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# Brain Tumors: Focus on Glioblastoma

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# Table of Contents

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## Abstract

Brain tumors are abnormal growths of tissue in the brain or the spine (central nervous system, CNS) that can disrupt proper functioning of the brain. Brain tumors consist of a diverse collection of neoplasms arising from different cells either within the brain (primary brain tumors) or from systemic tumors that have metastasized to the brain, usually through the bloodstream (metastatic brain tumors).

Primary brain and spinal cord tumors may be either benign or malignant. Benign brain and spinal cord tumors grow and press on nearby areas of the brain, but rarely spread into other tissues and once treated or excised may recur. Malignant brain and spinal cord tumors are aggressive and spread into other brain tissue, but they rarely spread to other parts of the body. Primary tumors are categorized as glial tumors or glioma (composed of glial cells) or non-glial tumors (developed on or in the structures of the brain, including nerves, blood vessels and glands).

While our understanding of the biological basis of brain tumor development has improved, and newer treatment modalities have been developed, survival for many types of malignant primary brain tumors has not improved significantly in the last decade[1, 2]. Nearly 30% of adults who have a malignant primary tumor at one site are likely to develop brain metastases and form malignant brain lesions throughout the brain [3]. Furthermore, in the case of children, brain tumors are the most common type of solid tumors and are the leading cause of cancer-related deaths in this population [4].


## Types of Brain Tumors

### Types of Benign Brain Tumors

- Chordomas
- Craniopharyngiomas
- Gangliocytomas,
- Glomus jugulare tumors
- Meningiomas
- Pineocytomas Pituitary adenomas
- Schwannomas

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### Types of Malignant Brain Tumors

Gliomas are the most prevalent type of brain tumor in adults. They arise from the supporting cells of the brain, called the glia**.** Glial tumors include the following:

- Astrocytomas
- Ependymomas.
- Glioblastoma multiforme (GBM) is the most invasive type of glial tumor.
- Medulloblastomas
- Oligodendrogliomas

**Other Types of Brain Tumors**

- Hemangioblastomas
- Rhabdoid tumors

## Glioblastoma

Based on data collected between 2009 and 2010, gliomas account for nearly 80% of malignant brain tumors, and the highest grade or grade IV glioma, glioblastoma, is one of the most lethal cancers in adults with an annual incidence of 5.26 per 100,000 population or approximately 16,300 new diagnoses per year in the United States (US), based on the US population at the time [3]. The World Health Organization (WHO) classification system groups gliomas into four histological grades defined by increasing degrees of undifferentiation, anaplasia and aggressiveness. This subcategorization is particularly influential in clinical settings, as it can assist in determining the types of treatment(s) for patients. Grade I tumors are neoplasms with low proliferation rates that can be cured by surgery alone. On the other hand, grade II tumors are invasive and often recur despite low proliferative potential. Grade III tumors are generally malignant tumors with histological confirmation that exhibit anaplasia and rapid mitotic cell division, while grade IV gliomas are of the most advanced grade and are malignant tumors that have the poorest prognosis, with high potential for fatal outcome [5 – 7].

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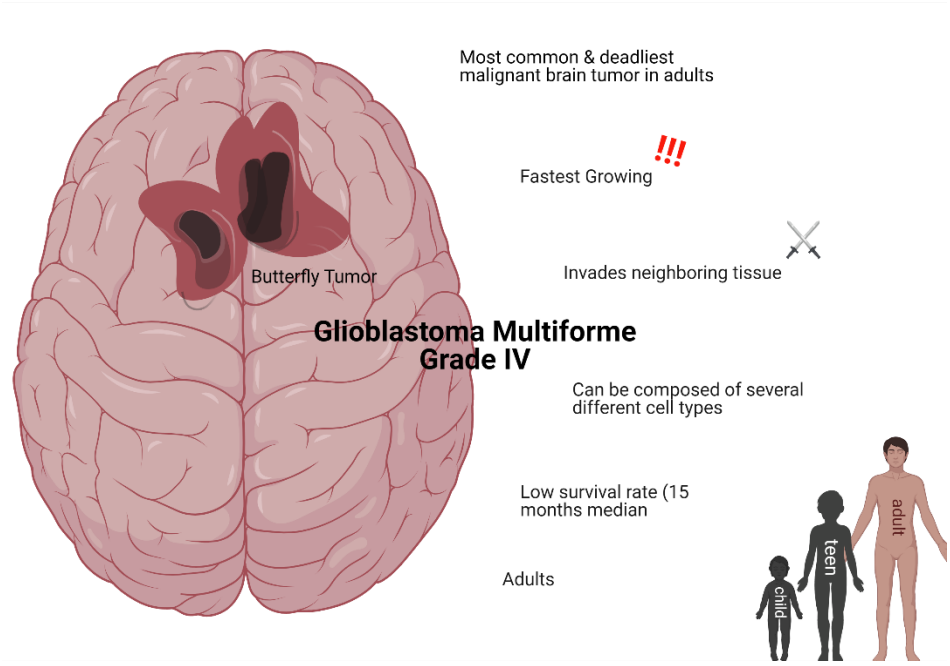


