte ablative, therapy by removing CD52 molecules, and reduce HLA able to inhibit NK removal by increasing expression of Siglec ligands of -E and G variants. To protect a patient from GVHD, allogeneic CAR-T cells can be engineered to lose TCR expression

circumvent transplant rejection in vivo showed its feasibility [275]. However, repeated dosing needs repeated immunosuppression of the patient, and repeated encounters with the host's immune cells increase allogeneic reaction risk, at least with antibody generation from past transfusions. Aiming for long-term persistence of lymphopenia is also an option, but it is necessary to generate CAR-T cells that can resist lymphopenic agents. To this end, ab-TCR-deficient CAR-T cells, rendered resistant to several purine nucleotide analogs with the deletion of the deoxycytidine kinase gene, can efficiently function in the presence of agents with lymphodepleting potential and can kill tumor cells [276]. In addition, CAR-T cells were rendered resistant to depletion by knocking out CD52 using an anti-CD52 monoclonal antibody (alemtuzumab) applied as a preconditioning regimen [277]. Regardless of the number of infusions or the strength of lymphodepletion, it is always desirable to reduce the allogeneic CAR-T cell's immunogenicity, and a direct method is the genetic nullification of class I MHC molecules. Although they are highly polymorphic molecules, they all are the same in having the b2 microglobulin protein and disruption of this subunit allows the removal of all surface MHC class I molecules on the T cell [278]. Second-level allogeneic rejection may be mediated by the presence of class II HLA on the CAR-T cells membrane. Indeed, activated human T cells express the MHC class II molecules DR, DP, and DQ, on their cellular membrane, that are regulated by MHC class II trans-activators (CIITA). Although the function of MHC class II molecules on T cells remains controversial [279], it is possible that they may induce allogeneic rejection through the recognition of CD4+T cells. This problem could be circumvented by genetic manipulation of transcription factor regulators CIITA and X [280]. Allogeneic anti-CD19 CAR T cells with a triple knockout of TCR, class I, and class II HLA outperform double knockout cells and showed good persistence in a study on a model of mouse tumor with antitumor activity but no GVHD [281]. Other cells that may mediate allogeneic responses are NK cells [282], but NKs are functionally impaired in some tumors, especially those of blood origin [283]. Expression or overexpression of inhibitory ligands could be a potential approach to prevent NK allo-rejection mediated by NK cells, HLA-E, or G ligands [284, 285] or Siglec 7/9 ligands [286] is one of the most promising choices. Finally, new workarounds are developed for the rejection of CAR-T cells. A promising solution is the latest generation of CARs that mediate the elimination of activated host NK cells and T cells through the extracellular expression of 4-1BB ligands in combination with intracellular CD3z signaling molecules [287].

Loss of antigen is a common mechanism of tumor resistance to CAR-T cell therapy [288] and a major cause of recurrence in GBM [236] and hematological malignancies [289] as well as in preclinical models of solid tumors [290]. An interesting method to overcome

