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# Current Trends in Glioblastoma Treatment

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## Abstract

Glioblastoma (also called glioblastoma multiforme – GBM) is a primary brain neoplasm, representing about 55% of all gliomas. It is a very aggressive and infiltrative tumor. Glioblastoma is usually highly malignant, with more than 90% 5-year mortality and a median survival of about 14.6 months. Compared to other cancers, the survival rate has not greatly changed over time and no current treatment is curative for this disease. Because the tumor has a heterogeneous cell population containing several types of cells, the treatment for GBM is one of the most challenging in clinical oncology. This chapter will discuss the current approaches in glioblastoma treatment, including resection techniques, chemotherapy and radiation therapy.

**Keywords:** glioblastoma, surgical resection, intraoperative guidance, radiation therapy, chemotherapy, intratumoral therapies, targeted therapy

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## 1. Introduction

Glioblastoma is the most common primary brain malignancy in adults. It is the most aggressive of the gliomas, largely resistant to conventional therapies, having a very poor prognosis. The global incidence is 2–3 newly diagnosed cases per 100,000 people per year in the United States and Europe. According to Central Brain Tumor Registry of the United States, GBM accounts for 14.9% of all primary brain tumors and 55.4% of all gliomas. It represents the highest number of cases of all malignant tumors, with an estimated 12,390 new cases predicted in 2017. Currently, the standard of care (SoC) for patients with GBM consists of maximal

safe surgical resection, followed by concurrent chemoradiotherapy and adjuvant chemotherapy with temozolomide (TMZ). New discoveries are being made in basic and translational research, novel therapeutic approaches have been tried and tested, some of them finding their way into clinical practice. Despite the synergistic multimodal strategies and individualized therapies, the available treatment is of limited utility, and patients have a poor prognosis, with a progression-free survival (PFS) of 7–8 months, a median survival of 14–16 months and 5-year overall survival (OS) of 9.8% [1]. This review focuses on the current treatment strategies and perspectives in the management of GBM.

## 2. Histology and classification

**The cellular origin** of GBM is unknown. Astrocyte, oligodendrocyte precursor cell and neural stem cell can all serve as the cell of origin for this type of brain tumor. For this reason, in the recent version of the 2016 World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS), which is the current international standard for the nomenclature and diagnosis, GBM is incorporated into the category of “diffuse astrocytic and oligodendroglial tumors”, being considered a grade IV tumor.

There are **some properties of the tumor cells that render GBM incurable**. First, the diffuse infiltrative nature of the cells makes complete tumor resection impossible, despite the advances in neurosurgical techniques. Glioma cells have the ability to migrate away from the main tumor mass, through the brain. Typical migration routes include white matter tracts, along the basal lamina of the blood vessels, perineuronal and in between the glia limitans and the pia mater. Tumor cells are still detectable at a distance greater than 4 cm away from macroscopic and radiologic margin of the tumor [2]. Second, there is a resistance of the glioma cells to conventional radiation therapy, chemotherapy and other therapies, as they are spared from eradication. This resistance is correlated with the heterogeneous character of the tumor itself, with its “multiforme” appearance [3]. GBM is multiforme macroscopically, featuring multifocal hemorrhage, necrosis and cystic areas. It is multiforme microscopically, demonstrating pleomorphic cell population, hypercellularity with mitotic activity, nuclear atypia, pseudopalisading necrosis and microvascular proliferation. And it is multiforme genetically, with various genetic abnormalities and heterogeneous subclones within the tumor cell population.

**The histological diagnosis** of GBM should be undertaken by a neuropathologist by standard histopathology methods and should include tumor type and tumor grade according to WHO Classification of Tumors of the CNS.

Progress has been made in knowledge of the GBM biology in relation to its microenvironment. Patterns of **molecular genetic alterations** have been associated with specific types of GBMs. Recent medical advances have indicated the importance of molecular typing in determining the prognosis and personalized treatment strategies for the patient. For this reason, in the recent version of the 2016 WHO Classification of Tumors of the CNS, molecular parameters are used in addition to histology to define diagnostic entities. This adds

a level of objectivity to diagnostic and should lead to improvements in determination of prognosis and treatment response. So, GBMs are further defined by the presence or absence of **isocitrate dehydrogenase (IDH) gene mutations**. IDH is an enzyme encoded by the IDH gene, whose mutations occur in gliomas. These mutations are oncogenic and they lead to a hypermethylation phenotype, as well as changes in cellular metabolism and response to hypoxic and oxidative stress [4, 5]. Mutated IDH can now be detected by immunohistochemistry and magnetic resonance spectroscopy (MRS). IDH mutation is identified as a genetic marker of secondary GBM. It can indicate a favorable prognosis and a relatively good response to radiation and/or alkylating chemotherapy.

**GBMs are divided into:** GBM, IDH-wildtype; GBM, IDH-mutant and GBM, NOS [6]. IDH-wildtype GBM corresponds with clinically described primary or de novo GBM. It represents about 90% of GBMs. It arises without clinical, radiologic or histologic evidence of a pre-existing less malignant lesions, in elderly patients (median age of 62 years), usually supratentorial. The mean length of clinical history is 4 months and the median overall survival after conventional surgery, radiotherapy and chemotherapy is 15 months, the prognosis being poor [6, 7]. IDH-mutant GBM corresponds with secondary GBM (approximately 10% of GBMs). It typically develops from lower grade diffuse glioma. It occurs in younger patients (median age of 45 years), preferentially in the frontal lobe. The mean duration of the clinical history of secondary GBM is 15 months and the median overall survival after multimodal treatment (including surgical resection, radiotherapy and chemotherapy) is 31 months, a significantly better prognosis than primary GBM [6, 7]. Primary and secondary GBMs carry distinct genetic abnormalities. Other common genetic alterations in secondary GBMs include TP53 mutations (~65%), ATRX mutations (~65%) and loss of heterozygosity (LOH) on chromosome 19q (~50%). In primary GBMs, there is a high frequency of EGFR amplification (~35%), phosphatase and tensin homolog (PTEN) mutation (~25%) and LOH on chromosome 10 (LOH 10p ~50%, LOH 10q ~70%) [7, 8]. There is now increasing evidence that primary and secondary GBMs are in fact different tumor entities that develop from distinct cells of origin [7]. Despite the differences in their phenotypic and genotypic profiles, these two subtypes of GBM are histopathologically indistinguishable, except that extensive necrosis is more frequent in primary GBM and oligodendroglioma components are more frequent in secondary GBM [7]. Recent findings in pediatric GBMs regarding mutations in the histone H3F3A gene suggest that these tumors may represent a third major category of GBMs, separate from adult primary and secondary GBMs [9]. The terminology NOS (i.e., not otherwise specified) is used for GBM when molecular information is insufficient, either because testing cannot be fully performed or the results do not fit within a defined category.

In the 2016 update of the WHO Classification of Tumors of the CNS, there are **three variants of IDH-wildtype GBMs**: giant cell GBM, gliosarcoma and epithelioid GBM. It is to be noted that variants are subtypes of accepted entities that are sufficiently well characterized pathologically and have potential clinical utility [6]. There are also **different patterns in GBMs**, including small cell GBMs, granular cell GBM and GBM with primitive neuronal component (previously referred as GBM with primitive neuroectodermal tumor (PNET)-like component). Patterns are histological features that are readily recognizable, but usually do not have clear clinicopathological significance [6].

### 3. Patient evaluation

**GBMs** are typically large tumors at diagnosis. They occur most commonly in the supratentorial compartment and are less common in the posterior fossa and brainstem. Lesions usually start within the deep white matter, but often infiltrate into cortex (**Figure 1**), deep nuclei or through commissural pathways into the contralateral hemisphere.

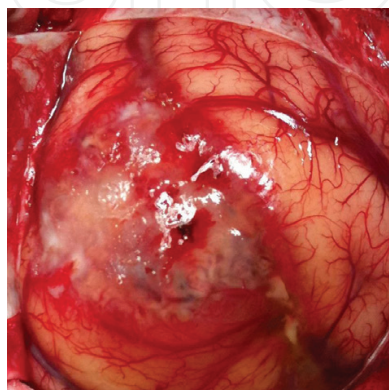
When a GBM spreads across the corpus callosum, there is a characteristic appearance of bihemispheric involvement, resulting in the classic “butterfly” pattern on imaging (**Figure 2**).

The vast majority of GBMs are solitary lesions, but cases of multiple GBMs were observed in 0.5–1% of cases. Multiple gliomas can be categorized as multifocal or multicentric. Multifocal disease consists of multiple tumors which result from dissemination along an established route of CNS, spreading through white matter tracts, cerebrospinal fluid pathways or through local extension by satellite formations. They can be separated by abnormal white matter tracts within the same hemisphere (demonstrated by contiguous areas of modified T2-weighted signal on cerebral MRI (**Figure 3**).

On the other hand, multicentric disease represents multiple tumors with normal intervening brain, so widely separated masses in different lobes or hemispheres (**Figure 4**).