The most common and yet most deleterious grade IV glioma subtype is glioblastoma multiforme (GBM) [8]. While the term multiforme is no longer in use, GBM remains the anachronym. According to the Central Brain Tumor Registry of the United States (CBTRUS) Statistical Report 2011–2015, glioblastomas constitute about 57% of the average annual age-adjusted incidence rate of all neuroepithelial tumors and about 48% of all malignant brain and CNS tumors. It has been noted that the incidence rate of glioblastoma tumors is 1.58 times higher in the male population compared to females in the US [8]. Glioblastoma accounts for 82% of cases of malignant glioma and is characterized histologically by considerable cellularity and mitotic activity, vascular proliferation and necrosis. Because cells in these tumors vary in size and shape, *i.e*., pleomorphic, glioblastomas are also called glioblastoma multiforme (GBM). Glioblastoma and other malignant gliomas are highly invasive, infiltrating surrounding brain parenchyma, yet they are typically confined to the central nervous system (CNS) and do not metastasize. The detrimental nature and quick progression (median survival of about 15 months) of glioblastomas, make it almost impossible to cure these patients despite aggressive multimodal treatment strategies. Moreover, the heterogeneous nature of glioblastomas makes it extremely challenging to develop an effective therapeutic approach with a uniform outcome for all patients [9].

Although GBMs occur almost exclusively in the brain, they can also appear in the brain stem, cerebellum and spinal cord. According to American Association of Neuroscience Nurses, sixty-one percent of all GBMs occur in the four lobes of the brain: frontal (25%), temporal (20%), parietal (13%), and occipital (3%). Originally, GBMs were thought to be derived solely from

glial cells; however, evidence suggests that they may arise from multiple cell types with neural stem cell–like properties. These cells are at multiple stages of differentiation from stem cell to neuron to glia, with phenotypic variations determined, in large part, by molecular alterations in signaling pathways rather than by differences in cell type of origin [10].

### Risk Factors

Efforts to identify specific correlations of this disease with environmental and occupational exposure have largely been inconclusive. Ionizing radiation is one of the few known risk factors that increase the risk of glioma development. Radiation-induced GBM is typically seen years after therapeutic radiation indicated for another tumor or condition [11]. Other environmental exposures to pesticides, smoking, petroleum refining, and synthetic rubber manufacturing have been loosely associated with the development of gliomas. Electromagnetic fields, formaldehyde, and nonionizing radiation from cell phones have not been proven to be associated with GBM. An increased risk of glioma development is seen in some specific genetic diseases, such as neurofibromatosis 1 and 2, tuberous sclerosis, Li-Fraumeni syndrome, retinoblastoma, and Turcot syndrome; however, less than 1% of patients with a glioma have a known hereditary disease [12].

## Pathogenesis

### IDH Mutation

Isocitrate dehydrogenase (IDH) is an enzyme that plays a central role in the citric acid cycle and in the conversion of isocitrate and NADP+ to -ketoglutarate (-KG) (a cofactor for several enzymes), NADPH, and carbon dioxide[13]. IDH mutations are found to exist in high numbers in secondary glioblastomas and grade II and III gliomas but are rare in primary glioblastomas [14]. The IDH mutation leads to a decrease in its binding affinity for isocitrate, preventing the conversion of isocitrate to -KG. In addition, IDH mutation also increases its binding affinity for NADPH, which results in incomplete reaction by only reducing -KG without carboxylation, forming 2-hydroxyglutarate (2-HG) instead of -KG. The abnormal accumulation of 2-HG, an oncometabolite, is responsible for cancerogenesis [15]. This led to the identification of mutant IDH (mIDH) inhibitors as novel targeted cancer therapies. The mIDH1 inhibitor AGI-5198 was found to successfully cause 2-HG inhibition and hindered the growth of mIDH1 glioma cells *in vivo*. Optimization of AGI-5198 led to the identification of AG-120 (ivosidenib), which became the first mIDH1 inhibitor to achieve clinical proof-of-concept in human trials [16].

