Review

Insights into molecular therapy of glioma: current challenges and next generation blueprint

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Abstract
Glioma accounts for the majority of human brain tumors. With prevailing treatment regimens, the patients have poor survival rates. In spite of current development in mainstream glioma therapy, a cure for glioma appears to be out of reach. The infiltrative nature of glioma and acquired resistance substantially restrict the therapeutic options. Better elucidation of the complicated pathobiology of glioma and proteogenomic characterization might eventually open novel avenues for the design of more sophisticated and effective combination regimens. This could be accomplished by individually tailoring progressive neuroimaging techniques, terminating DNA synthesis with prodrug-activating genes, silencing gliomagenesis genes (gene therapy), targeting miRNA oncogenic activity (miRNA-mRNA interaction), combining Hedgehog-Gli/Akt inhibitors with stem cell therapy, employing tumor lysates as antigen sources for efficient depletion of tumor-specific cancer stem cells by cytotoxic T lymphocytes (dendritic cell vaccination), adoptive transfer of chimeric antigen receptor-modified T cells, and combining immune checkpoint inhibitors with conventional therapeutic modalities. Thus, the present review captures the latest trends associated with the molecular mechanisms involved in glial tumorigenesis as well as the limitations of surgery, radiation and chemotherapy. In this article we also critically discuss the next generation molecular therapeutic strategies and their mechanisms for the successful treatment of glioma.

Keywords: glioma; surgery; radiation; chemotherapy; acquired resistance; proteogenomic characterization; gene therapy; cancer stem cells; immunotherapy

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Introduction
Central nervous system (CNS) tumors are considered to be the most devastating of all cancers since they predominantly affect the cells of the brain or spinal cord that are most vital in regulating neurological balance[3]. These tumors affect people of all ages due to either developmental abnormalities or inheritance (www.cdc.gov). Age-sex-race-specific prevalence data from the 2013 CBTRUS assessed 69720 new cases of brain tumors. The different cells involved in CNS tumors are glial cells, non-neuronal cells and Schwann cells. The classification of CNS tumors is depicted in Figure 1. Seventy percent of brain cancer and one-fifth of spinal cord cancer are glial-cell-specific[3]. The risk factors involved in glial tumorigenesis are exposure to chemicals, ionizing radiation, viral infection and genetic manipulation (TP53, PTEN, CDKN2A, EGFR, TSC, IDH, histone, and FGFR-TACC, etc). Glioma is characterized by high proliferative potential, infiltrative growth behavior, intratumoral heterogeneity and tumor recurrence. The location and size of glial tumors are the analytical factors that contribute to the monitoring and implementation of an appropriate treatment regimen. The mainstream treatment modality for glioma revolves around surgery, radiation and chemotherapy. These strategies are inadequate in comparison with the varied avenues of glioma progression. Surgical resection is futile due to regrowth of tumors, acute morbidity and the need for ventriculoperitoneal shunting. Radiation therapy is palliative because of normal tissue toxicity and resistance. Radiation oncologists hesitate to re-treat local recurrences, assuming loss in neuroregeneration potential. Chemotherapy is now a standard of care following surgery along with radiotherapy. Depending on the roles of different growth factors (PDGF, EGF, IGF, FGF, CNTF, VEGF, and TGF, etc) in brain tumor
Figure 1. Classification of CNS tumors. Molecular & genetic anomalies and involvement of growth factors in gliomagenesis. The CNS tumors are categorized on the basis of type of cells present in CNS and glioma is further classified on basis of type of glial cells present. The neural stem cells differentiate into different cell lineages of the CNS and putative cells of origin of glioma. Three main types of cells in the mature CNS, including neurons and glial cells (particularly oligodendrocytes and astrocytes; ependymal cells) originates during the differentiation process. The glioma originates from the direct transformation of neural stem cells or glial progenitor cells. Gial tumorigenesis is driven by upregulation or downregulation of various growth factor receptor signaling pathways. Several growth factor receptors, such vascular endhotelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), cyclin-dependent kinase 4 (CDK4), phosphoinositide 3-kinase (PI3K), isocitrate dehydrogenase 1(IDH1) and other growth factors receptors are overexpressed, amplified and/or mutated in gliomas. It also comprises of loss of tumor suppressor genes TP53, the retinoblastoma (Rb) gene, which are essential for cell growth, differentiation and function. Loss of heterozygosity (LOH) is most frequent genetic alteration in both primary and secondary GBMs.
development, a chemotherapeutic regimen can be designed. However, bypassing the blood-brain barrier (BBB), interaction with anti-seizure medications and/or steroids, and intrinsic or acquired resistance are the limiting factors for chemotherapy.

This review summarizes existing treatment regimens (surgical, radiation therapy and chemotherapy) for glioma and their prevailing limitations, and addresses a vision towards the development of new glioma therapeutics, including unconventional treatment strategies such as proteogenomic characterization, identification of molecular targets initiating metastasis, and gene/microRNAs (miRNA)/stem cell/immune therapy to curb glioma. The proteogenomic characterization of glioma implicates a positive correlation among genotype, proteotype and clinical phenotype, facilitating biomarker discovery, diagnosis and design of potential therapeutics. In gene therapy, using RNAi and siRNA delivery gliomagenesis genes are silenced and DNA synthesis is terminated by pro-drug-activating genes. miRNAs can be targeted because their up-regulation shows activation of both oncogenes and tumor suppressor genes. Cancer stem cells (CSCs) of the brain imitate the neural stem cell niche. The molecular characteristics exhibited by CSCs include the expression of multidrug-resistance genes (such as ABCG2 and BCRP1) and the promotion of drug efflux and CSC survival. Thus, selective annihilation of CSCs could be achieved through a combination of chemotherapy and RT with antiangiogenic drugs. Researchers hope that vaccines currently in clinical trials can effectively address the issue of tolerance so that cancer cells can be recognized by a patient’s immune system. Fascinating results have been observed in patients with malignant glioma, anaplastic astrocytoma and glioma who have been vaccinated using tumor lysate as an antigen source. An efficient depletion of tumor-specific CSCs has been observed with cytotoxic T lymphocytes (CTLs) generated by dendritic cell (DC) vaccination+CSC-derived tumor lysate. Studies of the SOX2 gene have led to the genesis of specific CTLs. These strategies will encourage effective glioma stratification.

Types of glioma and their histological features
A glioma is a tumor of the glial cells that maintain the brain and nourish nerve cells. Glioma accounts for 30% of brain cancer, and 80% of gliomas are malignant. The severity of a brain tumor is due to its infiltrative nature (www.dana.org). The WHO grading system relies on atypia, mitosis, endothelial proliferation and necrosis. Tumors with none of these features are grade I and with any of these features are grade II. Grade I and II tumors are considered benign; grade III are malignant and grade IV (glioblastoma) are the most aggressive and malignant. Low-grade astrocytoma is commonly found in children, and high-grade is more frequently found in adults. The classification of glioma on the basis of the type of glial cells and their specific histological features has been discussed in Table 1. Such a glioma classification based on a broad-scale omics study would be an attempt to obtain an integrative view of glioma biology. Knowledge obtained at the system level would aid in deciphering biological insights into the molecular mechanisms underlying the limitations in prevailing glioma therapy and radio/chemo resistance, biomarker discovery, diagnosis and the design of potential therapeutics. The common approaches employed in glioma proteomics are tissue preparation, protein/peptide enrichment and separation, mass spectrometry, quantification and data analysis[10, 11]. The insights provided by an omics study in search of protein signatures and biomarkers for glioma, highlighting the expression of specific proteins in different grades of glioma, have been tabulated in Table 2.

Low-grade glioma is most common in children, whereas in adults, diffuse high-grade gliomas (HGGs) are predominant. In children, diffuse HGGs are rare but have the same dismal prognosis as in adults and, in terms of histopathology, clinical behavior, genetic expression signatures and genetic abnormalities, are similar to WHO grade IV GBM. Pediatric glioma primarily arises in the pons, supratentorial locations and in midline structures (thalamus, cerebellum and spinal cord)[9]. The specific histopathological features of WHO grade IV GBM are shown in Figure 2. The location and size of glial tumors determines the clinical presentation. Supratentorial glioblastoma multiforme (GBM) in different parts of brain is displayed in Figure 3. This analytical factor plays a paramount role in the monitoring and implementation of an appropriate treatment regimen. Commonly reported symptoms for tumors located in or subjacent to cortical regions are headache or seizures or focal neurological alterations causing hemiparesis/hemiplegia or visuospatial alteration. An increase in intracranial pressure may also be observed due to perilesional edema. Infantile spasms are also reported in 20%–30% of patients.