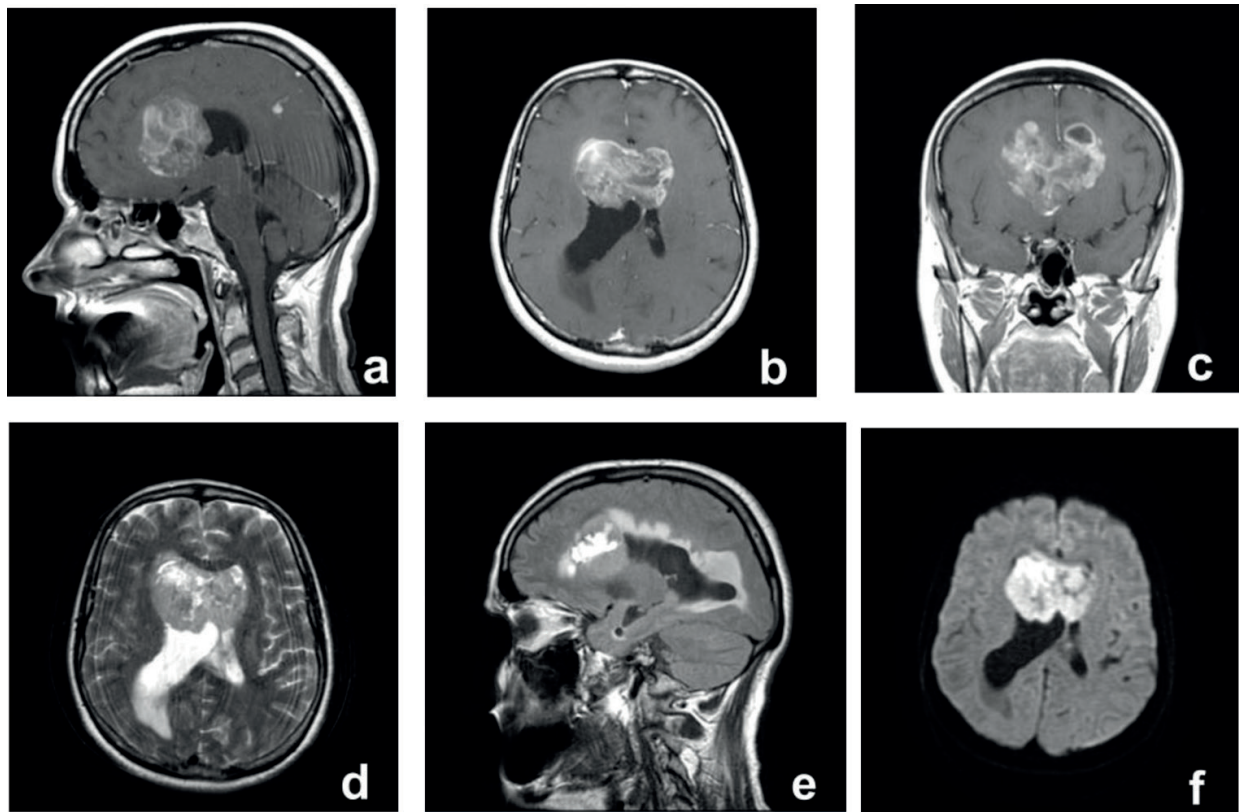
Although GBM is an invasive tumor, dissemination remains limited to the central nervous system and extracranial metastases are very rare (0.4–2%). GBMs usually appear like a mass with thick, irregular margins and a central necrotic core, sometimes with a hemorrhagic component. Tumors are surrounded by a vasogenic edema, characterized by extensive infiltration of tumor cells. This edema causes additional mass effect and leads to neurological disturbances.

The diagnosis of brain tumors must be evoked in any adult with symptoms of raised intracranial pressure, seizures or focal neurological deficit, the onset being usually weeks to months before. The **clinical presentation** is nonspecific and can vary widely, depending on the tumor localization and the rate of growth. Rarely, an intratumoral hemorrhage occurs and patient may present with sudden stroke-like symptoms. GBMs may occur at any age, but 70% of cases are seen between 45 and 70 years of age, with a mean age at the time of diagnosis being 53 years [10]. Men are more frequently involved (there is a sex ratio of 3:2).



**Figure 1.** Corticalized glioblastoma – Macroscopic appearance.

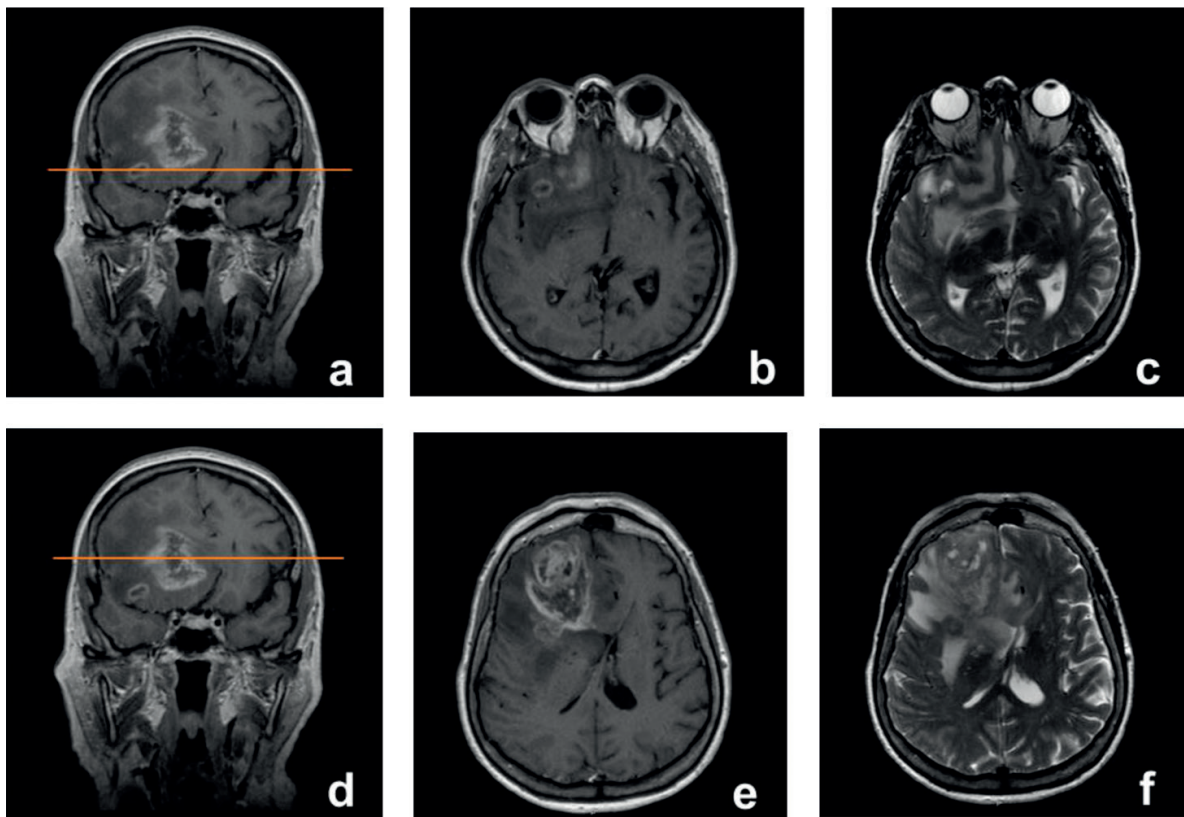




**Figure 2.** Butterfly glioblastoma – MRI features. Tumor involves both cerebral hemispheres by crossing the corpus callosum – Bifrontal localization. (a) Sagittal T1-weighted image with contrast; (b) axial T1-weighted image with contrast; (c) coronal T1-weighted image with contrast; (d) axial T2-weighted image; (e) sagittal fluid-attenuated inversion recovery (FLAIR) sequence; (f) axial diffusion-weighted image (DWI).

All patients, who are presented with symptoms that could be caused by an intracranial mass, require **neuroimaging** to establish the cause of these symptoms. **CT scan** is often the first examination, because it is widely available, fast and inexpensive. Typical findings for GBM on CT scan are a heterogeneous mass lesion, with an isodense to slightly hyperdense irregular thick ring and a hypodense core representing necrosis. There is an intense, irregular and heterogeneous contrast enhancement of the tumor mass (**Figure 5**). Images highlight a significant surrounding cerebral edema and a marked mass effect. CT scan is helpful in demonstrating the presence of intratumoral hemorrhage or calcification, which is thought to be related to a pre-existing oligodendroglial lesion.

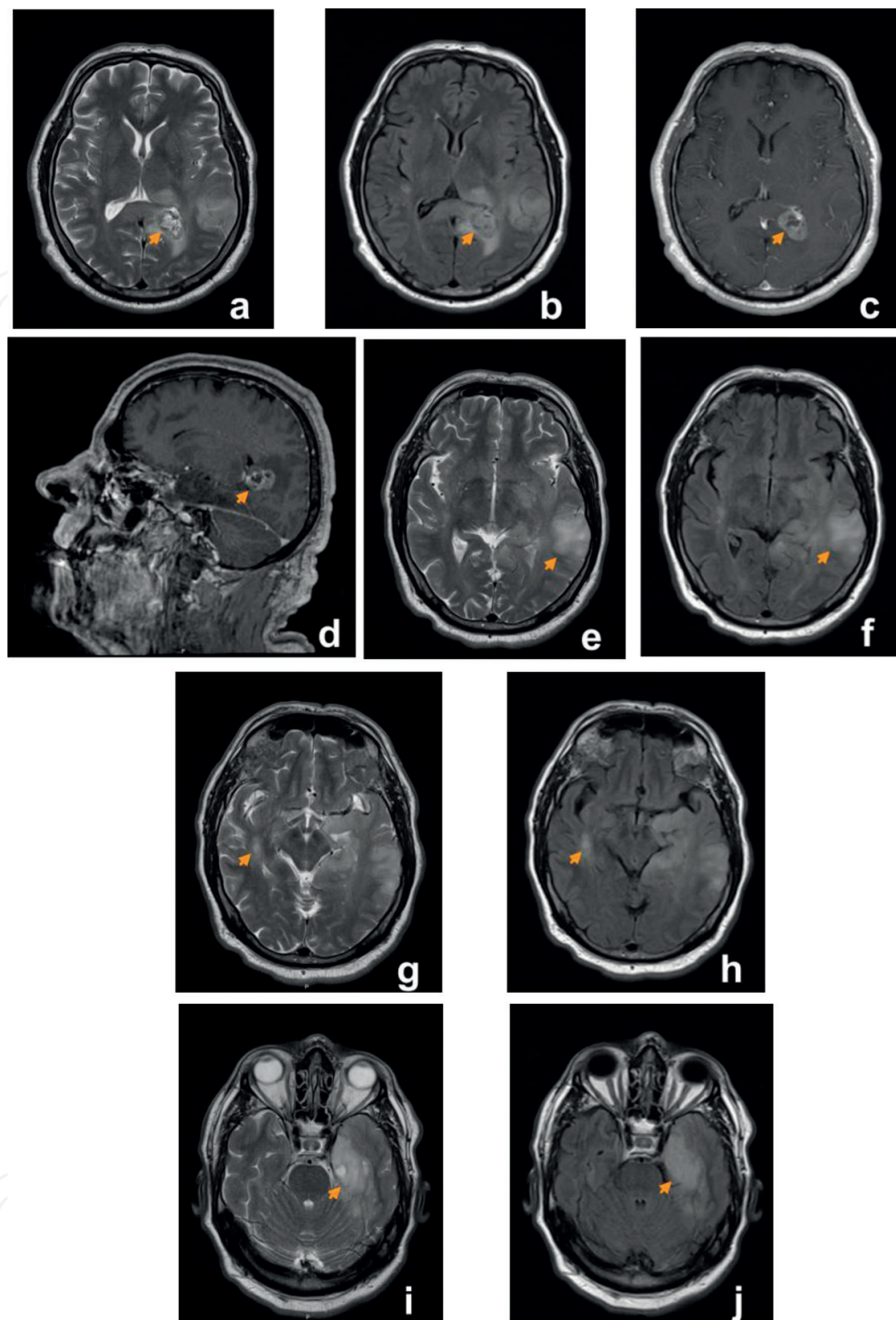
While CT scan provides initial data, **contrast-enhanced MRI** is the imaging modality of choice for GBMs, because of his greater accuracy and multi-planar imaging capabilities. All patients with a suspected brain tumor should have an MRI evaluation, unless it would be unsafe for them. It will confirm the diagnosis, will refine the diagnosis and will provide additional data needed for treatment planning. On T1-weighted images, GBMs typically appear as a hypo to isointense mass with central heterogeneous signal (necrosis, intratumoral hemorrhage and cysts), thick, irregular or poorly defined margins and peritumoral edema. After the administration of contrast medium, a heterogeneous or irregular ring-like enhancement is almost always present. T2-weighted and fluid-attenuated inversion recovery (FLAIR) images reveal a heterogeneous, hyperintense mass with adjacent tumor infiltration/vasogenic edema.