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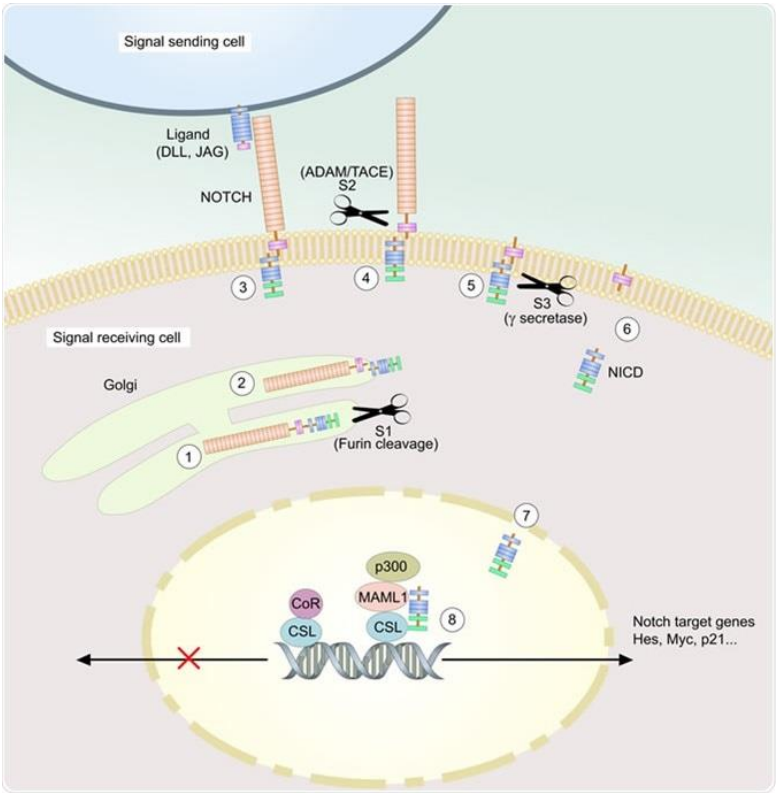
Subsequently a selective R132H-IDH1 inhibitor, AG-5198, was discovered which was found to completely block the ability of mIDH1 to produce 2-HG, and induced expression of genes involved in gliogenesis [17]. In addition, there is a potent, brain-penetrant mIDH1/2 inhibitor, AG-881 (vorasidenib) that is being tested in clinical studies in patients with glioma.

## Notch Pathway

Notch signaling plays an important role in cell differentiation, proliferation, and apoptotic events in different cell types and tissues, including neurons of the CNS. It is necessary to ensure that neural stem cells are promoted towards becoming glial cells instead of differentiating into another form. Due to its key role in cell processes, it is easy for Notch signaling to deviate towards tumorigenesis. There are four receptors involved in this pathway: Notch-1, Notch-2, Notch-3, and Notch-4. Notch-1 is found to be either a tumor suppressor or an oncogene based on the tissue type. Moreover, it has been found to be associated with glioma progression to determine the malignant phenotype of glioma. Notch-2, on the other hand, was identified as a prognostic marker for glioma along with Notch-3, which also promotes glioma cell proliferation. Lastly, Notch-4 was found to correlate with tumor aggressiveness [18]. Various studies have identified the Notch pathway to be a potential and effective target in stem-like glioma cells, which were found to express Notch family genes. One study demonstrated that all-trans retinoic acid (RA) can downregulate neurosphere cell expression of the Notch pathway targets Hes2, Hey1, and Hey2. When treated with RA, the Notch receptor intracellular domain (NICD1) is forced to rescue glioblastoma neurospheres, thus causing inhibition of Hes2, Hey1, and Hey2. The study concluded that this is an indication of RA affecting glioblastoma stem-like cells towards cell growth arrest, differentiation, and stem cell pool loss [19]. Furthermore, inhibition of the Notch



pathway, using gamma-secretase inhibitors, was found to reduce glioblastoma neurosphere engraftment *in vivo*, which prolonged the lifespan of the mice [20].

### Vascular Endothelial Growth Factor (VEGF) Signaling Pathway

Vascular endothelial growth factor (VEGF), a potent angiogenic cytokine, stimulates the growth of new blood vessels to restore oxygen supply. VEGF also plays a key role in promoting angiogenesis in glioma stem cells and optimizing the function and survival of its microenvironment. For survival of the glioblastoma, a vascular supply must be maintained, and early extensions in the growing tumor receive this vascular supply by angiogenesis [21]. Hence, blocking the VEGF pathway and thereby inhibiting angiogenesis is thought to be an effective strategy to treat the disease. Though anti-VEGF therapy has been widely used and has shown benefits in the reduction of vasogenic edema associated with this disease, the overall survival benefit remains low, and resistance to therapy is a major factor in patients on this group of drugs. However, several approaches using combination therapy with radiotherapy, immunotherapy, cytotoxic drugs etc., in addition to anti-VEGF therapy may improve outcomes. A recent study on combination therapy with platelet-derived growth factor (PDGF) inhibitors showed more promising results when combined with anti-VEGF therapy in terms of survival benefit and sensitization to therapy.