Anaplastic astrocytoma is characterized by tentacle-like projections towards surrounding tissue, which inhibits complete surgical excision. GBM comprises cysts, calcium deposits and blood vessels. In tuberous sclerosis complex (TSC) patients, static tubers are commonly found in the cortical parenchyma. Cortical tubers are nearly always benign hamartomas but are thought to elevate the rate of epilepsy in TSC patients. Based on MRI imaging, most TSC patients have subependymal nodules lining the ventricles. A successive neuroimaging technique helps to demonstrate succession from subependymal nodules to astrocytoma (SEGA). SEGAs exhibit both glial and neuronal features. Ultrastructure and immunophenotype studies have provided evidence of both neuronal and astrocytic differentiation. Glial fibrillary astrocytic protein (GFAP) expression is diffuse or focal compared to S100 protein expression. Neuronal markers (neuron-specific enolase (NSE) or neuron-associated cytoskeletal proteins such as β tubulin) and synaptophysin demonstrate focal positivity for some cells. However, many cells fail to be stained for either neuronal or glial markers[17, 18].

Molecular transformations as driving forces in glial tumorigenesis
The risk factors influencing the genesis of glioma include exposure to toxins such as vinyl chloride, ionizing radiation, electromagnetic radiation, infection with simian virus 40, gene
Table 1. Classification of glioma on the basis of type of glial cells and their specific histological features.

| Type of glioma | Location | WHO grading | Histology | Technique | Observations |
|---------------|----------|-------------|-----------|------------|--------------|
| Oligodendroglioma (10%–15% of the glioma) | Cerebrum (oligodendrocytes) | Grade II and Anaplastic grade III | Monomorphous cells with round, regular nuclei with perinuclear halos. Focal calcifications, interspersed delicate capillaries, nuclear labelling for S100 and diffuse background staining for S100 and GFAP are present. Rare or no anaplasia mitoses, microvascular proliferation and pseudo-palisading necrosis. | Light Microscopy | • Diffuse proliferation of spindle cells. • Intermediate sized polygonal cells. • Some giant cells in sheets, clusters or perivascular pseudorosettes like arrangement. • Eccentric, round nuclei with evenly distributed chromatin. • Small nucleoli. • Cytoplasmic invaginations. • Basophilic, fine granular material. • Well defined cell borders. • Foci of calcification. • Scattered mast cells. • Variable immunoreactivity for GFAP (50%) and S-100 protein (100%). |
| Ependymoma (2%–6% of glioma) | Intracranial (fourth ventricle, suprasellar region), posterior fossa luschka and magendie foramina, extending towards cerebellopontine angle and through magnum foramina into the spinal cord's upper cervical canal | Grade II and Anaplastic grade III | | Immunohistochemistry | • Neuron associated antigens were expressed (SMI33, TpNF1A3, TUJ1 and CL-300). • Cytoplasmic staining for somatostatin, metenkephalin, 5-HT, β-endorphin and neuropeptide Y. |
| Astrocytoma (7% of primary brain tumors and 80%–85% of all gliomas) Juvenile pilocytic, pleomorphic and subependymal giant cell astrocytoma (SEGA) | Monro foramina potentially obstructing cerebrospinal fluid (CSF) pathways | Anaplastic astrocytoma (grade III) and GBM (grade IV) | Hypercellularity, cytologic and nuclear atypia, mitoses, necrosis/pseudo-palisading necrosis and endothelial hyperplastic vascular proliferation. | Electron microscopy | • Similar to tubers giant cells. • Numerous intermediate filaments. • Frequent dense bodies (lysosomes). • Well-developed Golgi complexes. • Many lamellar mitochondria. • No definite synapse formation. • Microtubules within perikarya and cell processes. • Dense core granules of 105-225 nm within the cytoplasm. |
| Ultrastructure study+ Immunophenotype DNA study | | | | Ultrastructure study+ Immunophenotype DNA study | | • Divergent differentiation. | • Diploid DNA content with low proliferation rate. |
linked ailments (Li-Fraumeni syndrome, Turcot’s syndrome, and tuberous sclerosis) and chromosomal changes (chromosome 17, 7, 4 and 9). The literature also indicates that several genes such as TP53, PTEN, CDKN2A, and EGFR, etc are primarily mutated in glial tumorigenesis. It has been observed that mutation of TP53 occurs in astrocytoma, whereas amplification of EGFR and mutation of PTEN are the distinguishing features of high-grade gliomas. Multiple genetic mechanisms generate numerous mutations, which facilitate therapeutic resistance in the tumor cells via various signaling pathways. HGGs have a high mutational burden, and their frequency indicates differential selective pressure between dif-

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**Table 2. Protein signatures in different grades of glioma**[10–10].

| Glioma gradation | Proteins over-expressed | Proteins down-regulated |
|------------------|-------------------------|-------------------------|
| Low Grade        | AHSG, PDI A3, αB-crystallin, enolase, glutamate dehydrogenase I, phosphopyruvate hydratase, protein disulfide isomerase A3, cyclin-dependent kinase inhibitor 1 (p21), CDKN1A, glutathione S transferase P | cAMP response element-binding protein-1, GRP78, Rac1, and RhoA, cystatin B, MVP TTR |
| Grade II         | Astrocytic phosphoprotein PEA 15, UCHL1, CDKN1A | |
| High Grade       | Galectin-1, GFAP, IGFBP2/5, PBEF1/NAmPTase, PAI-1, Cathepsin-D, YKL-40, MMP-9, 1-CaD, RalA, Rab3B, nuclear GTP-binding proteins, GRP78, RhoA, Rac1, Ezrin, protein kinase C γ, MAPKa/ERK kinase 1, Rac 1, prohibitin, phosphoglycerate mutase 1, glutathione S-transferase M, RAB3A, Ras-related protein Ral-A, transforming protein Rho A | |
| Grade III        | Astrocytic phosphoprotein | |
| Grade IV (GBM)   | PEA 15, fatty acid binding protein 5, HSP 27, ferritin, eukaryotic initiation factor 4A (p37) | AHSG |
| Primary GBM      | CREB, peroxiredoxin 1 and 6, α-internexin, BTF3, calyculin, calpain light chain, tubulin-specific chaperone A, Calnexin, AnxA2, AnxA5, GFAP, transcription factor Sp2, DRP-2, large proline-rich protein BAT2, Cystatin B, MVP, HSP 27 eukaryotic initiation factor 4A (p37) | PDI A3, UCHL1; PKA |
| Secondary GBM    | Tenascin precursor, Enolase-1, centrosome associated protein 350, EGFR | |

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**Figure 2.** Histopathological examination revealing glioblastoma multiforme WHO grade IV. (A) Photomicrograph showing brisk mitotic activity (H&E, 200×). (B) Typical pallisading necrosis (H&E, 200×). (C) Glioblastoma with endothelial proliferation (H&E, 200×). (D) A case showing bizarre multinucleated tumor giant cells (H&E, 200×). (E) A case showing very high proliferation activity (Immunoreactivity to MIB-1) (IHC 200×). (F) Immunohistochemistry for p53 showing strong nuclear immunoreactivity (IHC 200×).
different locations of brain such as in the cortical region (histone H3.3 G34R or G34V mutations and BRAF-V600E mutations), midline region (histone H3 K27M mutations), pontine region (activin receptor type 1 (ACVR1) mutations) and thalamus [fibroblast growth factor receptor 1 (FGFR1) mutations]. The characteristic features of pediatric HGGs are complex genomic signatures, with significant copy number alterations (CNAs), single nucleotide variants (SNVs) and structural variants and a dorsal exophytic component mainly harboring BRAF-KIAA1549 gene fusions [in diffuse intrinsic pontine glioma (DIPG)]. The inherited predisposition factors in pediatric HGG are as follows: germline mutations in tumor suppressor genes TP53 and neurofibromin 1 (NF1); oncogenic NTRK fusions [tropomyosin 3 (TPM3)-NTRK1 and BTBD1-NTRK3]; PDGFRA mutation; EGFR mutation; focal amplification of CDK4, CDK6, cyclin D1 (CCND1), CCND2, or CCND3; histone H3.1 mutation; and K27M mutation. Thus, these mutants might be exploited as therapeutic targets in pediatric HGGs[16].

The major determinants of glial tumorigenesis are outlined below.

TSC manifestation in gliomagenesis

The TSC1/TSC2 complex performs a pivotal role in cortical evolution and growth regulation. A precise interface between TSC1 and TSC2 has a critical role in the development of the CNS, including morphogenesis, cell adhesion/migration and cell fate determination[17]. The detailed genetic arrangement of TSC1 and TSC2 is depicted in Figure 4. Mutational hotspots are absent from TSC1 or TSC2 genes.

TSC has a high penetrance induced by mutations and variability in TSC1/TSC2 tumor suppressor genes, which is responsible for more than 50% of deaths among children diagnosed with a brain tumor[17]. Distinctive TSC brain lesions comprise cortical tubers, SENs, and SEGAs[20]. In more than 90% of patients, SENs appear as tiny asymptomatic, intraventricular calcified protrusions in the lateral ventricles or proximate to the caudate nucleus. SENs positioned in the region of the Monro foramina can grow and transform into a SEGA. It has also been reported that solitary SEGAs even appear in the absence of any other TSC-related lesions[7, 18]. Multiple signaling cascades are involved in the focal abnormalities of different organs due to depletion in either of the TSC1/TSC2 gene’s second allele. These cascades culminate to control serine/threonine kinase mTOR, a critical regulator of many important cellular processes as depicted in Figure 4. Thus, mTORC1 inhibitors may potentially have a novel therapeutic role in the treatment of TSC patients[20].