**Figure 3.** Multifocal glioblastoma – MRI features. There are two separate tumor foci in the right frontal lobe: A smaller one in the basal frontal region and a bigger one in the anterior frontal region. The presence of connecting signal alteration in T2-weighted images defines multifocal lesions. (a) Coronal T1-weighted image with contrast, with section line at the level of smaller tumor; (b) axial T1-weighted image with contrast – Section level is indicated in (a); (c) axial T2-weighted image – Section level is indicated in (a); (d) coronal T1-weighted image with contrast, with section line at the level of bigger tumor; (e) axial T1-weighted image with contrast – Section level is indicated in (d); (f) axial T2-weighted image – Section level is indicated in (d).

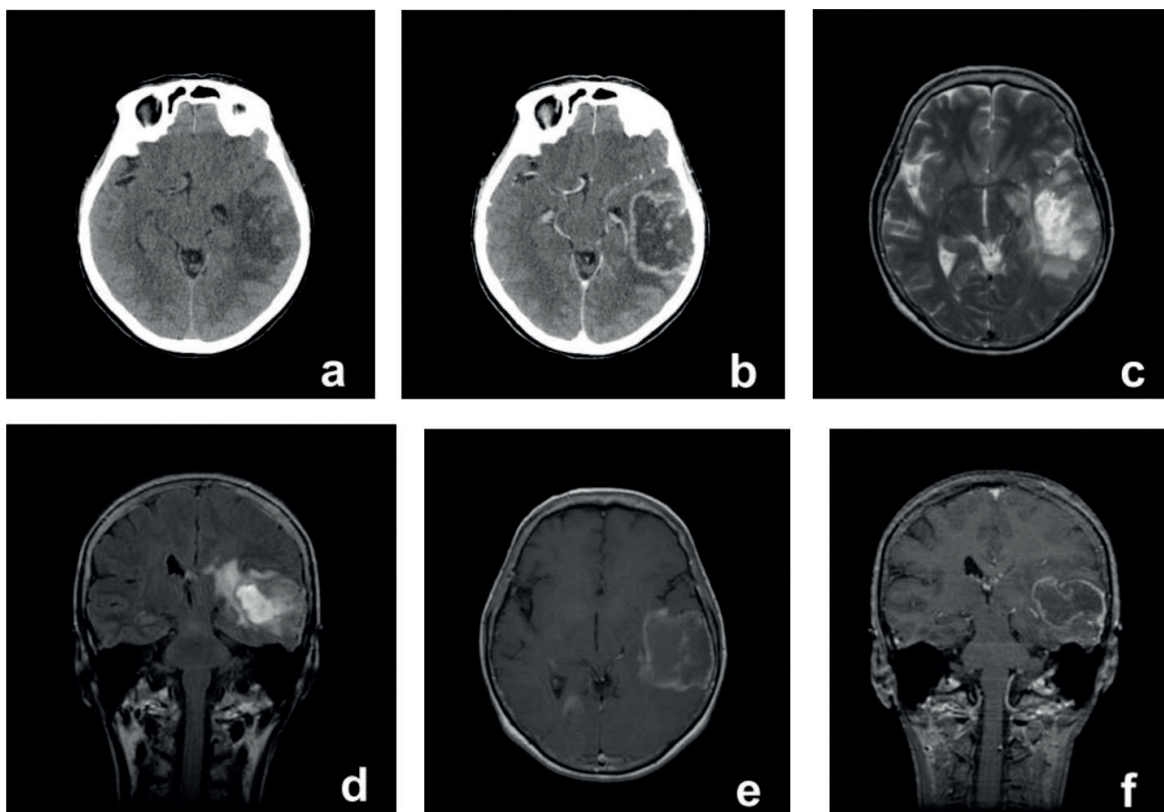
Surrounding infiltrative edema (which is a combination of increased interstitial water and neoplastic cells) is better appreciated in T2-weighted images as compared with T1-weighted images (**Figures 5 and 6**).

Advanced imaging technologies have been developed, including diffusion-weighted imaging (DWI), diffusion tensor imaging (DTI), perfusion-weighted imaging (PWI) and MR spectroscopy (MRS). Diffusion-weighted imaging (DWI) allows the calculation of the apparent diffusion coefficient (ADC) that is correlated with tumor cellularity and tumor grade. Diffusion tensor imaging (DTI) offers the possibility to identify and to characterize the white matter tracts. Perfusion-weighted imaging (PWI) provides useful information about the cerebral microcirculation and allows the development of cerebral blood volume maps. MR spectroscopy (MRS) allows in vivo measurements of certain tissue metabolites. These techniques focus on pathophysiological changes in disease and offer potential indications on differential diagnosis and individual anatomy. Post-therapeutic MRI examinations are used to monitor treatment response and to differentiate radionecrosis from residual or recurrent tumor.



**Figure 4.** Multicentric synchronous glioblastoma – MRI features. There are widely separated lesions, occurring in different lobes or hemispheres, with no connection between foci. They were already present at the time of initial radiological investigation. The figures (a), (b), (c) and (d) demonstrate a left paraventricular occipital tumor: (a) axial T2-weighted image; (b) axial FLAIR sequence; (c) axial T1-weighted image with contrast; (d) sagittal T1-weighted image with contrast. The figures (e) and (f) illustrate a left posterior temporal lesion: (e) axial T2-weighted image; (f) axial FLAIR sequence. The figures (g) and (h) present a right deep temporal lesion: (g) axial T2-weighted image; (h) axial FLAIR sequence. The figures (i) and (j) reveals a left temporomesial lesion: (i) axial T2-weighted image; (j) axial FLAIR sequence.

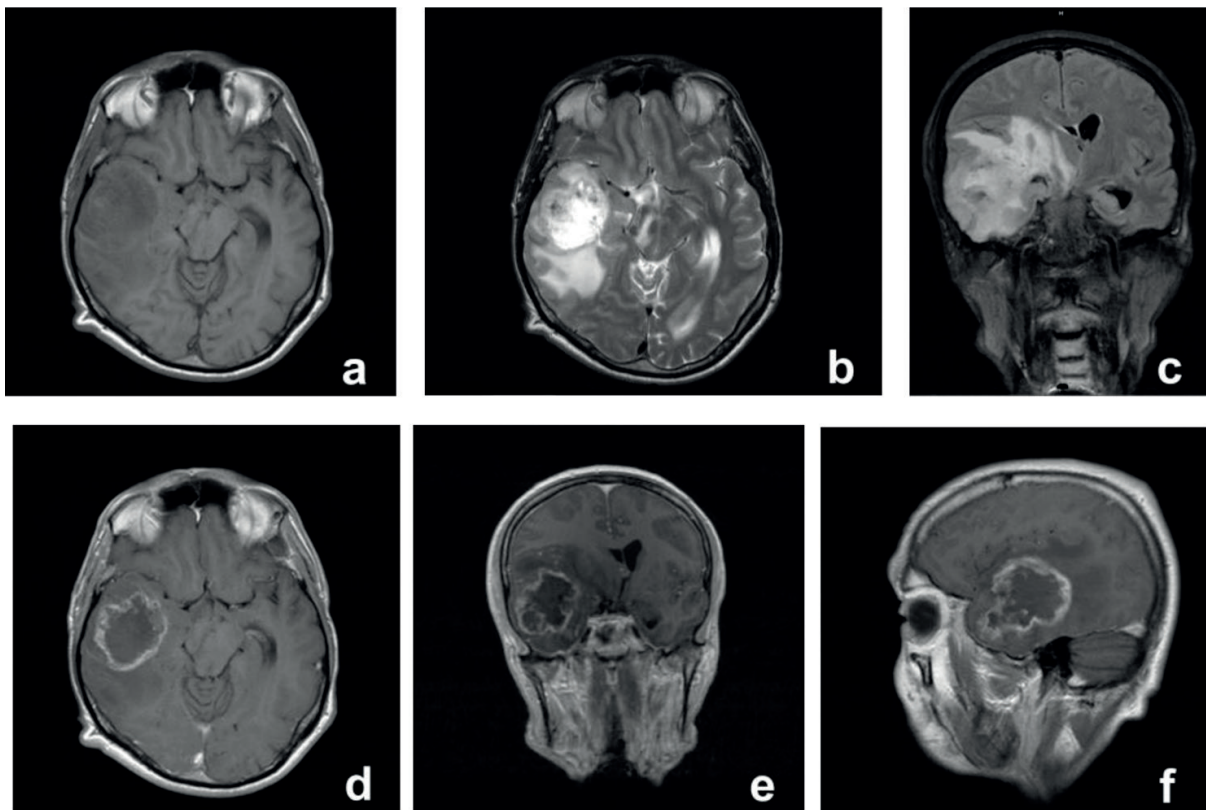




**Figure 5.** Left temporal glioblastoma. (a) CT scan without contrast; (b) CT scan with contrast; (c) MRI – Axial T2-weighted image; (d) MRI – Coronal FLAIR sequence; (e) MRI – Axial T1-weighted image with contrast; (f) MRI – Coronal T1-weighted image with contrast.