### Platelet-derived growth factor Signaling

Platelet-derived growth factor (PDGF) has been identified as a target in the treatment of glioblastoma due to its ability to promote glioblastoma proliferation and survival [22]. In normal glial cells, PDGF ligands bind to the platelet-derived growth factor receptor (PDGFRα or PDGFRβ) which upon binding, dimerize, allowing the subunits to cross phosphorylate several tyrosine residues in the receptor. This activated form acts as a docking site for multiple protein complexes to activate many signal transduction cascades, ultimately leading to DNA synthesis and cell proliferation. A PDGF autocrine loop is exhibited in glioblastomas which should be absent in normal brain tissue. PDGFRα gene is found to be amplified, mutated, or rearranged in glioblastoma tumors, playing a major role in oncogenesis. Similarly, PDGF and PDGFR were found to be overexpressed in glial tumor cell lines and samples correlating with a higher tumor grade. Autocrine signaling in glial tumor proliferation was tested *in vitro* where PDGF inhibitors were able to limit colony activity and cell growth. In clinical settings, several tyrosine kinase inhibitors (TKIs) such as tandutinib, crenolanib, sorafenib, sunitinib, and

pazopanib are currently being studied for PDGF and PDGFR inhibition in glioblastoma therapy.

### Epidermal Growth Factor Receptor (EGFR) Pathway

In normal glial cells, the EGFR pathway and its downstream cascade participates in DNA synthesis, cell proliferation, migration, and adhesion [23]. Mutations in EGFR are a common phenomenon in primary glioblastomas (40%) but rarely present in secondary glioblastomas [24]. Particularly notable is the EGFR variant termed EGFRvIII, which enhances tumorigenicity of glioblastoma through mitogenic signaling by inhibiting apoptosis. Recent studies are focused on both immunotherapy as well as tyrosine kinase inhibitors (TKIs) for inhibiting the EGFR pathway. Though TKIs are promising, further pre-clinical evaluation of drug delivery and activity of EGFR inhibitors is also required. Currently there are nearly 19 clinical trials ongoing which target the EGFR pathway which also include tyrosine kinase inhibition for glioblastomas. Some of these EGFR-targeting therapeutic agents under clinical trial include: dacomitinib, nimotuzumab, ABBV-321, AMG596, CART-EGFRvIII T cells, EGFR(v)-EDV-DOX, axitinib, cabozantinib, neratinib, afatinib, alectinib, and tesevatinib [25].

### PI3K/AKT/mTOR Pathway

Overactivation of the PI3K/AKT/mTOR pathway has been shown to reduce survival of glioblastoma patients and increase aggression of the tumor as it triggers signaling pathways responsible for cell proliferation, survival and migration in glioblastoma [26, 27]. Preclinical trials have reported that mTOR kinase inhibitors like CC214-1 and CC214-2 were capable of inhibiting glioblastoma growth by blocking mTOR2C2 activity both *in vitro* and *in vivo*. Currently approximately 11 clinical trials are ongoing which target and inhibit PI3k (BKM120, regorafenib, GDC-0084, and fimepinostat) and mTOR (temsirolimus, everolimus, CC-115, ABI-009, AZD2014, sapanisertib, and siroquine) in glioblastoma [25].

### HGF/cMet signaling pathway

Mesenchymal epithelial transition factor (cMET) is another deregulated tyrosine kinase receptor which promotes malignant phenotypes in GBM. Over-activation of cMET pathway can: (a) increase levels of VEGFA and VEGFR2 in endothelial cells and promote proliferation, metastasis, and angiogenesis, (b) prevent apoptosis through activation of phosphatidylinositol-3-kinase (PI3 kinase) and subsequent AKT activation, (c) upregulates the PI3K/AKT pathway which leads to the growth and survival of uncontrolled tumor cells through the transcription factor, NFκB that activates many cell survival and anti-apoptotic genes [28]. Monoclonal anti-

HGF antibodies like onartuzumab/MetMAb, AMG102/rilotumumab, ABT-700/telisotuzumab, etc. function by blocking HGF binding and thereby preventing cMET activation which results in inhibiting the activation of signaling pathways downstream of cMET. Though monoclonal antibodies are specific and effective, their larger size often inhibits their penetration across the blood-brain-barrier. In order to overcome this drug delivery obstacle, small molecule inhibitors (like AMG 337, PLB1001, APL-101) are currently being tested [29].

## Clinical Presentation, Histopathology & Diagnosis

The clinical manifestation of newly formed GBM in a patient can vary greatly depending on the size and location of the tumor and the anatomic structures of the involved brain. Patients are often found with symptoms of increased intracranial pressure, including headache and focal or progressive neurologic deficits. A seizure is the presenting symptom in as many as 25% of patients at the early stage and can occur at a later stage of the disease in as many as 50% of patients [30]. New-onset seizures or development of neurologic deficits are commonly followed by a neurologic workup that includes magnetic resonance imaging (MRI). Computed tomography (CT) with contrast enhancement is less sensitive in detecting the typical features of glioblastoma. Its use is restricted to acute situations, e.g., when hemorrhage is suspected, or when MRI is not available or not possible, e.g., in patients with cardiac pacemakers or other metallic implants. Prior to biopsies, amino acid positron emission tomography (PET) is performed to guide the site of biopsy to metabolic hotspots that may represent sites of higher tumor grade. MR spectroscopy, T1 contrast subtraction maps, as well as diffusion/perfusion- and susceptibility-weighted MRI sequences have refined radiographic diagnostics and enabled more reliable discrimination of glioblastoma from other contrast-enhancing lesions, such as abscesses, primary central nervous system lymphomas, and metastases from non-primary brain tumors. However, the appearance of glioblastoma on imaging scans can vary considerably and therefore tissue-based diagnosis is necessary [31].