Aspect of histone mutation in glioma

The interplay between genetic and epigenetic events implies that there is a mechanism behind the epigenetic alterations (histone mutation) in glioma. One study laid the groundwork for a focus on chromatin remodeling machinery. The existence of alternative lengthening of telomeres and explicit gene expression profiles associated with H3F3A/ATRX-DAXX (α-thalassemia/mental retardation syndrome X-linked-death-domain associated protein)/TP53 mutations have also been reported. The literature reports somatic mutations in 44% of tumor cases (site of mutation ~H3.3-ATRX-DAXX chromatin remodeling pathway) and recurrent mutations in 31% of tumors (site of mutation ~H3F3A) with amino acid substitutions at K27 or G34 and in H3.1 histone genes HIST1H3B and HIST1H3C[4]. A sequencing study revealed that this mutation targets key sites on the histone tail for post-translational modifications. Hence, pharmacologic inhibition of histone demethylation might help in glioma management[21, 22].
Significance of IDH mutation in glioma

The involvement of isocitrate dehydrogenase (IDH) genes in the molecular pathogenesis of glioma and their translational relevance with respect to IDH mutations is considered to be a putative prognostic marker in WHO grade III gliomas and GBMs. A genome wide analysis predicted that nearly 12% of glioma patients display somatic mutations at codon 132 of the IDH gene. IDH1 or IDH2 is usually mutated in WHO grade II/III glioma or secondary GBM patients. Mutation of NADP+-dependent IDH encoded by IDH1 and IDH2 occurs in patients who develop secondary GBM from low-grade glioma \(^{[19]}\). The important discovery of IDH mutations in glioma using next-generation sequencing for glioma as well as other human diseases has elucidated the diagnostic and prognostic significance.
of IDH mutations in neuro-oncology.

The candidate genes IDH1 on chromosome 2q33.3 (codon R172) and IDH2 on 15q26.1 associated with GBM are mutually exclusive. Histidine change has been observed in more than 90% of IDH1 mutations at R132 codon. IDH1-associated gliomas are located in the frontal lobe (73.5% of cases) and temporal lobe (41.78% of cases). Thus, the assessment of IDH mutation is of great diagnostic relevance (immunohistochemical evaluation of anti-mIDH1), prognostic significance (longer survival of GBM, grade III astrocytoma and oligodendroglioma patients) and therapeutic impact (IDH mutants possess enhanced therapeutic sensitivity; D-2-HG, an oncometabolite of mutant IDH enzymes, is a candidate for glioma therapy). Designing inhibitors of IDH mutant proteins that also penetrate the BBB might also aid in glioma stratification.

FGFR-TACC fusions: a novel mutation

Chromosomal rearrangements (translocations) result into gene fusion by fusing two separate genes to produce a new gene with oncogenic properties. Accumulating evidence has shown that drugs specifically targeting oncogenic fusion proteins have therapeutic success in leukemia and lung cancer. A particular study that sought to identify oncogenic gene fusions associated with GBM development demonstrated FGFR-TACC (fibroblast growth factor receptor-transforming acidic coiled coil) fusions, the first example of a dominant mutation responsible for aneuploidy in human cancer. It has also been found that, during mitosis, FGFR-TACC fusions trigger aberrant chromosome segregation, initiating chromosome instability (CIN) and aneuploidy, the two hallmarks of cancer. FGFR-TACC fusions have been frequently identified in pediatric and adult glioma, bladder carcinoma, squamous lung carcinoma and head and neck carcinoma. Since GBM is a markedly heterogeneous tumor, it is essential to determine whether such heterogeneity is also present in gliomas harboring FGFR-TACC translocations. This is reminiscent of other chromosomal translocations (BCR-ABL, EML4-ALK) and compatible with FGFR-TACC fusions in glioma. This behavior is essential for tumor maintenance, irrespective of secondary genetic alterations that occur during tumor progression. The structural heterogeneity of FGFR3-TACC3 fusions is more distinct at the genomic level, and each fusion event signifies genomic breakpoints for identical fusion transcripts. FGFR3-TACC3 positive samples harbor small, intragenic micro-amplification events classically incorporating only the exons of FGFR3 and TACC genes involved in the breakpoint.

A screening of glioma datasets confirmed that FGFR-TACC rearrangements occur in ~3% of GBM and revealed the presence of FGFR-TACC fusions in IDH1 wild-type lower grade glioma (grade II-III) subgroups. The results even demonstrated that in addition to mutual exclusivity between IDH1 mutations and FGFR-TACC fusions, patients with FGFR3-TACC3 rearrangements lack EGFR amplification and EGFRvIII. FGFR-TACC fusions involve the tyrosine kinase (TK) domain of FGFR and the coiled-coil domain of TACC proteins. A study involving tumor dependency on FGFR-TACC fusions in preclinical mouse models highlighted the anti-tumor effects of FGFR inhibition (AZD4547 and JNJ-42756493). The targeted inhibition of FGFR-TK in preselcted IDH wild-type FGFR-TACC-positive glioma may provide clinical benefits for recurrent glioma patients. These findings offer glioma scientists a better understanding of chromosomal instability in tumors and a novel therapeutic target.

Role of infections in gliomagenesis

The role of infections in gliomagenesis has always been questionable, and viral infections have been suspected of potentially being associated with glioma risk. These uncertainties necessitate the epidemiologic investigation of the role of viral infections in glioma etiology. Cytomegalovirus (CMV), a type of herpes virus, has been found in cancerous tumors. Recently, it has been postulated that CMV infection and GBM incidence are inversely associated with socioeconomic status. The association between CMV and GBM needs to be unraveled. Pandole et al critically reviewed the association between varicella zoster virus (VZV) immunity and glioma risk. Their study emphasized the comparison of VZV infection and immunity biomarkers with anti-VZV IgG levels for further studies. This neurotropic virus usually invades the host’s dorsal root ganglia and induces alterations in the serorevalence of VZV proteins (VZV ORF2p and IE63 proteins). Further comprehensive investigation of viral DNA, protein and RNA transcripts and cell-mediated immunity markers is essential to untangle the association between infections and glial tumorigenesis.

Challenges in glioma management: heterogeneity and recurrence in tumor microenvironment

GBM is one of the most malignant and invasive types of brain tumor. The cells actively migrate from the primary tumor site to narrow spaces within the brain. Indeed, before diagnosis, the single tumor cells may potentially create a hub in the brain. Usually, cancer patients have tumor tissue and normal tissue, but in the brain, there is a blend of normal and cancer cells. This is the most basic and confounding parameter for glioma therapeutics. Thus, conventional therapeutic strategies are not successful in the treatment of GBM, resulting in poor survival rates. The underlying reason for this ineffectiveness might be because of our superficial scientific and clinical approaches. The mainstream treatment for glioma revolves around surgery, radiation and chemotherapy. Tumor locations near eloquent sites and the infiltrative nature of glioma reduce the likelihood of complete surgical abscission of a tumor mass. Radiation therapy is employed in combination with surgical resection but is limited since the tumor center is hypoxic, and the presence of oxygen is essential for effective radiation therapy. Furthermore, tumor recurrence and radioresistance limit the effectiveness of radiation therapeutic approaches. The insensitivity of glioma cells to chemotherapeutic agents, the inability of such agents to breach the BBB, and the expulsion of such agents from cells due to multidrug-resistant protein expression restrain the prevailing chemotherapeutic strategies.
Moreover, radiation and chemotherapy lead to short-term memory deficit, physical fatigue, and weakness[37]. The clinical trial hurdles that impede the development of glioma therapies include the following: usually patients are not enrolled (fewer glioma patients than those with other tumors); the period between glioma diagnosis and clinical response dominates the disease prognosis rate; patients undergoing surgical debulking and external beam radiation are preferred. Conventional therapy results in median survival of only 10 to 12 months, and hence, it is rational to start with an investigational approach.

Modeling glioma in animals would aid in the identification of the genetic proceedings and molecular mechanisms contributing to tumorigenesis within the CNS and in the evaluation of potential therapeutic strategies[38]. The factors responsible for the failure of in vivo studies of glioma include the following:

The glioma models fail to reflect the biological properties of humans;

The pharmacokinetic profiles vary between the animals used and humans;

The tumors established differ from humans in terms of cellular heterogeneity.