**Positron emission tomography (PET)** can be used to provide additional metabolic information of the tumor. This technique is based on the detection of radioactivity emitted by biochemically active molecules labeled with radiotracers. Different molecular processes can be investigated including glucose consumption, expression of amino acid transporters, proliferation rate, membrane biosynthesis and hypoxia. The glucose analog  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$  FDG) is the most commonly used radiotracer for PET to measure the local metabolic rate of glucose. Increased glucose metabolism is a feature of high-grade glioma (HGG) and a positive correlation between glycolysis rate and malignancy was demonstrated. Radiolabeled amino acids (like  $^{11}\text{C}$  Methionine— $^{11}\text{C}$  MET) have been introduced as suitable tracers in brain tumors, because amino acid transport as well as protein synthesis were both demonstrated to be enhanced in HGG. Even more,  $^{11}\text{C}$  MET has increased specificity and sensitivity, highlighting areas of cellular proliferation correlating well with the Ki-67 labeling index of proliferation and with microvascular density. PET can help distinguish GBMs from other brain lesions pre-operatively, can reveal malignant transformation in low-grade gliomas (LGG), and can evaluate the tumor extension for an appropriate site for biopsy, for surgery planning or for radiation therapy planning. PET is also important in assessment of treatment response, being beneficial for differentiation of tumor tissue from post-therapeutic changes.

In patients with a suspected diagnosis of GBMs, **initial management** is intended to control symptoms and prepare the patients for surgery. Corticosteroid therapy reduces peritumoral edema and alleviates symptoms of raised intracranial pressure and neurologic



**Figure 6.** Right temporal glioblastoma – MRI features. (a) Axial T1-weighted image; (b) axial T2-weighted image; (c) coronal FLAIR sequence; (d) axial T1-weighted image with contrast; (e) coronal T1-weighted image with contrast; (f) sagittal T1-weighted image with contrast.

symptoms, making surgery safer. Anticonvulsants are necessary when a history of seizures exists. However, prophylactic use of antiepileptic drugs outside the perioperative phase is controversial.

Ideally, all patients with GBMs should be managed by a multidisciplinary team in a centralized neurosciences center. The neuro-oncology group should include specialists from neuro-radiology, neurology, neurosurgery, neuropathology, intensive care, medical and radiation oncology, neurorehabilitation, etc.

#### 4. Surgical management

Neuroimaging modalities provide a lot of data about mass lesion, but cannot reliably predict the diagnosis of tumor type and grade. Histological assessment is required. Thus, **a representative tissue sample should be obtained by biopsy or resection** to have a correct diagnosis before specific adjunctive therapies have been initiated. The neurosurgeon is involved in decision-making regarding the appropriate surgical procedure for patients with GBM. Based on preoperative evaluation, he must indicate either an open surgical resection for both diagnosis and treatment or only a biopsy for diagnosis. Special consideration should be given to some important factors, including age of the patient, location and size of the tumor, neurological status, functional impairment (quantified by Karnofsky Performance Status (KPS) Scale), significant comorbidity

and patient and family preferences. Patients with GBM should have **surgery for maximal tumor removal** whenever safe, because this could prolong their survival when compared with biopsy, subtotal or partial resection. **Biopsy** is only indicated in cases of “inoperability” of the tumor, because of the associated risks are minimal. The image-guided stereotactic techniques are preferred over open biopsy.

#### 4.1. Stereotactic biopsy

Stereotactic biopsy enables safe retrieval of sufficient material to allow pathologic diagnosis from precise targets in GBMs with the help of MRI or CT scan. Framed or frameless stereotactic biopsy can be performed. The main indications are inaccessible tumor location (deep-seated lesion), multiple or bilateral disease, potential unacceptable surgical morbidity because of eloquent adjacent brain areas, poor performance status (KPS < 60), lesion in a surgically poor candidate because of significant medical problems. When the diagnosis of GBM versus other space-occupying lesions is in doubt, biopsy may be a better initial step. Stereotactic biopsy is a minimally invasive technique, with low risk and good diagnostic accuracy, which can provide valuable information, guiding further treatment. However, an average morbidity rate of 4.1% (range, 0.7–7%) and a mortality rate of 0.9% (range, 0.2–2.3%) have been demonstrated [11]. Owing to histological heterogeneity, it leads to an inaccurate or imprecise diagnosis in about 10% of cases. There are approximately 15–20% of patients who undergo only biopsy as a surgical procedure [12].

#### 4.2. Open surgical resection

To date, surgery for resection remains **the first and the most important treatment modality** in GBMs. **The goal of surgery** should be to remove as much of the tumor as possible, while minimizing damage to surrounding healthy brain. Unfortunately, surgery is not curative. Because of the highly aggressive and invasive nature of the GBM, a complete resection is not possible. Despite the relative lack of appropriately designed trials, experience strongly supports the fact that gross-total resection (GTR) of the entire area of gadolinium-enhancement tumor is associated with improved outcome. Therefore, the current trend is to perform maximal tumor removal whenever possible, while minimizing the risk for unacceptable neurological deficit, aimed to both improve the quality of life and prolong survival.

There are **some reasons for surgery** on lesions thought to be GBMs. The first indication is to obtain a histological diagnosis. Owing to glioma histological heterogeneity, multiple biological samples from separate places of the tumor should be taken and examined. Open resection can provide a larger tissue specimen as compared to biopsy. Provision of tumor for research and scientific analysis also could be beneficial for the patient. The second indication is to perform a surgical decompression that can relieve intracranial hypertension, can improve neurologic functions and can prevent death due to brain herniation. The third indication is to reduce the tumor mass as much as possible. Reduction of the tumor burden provides a rapid drop in the global tumoral cell population, removes resistant cells and prolongs survival. Extensive resection of the tumor may potentiate or facilitate radiation therapy, chemotherapy, immunotherapy or other modalities of treatment. The fourth indication is to deliver adjuvant therapies, including intratumoral chemotherapy, intracavitary brachytherapy, gene therapy, immunotherapy, photodynamic therapy, etc. Radical surgery to the extent feasible

should be recommended whenever is possible, regardless of age. Relative contraindications include inaccessible or eloquent location, poor performance status and important comorbidities. Typically, tumors located in the basal ganglia, thalamus, corpus callosum, brain stem or multiple tumors are biopsied only.

**Proper patient selection and preoperative planning** are very important for the success of the surgical intervention. The decision to undergo radical surgery needs to be reasonable and the surgical approach must be individualized for each patient. Careful assessment of the preoperative MRI imaging studies is essential for preoperative planning. The tumor location determines the type of approach to be used and the optimal trajectory to the lesion. The neurosurgeon should measure the tumor dimensions in all three axes on the contrast-enhanced MRI and compare them with on-site measurements for a good estimation of the extent of resection (EOR). If there is concern regarding proximity of the tumor to eloquent areas, a functional MRI can help to highlight the location of critical brain regions. Consequently, the surgeon can plan the operative technique and can take the decision to perform intraoperative mapping (sometimes an awake craniotomy is needed). The blood oxygenation level-dependent functional MRI (BOLDfMRI) is used in the clinical practice for presurgical mapping of motor areas and language areas (lateralization and localization). It works by recording subtle changes in blood oxygenation and flow that occur in response to a particular neural activity. It produces activation maps.

Maximal safe tumor resection represents the mainstay in GBM treatment. **Tumor removal** involves standard neurosurgical techniques. A good knowledge of surgical anatomy and a meticulous microsurgical technique, while preserving brain functions are essential. To increase the precision and the safety of the surgery, the specialist can use various technologies which allow intraoperative guidance. Neurosurgery for patients with GBM should be conducted in accredited facilities, that have the appropriate neurosurgical equipment and trained staff and where there is a specific multidisciplinary team.

Ideally, **the extent of resection (EOR)** should be assessed after surgery. This must be carried out by a contrast-enhanced MRI within 24–48 hours postoperatively, in order to distinguish between residual GBM, postoperative reactive changes and parenchymal damage as a result of surgery. Postoperative contrast-enhancing tumor mass is typically used to delineate residual GBM and completeness of removal. It is better to use volumetric analysis of the preoperative and postoperative tumor to accurately measure EOR and residual volume (RV). Reactive postoperative changes can be seen as early as 18 hours on MRI, but usually does not appear in the first 3–4 days. The EOR was identified as a strong prognostic factor for survival in GBM, together with patient's age and patient's functional status. Surgical removal has a critical role in GBM management because the only potentially modifiable risk factor associated with survival is EOR. The gross-total resection is not always possible. Thus, several studies have been conducted to evaluate EOR threshold which may serve as minimum surgical goal to achieve. Other studies demonstrated that EOR is not an ideal indicator to the success of the surgery, because it is a percentage value, reported to initial volume of the tumor, which can vary widely. Contrast-enhancing RV is considered a more clinically relevant measure and a stronger predictor of survival than EOR, representing the tumor mass existing prior to starting medical therapy. Chaichana et al. in 2014 evaluated newly diagnosed GBM patients who



underwent surgery and found that the minimum EOR of 70% and the maximal RV of 5 cm<sup>3</sup> showed statistical significance for prolonged survival and delayed recurrence [13]. Grabowsky et al. in 2014 reported that RV of 2 cm<sup>3</sup> or less confers survival benefit to the patient [14]. There are models that argue for a continuous relationship between EOR and median survival, suggesting that there is a survival advantage associated with any degree of resection [15]. This is evident for the practice of maximal safe resection for GBM.