The most notable histopathologic features of glioblastoma are necrosis and microvascular proliferation. These qualify glioblastoma for the highest grade in the WHO classification of primary brain tumors, grade IV. Other signs of malignancy that are present in glioblastoma include anaplasia, high mitotic rates, and invasiveness, but these features are also present in anaplastic gliomas, which are assigned WHO grade III [32]. In addition, immunohistochemical markers are commonly assessed to ascertain the diagnosis of glioblastoma, including glial

fibrillary acidic protein expression to confirm astrocytic lineage differentiation and MIB-1/Ki-67 to aid quantification of proliferation.

Isocitrate dehydrogenase (IDH) mutations, (O6-Methylguanine-DNA methyltransferase) MGMT promoter methylation, (epidermal growth factor receptor) EGFR amplification/mutations, and vascular endothelial growth factor (VEGF) overexpression have been previously correlated with patient outcomes or treatment efficacy. IDH1/2 mutations are now part of the routine diagnostic pipeline, and bear not only diagnostic but prognostic relevance as well, as IDH mutant glioblastomas have better prognosis compared to IDH wild type. IDH mutation is currently thought to be involved in gliomagenesis through the accumulation of oncometabolite 2-hydroxyglutarate, which is believed to lead progenitor cells into gliomas via multiple processes. IDH mutations are present in only about 10% of glioblastomas, but when present, they indicate better survival [33]. MGMT promoter methylation is a predictive marker of response to alkylating agent temozolomide (TMZ), a routinely used chemotherapeutic agent in glioblastoma treatment [34]. Translation of the MGMT gene results in a DNA repair enzyme, and hypermethylation of the MGMT promoter region results in the silencing of the gene, thus allowing TMZ to exhibit its therapeutic effect. Overexpression and mutations (especially EGFRvIII mutation) is common in glioblastomas and are known to contribute to glioma invasiveness. In many other types of cancers, the inhibition EGFR signaling was found to be an effective method to fight cancer; however, its amplification in GBM has failed so far. The EGFRvIII mutation combined with other known factors (e.g., Ki67<20%, phosphatase and tensin homolog (PTEN) wild type, or MGMT hypermethylation could indicate better prognosis, and EGFRvIII positive tumors are candidates for vaccine-based novel experimental therapies [31]. VEGF is overexpressed in gliomas and is currently the only molecule suitable for targeted therapy in (recurrent) glioma [35]. These afore-mentioned markers are identified by immunohistochemistry, FISH or PCR-based methods. They are routinely available in clinical practice; however, these methods always require tumor samples, and they cannot be used in real-time monitoring of treatment response, in screening for tumor recurrence, or in differential diagnosis. There is a serious need for novel prognostic and predictive markers in glioblastoma treatment, which could signal early tumor recurrence or ineffective treatment.

In addition to the WHO classification of GBM, a molecular classification evolved from the extensive work of The Cancer Genome Atlas (TCGA) Network wherein recurrent genomic abnormalities were characterized. Based on this gene expression-based molecular classification, there are four subtypes of GBM – Proneural, Neural, Classical and Mesenchymal. Each subtype has a distinct genomic signature and can be used to better understand this disease. Importantly, this classification can provide the basis for molecular stratification to personalize therapeutic strategies for each patient.

Liquid biopsy is an increasingly developing field of research. Serum, urine, CSF and other bodily fluid contain several tumor-derived circulating nucleic acids (e.g., ctDNA, cmtDNA, mRNA, non-coding RNAs including miRNAs, long non-coding RNAs) that are linked to prognosis or treatment response. A wide range of these particles have been studied in glioblastoma and are now considered as novel markers of glioblastoma prognosis or treatment response. Some of these markers could prove useful in diagnosis, others could be used in early follow up after surgery or radio-chemotherapy, and still some could be used to identify tumor recurrence or the development of therapeutic resistance [36].

## Treatment

### Standard Therapy for Glioblastoma

The current standard of care for newly diagnosed GBM patients is resection followed by postoperative radiation therapy (RT) with concurrent and adjuvant temozolomide. Surgery serves to alleviate symptoms of mass effect, reduce tumor burden and provide adequate tissue for diagnosis and molecular profiling. Although randomized evidence for the efficacy of maximal surgical resection is limited, numerous retrospective studies and meta-analyses have found that gross total resection improves overall and progression-free survival relative to subtotal resection. Furthermore, fluorescence-guided surgery using 5-aminolevulinic acid, which allows for more complete resection, has been found to improve progression-free survival in a randomized trial. Based on this and other evidence, maximal safe resection is considered the current standard for GBM treatment.