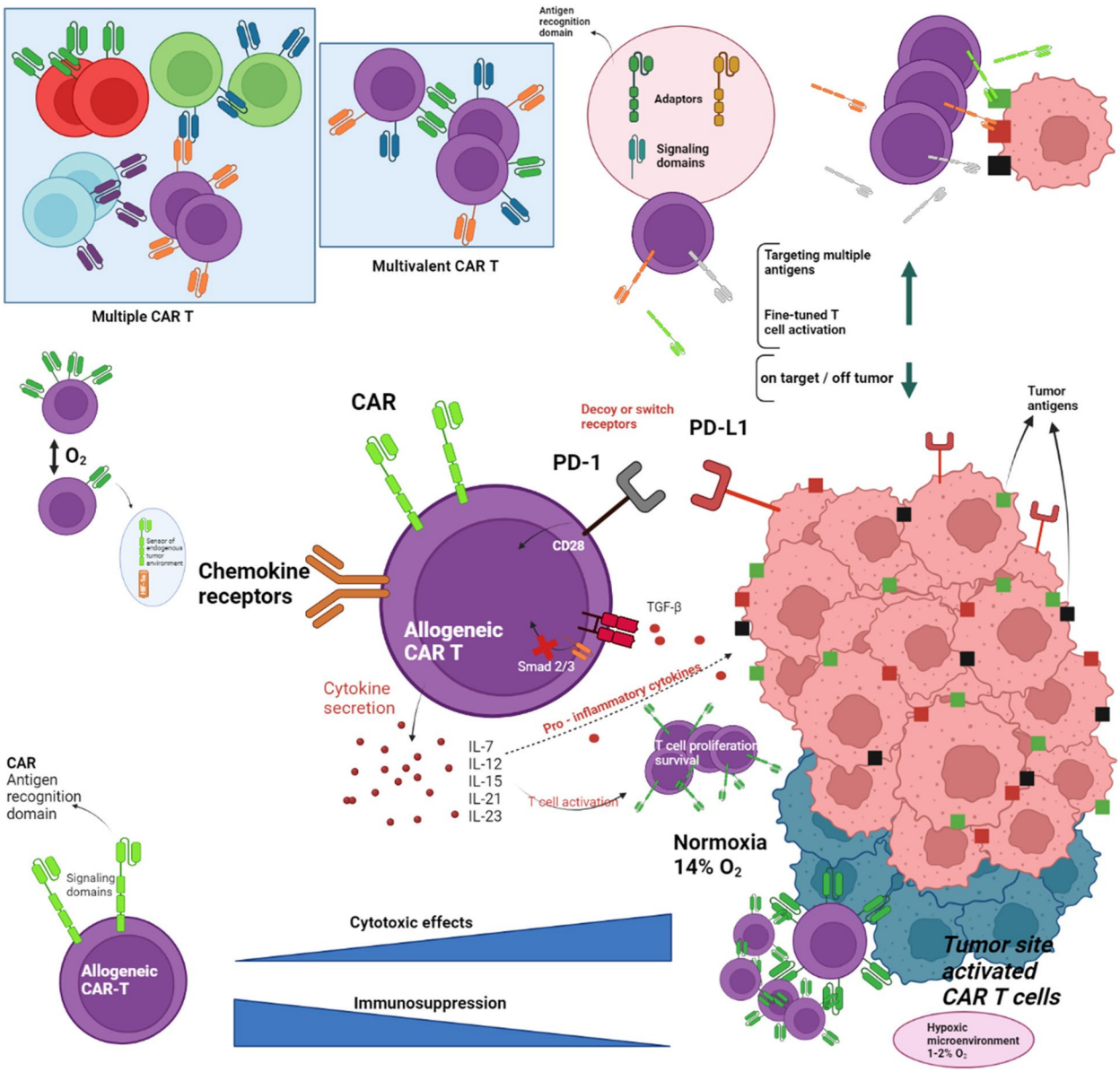


Fig. 8 Allogeneic CAR-T cells provide a versatile platform for attacking GBMs and their environment. GBM heterogeneity requires a multi-target approach. This can be achieved by allogeneic CAR-T cells using multiple CAR-T cell mixtures, multivalent CAR-T cells, or modular CAR-T combined with multiple adapters. To overcome the immunosuppressive TME of GBM, different strategies can be employed to develop allogeneic CAR-T cells. Secretion of proinflammatory cytokines (IL-7, IL-12, IL-15, IL-21, IL-23, etc.), expression of lock or switch receptors (to convert immunosuppressive signals into activating signals), expression of chemokine receptors (to convert CAR-T targeting cells to tumor sites), and generation of locally activated CAR-T cells (e.g., hypoxia-induced CAR-T cells)

antigen flight is the application of a "universal" modular CAR design. Here, a scFv recognizing a target antigen is fused to a soluble intermediate molecule (or adapter) to which a construct containing activation signals that are expressed by T cells can be attached (Fig. 8).

These CARs are designed based on the antibody-Fc receptor, streptavidin–biotin interactions, scFv against specific tags, or other possible combinations [291, 292]. Two of the most popular universal modular CARs are the Universal CAR (UniCAR) and the Shared Universal and Programmable (SUPRA) CAR. The SUPRA CAR consists of a receptor containing a leucine zipper on T cells and another scFv with a leucine zipper adapter molecule that targets a specific antigen [293]. The UniCAR system also has two components: the first one is a CAR-T cell expressing a CAR against the peptide E5B9 derived

from core antigen La-SS/B and the other is a so-called targeting module from E5B9 peptide. A tumor-specific antigen-binding domain usually is fused to scFv [294]. The UniCAR system could also target multiple antigens and combine various signaling domains to provide an on/off switch that improves control of activation of CAR T cells [294–296]. In general, adapter molecules allow it to control CAR T cell activity by target selection, addressing one or more of these targets sequentially or simultaneously. Furthermore, effector activity could be individually activated or deactivated for each target by removing or adding soluble adapters without CAR-T cell depletion. Universal modular CAR-T cells, therefore, offer an opportunity to target several tumor antigens with lower toxicity. Along with these desirable properties, adjusting adapter doses could mitigate potential complications of modular CAR-T cells [297]. However, modular CAR-T cells still have some drawbacks related to the exogenous nature of the adapter molecules that can produce neutralizing antibodies within the host. Moreover, each new adapter may require clinical validation, manufacturing development, and approval from regulators for efficacy and safety [298]. Universal standard and modular CARs can be combined to obtain a ready-to-use "fully universal" CAR for modifying target specificity while allowing fine-tuned control. This approach may be particularly suitable for a solid tumor like GBM, that has a high heterogeneity.