Surgery is associated with some **risk**. Complications encountered in open surgery for GBM are those of craniotomy in general. There are reported morbidity rates ranging from 5 to 15% and mortality rates from 1 to 5% [8].

### 4.3. Intraoperative neurosurgical guidance

The key issue for glioma surgery is to accurately delineate the tumor into the operative field, which can be a challenge for the neurosurgeon. Many useful tools have been created to help the surgeon differentiate between tumor and normal tissue. It is important to adapt modern technologies to successfully guide maximal surgical resection without postoperative neurological deficit. Multiple studies suggest that extensive resection is beneficial for the patient. But an excessive excision should be avoided, since it can induce permanent neurological dysfunction. At the same time, an incomplete resection can be therapeutically ineffective. The ideal goal of neurosurgery is to maximize the resection of the tumor mass safely, without impairing eloquent functions and quality of life. For higher efficacy and lower risk, the current concept of neurosurgery is an “information-guided surgery”, using multimodal intraoperative information to identify the positions of the eloquent brain areas accurately and in real-time [16, 17]. Anatomical information from navigation, ultrasonography and intraoperative MRI, functional information from mapping and monitoring and histopathological data must all be considered to prevent unexpected deficits and promote extensive resection.

#### 4.3.1. Image-based navigation

**MRI neuronavigation (frameless stereotactic navigation)** is based on preoperative MR-imaging data, taken with fiducial markers that are left in place on the scalp. This data is projected into the operative field for better anatomical orientation. It is useful for surgical planning and image guidance, particularly when the tumor cannot be seen on the cortical surface of the brain. However, it is rendered unreliable when variations in brain volume or shifts of the intracranial content appear during the surgery, because this technology is based on a preoperative set of images, without updating during surgery.

**Intraoperative ultrasonography** is helpful when the tumor is not isoechoic with the brain or the density difference is greater (when there is a hematoma or a cystic component into the mass lesion). It is a dynamic imaging modality that can guide the neurosurgeon in real-time during resection. It has the advantage that brain shift and brain relaxation that occur during the excision of the lesion do not influence the accuracy of the procedure. Three-dimensional sonography with navigation software solves any orientation problems.

**Intraoperative MRI** systems are available, but the equipment is expensive and therefore the access is somewhat restricted for many neurosurgeons and patients alike. It has the advantage to avoid potential errors caused by brain shift. It provides information about the completeness of tumor resection during surgery and allows the surgeon to perform an additional tumor excision, if needed. However, it has a limited ability to delineate between residual glioma and adjacent normal brain. The system has been shown to improve the extent of tumor removal.

#### 4.3.2. *Intraoperative functional mapping and monitoring*

Intraoperative functional mapping and monitoring are essential for safe excision of GBM localized near eloquent cortex. It can accurately identify individual eloquent brain areas, including somatosensory cortex, motor cortex and language cortex, enabling the neurosurgeon to avoid these regions during tumor resection and thereby minimizing the risk of neurological morbidity. One of the most important advantages of this method over the imaging techniques is allowing assessment of the cortical and subcortical function in real-time. In addition, continuous monitoring of the patient's neurological findings during surgery is very useful for intraoperative feedback to the surgeon. Using these functional methods, the edge of resection can exceed the anatomical borders of the tumor (contrast-enhancing regions) to reach the functional border of the tumor (placed in the peritumoral tissues, invaded by the tumoral cells) [18].

**Localization of the primary somatosensory cortex** can be achieved by somatosensory evoked potentials (SSEPs) mapping, performed under general anesthesia or in awake patient. Techniques are similar to those used for routine diagnostic studies. Evoked potentials are recorded by stimulating peripheral afferent nerves (median nerve, posterior tibial nerve, etc.), usually electrically. Recording electrodes are placed on the cortical surface (typically proximal to the lesion). When recording SSEPs, the primary sensory cortex and primary motor cortex generate potentials that are mirror images of each other. This "phase reversal" across the central sulcus aids in the localization of the primary motor cortex. Localization of the primary somatosensory cortex can also be performed by direct cortical electrical stimulation of the postcentral region. The awake patient communicates the presence or absence of the sensory symptoms triggered by stimulation.

**Localization of the primary motor cortex** can be accomplished using the SSEPs "phase reversal" technique or by direct cortical electrical stimulation (with patient under general anesthesia). It is recommended to use both techniques, starting with central sulcus identification and continuing with cortical stimulation of precentral regions and recording the muscle motor evoked potentials (mMEPs) from the corresponding muscles or observing clinical movements [19]. The former technique is preferred, because the stimulation threshold for obtaining mMEPs is smaller than that for obtaining clinical movements [8, 17, 19]. Thus, the risk of eliciting local or generalized seizure activity is decreased. During the cortical stimulation, simultaneous electrocorticogram (EcoG) recording is required for the safety of the patient. It is used to identify spontaneous or stimulation-induced epileptic discharges (after discharges), marking a subclinical seizure activity. It is important to have an adequate serum anticonvulsant level pre-operatively and, if necessary, additional intravenous boluses of antiepileptic drugs may be considered [8]. It is of paramount importance to distinguish primary from

supplementary motor areas as it is known that damage of the motor strip will cause a permanent postoperative motor deficit, while damage of the supplementary and premotor areas will result in a temporary postoperative deficit. Once the motor strip was identified, direct cortical stimulation or subcortical stimulation can be used for continuous evaluation of the functional integrity of the motor pathways during glioma resection.

**Localization of the language cortex** is performed under awake craniotomy (AC), by cortical and subcortical direct electrical stimulation (DES). A “positive mapping” strategy can be used: a large craniotomy exposes the brain a good distance from the tumor and makes it possible to identify “positive” language sites (areas where a cortical stimulation induces a language function) prior to excision. Lately, a “negative mapping” strategy emerged as preferable. It supposes to identify “negative” language sites, meaning regions where a cortical stimulation blocks a language function. This technique allows a smaller, tailored craniotomy, with a minimal cortical exposure around the tumor (up to 2 cm of surrounding brain) and a less extensive mapping. It is a more time-efficient neurosurgical procedure [17, 20, 21].

It is important to emphasize that stimulation mapping is used to identify essential language cortex, whose injury will lead to permanent deficit. But there are also multiple nonessential speech areas. The essential language cortex is obviously different from involved language cortex identified by functional imaging techniques, such as functional MRI (fMRI) and positron emission tomography (PET). Although these imaging techniques have advanced considerably, they have some limitations and cannot replace intraoperative mapping. Patients who speak multiple languages have separate language sites for each of their different languages [8]. Different language tasks performed by a patient may lead to delineate distinct language sites. There is significant individual variability in the location of the language areas, sometimes the Broca area or the Wernicke area having a location beyond the classic anatomical boundaries or more than two essential speech areas being identified. Quinones-Hinojosa et al. found a variability of more than 4 cm in the location of speech arrest when using classical neuroanatomic landmarks [22, 23]. Furthermore, cerebral topography is distorted by the tumor mass effect and brain plasticity can induce a functional reassignment [17, 20, 23]. Thus, intraoperative identification of the language areas is essential for extensively and safely removing GBMs located near these eloquent regions in the dominant-hemisphere. It is best to continuously monitor the patient’s ability to speak, especially during the part of the excision which is close to the identified language sites (within 2 cm). If the distance between resection border and the nearest language area is more than 1 cm, significantly fewer permanent language deficits occur [20, 24]. A subcortical stimulation can be used into the resection cavity to guide the removal technique (when stimulation block the language function, the location is very close to the subcortical language pathways – 5 mm or less) [17, 25].

A new intraoperative method to assess integrity of functional interconnections between language areas during surgery was proposed by Yamao et al. [17, 26, 27]. The authors monitored the integrity of the dorsal language pathway (arcuate fasciculus) using cortico-cortical evoked potentials (CCEPs). The technique is based on the electrical stimulation of the anterior perisylvian language area while recording the average response from posterior perisylvian language area. It is clinically useful for evaluating the integrity of the language network and have the advantages that is task-free, do not require the cooperation of the patient and therefore can be performed also under general anesthesia [27, 28].

#### 4.3.3. Enhanced visual tumor demarcation

The ideal technique for sharper intraoperative delineation between tumor and the surrounding cerebral tissue should provide real-time information during resection, without concern about changes into the operative field and still be affordable.

Intraoperative tumoral tissue fluorescence due to specific enhancing agents provides a real-time GBM discrimination *in situ*. The differences are visualized using specially designed microscopes, equipped with appropriate filters to detect fluorescent light emission. Fluorescence is the emission of light with a short wavelength by a substance that has absorbed light of a longer wavelength. Fluorochrome is a fluorescent dye, used to stain biological material before microscopic examination. In neurosurgery of the gliomas, a specific fluorochrome is associated with glioma tissue (selectively if possible) and then illuminated by light. Fluorescent dye will emit light, which will be perceived by the surgeon using special filters. There are four types of approaches to intraoperative fluorescence: (1) tissue fluorescence induced by specific metabolic activity; (2) tissue fluorescence-based on passive permeability; (3) tissue fluorescence due to targeted fluorescent probes accumulated into the tumor tissue; and (4) autofluorescence [29].