Tumor heterogeneity

GBM comprises pathological and phenotypic blends of cells exhibiting cellular and nuclear polymorphism. The heterogeneous nature manifests as mixed cytological subtypes, regional differences in gene expression, and non-uniform representations of key gene mutations and genomic alterations[39–41]. Whether the inherent interactivity between tumor cells, genomic instability, or stochastic noise at the level of transcription, translation, or post-translational modifications has any influence on intratumoral heterogeneity has yet to be unraveled. An examination of dynamic heterogeneity at the cellular level is essential for understanding the origin of cells, potential therapeutic targets and source of tumor recurrence as well as for the identification of optimal cell-specific therapies. Recently, single-cell RNA-sequencing methods have confirmed intratumoral heterogeneity with different morphological, self-renewal and proliferative capacities. Differing treatment responses based on patient-specific dynamics have also been reported. Clonal evolution, CSCs and interclonal cooperativity promote tumor evolution and heterogeneity. Heterogeneity contributes to the failure of targeted therapy owing to the survival of genetically mutated heterogeneous populations of malignant cells. Tumoral heterogenic patterns might stratify patients individually, enabling the selection of appropriate therapeutics. Hence, intra-tumoral heterogeneity significantly contributes to the development of prognostic/predictive biomarkers and personalized treatment regimens[42–46].

Tumor recurrence

The high propensity for tumor recurrence is the critical parameter responsible for unfavorable prognosis in glioma. The challenges of recurrent GBM are: 1) uniform definition and criteria regarding recurrence is indefinite due to newly formed lesions and infiltrative nature; 2) institutional variation in therapeutic strategy; and 3) tumor heterogeneity. Recurrence often occurs as a local continuous growth within 2–3 cm of the lesion margin, at the original tumor site, through newly formed parenchymal lesions, or as unusual relapse patterns in midline tumors[47]. GBM recurrence after treatment occurs either from the bulk of the mass or within 20 mm of its boundary as detected by T1-weighted MR imaging (~97% of cases)[48]. Gadolinium-enhanced MR imaging, PET and MR spectroscopy are also used in surveillance of recurrent GBM[47]. PET demonstrates that high regional glucose metabolism correlates with cellularity, patient survival and radiation necrosis[49]. MR spectroscopy discriminates between localized radiation necrosis and recurrent tumors through high Cho levels[50].

Current therapeutic strategies and their limitations in glioma treatment

Glioma therapy involves multidisciplinary approaches comprising treatment, diagnosis and monitoring of aggressive malignant states. In low-grade tumors, the possibility of recurrence should be monitored, and in high-grade tumors, differential recurrence resulting from treatment-instigated alterations (radiation necrosis) should be monitored. For TSC individuals <20 years, age-dependent monitoring should be performed every 2 years. Stable glial tumors require no monitoring, but growing glial tumors require continuous monitoring. Tumors >1 cm require MRI scanning every 6 months. During pre- and post-treatment, neuroimaging techniques are used to diagnose and examine the site, extent and biological activity of the tumor[50]. Different neuroimaging techniques that are used for glioma are listed in Table 3.

The early detection of tumors is subtle due to a lack of precise symptoms. Patients usually report positional headache (worse in a dependent position), visual obscurations, exacerbation of focal symptoms, or sudden aggravation of seizures possibly followed by lethargy, nausea, vomiting, and diplopia[51, 52]. Conventional treatment regimens revolve around surgery, radiation therapy and chemotherapy. These treatment strategies are inadequate in comparison with the versatile avenues of cancer progression. Typically, intermittent neuroimaging and surgical abscession enable glioma management. Hydrocephalus may be easily avoided by early surgical intervention. Surgical resection is usually unsuccessful due to regrowth of the tumor, acute morbidity and the need for ventriculoperitoneal shunting. Surgical excision is generally followed by fractionated radiotherapy (up to 54 Gy)[53]. Radiation therapy (RT) in combination with surgery has shown better results for glioma control, but the drawbacks of RT include damage to adjacent normal tissues and acquired radioresistance. Selecting an appropriate medical regimen for glioma is difficult, particularly when the question arises amid surgery and chemotherapy. Gliomas exhibit high VEGF and dense vasculature. Most of the astrocytic tumor cells show an
elevated level of indicators of mTOR activation (phospho-S6K, phospho-S6, and phospho-Stat3), which are also the cause of tumor proliferation and energy metabolism. The first chemotherapy substitute for surgery for tumors was launched in a recent clinical study of the function of angiogenic and mTOR inhibitors in inducing regression of glioma and astrocytoma associated with TSC. Regardless of continuous advancements in chemotherapy, bypassing the BBB and acquired resistance due to transporter protein up-regulation in cancer stem cells are the key hurdles.

Surgery
For nearly all glioma patients, surgery is considered the benchmark for restoring and relieving the symptoms of mass effect. Neuroimaging confirmation of tumor progression and symptoms of increased intracranial pressure are the indications for surgical resection. The different indications for surgery in glioma are listed in Table 4. In a case of cerebellar pontine angle (CPA) tumor with right-sided ventriculoperitoneal shunt, a subtotal tumor was excised by employing a left retroauricular suboccipital approach. The respective images are

Table 3. Different neuroimaging techniques implemented for glioma.

| Neuroimaging techniques | Condition | Observation |
|-------------------------|-----------|------------|
| CT Scan | Glioma | Increased tissue cellularity, heterogeneous |
| Subependymal nodules (periventricular region) | | Less than 6 in number; Calcified nodules |
| Subependymal astrocytoma | | Isodense nodules obstructing the foramen of Monro, resulting ventricular dilatation |
| Oligodendroglioma | | Well defined tumor calcification |
| Non-contrast CT Scan | Subependymal astrocytoma | Calcification and small cysts |
| MRI | Glioma | III defined margins, surrounding edema, hemorrhage, necrosis |
| | Ependymoma | Rise in choline levels, reduced N-acetyl aspartate |
| | | Heterogeneous, cysts, calcification, occasional hemorrhage |
| | Subependymal astrocytoma | Hypointense to isointense, pattern with heterogeneous enhancement on contrast infusion, ring enhancement is sometimes seen but less common |
| | | Isointense with cerebral cortex |
| | | Isointense with the white matter |
| T1-weighted image | Glioma | Hyperintense |
| | Subependymal astrocytoma | Isointense with cerebral cortex |
| | | Isointense with the white matter |
| | | Homogeneous or heterogeneous enhancement |
| T2-weighted image | Glioma | Isointense with cerebral cortex |
| | Subependymal astrocytoma | Isointense with the white matter |
| | | Homogeneous or heterogeneous enhancement |
| 1H MRS | Parenchymal lesions | Multiple parenchymal lesions showing increased signal intensity |
| | Glioma | Increasing choline/creatine/lactate and decreasing N-acetylaspartate correlate with tumor progression, helpful in cases of recurring tumor |
| Echo Planar MRI | Glioma | Mapping of tumor blood flow and extends better resolution of tumor versus surrounding edema at tumor border |
| SPECT Thallium 201 | Glioma | Distinguishes benign from malignant lesions |
| | | Amount of thallium uptake correlates with grade of tumor |
| 18F SPET | Glioma | Employs Iodine-123α methyl L-tyrosine for detecting tumors recurrence |
| 18F-FDG PET | Glioma | Differentiate between recurrent or residual tumor and radiation necrosis |
| 18F-Fluoromisonidazole PET | Amino acid and amino acid analog PET tracers | Physiologic marker for tumor progression and radioresistance |
| | | Amino acid uptake is mediated by type L amino acid carriers, upregulated in tumor vasculature |
| 18F-FLT | Glioma | Biomarker for differentiating between radiation necrosis and tumor recurrence |
| | | Marker for tumor proliferation |
depicted in Figure 5. A neurosurgeon’s perspective regarding surgical removal of the tumor relies upon the following four parameters: the nature of the lesion; neurological condition of the patient/Karnofsky performance status; arresting tumor growth; and arresting malignant transformation. Various technical aids, such as neuronavigation and intraoperative MRI (iMRI), can be used to maximize the extent of resection in gliomas. Resective surgery for malignant glioma aids in decompressing tumor bulk, relieving pressure (vital for neurological improvement), reducing neoplasm volume (enhances adjuvant postoperative management), and defining a specific histopathological diagnosis (for selecting an appropriate therapy and predictive prognosis).