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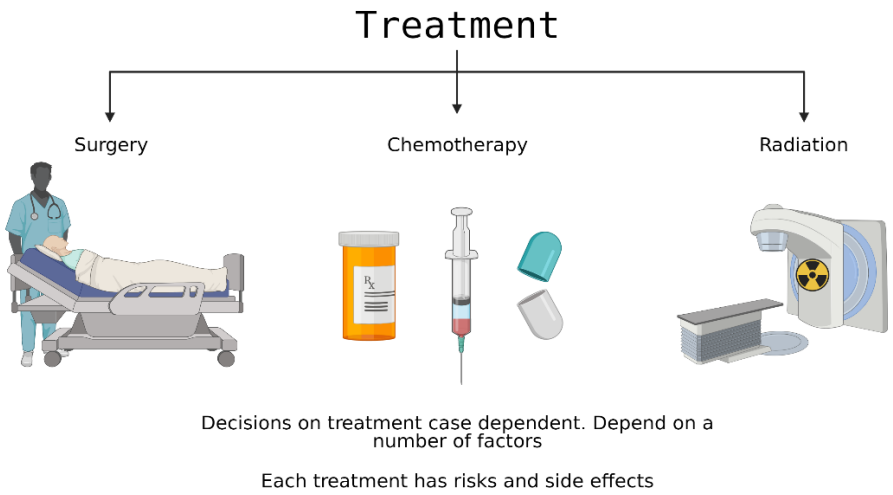



Postoperative radiation was established as the cornerstone of adjuvant therapy for GBM by the Brain Tumor Study Group (BTSG) 69–01 trial, which showed that whole brain radiation improved overall survival. The pooled analysis of BTSG trials established radiation dose of 60 Gy as the optimum dose for longer survival, which was confirmed in a subsequent randomized trial by the Medical Research Council, making 60 Gy the standard of care. Further dose escalation failed to improve survival, as did stereotactic radiosurgery boost. Modern guidelines typically call for the treatment of the postsurgical resection cavity, any gadolinium-enhancing residual tumor on magnetic resonance (MR) T1 imaging, and T2/FLAIR edema with a 2 to 3 cm anatomic expansion. This practice is based on studies showing that nearly 90% of recurrences occur within a 2 cm margin of the primary tumor site. Administration of the oral alkylating agent temozolomide (TMZ) during and following radiation was established as the standard of care by the EORTC 22981/26981 and NCIC CE.3 trial. In this trial, 573 GBM patients were randomized to 60 Gy radiation with or without concurrent and adjuvant temozolomide. The addition of temozolomide improved median survival as well as 5-year overall survival from. The relative benefit from concomitant versus adjuvant temozolomide remains unclear. Notably, intensifying post-radiation temozolomide dose in another randomized trial, RTOG 0525, failed to further improve survival. Contemporary randomized

trials have utilized postoperative radiation with concurrent and adjuvant temozolomide as a backbone on which to add investigational therapies.

## Current Chemotherapeutic Development

Currently, three chemotherapeutic agents (TMZ, bevacizumab, and carmustine) have been approved by the FDA to treat patients with glioblastoma. The same clinical study with 573 patients that showed addition of TMZ to radiotherapy significantly increases overall survival (OS), also found that $O^6$-methylguanine-DNA methyl-transferase (MGMT) gene methylation is a positive prognostic indicator for TMZ chemotherapy for newly diagnosed patients. Notably, bevacizumab is an anti-VEGF monoclonal antibody that has been approved by the FDA for the treatment of recurrent glioblastoma. It has been clinically observed that bevacizumab has anti-glioma activity with improvement in progression-free survival (PFS); however, it has no significant activity in terms of OS. A clinical trial on newly diagnosed glioblastoma patients with bevacizumab has shown no significant activity in terms of OS but longer PFS compared to the placebo group. Carmustine, a nitrosourea compound which is used in the treatment of the disease, is now avoided due to clear demonstration of severe bone marrow, liver and kidney toxicity. However, local delivery of carmustine in the form of an implant in the resection cavity followed by surgery can reduce systemic adverse events, and can improve median survival of the patients both in recurrent and newly diagnosed glioblastoma.