Oncolytic virus in GBM therapy

The concept of viral therapy for malignant tumors was first reported in a case report published in 1912 in which a female with cervical cancer experienced regression of tumor regression being vaccinated against an attenuated rabies virus. Since that time, case studies have reported spontaneous recovery in patients treated with the virus, particularly in leukemia and lymphoma cancers [299, 300]. Nevertheless, concerns about significant side effects and the development of chemotherapy stalled early advances in oncolytic virus therapy [301]. Its potential was reassessed by the late twentieth century, with advances in viral molecular biology and the advent of reverse genetics systems that enabled viral engineering [302]. GBM has been proposed because the tumor is confined to the brain, has no distant metastases, and post-mitotic cells primarily surround growth, allowing the application of viruses that require a replication-active cell cycle. In particular, it is suitable for oncolytic virus (OV) therapy [303]. Currently, immunotherapy in the treatment of GBM, with oncolytic virus, has been promisingly considered and it consists of two groups: (a) OVs with the replication ability, which are selectively replicated in infected cancer cells and suppress tumor cells. (b) Specific OVs are now genetically engineered against pathogen-specific receptors expressed on tumor cells and thus can replicate efficiently and selectively. In addition, viral vectors with replication defects are used as a means to transfer therapeutic genes. Viral infection and amplification ultimately trigger the host's immune response against tumors and eliminate cancer cells. More than 20 so far, such as adenovirus (Ad) [304], herpes simplex virus type 1 (HSV-1) [305, 306], measles virus (MV) [307], reovirus [308], poliovirus [309], and Newcastle Oncolytic virus candidate disease viruses [310] are being tested in clinical studies as anti-GBM therapies. In addition, new developments in OV delivery techniques are also underway to overcome the limitations caused by the BBB. PVSRIPO, a live attenuated poliovirus type 1 vaccine, replaces the corresponding internal ribosomal entry site with that of human rhinovirus type 2 to limit neurotoxicity. PVSRIPO targets GBM via CD155, which is a high-affinity ligand for immunoglobulins and T cell immunoreceptors with immunoreceptor tyrosine-based inhibitory motif domains that are hugely overexpressed on malignant cells. A phase I clinical trial confirmed the absence of neurotoxic potential with intratumoral IBD of PVSRIPO in cases with recurrent GBM and FDA gave it breakthrough therapy designation in May 2016. Relatively, PVSRIPO immunotherapy was higher than in previous treatment periods at 24 and 36 month controls. Based on phase I results, a randomized phase II clinical trial on PVSRIPO alone or its combination with single-cycle lomustine in cases with relapsed GBM is ongoing. The therapeutic efficacy of this new treatment in GBM patients is awaited. The pace of clinical activities has accelerated significantly [311], since the first time that viral engineering to oncolytic HSV in a murine glioblastoma model is used [312], with several completed or ongoing studies being conducted. In addition, several studies have used genetically engineered oncolytic adenoviruses combined with immune checkpoint inhibition or standard therapy. Phase I and II studies are currently being performed, currently in GBM patients and are expected to yield positive results. Adenoviruses have also been modified into an adenoviral vector, agratimagene besadenovec (AdV-tk), containing the HSV thymidine kinase gene, which is then modified with antiviral agents like valacyclovir, which act as toxic nucleotide analogs that can eliminate tumor cells. Herpes drug prodrugs have also been modified [313]. This approach, known as gene-mediated cytotoxic immunotherapy, proved safe in a clinical phase Ib study in new-onset malignant gliomas [314]. A phase II trial was subsequently conducted and showed a significant increase in malignant glioma survival in association with AdV-tk-based therapy [313]. Although clinical trials have demonstrated the

efficacy and safety of OV therapy in GBM, few of them have progressed to phase III trials. A phase III ASPECT study evaluated a gene therapy using adenovirus with Citimagen Seradenovec followed by intravenous injection of ganciclovir in cases with new onset resectable GBM. The safety and efficacy of internal administration were evaluated. ASPECT did not find significant effects on the OS [315]. Recently, a phase III clinical trial with Toca511 and Toca FC was stopped for unknown reasons. Toca 511 consists of a purified retroviral replicating vector which encodes a modulated yeast cytosine deaminase (CD) gene. This gene converts 5-flucytosine (5-FC) to the anticancer drug 5-FU in Toca 511 vector-infected tumor cells. In particular, several phase III studies combined cancer immunotherapy and OV have shown clinical potential for various cancer types [316]. Oncolytic virus therapy in GBM remains promising approach and may affect the patient care future. Recent investigations have found that the Zika virus (ZIKV) exhibits oncolytic activity against GSCs, which suggests that the development of ZIKV may be a therapeutic approach for glioblastoma [317–319]. ZIKV could selectively infect and destroy GSCs compared to normal neurons, making it a potential treatment choice for GBM. Of note, even though the overall safety of OV use has been shown by preclinical and clinical studies, modest clinical efficacy still lives up to the preclinical promise obtained in laboratory studies.