**Radiation therapy**

Radiation therapy (RT) is usually implemented after surgery to treat tumors in vulnerable sites and for recurrent gliomas. A large randomized trial showed an increase in time to progression after early RT compared to RT at the time of progression. Early RT (dose of 54 Gy in fractions of 1.8 Gy) improved median progression-free survival from 3.4 to 5.3 years, indicating that the timing of RT is less relevant as long as it is given. Reirradiation is frequently employed in recurrent GBM. Fractionated stereotactic radiotherapy also benefits recurrent GBM patients. Radiation oncologists hesitate to re-treat local recurrences of GBM because of the inability to regenerate or restore CNS tissues after radiation injury. A significant restoration of critical CNS structures has been observed with the use of modern high-precision radiotherapy equipment and enhanced imaging techniques. To limit the exposure of normal brain tissue outside the intended treatment area and to deliver very high doses of focused radia-

### Table 4. Indications for surgery

| Type of lesion                      | Surgical Indication                                      | Surgical intervention | Success | Complications | Recommendations                                      |
|-------------------------------------|----------------------------------------------------------|-----------------------|---------|---------------|-----------------------------------------------------|
| Small and asymptomatic             | - Predictable tumor growth                               | Complete resection    | Good-excellent | -            | -                                                   |
| - Ventricles enlargement            |                                                          |                       |          |               |                                                     |
| • Infiltrative lesion               |                                                          |                       |          |               |                                                     |
| - Predictable tumor growth         |                                                          |                       |          |               |                                                     |
| • Intraventricular lesions in the region of the foramen of Monro | |                       |          |               |                                                     |
| • Unilateral obstructive hydrocephalus |                                                          |                       |          |               |                                                     |
| Large and symptomatic              | - Early symptoms include restrained behavioural changes or worsening of seizures | Complete resection    | 66%     | Tumor regrowth 34% and postsurgical complications 49% | Regular neuroimaging monitoring is required |
| - Later symptoms include increased intracranial pressure | |                       |          |               |                                                     |

| [6–8, 17, 57–60] | | | | | |
tation, intensity modulated radiation therapy (IMRT), stereotactic RT, gamma knife, cyber knife and proton beam techniques are being employed\[69\]. However, RT is palliative because of radioresistance. The clinical response assessment criteria for glioma (disease progression and response) on the basis of the MacDonald and Response Evaluation in Solid Tumors (RECIST) 1.1 criteria comparison has validated the one-dimensional approach for solid tumor measurement and addressed the key issues for partly necrotic tumors and distinct cystic lesions\[67\].

The following have been proposed as possible mechanisms underlying radioresistance in glioma\[68–73\]:

- Increased DNA damage response
- Differential cyclooxygenase response
- Elevated HSP 70 and 90
- Increase in DNA double strand breakage reassembly in association with micronuclei
- Varying interferon-β response
- Divergent cell cycle arresting patterns
- Modulating cyclin-dependent kinase inhibitor expression and autophagy
- BCL-family protein modulation
- Aberrant p21 regulation in wild-type p53 radioresistant GBM cells
- Enriched CD133 (Prominin-1) marker
- Failure of p53 to induce p21bax expression
- Wnt activation
- Alteration in Notch signaling
- Radiosensitivity critically regulated by various kinases (Akt, BCR-ABL, EGFR, Erb-B2, VEGFR2)
- Ionizing radiation enhancing MMP-2 secretion, leading to increased invasiveness and malignancy of glioma cells.

Chemotherapy
Chemotherapy is an important adjuvant to radiotherapy following surgical resection of gliomas. The growth factors that play a pivotal role in brain tumor development are platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factors (IGFs), fibroblast growth factor 2 (FGF2), ciliary neurotrophic factor (CNTF), hepatocyte growth factor/scatter factor (HGF/SF), vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). EGF and other ligands such as TGF-α activate the members of the EGF receptor family (ErbB/HER1-4). In gliomas, EGF (HER1 or c-erbB1) is the most studied receptor. Overexpression of EGFR has been found in approximately half of GBMs, and approximately 40% of GBMS have EGFR deletions. Molecular and genetic anomalies and involvement of growth factors in gliomagenesis is depicted in Figure 1. In newly diagnosed GBM cases, temozolomide (TMZ) adjuvant to RT has clinically and statistically significant effects on survival without affecting toxicity levels\[59, 74\]. The different growth factors involved in gliomagenesis discussed in Table 5, the signaling pathways involved in glioma, and the roles of AKT, PI3K, ERK and mTOR in Figure 4 indicate that Akt, PI3K, ERK and mTOR inhibition might be promising targets for glioma treatment. Deletion of NFKBIA (encoding nuclear factor of K-light polypeptide gene enhancer in B-cell inhibitor-α), an EGFR inhibitor signaling cascade, promotes glial tumorigenesis but does not induce any EGFR alterations. Deletion of NFKBIA and amplification of EGFR actually show a pattern of mutual exclusivity\[86\]. Notch signaling influence on brain CSC’s and the key role of these tumor-initiating cells in glioma maintenance indicates that targeting these cells by Notch cascade inhibition may be worth further investigation\[87\]. Additionally, MMP inhibition may also be a potential antiangiogenic therapeutic modality. The chemical structure, mode of action and effect on glioma of different EGFR, VEGF, PDGF, PI3K/AKT, mTOR and MMP inhibitors are listed in Table 6.

TMZ is one of the leading compounds in glioma chemotherapy. It is an alkylating agent that potentially enters the CSF, bypassing hepatic metabolism for activation with predictable bioavailability and minimal toxicity. It has been approved in the US for refractory anaplastic astrocytoma and in the EU for recurrent tumors. TMZ administration in both concomitant and adjuvant phases prolongs survival and delays progres-
sion. Time to progression and QoL benefits have been observed in recurrent glioma cases. In an evidence-based clinical study, Olson et al recommends TMZ over procarbazine for first relapse of GBM. In a randomized phase III trial by Stupp et al, a TMZ+radiation regimen proved to be statistically significant and clinically meaningful therapy, with a median follow-up of more than 5 years.

However, the major constraints of chemotherapy are bypassing the BBB, its interaction with anti-seizure medications and/or steroids, intrinsic or acquired resistance, and cases of recurrent glioma. Bevacizumab (an anti-VEGF inhibitor) and bevacizumab+irinotecan/etoposide/CCNU are employed for recurrent glioma. Other agents that have been tested for recurrent GBM are cediranib (pan-VEGFR), erlotinib/gefitinib (EGFR), cilengitide (α and β integrins), rindopepimut (EGFR-vIII), vorinostat (HDAC), XL-184 (EGFR, C-MET), Tipifarnib (farnesyltransferase), enzastaurin (PKC) and temsirolimus (mTOR).

Combination therapy
Human malignant gliomas seldom show any dependency on a single oncogene or tumor suppressor. This might be responsible for the failure of agents targeting only one oncogenic pathway in clinical trials. It has also been revealed that EGFR pathway hyperactivation is associated with resistance to treatment with RT and chemotherapy. There are two important considerations that affect glioma therapy; first, numerous RTKs are co-activated in glioma cells, and second, issues of acquired resistance. Thus, a combination of surgery, chemotherapy and RT are essential for sensitizing the glioma cells to treatment.

Table 5. The different growth factors involved in gliomagenesis.

| Growth factors                                                                 | Role in glioma                                                                 |
|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Vascular endothelial growth factor (VEGF) and VEGFRs directed monoclonal antibodies | - Upregulation of VEGF and its receptors VEGFR-1 and VEGFR-2 activated by VEGF-A is often allied with cell proliferation, tumor invasion, migration and permeability  
- Proliferating and migrating endothelial cells are regulated by VEGFR incited Ras/Raf/mitogen-activated protein kinase and phospholipase Cγ/protein kinase C signaling cascades  
- VEGF ligand promotes tumor growth by both autocrine and paracrine manner |
| Platelet-derived growth factor (PDGF)                                       | - Correlation exists between abnormal PDGF signalling and glioma  
- Both autocrine and paracrine mechanisms involvement in gliomas (downregulation of PDGF-mediated signaling) |
| Epidermal Growth Factor Receptors (EGFRs)                                   | - Involved in tumorigenesis, differentiation, migration, proliferation, neural cell survival, neural cell fate and astrocyte differentiation  
- EGFRvIII, the most common mutation in gliomas enhancing tumorigenic behavior and causing genetic instability |
| PI3K and Akt pathway and PI3K/Akt                                           | - During downstream of RTK signaling, Ras/Raf/MAPK and AKT/PI3K are activated. The PI3K-Akt is generally up-regulated in malignant gliomas and GBM  
- Akt’s up-regulation enable glioma cells to grow continuously, evade apoptosis, and augment tumor invasion  
- PI3K dependent activation of Akt is inhibited by PTEN, AKT/PI3K is constitutively activated by mutation or loss of PTEN  
- Combination of activated Akt and constitutively active EGFR signaling induces glial tumor formation along with genetic instability |
| Mammalian target of rapamycin (mTOR)                                       | - Cancer growth eventuates by mTOR activation of lipid and protein biosynthesis during which signal transduction gets deregulated and mTORC1 effectors (S6K1 and eIF4E) gets up-regulated  
- TSC syndrome leads to upregulation in mTOR pathway and subsequent downstream kinase signalling cascade alters cell processes  
- Increased mTOR activity with upregulation of p70S6K has been observed in both healthy and lesioned skin biopsies of TS patients. The secretory agents from endothelial cells of brain maintain GBM stem-like cell growth by mTOR pathway |
| Notch pathway                                                              | - Influences neural stem cell renewal, progenitor cell differentiation, learning, memory, and gliogenesis  
- Notch1 and its ligands Delta-like 1 and Jagged1 are crucial for GBM cell growth |
| Matrix metalloproteinase (MMP) family                                       | - MMP-2 & -9 manifests a significant role in extracellular matrix degradation, neoangiogenesis and tumor vascularisation |
Table 6. Chemical nature, mode of action and effect of different EGFR, VEGF, PDGF, PI3K/AKT, mTOR and MMP inhibitors in glioma.