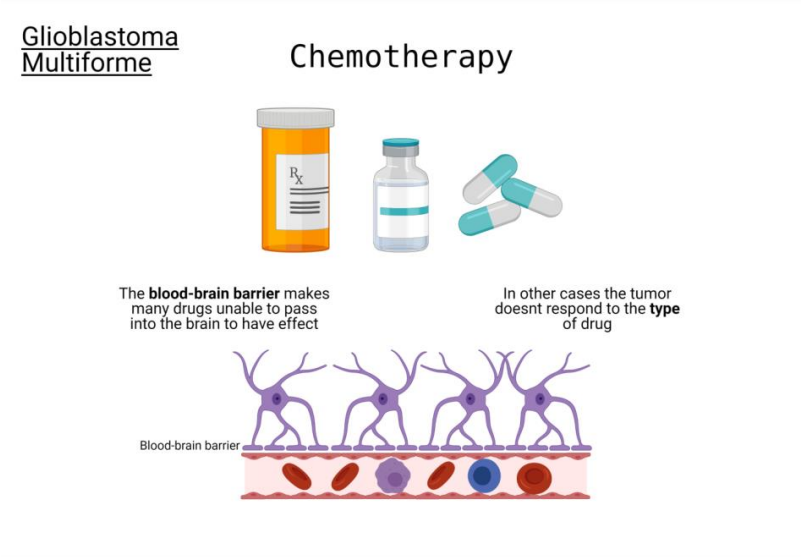
Currently, several drug candidates are in the pipeline at different stages of clinical development. For example, Alisertib, Lomustine, Ortataxel, Buparlisib, Palbociclib, Iniparib, Depatuxizumab mafodotin, Fotemustine, a combination of Temsirolimus and sorafenib, Vorinostat and bevacizumab, respectively etc.

## Novel Therapies

A prime example of both the unique biology and the anatomical challenges of treating brain tumors is the blood–brain barrier (BBB), the neurovascular unit that maintains brain homeostasis and acts as a ‘gatekeeper’, controlling the crossing of molecules and cells from

the blood into the brain. Although the BBB is often disrupted in brain tumors, effective delivery of chemotherapeutic drugs through this blood–tumor barrier remains a challenge.

Additionally, common setback with chemotherapy is that it induces severe side effects such as nausea, vomiting, hair loss, and a weakened immune system. Therefore, studies have been conducted to look for alternate therapies. Listed below are few novel therapies that are emerging for the treatment of glioblastoma [25].




## Laser Interstitial Thermal Therapy (LITT)

When surgical removal of a tumor is not possible, LITT offers treatment to glioblastoma patients by destroying the tumor cells with localized elevated temperature. This kind of thermal therapy can also be achieved using radiofrequency, ultrasound, microwave, and magnetic nanoparticle (MNP) treatments. However, the major advantage of laser-induced thermotherapy is minimal invasiveness. Studies have found that MRI-guided LITT is safe, and it can also disrupt the peritumoral blood–brain barrier (BBB) which improves therapeutic permeability; however, it should be used with caution. Moreover, it has also been observed that the efficacy of LITT in recurrent glioblastoma is an alternative to surgery. Retrospective analysis also found that LITT enhances the PFS of difficult-to-access high-grade gliomas. However, further studies are required to establish LITT as a substitute to standard surgical removal of the tumor.

### Tumor Treating Fields (TTFields)

TTFields is a technology which creates alternating electric fields of low-intensity (1–3 V/cm) and intermediate frequency (100–300 KHz) that interrupts the aggressive cell division of cancerous cells but does not affect the quiescent and non-dividing cells in the human body. Optune®, a device made by Novocure, is the commercial example of TTFields. It has been approved by the FDA for the treatment of supratentorial and histologically confirmed glioblastomas, both recurrent (in 2011) and newly diagnosed (in 2015). For recurrent glioblastomas, it is approved as monotherapy while for newly diagnosed glioblastomas it has been approved to be used along with adjuvant chemotherapy. The device is mounted on the shaved scalp of the patient with the help of an insulated transducer array. The results from various randomized clinical trials have confirmed that the combination of TTFields and adjuvant TMZ chemotherapy significantly increases OS and PFS and it does not have any serious negative effect other than itchy skin. Since the side effects related to TTFields are comparatively low, it is likely to be of benefit to patients; however, its use is limited by the high cost of the technology.

### New targeted Therapies

The prognosis of patients with primary glioblastoma treated with the current standard of care, surgery followed by radiation therapy and auxiliary temozolomide, remains poor. Integrative genomic analyses have identified essential core signaling pathways and frequent genetic aberrations, which provide potential drug targets for glioblastoma treatment. Van *et al.* summarized advances in drug development that have occurred prior to 2020 for targeted glioblastoma therapies and revealed the major challenges encountered in clinical trials or treatment. Many of these new alternatives have been discussed earlier in this paper. Suffice it to say that scientists now are targeting core signaling pathways in glioblastoma, including without limitation EGFR, the PI3K/AKT/mTOR signaling pathway, the p53/ARF/MDM2 signaling pathway and the Rb signaling pathway. Several drugs that appear to disrupt the signaling pathway required for the tumor to grow have been introduced into clinical trials evaluating their efficacy in treating this pernicious disease.

### Immunotherapy

Immunotherapy involves deploying the patient's own immune system to mount a response against tumor cells. The concept that originated in the nineteenth century has been refined by significant improvement of our understanding of immune biology and cell engineering. There

are several modes for activating an immune response such as the use of immune checkpoint inhibitors (CPIs), oncolytic virus therapy, vaccine therapies, modified T cell therapies, and gene therapies. These may be categorized as active or passive immunotherapy based on their mechanism of actions. The different types of immunotherapy are discussed below.