Vaccination for GBM therapy

Cancer vaccine as a promising approach has offered both curative and preventive potential [320, 321]. In GBM, cancer vaccines target tumor-specific antigens and aim to induce the development of an immune response against the tumor. Because antigens specific to GBM are rare, the targets of GBM antigens are mostly tumor-associated antigens, which leads to limited patient participation. Already, only a few vaccine-based approaches have reached clinical phase III in GBM cases, and many others are in the early stages of clinical investigations. The best-studied tumor-associated antigen is EGFRvIII. It is a constitutively active mutant form of EGFR, 25–30% of GBM [322]. Lindopepimto, a peptide vaccine that targets EGFRvIII, has been studied in several clinical investigations. In three uncontrolled phase II trials, Lindopepimto vaccination in patients with GBM who underwent radiochemotherapy and gross-total resection suggested a 24-month improvement in median survival compared with historical controls [323, 324]. Based on these promising results, an international phase III clinical trial ACT IV was performed to further evaluate the efficacy of lindopepimto in recently diagnosed subjects with EGFRvIII-positive GBM. Despite the patient's robust immune response against EGFRvIII, the primary analysis of the results showed a survival benefit in patients with minimal residual disease who were injected with lindopepimto in combination with TMZ compared to those who were injected with TMZ alone [325]. Notably, spontaneous antigen loss was observed in both treatment and control groups, raising questions about the application of immunotherapy to target single-tumor antigens with heterogeneous expression in tumors [325]. Recent research from a randomized, double-blind phase II trial in a small group of recurrent EGFRvIII-positive GBM patients showed favorable results with lindopepimto plus standard bevacizumab compared with bevacizumab alone [326]. In conclusion, the positive findings of lindopepimto in recurrent GBM in ReACT and the negative findings of ACT IV in recently diagnosed GBM suggest the need for further clinical studies using combined therapeutic strategies such as angiogenesis inhibition and immunotherapy. ICT107 is a six-synthetic peptide-induced DC vaccine specifically developed for GBM, already in phase III clinical trials. A phase I trial showed the safety of ICT-107 and demonstrated benefits for HLA-A2-positive patients [327]. A phase II study suggested potential therapeutic activity for ICT-107 in HLA-A2-positive cases. Given the encouraging results in preclinical studies and early clinical investigations [328], a phase III trial on utilizing DCVax-L in patients with newly diagnosed GBM was conducted. In this study, the mean OS for the entire intent-to-treat population was 23.1 months, which was more than the mean OS of 15–17 months in previous trials and clinical practice [329]. Nevertheless, this process was later discontinued for unknown reasons. In summary, the results of current clinical trials of vaccines against GBM are disappointing. Lack of antigens specific for GBM and high tumor heterogeneity make challenges for vaccine therapy in GBM patients, but recent advances in next-generation sequencing and new bioinformatics tools have resulted in the discovery of tumor-derived somatic mutations. It is now possible to systematically discover tumor neoantigens that it is therefore tumor-specific [321, 330]. Because neoantigens are extremely specific to individual patients, neoantigen-targeted tumor vaccines efficiently induce novel responses by T cell against neoantigens, enabling precise personalized therapy. Early investigations of personalized neoantigen-based vaccines have shown preliminary evidence of strong tumor-specific immunogenicity and antitumor activity in cases with high-risk melanoma and other cancers [330]. Based on these promising results, a phase I/Ib trial of a personalized neoantigen vaccine was conducted in 10 patients with recently diagnosed MGMT unmethylated GBM after conventional radiation therapy and surgical resection. Patients who did not receive dexamethasone produced circulating multifunctional

neoantigen-specific CD4+and CD8+T cell responses that were enriched for the memory phenotype and showed increased numbers of T cells infiltrating tumors [331]. Although intratumoral and systemic immune responses against neoantigen were generated after vaccination, all patients experienced recurrence of tumors and eventually died because of progressive disease. This suggests that induced responses by T cells still overcome significant challenges to generate clinically relevant activity against tumor, including tumor-intrinsic anti-tumor activity. Given that neoantigen-targeted vaccines can positively alter the immune environment in glioblastoma, it may be beneficial to combine vaccination with other therapies like immune checkpoint blockade.