| Drug                                      | Chemical nature            | Mode of action | Tumor type                                      | Effect                                                                                           | Reference |
|-------------------------------------------|-----------------------------|----------------|-----------------------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| Catanionic solid lipid nanoparticles (SLNs) loaded with doxorubicin | Anthraccline derivative. Dox-loaded catanionic SLNs (Dox–CASLNs) with surface anti-EGFR | Anti-EGFR       | U87MG cell line                               | Reduced the cytotoxicity to human brain-microvascular endothelial cells and high targeting efficacy against the growth of GBM | [88]      |
| DOX-loaded FA-PMs                          | Smart thermoresponsive micelle | Inhibit BRAF V600E | C6 glioma rat tumor model                      | Significant accumulation of drug in tumor sites inhibiting tumor volume by ~83.9%                   | [89]      |
| PLX4032 or Vemurafenib dabrafenib          | Phenylpyridine              |               | Pilocytic astrocytoma                          | 80% of patients showing either partial or complete remission, 74% reduction in disease progression or death, and a survival advantage over patients treated with dacarbazine alone Decreased plasma levels of endoglin, a marker of tumor-associated endothelial cells | [82]      |
| Sorafenib                                  | Indoline                    | VEGF inhibitor  | Pediatric tumor                               | Phase II study                                                                                                                                               | [76, 82]  |
| Sunitinib                                  | Diarylether                 | VEGF inhibitor PDGFR Raf | Pediatric patients with low grade astrocytomas |                                                                                                                                                              |           |
| Thalidomide                                | Isoindolone                 | Antiangiogenic  | High grade glioma                             | Withdrawn from clinical use due to well characterised teratogenic effects Increased haematological toxicity                                              | [82]      |
| Lenalidomide                               | Isoindolone                 | Antiangiogenic  | Recurrent primary CNS tumor                   |                                                                                                                                                              |           |
| PBTC-018                                   | Phase I Trial of Lenalidomide (pediatric brain tumor consortium study) | Antiangiogenic | 51 pediatric patients with recurrent, refractory, or progressive CNS tumors | Objective responses observed in children with low grade glioma                                                                                                                                                   |           |
| Imatinib mesylate (Gleevec®)               | N-phenylbenzamide           | PDGF inhibitor | Recurrent glioma                               | Penetrate BBB Decrease high interstitial fluid pressure in the tumor Shows little effect on cells expressing the EGFRVIII mutation Promising effects in GBMs where EGFRVIII and PTEN are coexpressed Strong antiedema effect and favorable PFS-6 Prevent EGFR-mediated signal transduction by interfering with ligand binding and EGFR extracellular dimerization Increase in overall survival, but only in wild-type EGFR amplified GBM | [74]      |
| Gefitinib (Iressa®)                        | Naphthyridines class Quinazolinamine | EGFR inhibitor  | 53 Recurrent GBM patients GBM                 |                                                                                                                                                              |           |
| Erlotinib (Traceva®)                       | Quinazolinamine             | EGFR inhibitor  | 53 Recurrent GBM patients GBM                 |                                                                                                                                                              |           |
| AZD2171 (cediranib)                        | Diarylether                 | VEGFR inhibitor | Recurrent GBM GBM                             |                                                                                                                                                              |           |
| Cetuximab                                  | Monoclonal antibody. Peptide derivative | EGFR inhibitor Targeting RTKs | U87 and LN827 cell line |                                                                                                                                                              |           |
| RO4929097                                  | Dibenzazepin Naphthyridines class. Quinazolinamine | γ Secretase inhibitor EGFR+Erb-B2 | Types of glioma U87 and M059K glioma cells | Clincial trials Considerable effects on proliferation, apoptosis and migration of glioma cells were observed Unacceptable toxicity and minimal activity Autophagy was observed In orphan status | [76]      |
| Lapatinib (GW572016)                       | Naphthyridines class. Quinazolinamine |               |  |                                                                                                                                                              |           |
| AEE788                                     | Pyrimidin-4-amine           | EGFR+VEGFR     | GBM patients                                   |                                                                                                                                                              |           |
| ZD6474 (Vandetanib)                        | Quinazolinamine             | EGFR+VEGFR+RET | U251 cell line, xenograft                      |                                                                                                                                                              |           |
| Nimotuzumab                                | Humanized monoclonal antibody | EGFR           | Gioma                                          |                                                                                                                                                              |           |
| Nilotinib                                  | N-phenylbenzamide           | PDGFR           | U87 and LN827 cell line                       | Oral drug that has greater potency and selectivity for BCR-ABL than imatinib                                                                                       | [76, 90]  |

(To be continued)
| Drug             | Chemical nature           | Mode of action          | Tumor type                   | Effect                                                                                                           | Reference |
|------------------|---------------------------|-------------------------|------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------|
| Vatalanib        | Phthalazine               | PDGFR+VEGFR              | Glioma                       | Influence angiogenesis and tumor growth through multiple targets and are currently in various stages of preclinical and clinical investigation. | [76]      |
| Dasatinib        | Anilide                   | PDGFR+Src+cKit+Bcr-Abl   | Malignant glioma             | Phase trials                                                                                                   | [76]      |
| Tandutinib       | Quinazolinamines          | PDGFR+cKit+FLT-3         | Malignant glioma             | Phase trials                                                                                                   | [76]      |
| CP-673,451       | Triazolo[4,5-d] pyrimidine| PDGFR                   | Rat glioma (C6 cell line)    | Inhibits tumor PDGFR-beta phosphorylation, selectively inhibits PDGF-BB-stimulated angiogenesis in vivo, and causes significant tumor growth inhibition in multiple human xenograft models. | [76, 91] |
| AMG706           | Nicotinamide              | PDGFR+VEGFR+cKit+Raf     | Glioma                       | Inhibits angiogenesis and induces regression in tumor xenografts.                                                | [92]      |
| Pazopanib        | Aminobenzenesulfonamide   | PDGFR+VEGFR+cKit         | Malignant glioma             | Influence angiogenesis and tumor growth through multiple targets and are currently in various stages of preclinical and clinical investigation. | [76]      |
| SU011248         | Indoline                  | PDGFR+VEGFR+cKit         | Malignant glioma             | Phase I trial                                                                                                  |          |
| OSI-930          | Thiophene derivitive      | PDGFR+VEGFR              | Malignant glioma             | Clinical investigation                                                                                           |          |
| TKI258           | Benzimidazole-quinolinone | PDGFR+VEGFR              | Malignant glioma             | Clinical investigation                                                                                           |          |
| Aflibercept      | Peptide                   | VEGF-A/B Ab              | GBM patients                 | 30% Therapeutic response                                                                                       |          |
| XL184            | Quinoline                 | VEGF+Rc-Met              | GBM                          | Phase II study                                                                                                  |          |
| Pazopanib        | Aminobenzenesulfonamide   | VEGF, PDGFR, c-Kit       | Malignant glioma             | Phase II trials                                                                                                 |          |
| Cediranib        | Quinazoline               | VEGFR+PDGFR+cKit         | CNS tumor                    | Phase I trials                                                                                                  | [76]      |
| Bay549805        | Quinazoline               | Raf, VEGFR               | Glioblastoma cell lines      | Anti-proliferative activity                                                                                        |          |
|                   |                           |                         | and intracranial glioblastoma xenograft designs                                                            |          |
| Torin-1          | Pyridinonequinoline       | mTORC kinase inhibitor   | Glioma                       | Preclinical studies                                                                                             | [82]      |
| WYE-354          | Piperidinecarboxylate     | mTORC kinase inhibitor   | Glioma                       | Preclinical studies                                                                                             |          |
| XL765            | Quinoxalinyl             | Dual PI3K/mTOR inhibitor | Intracranial xenograft mouse model of high grade glioma                                                      | Potential antineoplastic activity |          |
| Substituted      | Heterocyclic aromatic     | Raf/MEK/ERK inhibitor     | Astrocytoma                  | Antitumor activity                                                                                              | [93]      |
| benzimidazole     | organic compound          |                         |                              |                                                                                                                 |           |
|                   | (benzene and imidazole)   |                         |                              |                                                                                                                 |           |
| NVP-BEZ235       | Imidazoquinoline          | Dual PI3K/mTOR inhibitor | (GBM) cells in vitro         | Blocked the growth of GBM elicited a prodifferentiation effect on A172 CSLCs 55% PFS-6                           | [75, 76] |
| Enzastaurin       | Bisindolylmaleimide       | PKC-b2+Akt               | Malignant glioma             | Induce cell death and reduce proliferation                                                                       |          |
| Perifosine        | Piperidinium              | Akt                      | Malignant glioma             | 46% to 63% reduction in SEGA volume                                                                              | [7, 18]  |
| Rapamycin (sirolimus) | Macrolide               | mTOR inhibitor            | SEGA                         | 52% to 82.6% reduction of tumor volume with bilateral SEGA                                                       |          |
| Everolimus (Afinitor RAD001) | 40-O-(2-hydroxyethyl)  | mTOR inhibitor            | SEGA                         | SEGA tumor volume was reduced more than 30% relative to baseline in 75% of patients, there was 50% or more reduction in tumor volume in 32% of patients with decrease in ventricular volume. |          |