### *Immune Checkpoint Inhibitors*

Immune checkpoint inhibitors or CPIs have the ability to cross the blood brain barrier, which makes them promising candidates for recurrent glioblastomas. Drugs like nivolumab, pembrolizumab, durvalumab, atezolizumab, and pidilizumab have been under investigation for both combination therapy as well as monotherapy [25].

### *T-Cell Therapy*

Engineered T cells to express chimeric antigen receptors or CARS are a major backbone to T cell therapy. It is a new branch of therapeutic science, which is a rapidly evolving to treat glioblastomas. Recent studies are targeting EphA2, CD70, EGFR, HER2 and IL-13R or receptors to trigger cell mediated immune clearance of cancer cells. To date, FDA has not approved T-cell therapy for glioblastomas. However, T-cell therapy is being considered as part of a combination of other therapies [25].

### *Viral Therapy*

Novel to immunotherapy, an immunogenic oncolytic virus is engineered to exert its lytic cycle on cancer cells resulting in selective oncolysis or lysis of cancerous cells. The mode of oncolysis includes direct oncolysis, virus-induced anti-tumor immunity along with immunoregulatory inserts such as interleukin 12 (IL-12) and OX40 ligand. In case of glioblastoma, the immunostimulatory effect of oncolytic viruses is becoming a promising line of therapy. Currently there is no FDA approval for using viral constructs for treatment of glioblastomas, although there are promising results from clinical trials on viral therapy in combination with immunotherapy, radiotherapy or chemotherapy showing better patient outcomes. Moreover, many studies are ongoing at different stages of clinical trials as monotherapy as well as combination therapy [25].

### *Vaccine Therapy*

Vaccines are not thought as preventive measures rather as active immunotherapeutic strategies in cancer treatment. Vaccines as immunotherapy can provide high specificity along with low toxicity in comparison to other conventional therapeutics like chemotherapy and radiotherapy.

Glioblastoma vaccines can trigger an immune response against tumor associated antigens. The vaccines may be patient-derived dendritic cells and autologous tumor cells which would evoke an immune response after re-engineering and reinsertion into the body. There are other non-cell based vaccines such as heat shock protein and other peptide vaccines. These non-cell based vaccines are engineered peptide sequences that target immunity against major histocompatibility complexes (MHC) bound tumor antigens and escalate the adaptive immune response .All such peptides are used in parallel with an immunostimulant adjuvant for antigen presentation. Heat shock protein vaccines are specifically designed to trigger a specific antitumor inflammatory response. Whereas, autologous tumor cell vaccines are employed where cytotoxic T cells are induced in patient derived tumor cells *in vitro* and reintroduced into the body to elicit a specific antitumor immune responses. Designing dendritic cells (DC) as specific antigen presenting cells (APC) has been evaluated to stimulate the brain immune system, through culture *ex vivo* loading of glioma cell antigens to proliferate the DC. The "cocktail" is reintroduced to the body of the patient to activate both T helper cells and CD8+ T cells, resulting in specific tumor cell death [25]. Early clinical trial data is promising for this latter approach.

## Meta-analysis and Systemic reviews

Scientists and researchers attempted to draw conclusions from different potential studies on associative factors of glioma and related tumors through systemic reviews and meta-analysis from articles across the globe. Brain tumor is one of the most frequent types of tumors in young individuals below 25 years of age. After a systemic review of 70 full text articles, Zumel-Marne *et al.*, 2019 tried to elucidate specific environmental factors, for example: exposure to heavy metals, tap water use, cigarette smoking of parents, pesticide exposure, air pollutants as well as non-ionizing radiation associated with brain tumors in children and young adults. However, because of the small size of the study population, no conclusions could be drawn. The meta-analysis was performed mainly from studies in the US, Canada, Australia and European countries [37]. Another study spanning a period of 36 years and multiple publications, attempted to elucidate the role of mobile phone use with the risk of developing gliomas. The meta-analysis study was completed and eleven potential studies showing a significant pooled odds ratio of 1.44 (95% confidence interval, 1.08-1.91) indicating some association between long-term mobile phone use(minimum 10 years) and glioma [38]. In addition a meta-analysis of 19 publications from Europe, North America, Middle East, Asia and Australia was

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completed to determine a correlation between alcohol consumption and brain tumors. Unfortunately, the analysis failed to draw any meaningful correlation between alcohol consumption and adult brain cancer including gliomas and glioblastomas [39].

## Conclusion

While advances in our understanding of brain tumors and in particular, glioblastomas, has improved, the impact of our understanding on clinical outcomes has been limited. Future clinical benefit depends on the success of the aforementioned programs as well as identifying other novel biomarkers that allow our scientists to target the disease more precisely.

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