Role of ultrasound for GBM therapy

Despite gradual progress in treating GBM, little new and existing drug therapy has been developed for recurrent GBM [332]. The last agent that significantly improved OS in GBM was TMZ, introduced around 2 decades ago [333]. After years of development, a humanized monoclonal antibody called bevacizumab, which is a vascular endothelial growth factor (VEGF) inhibitor approved by FD for cases with recurrent GBM even without completing a randomized phase III trial. This makes bevacizumab the third treatment for GBM that is approved by the FDA [334]. Subsequently, bevacizumab was investigated in two large randomized phase III clinical trials [335, 336]. Despite impressive median progression-free survival (PFS) in both investigations, utilizing bevacizumab as a first-line therapy did not boost OS in glioblastoma patients. Correspondingly, a systematic analysis revealed that concomitant bevacizumab in newly diagnosed GBM was profitable in prolonging median PFS but not OS [135]. Therefore, innovative therapeutic approaches are needed to improve ultimate outcomes for glioblastoma patients. One of the main limitations of novel GBM treatments is due in part to the inefficiency in the delivery of drugs across the blood–brain barrier. The blood–brain barrier consists of endothelial cells lining the brain's microvasculature and poses special challenges for drug delivery [337]. Recently, focused ultrasound as a solution for this problem has made this technique a viable new option for CNS targeting [338]. Preclinical studies have shown that low-intensity pulsed ultrasound improves levels of systemically administered drug therapy in the parenchyma of the brain in animal models which resulted in prolonged survival in preclinical models of GBM [339–343]. After decades of preclinical research, centralized ultrasound has recently been applied to clinical trials of GBM [344]. In 2016, the first human single-center, single-arm study was started to investigate the feasibility and safety of repeated pulse ultrasound in patients with recurrent GBM [337]. Results of the study suggested that focused ultrasound as a new technique to treat patients with GBM is safe and less burdensome [333, 337]. More importantly, the additional treatment of pulsed ultrasound reported in this study can be enhanced and combined with further treatments to improve the penetration of the drug in GBM patients [333]. A prospective, open-label, single-arm, study was performed to investigate the effect of continuous magnetic resonance-guided focused ultrasound (MRgFUS) combined with adjuvant TMZ in GBM patients. This first human proof-of-concept study demonstrated that MRgFUS enhances the signals of circulating brain-derived biomarkers and provides data on the feasibility of focused ultrasound frames for liquid biopsy in patients with neuro tumors [345]. Temporary opening of the BBB in tumors by non-invasive low-intensity MRgFUS and systemic chemotherapy is safe and feasible [346]. Meanwhile, it is recommended that photodynamic therapy (PDT) be considered in this category [347].

Role of nucleic acid-based GBM therapy

Oligonucleotide-based therapies offer a wide range of treatment options for cancer. Oligonucleotides can be precisely engineered to have gene sequences that are unique to tumors rather than natural cells, allowing this type of drug to have precise specificity during treatment. One such agent recognizes the insulin-like growth factor-1 (IGF-1) receptor as a target, an oncogenic receptor constitutively overexpressed in GBMs that confers tumor cell resistance to radiation and apoptosis; IMV-001, a siRNA antisense oligonucleotide [348]. This drug in combination with autologous GBM cells was studied in a phase I trial of 33 newly diagnosed GBM patients [349]. Analysis revealed a mean overall PFS of 11.6 months and 17.1 months for patients who received the highest dose. The median overall survival of patients eligible for the protocol was determined to be 38.2 months [349]. In this way, a phase I study using two engineered DNA plasmids combined with the anti-PD-1 antibody semiplimab for treatment is a plasmid encoding hTERT, PSMA, and WT-1 and INO-9012 encodes IL-12 and is delivered by electroporation to ensure its uptake [350]. Importantly, hTERT, the human telomerase reverse transcriptase gene, is frequently mutated and hyper-activated in GBM cells [351]. WT-1 or Wilms tumor 1 is an overexpressed oncoprotein in GBM which is considered a tumor-associated antigen [352, 353]. PSMA, a prostate-specific membrane antigen, is found on new blood vessels within the GBM structure [354]. This grouping and semiplimab are thought to induce the response of the