**Tissue fluorescence induced by specific metabolic activity** is the basis to use of 5-aminolevulinic acid (5-ALA) in fluorescence-guided surgery. 5-ALA is an endogenous amino acid, the first compound in the porphyrin synthesis pathway. It is finally converted to protoporphyrin IX (PPIX), which chelates with iron in presence of enzyme ferrochelatase to produce heme (component of hemoproteins). GBM cells lack or have reduced ferrochelatase activity and this results in accumulation of protoporphyrin IX into the tumor tissue after oral administration of 5-ALA. Protoporphyrin IX is clearly visualized by its red fluorescence under blue-violet light conditions, enabling differentiation of viable tumor from normal adjacent brain. 5-ALA is the only agent that has been approved in fluorescence-guided neurosurgery in Europe, Canada and Japan, and is commonly used in surgery of GBMs. It induces GBM tissue fluorescence, having high sensitivity, specificity, and positive predictive values for identifying malignant glioma tumor tissue [30, 31]. In recurrent malignant gliomas, fluorescence is observed in anaplastic foci, in regions of gliosis or invaded by inflammatory cells, but not in normal brain. Prior alternative treatment such as radiotherapy or chemotherapy does not invalidate 5-ALA-induced fluorescence [32]. Fluorescence can discriminate malignant glioma cells down to a tumor cell density of approximately 10% [29, 33]. It is now demonstrated that visible fluorescence clearly extends beyond the border of preoperative MRI contrast-enhancement, PPIX accumulation being more sensitive than gadolinium enhancement [33, 34]. Thus, an extensive glioma resection beyond radiologically evident tumor can be performed. 5-ALA-guided resection of GBM was found to be beneficial, enabling surgeons to achieve a double rate of complete resections of malignant gliomas in comparison with conventional techniques [31, 35]. A randomized controlled multicenter phase III trial conducted by Stummer et al. involving 270 patients in 17 centers has examined a group undergoing 5-ALA fluorescence-guided surgery and a group undergoing conventional white light-guided surgery. The authors reported that gross-total resection evaluated on postoperative imaging was 65% in cases undergoing fluorescence guidance compared with 36% in the white light group ( $p < 0.001$ ), and progression-free survival was 41 versus 21.1% ( $p < 0.003$ ) [35].



**Tissue fluorescence based on passive permeability** uses fluorescein or indocyanine green, which has not been approved for intracranial use. Fluorescein is a typical marker of compromised blood-brain barrier (BBB), rather than a selective tumor marker, therefore its presence is highly nonspecific. It displays a yellow-green fluorescence visualized by the naked eye. Given its limited specificity, there is a great risk to remove normal, functional brain tissue, and given its sensitivity concerns, there is a risk of leaving residual tumor [36]. Recently, a dual-labeling approach has been proposed, using both PPIX and fluorescein fluorescence simultaneously. The advantage is that PPIX provides a reliable tumor detection and fluorescein gives a better background visualization, as it would be expected to accelerate surgery, while maintaining safety and efficacy [37]. Indocyanine green enables evaluation of tumoral and peritumoral blood flow and vascularization. It has the advantage of emitting light in the near-infrared region of the spectrum, therefore the fluorochrome can be visualized deeper in the tumoral tissue. However, the visualization requires special technologies.

**Tissue fluorescence due to targeted fluorescent probes** accumulated into the malignant tumor tissue is an ongoing subject of research. There are some fluorescent agents targeted or being retained by brain tumor cells undergoing clinical testing. Their effective application in clinical settings requires development of detection instrumentation and additional studies. Agents that show promise for intraoperative discrimination of GBM include Tumor Paint (chlorotoxin linked to a fluorophore), Angiopep-2 targeting agents, epidermal growth factor receptor (EGFR)-targeted agents, PTP $\mu$ -targeted SBK agents, the fluorescently labeled poly (ADP-ribose) polymerase 1 (PARP-1) inhibitor (CLR1502) and  $\alpha v\beta 3$  integrin-targeted agents [38, 39].

**Microspectrofluorometry** can be used to measure the autofluorescence spectrum of biological tissues both *ex vivo* on resected samples and *in vivo*, during surgery, by means of fiber optic probe. It is a dye-free method, based on the intrinsic autofluorescence properties of a tissue. In glioma, the autofluorescence profile is distinct from normal brain, due to changes of biochemical composition and histological organization. There are differences in both spectral shape and signal amplitude relative to normal cortex and white matter. These differences allow the use of autofluorescence *in situ* as a parameter for distinguishing neoplastic from normal condition and so to better delineate GBM resection margins [40–42].

**Confocal microscopy (laser scanning confocal microscopy – LSCM)** may provide *in vivo* images by optical sectioning, characterized by higher resolution and contrast, with magnification up to 1000x. These images enable intraoperative visualization of tumor histopathological features and cell morphology in real-time, in three dimensions, without the need for extensive traditional tissue processing [36]. Intraoperative confocal imaging correlates with histopathological analysis, the diagnostic accuracy being of up to 93% [43]. The major application of confocal microscopy is for imaging tissues labeled with fluorescent probes. In GBM surgery, confocal microscopy combined with tissue fluorescence provides a reliable identification of tumor cells and tumor-brain interfaces.

#### 4.3.4. Intraoperative sampling

The diagnostic of GBM is usually confirmed by standard postoperative histopathological examination of tissue sections with results only available several days after the surgery has

finished. But the maximal removal of the glioma is the key component in the specific treatment, a smaller volume of postoperative residual tumor being associated with an improved prognosis. One of the difficulties of achieving an optimal excision is failure to delineate the resection margins. Nevertheless, histopathological assessment is also available during the surgery, providing important diagnostic information. Even if such information is less reliable compared with that of postoperative approaches, sometimes **intraoperative sampling** is the sole source of diagnostic arguments for deciding the extent of resection. Precision increases with the number of tissue sections. Traditional histopathological techniques made intraoperative include frozen section and imprint cytology. They are time-consuming (requiring nearly 30 mins), laborious and subjective. It is desirable that they are performed by a skilled pathologist.

**Mass spectrometry-based molecular analysis** can rapidly provide detailed molecular information about tumor and adjacent brain tissue, allowing an intraoperative diagnosis and guidance in detection of the boundaries between glioma and normal brain. The desorption electrospray ionization mass spectrometry (DESI-MS) is a mass spectrometric imaging technique for characterizing lipid profile within tumor specimens. Because DESI-MS can be performed rapidly (minutes) and routinely, in the ambient conditions, with minimal pretreatment of biological samples, it can be used during surgery. It quickly provides a valuable diagnosis of tumor type based on lipid pattern [44]. It can also detect oncometabolites: 2-hydroxyglutarate and N-acetylaspartate. 2-hydroxyglutarate is present in small amounts in normal brain tissue, but its concentrations are extremely high in gliomas with mutations in IDH1 and IDH2 [45–47]. It could be used as a biomarker and serve as an important prognostic indicator. Detection of 2-hydroxyglutarate in operative field with precise spatial distribution could also help define surgical margins. DESI-MS provide valuable information that is unattainable by traditional histopathological techniques.

#### 4.4. Intratumoral therapies

Given that GBM is typically a solitary tumor, with local recurrence and very rare metastases, the disease is a proper candidate for local treatment. On the other hand, availability of drugs which can cross the BBB has severely limited the effective therapies against GBM. Strategies to bypass this barrier have been developed. Localized drug delivery into a postoperative tumor bed is an attractive option for administration of therapeutics while avoiding systemic side effects. Furthermore, this way provides a means for administration of new, tumor-selective molecules that are often largely excluded by brain.

Controlled-release polymer systems, like carmustine wafers (Gliadel wafers) can be implanted in the resection cavity. Another local approach is catheter-based convection-enhanced delivery (CED) of conventional or novel agents through continuous low-positive-pressure bulk flow. Intracavitary delivery of highly localized doses of irradiation is feasible through Gliasite system brachytherapy.

#### 4.5. Recurrence

Standard therapy in newly diagnosed GBM involves maximal safe surgical resection followed by radiotherapy (RT) with concurrent and adjuvant TMZ. Despite this first-line treatment, recurrence inevitably occurs, most patients experiencing it after 7–8 months of primary

treatment. There are no well-defined management protocols for recurrent GBM. Options for second-line treatment are limited and include repeat surgery, re-irradiation, chemotherapy, novel therapies, supportive care or, better, a combination of these.

**The standard neuroimaging modality** for the follow-up of GBM is contrast-enhanced MRI, which is performed every 2–3 months while the patient is on therapy. Criteria to assess treatment response and progression have been established by the Response Assessment in Neuro-Oncology (RANO) Working Group [48]. Progression is defined as at least 25% increase in the contrast-enhancing MRI lesion (the product of the maximal cross-sectional enhancing diameters of tumor area). Diagnosing a true progressive tumor growth after chemoradiation by MRI alone remains a challenge, because it is very difficult to distinguish between post-treatment radiation effects (such as pseudoprogression or radiation necrosis) and tumor recurrence. Post-treatment radiation effects can be divided into pseudoprogression and radiation necrosis. Pseudoprogression appears several weeks up to 3 months after RT (5.5–31%), whereas radiation necrosis occurs 3 months to years after irradiation (3–24%) [49]. Radiation necrosis is a space-occupying necrotic lesion, with mass effect and neurological dysfunction. It is irreversible and progressive. Its features on MRI are often identical to that of recurrent GBM. The differentiation is very important, because the management is different. Advanced MRI techniques such as DWI, DTI and PWI provide additional information. Metabolic imaging techniques like PET, single-photon emission computed tomography (SPECT) and MR spectroscopy (MRS) are helpful in differentiating between tumor recurrence and therapy-related changes. Tumor recurrence appears as a lesion metabolically active, while radiation necrosis appears metabolically inactive. However, no imaging modality has sufficient specificity and tissue biopsy remains the gold standard to obtain a definitive diagnosis.

GBMs typically recur focally and in many cases surgery is possible. **Repeat surgery** is performed in approximately 25% of cases. Although a repeat surgery is associated with a higher complication rates than the initial surgery, this increase is rather small and clearly acceptable [50, 51]. However, its efficacy is debated. Many recent studies reported a survival benefit and an improvement of quality of life resulting from repeat resections in selected patients. Performing an overview of the current literature on second surgery for recurrent GBM, Montemurro et al. found the median overall survival from diagnosis being 18.5 months and the median survival from second surgery being 9.7 months [51]. Extent of resection at reoperation has been demonstrated to improve overall survival, thus a maximum safe excision should be the surgical goal. The decision of a second surgery should be individualized and should involve a multidisciplinary team approach. The age and the preoperative performance status are the most important predictors of a prolonged survival. A more favorable prognosis following surgery for recurrence is associated with a younger age (< 60 years) and a good preoperative performance status (KPS  $\geq$  70) [51]. Reoperation is not recommended for patients with involvement of eloquent brain regions.

Thus, patients with recurrent GBM may benefit from resection of tumor whenever safely possible. Repeat surgery can help in providing symptom relief and differentiating tumor recurrence from pseudoprogression, radiation necrosis, respectively. But surgery should be followed by adjuvant therapies.