(To be continued)
Next generation therapeutic strategies to combat glioma

Despite various treatment modalities, such as surgery/RT/chemotherapy and their prevailing limitations, glioma patients have a dismal prognosis. A different approach to recurrent GBM therapy uses medium frequency electrical fields. In 2011, a novel device NovoTTF100A (Novocure, New Hampshire, USA) was employed for arresting dividing cells in mitosis. The device was as effective as chemotherapy, and quality of life was better compared to systemic therapy. Researchers also found that the device might aid in potentiating the effects of chemotherapy (www.clinicaltrials.gov, NCT00916409). Progress in glioma therapy could be attained by improved comprehension of glioma biology, identification of relevant targets and signaling pathways for treatment interventions, development of personalized medicine, optimization of surgery and RT, and innovative neuroimaging techniques. Proteogenomic characterization is a potential strategy that could lead to identification of molecular drivers, molecular classification of disease subgroups and glioma treatment. The ultimate goal of targeted therapy should be “selectivity,” i.e. inhibiting only tumor cells. The targeted approaches currently in clinical trials or in laboratory development include drugs, monoclonal antibodies, immunotherapy, small molecules inhibiting specific proteins and specific targeting of CSC’s. Thus, there is a need for unconventional treatment strategies to curb glioma. Strategies such as gene therapy, microRNA (miRNA) therapy, stem cells, and immunotherapy may potentially lead to effective GBM treatments.

Proteogenomic characterization of glioma

Next-generation sequencing is being widely employed to characterize developed genomic and transcriptome alterations in human diseases. The insights provided by omics studies in search of protein signatures and biomarkers for glioma that highlight the expression of specific proteins in different grades of glioma have been already discussed in Table 2. Transcriptome profiling for gene expression fails to correlate with protein expression and posttranslational modifications (PTMs). Hence, advancements in proteomic platforms with inclusive proteome arrays would aid in providing systematic and analogous protein expression evidence that is complementary to
DNA and RNA profiles\footnote{110}. An initiative integrating genomic, proteomic, and phosphoproteomic dimensions might aid in identifying differential signaling pathways and functional modules exhibiting substantial associations with patient outcomes. Such methods would likely identify PTMs, revealing a strong association between histone acetylation and the homologous recombination deficiency (HRD) phenotype\footnote{111}.

Recently, Zhang et al provided a comprehensive analysis of the molecular components and underlying mechanisms of ovarian cancer. They performed an inclusive mass-spectrometry-based proteomic characterization of 174 ovarian tumors of the high-grade serous carcinoma (HGSC) category. The insights provided by the study include the following: the influence of different copy-number alternations on the proteome; proteins associated with chromosomal instability; specific protein acetylations associated with HRD; the influence of the somatic genome on the cancer proteome; and associations between proteins and PTM levels and corresponding clinical outcomes in HGSC\footnote{110, 115}. The complex proteome analysis was primarily carried out through a mass spectrometry (MS)-based shotgun proteomics approach. The resultant peptide mixtures obtained from protease-digested complex protein samples were fractionated on HPLC columns, followed by tandem MS analysis. The subsequent MS/MS spectra were compared to a protein database for protein identification and PTMs. A study of Alzheimer’s disease (AD) by Wang et al highlighted that gas-phase fractionation of peptide ions enhanced peptide identification by \textasciitilde 10\%. The identification of 96,127 peptides and 10,544 proteins at a 1\% protein false discovery rate was enabled by combining basic pH liquid chromatography (LC) prefractionation with a long gradient LC-MS/MS platform\footnote{110}. This study contributed to the systematic optimization of long gradient chromatography MS for a profound study of the brain proteome. Li et al used proteogenomics to improve gene annotation and interpretation of proteomics data. They employed an integrative proteogenomics pipeline JUMP\(^\text{\textregistered}\) for processing a label-free MS data set of AD, recognizing 496 new peptides (amino acid substitutions; alternative splicing; frameshift; non-coding gene translation), and analyzing a stable-isotope-labeled data set of multiple myeloma cells, revealing 991 sample-specific peptides (protein sequences in the immunoglobulin light chain variable region). The multistage strategy included a modified database structure, tag-based database probe, peptide-spectrum match sieving, and data conception. Their study highlighted expression of a novel protein PNNMA6BL in the brain and the use of the JUMP\(^\text{\textregistered}\) program in proteogenomics for multi-omic data integration\footnote{112}.

Thus, understanding the molecular basis of glioma can be enhanced by an in-depth evaluation of pathway activity by using a proteogenomic approach to show the correlation between genotype and proteotype and ultimately clinical phenotype.

**Gene therapy**

In brain tumors, gene therapy transfers genetic material into the tumor cells. Gene therapy has the ability to target invasive tumor cells resistant to conventional therapy. The different gene therapy strategies for glioma include the following: (a) *Suicide gene therapy* - DNA synthesis is terminated by a prodrug-activating gene. This method results in gene expression for a shorter period and enhanced sensitivity, but *in vivo*, the gene transfer rate is poor and fails to target dispersed tumor cells. (b) *Oncolytic viral therapy* - viral replication lyses tumor cells. For this method, transduction efficiency and viral titers are high, but there is a possibility of host immune rejection, and local administration during surgery is required. (c) *Immuno-modulatory therapy* - involves stimulation of an antiglioma immune response and regulation of the tumor microenvironment. However, the limitations are immunosuppression and lack of antigen-presenting dendritic cells. (d) *Synthetic vectors* (nanoparticles) - is a safer approach for silencing gliomagenesis genes by RNAi and siRNA delivery. A sustained release pattern is an added advantage, but reduced intratumoral distribution and transduction efficiency have also been observed.

Gene therapy has demonstrated significant therapeutic efficacy in preclinical and phase I trials but has failed in phase III trials because of the heterogeneity and invasiveness of GBM, anatomical features of the CNS, host immune system and inadequacy of GBM animal models\footnote{113}.

**miRNAs-anti-oncogenic therapy**

The ‘oncomirs’ or miRNAs have been found to be associated with different human cancers and are also viewed as promising therapeutic targets in cancers. Some miRNAs show oncogenic activity by upregulating miRNAs in cancer and targeting tumor suppressor genes, while others illustrate tumor suppressor activity by downregulating miRNAs in cancer. This distinct activity of miRNAs depends on the biological context and tissue type. The potential role of miRNAs in CSCs, to curb resistance, has been described in recently published studies. In GBM, several miRNAs regulating oncogenic and tumor suppressor proteins have been identified. Identification of dysregulated miRNAs in GBM that are potential participants in glioma genesis, such as miR-21, miR-196, miR-10b, miR-128-1, and miR128-2, has led to more accurate predictions of clinical outcome than mRNA profiles. A stronger correlation between clinical outcome and miRNA-mRNA expression signature has also been acknowledged\footnote{114, 115}.

**Cancer Stem Cell (CSC) therapy**

Research regarding CSCs and their role in GBM survival and relapse is being carried out on a larger scale. It seems that the heterogeneity of brain tumors is dependent on the heterogeneity of their CSCs, and their involvement in complex mechanisms largely depends upon their microenvironment. Consequently, CSCs could also be a potential therapeutic target in GBM. According to Binello and Germano, direct targeting refers to augmenting CSC functions via EGFR/PI3K/Akt inhibition and inducing differentiation to curb resistance to standard treatments, whereas indirect targeting addresses perivascular niches, hypoxic niches and immune evasion. The molecular characteristics exhibited by CSCs include the...
expression of multidrug-resistance genes (such as ABCG2 and BCRP1) and the promotion of drug efflux and CSC survival. In GBM-tumor sphere cells, the expression of multidrug resistance genes has been found to be enhanced. Expression of the stem cell-associated protein CD133 helps in the identification and isolation of GBM CSCs. It has been recently determined that CD133+ GBM cells are more radioresistant than CD133- cells[79]. In spite of having an intact G2 checkpoint, CD133+ cells lack the intra-S-phase checkpoint. Compared to GBM cell lines, in vitro CD133+ GBM CSCs are more radiosensitive with a reduced capacity to repair DNA double-strand breaks[116]. Hedgehog-Gli signaling inhibitors have been used to treat tumors and are associated with CSCs and the regulation of proliferating CSCs[117]. These inhibitors induce GBM-derived neurosphere cells to lose their tumorigenicity, reduce stem cell marker expression and target radiation to unaffected GBM cells. Therefore, Hedgehog blockade potentially offers a new therapeutic in combination with chemotherapy or RT.

Akt inhibitors play a substantial role by sensitizing brain CSCs to radiation for inducing apoptosis and directly targeting CSCs. The CSCs of the brain are maintained within vascular niches that imitate neural stem cell niches[118]. Thus, selective annihilation of CSCs could be achieved by employing a combination of chemotherapy and RT with antiangiogenic drugs.