immune system to these specific antigens that are not expressed in healthy cells in normal conditions. The recent success of an mRNA vaccine against COVID-19 highlights the potential of mRNA technology in immunotherapy for cancer [355]. Much remains to be found out about these therapeutics and their potential in vivo efficacy and/or complications in humans. Numerous clinical trials are likely to begin over the next decade as more and more biotech companies acquire and perfect their capabilities to manufacture these compounds. Various immunotherapeutic approaches have shown promising findings in preclinical studies, but many have not produced effective or sustained responses in the clinical setting. Several interventions focused on combined immunotherapy could be applied to enhance the response of the immune system. These approaches in GBM have been discussed elsewhere [356]. The principles and practices of combined immunotherapy in general oncology have also been extensively reviewed in recent research [357]. Below are some important examples of preclinical studies and active clinical investigations in the area of immunotherapy and GBM. This strategy showed higher survival rates and responses of T cell memory upon tumor reclamation [358]. A combination of anti-CTLA-4 and anti-PD-1 antibodies was used with IL-12-expressing oncolytic herpes simplex virus to treat the GSC-based mouse model of GBM [359]. Several clinical studies investigating combination immunotherapies are currently underway. In a TEM-GBM study, hematopoietic stem cells are transduced with a lentivirus that directs expression of IFN-α in Tie-2-positive monocytes [360]. In addition to therapies using immunomodulatory cytokines, novel cell-based therapies are combined with gene therapy viral administration. No dose-limiting toxicity was reported in this study [360]. In the future, autologous glioma cell lysates and other personalized medicine strategies will be applied to target tumors at an individual tumor-specific level. As this technology advances, the ability of modern oncology to extend the survival of patients with glioma will increase accordingly. In addition, advances in oligonucleotide and other nucleic acid-based therapeutics, including prevalent mRNA vaccine technology in the current pandemic's coronavirus vaccines, will open up opportunities for personalized medicine. With the rise of immunotherapy and several new agents, physicians must exercise great caution in evaluating all available clinical studies and weighing potential benefits compared to conventional radiation and chemotherapy. The aim is to identify patients who are interested and suitable for participation in clinical studies. Key factors influencing clinical trial participation include the presence of American Society of Clinical Oncology (ASCO)-related comorbidities. The primary responsibility for recruiting patients interested in clinical trials rests with clinicians. Knowing about the various clinical trial options, their possible side effects, and the centers that offer them, can help physicians help patients make more informed decisions about treatment.

Role of MEK in GBM therapy

Extracellular signal-activated kinases (MEKs) can be classified into two distinct forms: in the same vein, important genes such as MEK1 and MEK2. MEK, which is a downstream kinase and part of the RAS cascade, is responsible for signaling related to important cellular functions such as cell proliferation, survival, and differentiation [361, 362]. Dysregulation of the Ras-Raf-MEK-ERK signaling pathway is therefore common in oncogenesis. It is activated by both growth factors and changes in several proteins involved in this cascade. Mutation prevalence decreases along the pathway, making it most common in RAS (22%) and BRAF (7%), least common in MEK (less than 1%), and less common in ERK (very rare). At the initiation of the Ras-Raf-MEK-ERK cascade mechanism, growth factors bind to tyrosine kinase receptors (TKR). Epidermal growth factor receptor (EGFR) leads to receptor activation and subsequent activation of Ras small GTPase. This leads to the formation of Ras-GTP and subsequent activation of Ras. The Raf serine/threonine kinase then becomes a downstream effector target of Ras, and finally, the activated Raf protein, upon phosphorylation, activates MEK1 and MEK2 hyperactivity of this cascade is significantly correlated with tumor cell proliferation and cancer progression. Therefore, molecular therapeutics targeting the Ras-Raf-MEK-ERK signaling pathway are being actively developed [363]. Dysregulation of signaling through the Ras signaling pathway has been observed in approximately 90% of GBM [364]. MEK1/2 is involved not only in tumorigenesis but also in the inhibition of apoptosis. Therefore, MEK1/2 inhibitors are a suitable treatment option [365]. There is evidence that blocking MEK signaling in GBM is associated with antiproliferative effects by blocking cell division and reducing the percentage of Ki67-positive cells. As a MEK1/2 inhibitor, trametinib has been shown to inhibit the Ras-Raf-MEK-ERK pathway and block its downstream extracellular kinases. In addition, it can limit the proliferation, migration, and invasion of GBM. Trametinib monotherapy has been approved by the FDA for the treatment of melanoma to date [366, 367]. In addition to trametinib, other MEK inhibitors such as cobimetinib are also considered novel treatments for GBM. They are generally well tolerated and their efficacy can be judged by a reduction in tumor