## 5. Radiation therapy

**The SoC for newly diagnosed GBM** consists of maximal safe surgical resection followed by radiotherapy (RT) plus concomitant and adjuvant TMZ.

Following gross-tumor removal, the final histological diagnosis is established, and RT should start. **The optimal time to initiate** radiation is controversial. There are studies showing worse outcomes and even decreased survival when radiation is delayed [52, 53]. Irwin et al. found that a 6 weeks delay (from 2 weeks postoperative to 8 weeks) reduces median survival by 11 weeks for a “typical” patient [52]. But there also studies showing no association between timing of radiation initiation and outcomes [54, 55] and studies suggesting a possible benefit of delay (however, up to a reference range of time) [56, 57]. Blumenthal et al. analyzed the relationship between the delay of RT and the outcome on a large cohort of more than 2800 patients. They observed no obvious reduction in survival with increasing delay (within relatively narrow temporal limits—6 weeks). Indeed, median survival time was unexpectedly greater in the group with the longest interval (>4 weeks) than in those with the shortest delay ( $\leq 2$  weeks), respectively, 12.5 months versus 9.2 months ( $P < 0.0001$ ). The authors do not exclude the possibility that an adjuvant treatment initiated beyond 6 weeks postoperatively may be detrimental [56]. In other studies, Han et al. found a narrow range of time (from 30 to 34 days after surgery) where there is prolonged overall survival and prolonged progression-free survival compared with early initiation of concurrent chemoradiation [57, 58]. In common practice, the patient commonly waits about 4 weeks before adjuvant therapies. It is generally agreed that a postoperative delay of 6 weeks may not be critical.

**Concomitant TMZ and RT** (known as the Stupp regimen) have been shown to be more effective than radiation alone with minimal additional toxicity. **After the end of radiation, an adjuvant treatment with TMZ is indicated.** Patients who received RT and concurrent TMZ presented a median survival of 14.6 months versus 12.1 months with RT alone [59]. Furthermore, the two-year survival rate was 26.5% with RT plus TMZ versus 10.4% with RT alone. This is the current SoC for patients with newly diagnosed GBM up to age 70, with a good performance status (Karnofsky Performance Status (KPS)  $\geq 60$ ).

RT using three-dimensional conformal beam or intensity-modulated RT is used now. The typical total dose delivered is 60 Gy in 2 Gy fractions, administered 5 days per week for 6 weeks and there is no evidence that higher doses improve outcome [60, 61]. The RT involved fields should include the tumor bed with a 2–3 cm margin, based on the observation that GBM commonly recurs within 2 cm of the original tumor location in 80–90% of cases.

The optimal management of **elderly patients** is controversial. In practice, for patients >70 years old or for patients <70 years old with a poor performance status (KPS < 60), an alternative hypofractionated regimen can be considered. For elderly not suitable for radiation, chemotherapy alone may be an option.

Despite maximal multimodal treatment, GBM invariably recur, disease progression occurring within the first year in about 70% of cases. In selected cases of **recurrences**, a second course of radiation may be possible, but tolerance of local brain tissue to radiation is limited and there



is an increased risk of radiation necrosis. This may lead to neurological dysfunction, edema and mass effect. Radiation necrosis is very difficult to distinguish from progressive disease solely by imaging techniques. Histology remains the gold standard for diagnosis. Combs et al. investigated the role of re-irradiation using the fractionated stereotactic approach and demonstrated a median survival of 8 months and a progression-free survival of 5 months for patients with GBM [62].

**Stereotactic radiosurgery** offers the potential of providing a “boost” radiation to a portion of the radiation field in newly diagnosed patients or treating a small recurrence, being an alternative to open surgery [63]. However, its applicability remains very limited in absence of studies which could demonstrate a statistically significant benefit.

**Intracavitary brachytherapy** using the GliSite system can be used in selected newly diagnosed patients or in recurrent disease, intending to deliver an additional radiation to the surgical cavity wall. It is a medical device, composed of a balloon that will contain a radioactive solution with  $^{125}\text{I}$  during the period of irradiation, connected through a catheter to an infusion port. The balloon is placed in the resection cavity during surgery and the radioactive solution is injected later. Re-irradiation of recurrent GBM with GliSite Radiation Therapy System after resection seems to provide a median survival of approximately 9 months [64, 65].

## 6. Chemotherapy

For the time being, **TMZ** is considered the first-line chemotherapy drug in GBM. It is an oral systemic drug with a good penetration of the BBB and limited side effects. The mechanism of action is based on its ability to alkylate/methylate DNA. This alkylation damages the DNA and triggers the death of tumor cells. **MGMT** (O<sup>6</sup>-methylguanine-DNA methyltransferase) is a DNA-repair enzyme that rescues glioma cells from damages induced by alkylating agents like TMZ or carmustine. High activity of MGMT in tumor cells creates resistance to chemotherapy with alkylating agents and may determine treatment failure. Epigenetic silencing of the MGMT gene by promoter methylation is associated with decrease of DNA-repair activity and thus tumor cells will be more responsive to TMZ. In other words, the methylation status of MGMT promoter is associated with a benefit from alkylating agent-based chemotherapy in GBM. Numerous studies have confirmed that carriers of the methylated form of MGMT promoter with GBM treated with TMZ and RT have a prolonged overall survival [66–68]. Hegi et al. found that their median survival was 21.7 months as compared with 15.3 months among those who were assigned to only RT [69]. Furthermore, assessing MGMT methylation status in a cohort of patients with GBM who underwent radiation treatment but did not receive chemotherapy, Rivera et al. have demonstrated an 50% reduction in the rate of tumor progression during RT in methylated tumors versus those that were unmethylated. These data suggest that MGMT promoter methylation may predict a better response to any form of therapy, including RT [70]. Consequently, MGMT promoter methylation status has been established as an important prognostic biomarker, helping in performing a risk stratification of cases. National Comprehensive Cancer Network (NCCN) guidelines consider MGMT promoter methylation status in clinical management of the patients with GBM.

**For newly diagnosed GBM**, TMZ is typically given following surgical resection, concurrent and adjuvant, in addition to RT. It is administered daily at a dose of 75 mg/m<sup>2</sup> for 6 weeks during irradiation, followed by a rest period of about 1 month after RT is completed (concurrent treatment). When restarted, TMZ is dosed at 150 mg/m<sup>2</sup> daily for 5 days every 4 weeks for 6 cycles (adjuvant treatment). If tolerated, the dose of the adjuvant treatment can be escalated up to 200 mg/m<sup>2</sup> daily. This is the well-known Stupp regimen. In common practice, some medical centers have attempted to prolong TMZ administration for 12–18 months. Some evidence suggests that long-term therapy with TMZ in selected patients is superior to Stupp regimen [71–73], but there is no definitive data to prove this.

The use of standard or hypofractionated RT plus concomitant and/or adjuvant TMZ has been extended to **elderly** (> 70 years old) with a good performance status (KPS ≥ 60). For patients >70 years old with a poor performance status (KPS < 60), TMZ alone can be an option.

At the time of **recurrence**, reoperation should be proposed if the tumor is resectable and if prognostic factors suggest a benefit. Local chemotherapy can be administered during surgery by implantation of Gliadel wafers. Second-line chemotherapy is indicated based on MGMT promoter methylation, time to disease recurrence and toxicity profile. The nitrosourea-based regimen is the preferred choice. Restarting therapy with TMZ may be an option in MGMT-methylated patients. Other agents, such as carboplatin, etoposide, irinotecan may be tried as single agents or in regimens.

**Gliadel wafers** are composed of a biodegradable polymer impregnated with carmustine (BCNU), an alkylating agent of the nitrosourea family. During the surgery, after removal of the tumor, up to 8 wafers (containing a maximum of 61.6 mg BCNU) are deposited along the wall of the resection cavity and left in situ. BCNU will be release over a period of 2–3 weeks, the tumor cells being directly and efficiently exposed to high levels of drug starting immediately after surgery. Gliadel has received FDA (USA) approval for use in both newly diagnosed GBM and recurrences. Studies have consistently reported an increase of median survival by about 2 months [74–76]. Local delivery of carmustine reduces systemic adverse events, but sometimes induces complications: cerebral edema, seizure, poor wound healing, cerebrospinal fluid (CSF) leak, infection, headache, hemiparesis, hydrocephalus, particularly in patients with recurrent GBM. Combining local and systemic chemotherapy offers advantages that may explain the prolonged survival. First, systemic TMZ is most effective in regions of the tumor that are most vascular, whereas local release of BCNU allows direct access to relatively avascular areas of walls of the surgical cavity. Second, following the Stupp protocol, between surgery and chemoradiotherapy there is a period without treatment. Gliadel allows treatment of residual tumor cells within this period. Therefore, the combination of different treatment modalities allows continuous therapy up to 9 months, beginning immediately following surgery [77].

## 7. Other therapies

**Optune (formerly NovoTTF-100A) is a device that delivers tumor-treating fields (TTFields)**, meaning low-intensity, intermediate-frequency, alternating electric fields that have

antiproliferative properties with minimal toxicity. It has been approved (FDA 2015) as an alternative treatment for adult patients having a newly diagnosed supratentorial GBM following debulking surgery and completion of RT, with concomitant SoC chemotherapy. It has also been approved (FDA, 2011) for the treatment of adult patients with supratentorial confirmed recurrences of GBM, to be used as a monotherapy, as an alternative to standard medical therapy after surgical and radiation options have been exhausted (Novocure, 2017). Current evidence supports the use of TTFs as a therapeutic option. Stupp et al. analyzed 315 patients with GBM who had completed standard chemoradiation therapy, adding TTFs to maintenance TMZ chemotherapy and found a significantly prolonged progression-free survival and overall survival. Median progression-free survival was 7.1 months in the TTFs plus TMZ group and 4 months in the TMZ alone group. Median overall survival was 20.5 months in the TTFs plus TMZ group and 15.6 months in the TMZ-alone group [78].

## 8. Emergent treatments strategies

As the field of neuro-oncology continues to progress, numerous novel therapies have been tried and tested. Results from preclinical and clinical studies applying new treatments alone or in combination with conventional methods are promising.