Immunotherapy

Immunotherapy provides a durable and targeted treatment against cancer by harnessing the body’s adaptive immune mechanisms. The principal mechanisms employed are the improvement of the immune response and targeting of specific antigens. The immune system in the brain is highly active and interacts with brain tumors. However, the diffuse and infiltrative nature of glial tumors poses a challenge to effective immunotherapy. An invasive tumor residing behind the BBB is isolated from effective immunosurveillance and ultimately leads to “immunologically silent” tumor peninsulas. The ability of adoptively transferred T cells to migrate and mediate regression in areas of invasive GBM is unclear[119].

The prevailing multimodal therapy is non-specific and is limited by tissue toxicity. In contrast, immunotherapy research has shown substantial evidence of T cells’ ability to eradicate large, well-established tumors in mice and humans while sparing the normal brain cells. Glioma cells express and secrete numerous immunosuppressive molecules regulating immune cell functions. The true mechanism of immunosuppression involves a combination of factors, including regulatory T cells (Tregs), tumor-associated PD-L1 expression, and CTLA-4 signaling[120-122]. The current immunotherapeutic approaches focus on enhancing T-cell function by generally stimulating the immune system or by attacking specific tumor cell antigens. These strategies include the use of vaccines, adoptive cell transfer and immune checkpoint inhibitors.

Vaccines

The use of vaccines is an active immunotherapeutic approach that is intended to activate and expand tumor-specific T cells to induce an anti-tumor response. Various cancer vaccines are made by expanding and stimulating dendritic cells (DCs). Currently, all vaccines in clinical trials are struggling with the challenge of tolerance so that the cancer cells can be recognized by a patient’s immune system. The steps involved for efficient vaccination are, first to identify the specific tumor antigens and, second, to generate an anti-tumor response and to block all the inhibitory immune mechanisms by adopting proper immune strategies. Naive immune system vaccination is stimulated by antigen presenting cells known as dendritic cells (DCs), which help maintain self-tolerance. In vaccination studies, DCs are usually loaded with specific tumor-associated peptides, tumor RNA, cDNA, cell lysate, or apoptotic cells. DC generation, maturation, subtype, dosing, vaccination schedule, route of administration, and antigen loading approaches are the factors that must be standardized before DC vaccination can enter the clinical phase. In a phase I study of a DC vaccine in high-grade glioma, patients exhibited longer survival time, and a positive cytotoxic T-lymphocyte (CTL) response was induced. Fascinating results have been observed in patients with malignant glioma, anaplastic astrocytoma and GBM who have been vaccinated using tumor lysate as an antigen source[123]. An efficient depletion of tumor-specific CSCs has been observed by CTLs generated by DC vaccination+CSC derived tumor lysate in mouse glioma GL261 neurospheres (GL261-NS)[124]. Studies of the SOX2 gene have led to the genesis of specific CTLs against HLA-A0201-restricted SOX2-derived peptide (TLMKKDKYTLY)[125]. This remarkable discovery helped in lysing glioma cells and developing T cell-based immunotherapy of brain CSCs.

In recurrent glioma patients, the antiEGFRvIII vaccine strategy is also being evaluated, with randomization of first or second recurrent patients to receive either bevacizumab+vaccine or placebo (bevacizumab naïve patients) or bevacizumab+vaccine (antiVEGF refractory tumors) (www.clinicaltrials.gov, NCT01498328).

Adoptive cell transfer

Adoptive cell transfer mainly involves the transfer of tumorigenic immune cells to cancer patients. Lymphocytes are isolated from the blood, followed by transferring molecules that recognize tumor antigens (artificial T-cell receptors) onto the lymphocytes, providing them with a new and enhanced function. This process is called the chimeric antigen receptor (CAR) method[126]. CAR enhances the ability of T cells to specifically target antigens and efficiently kill tumor cells by combining the specificity of an antibody and the cytotoxicity of CTL. CAR consists of single chain variable fragment (scFv) of a tumor antigen-specific antibody and the signaling domains of the T cell receptor (TCR)[127]. In fact, CAR bypasses the mechanisms (down-regulation of MHC and costimulatory molecules and induction of suppressive cytokine and regulatory T cells) by which tumor cells escape immunorecognition. Recently, clinical trials of CAR-mediated adoptive immunotherapy in a variety of tumor systems such as renal cell
cancer[128], indolent B-cell and mantle cell lymphoma[129], neuroblastoma[130], acute lymphoblastic leukemia[131], and chronic lymphoid leukemia[132] have established their significant potential. However, some adverse events have also been reported resulting from the administration of CAR T cells against tumor antigens that are simultaneously expressed on normal tissues[133].

Accumulating evidence regarding enhanced antitumor activity due to the activation, proliferation and survival of CAR T cells comprising co-stimulatory molecules has led to new innovations in glioma therapy. The most commonly used co-stimulatory molecule is CD28[134]. The prerequisite for attaining a significant response in CAR-mediated immunotherapy is the CAR architecture and the choice of tumor associated antigen. EGFR variant III (EGFRvIII) is an oncogenic variant frequently expressed in glioma and other types of cancer[135]. EGFRvIII expression in association with survival, invasion, angiogenesis and radio/chemo resistance makes it an attractive target. Recent reports have demonstrated that systemically administered EGFRvIII+CAR T cells potentially migrate to the invasive edges of tumors, suppress tumor growth and enhance survival of intracranial D-270 MG tumor-bearing mice[136].

**Immune checkpoint inhibitors**

Immune checkpoint activating molecules (CD200, a positive regulator of MDSC[52], and the immunosuppressive FgI2) and immune checkpoints [PDL-1 (Programmed cell death protein 1 ligand), IDO (indoleamine 2,3-dioxygenase) and CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4)] are upregulated within the tumor tissue and in the serum of patients with high-grade glioma. Therefore, immune checkpoint inhibitors might play a crucial role in glioma immunotherapy. Immune checkpoint therapy has emerged as a potent addition to glioma therapy. Clinically successful checkpoint blockades such as CTLA-4 and PD-1 both alone and together have shown promising outcomes. Other targets are LAG-3, TIM-3, KIR, and GITR. Checkpoint inhibitors may be effective as monotherapy or in combination with chemotherapy and/or radiation therapy. Significant improvements in tumor regression and overall survival have been attained due to the synergy between the antibodies and either of the two conventional modalities[137, 138]. The key immune checkpoints that play a role in gliomagenesis, such existing preclinical and clinical data, antitumor efficacy, and clinical applications for each checkpoint and in combination with chemotherapy and radiation, are listed in Table S2.

**Molecular targets initiating metastasis**

Glioma is among the most vascularized and invasive cancers. In GBM, angiogenesis is correlated with malignancy grading and inversely correlated with patient survival. Glioma cells infiltrate and disrupt physical barriers (such as basement membranes, extracellular matrices and cell junctions). The overexpression of several members of the zinc-based proteinase family (metalloproteinases) is a hallmark in the process of invasion. The migratory potential throughout brain structures, infiltrative nature and rapid tumor progression of glioma cells make them elusive targets for effective treatment. Moreover, inadequate results with chemotherapy have led to the study of molecules targeting specific pathways or proteins involved in glioma progression. Therefore, the migratory behavior of glioma cells could potentially be efficiently managed through the identification of the molecular targets that induce metastasis, which could be achieved through a better comprehension of glioma biology.

**Concluding remarks and future directions**

Tumor location, potential symptoms and the risks/benefits of the various treatment regimens (surgery/radiation/chemotherapy) are the parameters that are taken into consideration in the clinical management of glioma patients. The migratory potential of glioma cells over relatively long distances through brain structures make them elusive targets for effective surgical management. Despite the continuous development of new chemotherapeutic agents, the ability to bypass the BBB and acquired drug resistance are still constraints. Rather than attempting to control the migration of diffuse glioma, interventions that specifically target the invasive phenotype should be developed. However, recent advancements in neuroimaging will contribute to early diagnosis and initiation of glioma management. Advancements in non-invasive imaging protocols and a better understanding of glioma biology will enable neuro-oncologists to decipher the molecular, cellular, genetic and epigenetic makeup of tumors. This information might pave the way to personalized glioma therapies. Nanotechnology, transplantation of stem cells, drug dosing or timing variations, mitigation of immunosuppressive mechanisms and a better understanding of miRNA and mRNA interactions are also some of the strategies that may facilitate GBM stratification. A better understanding of the complex biology of glioma and identification of the molecular targets that initiate metastasis will facilitate the development of a novel class of anticancer therapeutics with improved efficacy and safety. Additionally, proteogenomic characterization may also identify molecular drivers and lead to molecular classification of glioma subgroups. The insights provided by omics studies will facilitate identification of glioma protein signatures and biomarkers as well as the design of potential treatment regimens. Next generation therapies comprising progressive neuroimaging techniques, termination of DNA synthesis by prodrug-activating genes, silencing gliomagenesis genes, targeting miRNA oncogenic activity, sensitizing cancer stem cells by Hedgehog-Gli/Akt inhibitors+radiotherapy and employing tumor lysates as antigen sources for efficient depletion of tumor-specific CSCs by cytotoxic T lymphocytes along with conventional strategies will provide a new paradigm in glioma therapeutics with a focus on early diagnosis and successful management.

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