size [368]. Phosphatidylinositol-3-kinases (PI3Ks) represent a family of lipid kinases that are activated by numerous receptor tyrosine kinases. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that can sense and transduce signals from a variety of stimuli and belongs to the family of PI3K-related protein kinases. The PI3K/mTOR signaling pathway plays a key role in several key cellular functions such as cell metabolism, growth, survival angiogenesis, and motility [369]. In addition, aberrant activity of this signaling pathway has also been implicated in the development of GBM. PI3K mutations are found in 1 in 4 of GBM patients [370]. The most common mechanisms associated with the overactivation of the PI3K signaling pathway include mutations in the catalytic α subunit of phosphatidylinositol-4, 5-bisphosphate-3-kinase (PIK3CA), phosphatase, and tensin homologs (PTEN). Loss of function and epidermal growth factor receptor (EGFR) gene [371] are important in the mechanism of the PI3K/Akt/mTOR signaling pathway. PI3K migrates to the plasma membrane and catalyzes the production of phosphatidylinositol-3,4,5-triphosphate (PIP3), which in turn activates phosphoinositide-dependent serine/threonine kinase 1 (PDK1) or Akt, which the result leads to the inhibition of apoptosis. Activated Akt then activates mTOR (a downstream target of PI3K) by mediating protein synthesis [372]. Rapid tumor growth and multidrug resistance result from over-activation of the PI3K/Akt pathway in GBM. Consequently, inhibition of PI3K alone or in combination with other targets may lead to cellular apoptosis and slow progression of GBM. PI3K inhibitors include pan-PI3K inhibitors. Furthermore, PI3Ks can be divided into three classes, and Class I PI3Ks consist of a catalytic subunit and a regulatory subunit [373]. Pan-PI3K inhibitors and isoform-selective PI3K inhibitors suppress the activity of p110 catalytic isoforms, whereas dual PI3K/mTOR inhibitors act on both p110 and mTOR complex 1/2. Over 50 PI3K inhibitors have been discovered in cancer therapy, but only a few are currently being tested in clinical trials [374]. PI3K inhibitors that have been shown to have the ability to enter the brain to date include NVP-BEZ235, XL765, GDC-0084, and PQR 309. The development of targeted therapies that focus on the PI3K signaling pathway will have wide-ranging and clinically important applications.

Role of FGFR in GBM therapy

Key regulators of tissue growth, metabolism, differentiation, and repair are fibroblast growth factors (FGFs). FGF signaling is induced by acting through tyrosine kinase receptors known as fibroblast growth factor receptors (FGFRs). Four transmembrane receptors can be distinguished in the FGFR family. FGFR1-4 [375]. FGF–FGFR induces cell signaling pathways such as RAC/JNK, RAS-MAPK (both related to cell proliferation), and PI3K/AKT. Furthermore, gene expression analysis showed a correlation between somatic FGFR mutations and GBM progression [376]. With a prevalence of approximately 6%, FGFR genomic alterations are less common in GBM [377]. The FGFR3-TACC3 fusion has been identified as the most common FGFR alteration underlying IDH wild-type GBM [378]. FGFR1 has been implicated in the development of cytotoxicity and resistance to hormone therapy in various types of tumors [379, 380]. FGFR1 can also modulate the tumor microenvironment and angiogenic response, resulting in decreased GBM radiosensitivity [381]. FGFR1 levels increase with tumor progression, whereas FGFR2 expression steadily decreases with increasing GBM grade [382]. Decreased expression of FGFR2 is closely associated with poor patient prognosis and worse outcomes [383]. Higher levels of FGFR2, as measured by Ki-67 core antigen expression, may correlate with decreased proliferation. The fusion of FGFR3 and TACC3 genes, which occurs in 3% of GBM, leads to the formation of oncogenic FGFR3 [376]. In these cases, increased oxidative phosphorylation and mitochondrial activity are observed, which play a key role in GBM [384]. Recent studies have shown that FGFR4 contributes to GBM cell viability, adhesion, migration, and clonogenicity. Furthermore, FGFR4 has been reported as a predictor of shorter survival in patients with this type of brain tumor [385]. Considering the above points, FGFR inhibition is a potential therapeutic target in GBM patients [386]. Selective inhibitors like nintedanib and pemigatinib are already being tested in clinical studies.

Role of VEGF in GBM therapy

The vascular endothelial growth factor (VEGF) family includes proteins associated with specific receptors such as neuropilin-1, neuropilin-2, VEGFR-1, VEGFR-2, and VEGFR-3 [387]. VEGF is a prognostic angiogenesis marker that has been shown to play an important role in the pathobiology of GBM [388]. Necrotic and hypoxic conditions activate GBM cells and induce the pro-angiogenic factors to release like VEGF [389]. VEGF is generated by GBM tumor cells, stromal cells, and inflammatory cells and stimulates VEGF receptors, leading to endothelial cell proliferation, migration, and survival. This contributes significantly to increased tumor perfusion and increased interstitial pressure. This leads to the blood–brain barrier loss and the development of mass-effect vasogenic edema, a major etiology of morbidity in GBM patients [390]. GBM is one of the most vascularized solid tumors. It is characterized by strong vascular proliferation, leading to the formation of dilated, tortuous, impermeable, and hyperpermeable vessels. Abnormal and dysfunctional vasculature can result in limited

delivery of chemotherapeutic agents to the tumor mass. Malignant vasculature is closely associated with the development of the GBM. Therefore, the degree of angiogenesis strongly correlates with prognosis. Antiangiogenic drugs are currently being investigated as potentially potent anti-GBM therapies [389, 391]. Bevacizumab is a humanized monoclonal antibody that targets VEGF-A. The only FDA-approved VEGF inhibitor therapy for recurrent GBM was Bevacizumab monotherapy [392]. Anti-angiogenic therapies evaluated in clinical trials offer complementary or alternative options to conventional treatment of GBM. It has been considered in the treatment of GBM [393].