GBM has abnormalities in **cellular signal transduction pathways**. All these pathways include receptor tyrosine kinases (RTKs) like vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), etc., and share common mechanisms of activation and intracellular signaling, meaning RAS or phosphoinositol-3-kinase (PI3K) pathways. Genetic alterations of RTK/RAS/PI3K occur in about 88% of primary GBMs; the pathways are overactivated, allowing uncontrolled cellular proliferation, survival and invasion [79, 80]. Targeted molecular drugs have been developed to inhibit aberrantly activated signaling pathways in the clinical setting.

**Increased epidermal growth factor receptor (EGFR) signaling** has been reported in approximately 45% of GBM [79, 80]. It results in tumor cell proliferation, invasiveness, migration, angiogenesis and inhibition of apoptosis. Moreover, in the clinical setting, EGFR overexpression is associated with resistance to RT while EGFR inhibitions increased sensibility to RT [81]. Inhibitors of EGFR have been developed to block-specific pathways, but the results are disappointing. These include: monoclonal antibodies (cetuximab and nimotuzumab), small molecule tyrosine kinases inhibitors TKIs (gefitinib and erlotinib), a dual EGFR and ErbB-2 inhibitor (lapatinib), a pan-ErbB inhibitor (canertinib), a dual EGFR and vascular endothelial growth factor receptor (VEGFR) inhibitor (vandetanib), irreversible EGFR inhibitors (BIBW 2992 and PF-00299804), etc. It is to be noted that the ErbB family of proteins contains four receptor tyrosine kinases, structurally related to EGFR, marked as ErbB-1 to ErbB-4. ErbB-1 and ErbB-2 are found in many human cancers.

**Overexpression of alpha subtype of PDGF receptor (PDGFR)** occurs in about 13% of GBM [79]. Imatinib mesylate and tandutinib are inhibitors of PDGFR and other molecules involved in intracellular signaling pathways.

**VEGF** is a key factor implicated in tumor neoangiogenesis. GBM is a highly vascular tumor, that depends on vascular proliferation for growth. Recent evidence suggests vasculogenic mimicry in GBM, meaning formation of vessel-like network by tumor cells, allowing a blood supply for tumor growth. This process differs from angiogenesis, it is happening without the presence of endothelial cells. Angiogenesis is driven primarily by tumor-secreting VEGF-A (one member of the VEGF family), but there are many secreted proangiogenic factors [82]. The level of VEGF in HGG is greater than 10-fold compared with LGG [83]. Thus, drugs have been developed to interfere with angiogenesis by directly blocking ligand (VEGF) or receptor (VEGFR) or by targeting proangiogenic molecules that function by alternative mechanisms [84]. Of all targeted biological agents, only **bevacizumab** (Avastin) has demonstrated efficacy. It is a humanized monoclonal antibody that selectively blocks VEGF and so the BBB becomes more stable, with a resultant decrease in vascular permeability and edema, such that the corticosteroid doses can be reduced or suspended. Bevacizumab may be useful during and after RT, because of reduction of peritumoral edema, sometimes refractory to corticosteroid drugs and because of reduction of radiation necrosis rate following improving oxygenation. It has been approved by FDA (2009) as a single agent in the treatment of recurrent GBM following prior therapy, based on improvement in progression-free survival (that however did not translate into an improvement in overall survival) and a modest toxicity profile. Patients treated with bevacizumab inevitably relapse and sometimes an aggressive, invasive “gliomatosis” pattern of recurrence, unresponsive to subsequent therapy is observed. In addition to bevacizumab, there are many inhibitors of VEGF/VEGFR and other relevant targets under investigation, including: vatalanib, cediranib, sunitinib, sorafenib, vandetanib, VEGF trap, ramucirumab, pazopanib, etc. Dually targeted VEGFR/PDGFR inhibitors may prove useful, because of role of PDGFR in pericyte recruitment.

Other antiangiogenic approach targets **the integrins  $\alpha v\beta 3$  and  $\alpha v\beta 5$**  that are overexpressed by tumor endothelial cells. They are transmembrane receptors that interact with extracellular matrix proteins to facilitate angiogenesis and invasion. Cilengitide inhibits these integrins.

There are clinical studies focused on **substances that inhibit intracellular signaling molecules**. Overactivation of the PI3K/Akt/mTOR signaling in GBM has been observed, because of receptor tyrosine kinase overactivity, mutated oncogenic PI3K subunits, and/or loss of PTEN tumor suppressor activity. Several mTOR inhibitors are currently tested, including sirolimus, temsirolimus, everolimus, and ridaforolimus. Enzastaurin is an inhibitor of protein kinase C- $\beta 2$  that suppresses PI3K/Akt pathway. Overactivation of RAS/RAF/mitogen-activated protein kinase pathway in malignant glioma has provided the rationale to study farnesyl transferase inhibitors (farnesylation is a critical step in activation of RAS). Tipifarnib, lonafarnib and sorafenib may inhibit farnesyltransferase. Histone deacetylase inhibitors (vorinostat, romidepsin, valproic acid, etc.) prevent gene transcription, resulting in cell cycle arrest, differentiation, and/or apoptosis of tumor cells. Clinical trials are in progress.

**Immunotherapy** has become a promising cancer treatment, which allows for synergistic multimodal strategies. There are different immunotherapeutic approaches in GBM, including active immunotherapy (tumor vaccination therapy) and passive immunotherapy (antibody-based immunotherapy, adoptive cell therapy and other immune-modulatory therapy). **Tumor**



**vaccination therapy** uses administration of the antigens to activate an antitumor immune response. There is a vaccine targeting the mutant of epidermal growth factor receptor EGFRvIII (only expressed in GBM cells in about 20–25% of cases) – rindopepimut. Vaccination with dendritic cells is based on their ability to absorb all kinds of antigens and to secrete interleukin-2, thus activating T lymphocytes and initiating an efficient and specific immune response [85]. The application of tumor-derived heat shock proteins as tumor antigen carrier may be effective in boosting immune response. **Antibody-based immunotherapy** refers to the use of specific interaction between antibodies and antigens to block negative immune regulatory molecules that would have been preventing activated T cells from attacking the cancer cells. The most promising class of drugs that emerged is based on immune checkpoint inhibitors. Nivolumab and pembrolizumab are antibodies targeting the receptor programmed cell death-1 (PD-1) receptor of lymphocytes. Ipilimumab is an antibody that binds cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which would have inhibiting cytotoxic T lymphocyte to destroy cancer cells. **Adoptive cell therapy** is based on infusion of activated immune effector cells into cancer patients with the goal to enhance antitumor immunity. Immune effector cells include lymphokine-activated killer (LAK) cells, natural killer (NK) cells, T cells, tumor-infiltrating lymphocytes (TILs), cytotoxic T lymphocyte (CTLs), tumor antigen-specific TCR-transgenic T cells and chimeric antigen receptors-modified T cells (CAR T) [86]. This approach would be beneficial to non-responsive patients and non-immunogenic tumors.

**Gene therapy** is designed for delivery of genetic material, usually transgenes or viruses, into cells for therapeutic purposes. There are four types of gene therapy proposed for the treatment of GBM: suicide gene therapy, immunomodulatory gene therapy, tumor-suppressor gene therapy, and oncolytic virotherapy. **Suicide gene therapy** uses genes that encode enzymes able to convert a non-toxic drug into an active cytotoxic compound. The herpes simplex virus (HSV) type 1 thymidine kinase (tk) gene has been used as a “suicide gene”, allowing to tumor cells to produce high levels of tk. Ganciclovir is the systemically injected prodrug, that will be converted by tk into a toxic metabolite. This compound is incorporated into DNA of actively proliferating tumor cells, and consequently blocks DNA replication and inhibits cell division. Apoptosis underlies the mechanism of cytotoxicity [87]. Another “suicide gene” is cytosine deaminase (CD), an enzyme capable to convert an antifungal drug, 5-fluorocytosine (5-FC), into the highly toxic compound 5-fluorouracil (5-FU). This is again converted into molecules which interfere with RNA processing and DNA synthesis and apoptosis invariably occurs. **Immunomodulatory gene therapy** induces or augments an enhanced specific antitumoral immune response, overcoming the tumor-induced immunosuppression. **Tumor-suppressor gene therapy** aims to restore the function of a tumor-suppressor gene lost or functionally inactivated in cancer cells. They play a critical role in maintaining genome integrity and in regulating cell proliferation, differentiation, and apoptosis. In GBM, there is at least one tumor-suppressor gene mutated or deleted in all cases; in 91% of patients, 2 or more of these tumor-suppressor genes are inactivated [88]. Correcting the genetic abnormalities in the glioma cells has been demonstrated to suppress tumor growth via induction of apoptosis and cell cycle arrest. Genes encoding p53, p16, or phosphatase and tensin homolog (PTEN) can be candidates for this type of therapy. **Oncolytic virotherapy** employs replication-competent viruses with natural or engineered tropism and activity against tumors. They specifically infect and replicate in target tumor cells. During progeny particle release, tumor

host cells are destroyed, and tumor-associated antigens are released, while progeny virions infect neighboring tumor cells. Finally, a complete destruction of the tumor can be achieved, multiple mechanisms being involved together with direct oncolysis, meaning induction of an effective antitumor immune response, cancer cell starvation by destruction of tumor vasculature, and sometimes the activity of virally encoded therapeutic transgenes. The two most studied oncolytic virus types are adenoviruses and herpes simplex viruses. **Another strategy of gene therapy targets genes that may modulate the tumor microenvironment**, to create unfavorable conditions for tumor growth or enhance the efficacy of therapy. Although there is a limited evidence of a therapeutic benefit of gene therapy to date, it is important to note that these therapies appear to be safe.

## 9. Conclusions

Effective treatment in GBM remains one of the most formidable challenge in neuro-oncology. Treatment is multimodal and despite significant advances in diagnostic technology, surgical technique, radiation, chemotherapy and targeted therapy, the prognosis remains poor. Large-scale research efforts are required to understand the molecular biology of brain tumors and to discover novel therapies. Synergistic multimodal strategies and individualized treatments are likely to be the best approach of these complex tumors to finally improve survival and quality of life of the patients.

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