Role of pharmaceutical applications for GBM therapy

The main potential antineoplastic agents are important in GBM therapy. These include BRAF inhibitors (dabrafenib and vemurafenib), MEK inhibitors (cobimetinib and trametinib), PI3K inhibitors (paxalisib), FGFR inhibitors (pemigatinib and nintedanib), mTOR inhibitors (everolimus), VEGF inhibitors (Bevacizumab and Vercept), and VEGFR inhibitors (pazopanib, nintedanib, sorafenib, lenvatinib, sunitinib, regorafenib, and apatinib). Based on recent investigations, drug treatment efficacy as measured by progression-free and overall survival was compared in patients who were treated with selected targeted agents.

Conclusions

Cancer immunotherapy is considered one of the most new and practical types of cancer therapy particularly for GBM. Notably, this molecular and practical structure of GBM induced scientists to consider strongly to particular and alternative procedures, for the most impressive result in therapy and with fewer side effects. In this account, immune checkpoints in GBM therapy pursue to exceed the tolerance of induced tumors by the reversal of T cell rebuilding and exhaustion of anti-tumor immunity, and many different clinical investigations have recently been done for patients with brain tumors, particularly GBM. The progression of an applied and effective vaccine for GBM is one of the most challenging discoveries for researchers. Notably, most novel cancer immunotherapies are centralized on the significance of all cytotoxic T cells. Predictably, it can be concluded to underestimate the importance of innate immune structures in the microenvironment of the tumor, comprising TAMs. Tumors strongly have adaptive properties and conserve abundant non-cancerous cells. Consequently, concurrent therapies including multiple attitudes that synchronously target tumor cells, T cells, and TAMs must be investigated. In this account, blocking TAM-mediated immunosuppression has been shown to hold great promise in increasing the efficacy of gene therapy-mediated immunotherapies for GBM. In the treatment of GBM, CAR T cell therapies, especially second and third-generation CAR T cell therapies, have achieved promising preclinical efficacy in prolonging the survival of the patient. Meaningly, CAR T cell therapies are transforming the treatment of hematological malignancies and have the potential to do the same for solid tumors. However, despite some evidence of anti-tumor effects, CAR T cell therapies against GBM have not yet demonstrated their efficacy as a viable and impactful treatment option. A large portion of immunosuppressive cells (like Tregs, TAMs, and MDSCs) enter the GBM microenvironment, upregulating several immune checkpoints (e.g., PD-1, Tim-3, CTLA-4, and IDO-1) as well as immunosuppressive ligands (e.g., PD-L1, on GBM and tumor-infiltrating myeloid cells) and GBM tumor antigen masking. These factors lead to the GBM's immunosuppressive environment and cause inhibition and dysfunction of the proliferation of infiltrating T cells. Thus, reducing or eliminating immunosuppressive cell infiltration and increasing the number and activity of effector T cells are key to the success of GBM immunotherapy. In summary, degradation of the immunosuppressive environment, activation of tumor killer cells in the tumor microenvironment, and release of tumor antigens are the most promising immunotherapeutic strategies for the treatment of tumors. GBM therapy and a combination of two or three strategies will inhibit GBM tumor growth and improve treatment.

Acknowledgements

We express our gratitude and appreciation to all the people who contributed to this study. In this way, this review article is written according to the project with the ethical code: IR.SEMUMS.REC.1402.035, Semnan University of Medical Sciences, Semnan, Iran, and also Guilan University of Medical Sciences, Rasht, Iran.

Authors' contributions

AAS, ShYCh, and AR conceptualized, wrote, and edited the manuscript. SEN, BYG, and FN accompanied the writing of many sections of the manuscript and were involved in other parts of this project. All authors have read and confirmed the final revised version of the manuscript.

Funding

It is a narrative review article.

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

This review article is written according to the project with the ethical code: IR.SEMUMS.REC.1402.035, Semnan University of Medical Sciences, Semnan, Iran, and also Guilan University of Medical Sciences, Rasht, Iran.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 26 January 2024 Accepted: 21 September 2024
Published online: 28 October 2024

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