**Universidade de Lisboa**

**Faculdade de Farmácia**

# Therapeutic Options in Glioblastoma Multiforme:

## Current Trends and Future Perspectives

**Patrícia Nunes Gonçalves**

Monografia orientada pela Professora Doutora Cristina Maria Leitão de Carvalho, Professora Associada

**Mestrado Integrado em Ciências Farmacêuticas**

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## Resumo

O glioblastoma é um tumor cerebral do Sistema Nervoso Central extremamente agressivo, com sobrevivência limitada e poucas opções terapêuticas. A terapia convencional consiste na ressecção cirúrgica, seguida de radioterapia e quimioterapia adjuvante com Temozolamida. Os doentes com glioblastoma têm pouca esperança de vida e mesmo depois do tratamento, a recorrência do tumor é frequente. Novas terapias que podem melhorar o tratamento e prognóstico atuais estão sob desenvolvimento.

As terapias pioneiras englobam imunoterapia, viroterapia e terapias direcionadas. A imunoterapia estimula o sistema imunitário do doente contra o tumor, o que pode ser conseguido através de imunoterapia adotiva, vacinação e inibição dos *checkpoint* imunitários. A viroterapia reside na atividade oncolítica dos vírus para detetarem células tumorais e destruírem-nas. As terapias direcionadas têm em consideração as várias vias que se encontram normalmente desreguladas e que o tumor utiliza para crescer e sobreviver, como as vias dos recetores das tirosina quinases/fosfatidilinositol 3-quinase/proteína quinase ativada por mitogénios (RTK/PI3K/MAPK), p53 e retinoblastoma. Ao identificar as proteínas e os genes que contribuem para a proliferação tumoral, podemos restringir o crescimento tumoral com a ajuda de pequenas moléculas inibidoras, anticorpos monoclonais e conjugados de fármaco-anticorpo.

Uma vez que o glioblastoma se trata de uma doença fatal, novas opções de tratamento são imperativas para melhorar a qualidade de vida e a sobrevivência dos doentes. O objetivo desta monografia é analisar o futuro dos tratamentos contra o glioblastoma e abordar as terapias inovadores que se encontram em investigação. Apesar de promissoras, assuntos relacionados com a barreira hematoencefálica, o microambiente e heterogeneidade do tumor são grandes desafios ainda a superar e que serão também abordados mais à frente.



**Palavras-chave:** Glioblastoma, quimioterapia, radioterapia, imunoterapia, terapias direcionadas, novas terapias

## Abstract

Glioblastoma is an extremely aggressive brain tumor of the Central Nervous System with limited survival and poor treatment options. The standard therapy consists of surgical resection followed by radiotherapy and adjuvant chemotherapy with Temozolomide. Patients with glioblastoma have a low life expectancy and even after treatment, recurrence is often observed. New therapies that can improve the standard treatment and prognosis are under development.

The pioneering therapies comprise immunotherapy, virotherapy, and targeted therapies. Immunotherapy stimulates the immune system of the patient against the tumor, which can be achieved by adoptive cell therapy, vaccine therapy, and immune checkpoint inhibition. Virotherapy relies on the oncolytic activity of viruses to target tumor cells and destroy them. Targeted therapies consider the many pathways that are more commonly dysregulated and that the tumor uses to grow and survive, such as receptor tyrosine kinase/phosphoinositide 3-kinase/mitogen-activated protein kinase (RTK/PI3K/MAPK) pathway, p53, and retinoblastoma pathways. By identifying which proteins and genes contribute to tumor proliferation, we can target cancer cells and restrain tumor growth through small molecule inhibitors, monoclonal antibodies or even antibody drug conjugates.

Since glioblastoma is a fatal disease, more treatment options are imperative to improve quality of life and the overall survival of patients. The purpose of this monograph is to analyse the future of glioblastoma treatments and to look into the innovative therapeutic approaches that are under development. Although these new therapies look promising, issues related to the blood-brain barrier, tumor microenvironment and heterogeneity are a huge challenge that will be further discussed.

**Keywords:** Glioblastoma, chemotherapy, radiotherapy, immunotherapy, targeted therapies, novel therapies

## Acknowledgments

*“Education is power”*

Upon reaching the end of this 5-year journey, I cannot help to thank a special group of people who made this journey more enjoyable, and to whom I dedicate this monograph. I would like to thank my family for the unconditional support since day one, for celebrating the successes and comforting me in the hard times, for always supporting and encouraging me to give my best and to be the best version of myself. To my friends, Sara Fonseca, Mafalda Cruz, Pedro Costa, Catarina Calado, Isabel Pedrosa and Madeira loves, João Paulo and Mafalda Pereira and Andreia Jesus, I thank for all the memories and friendship. You made these 5 years the best of my life and I will forever treasure our Ameixoeira nights and Madeira trip in my heart. I also would like to thank my boyfriend, António, for showing me that whatever happens in life, there is always going to be someone willing to love you.

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## Abbreviations

5-ALA - 5-Aminolevulinic Acid

AbDC - Antibody Drug Conjugate

ADC - Apparent Diffusion Coefficient

AGT - O6-Alklyguanine-DNA Alkyltransferase

APC - Antigen Presenting cell

ASCO - American Society of Clinical Oncology

ATM - Ataxia Telangiectasia-mutated

ATR - Ataxia Telangiectasia and Rad3-related

BBB - Blood-Brain Barrier

BCNU - 1,3-Bis(2-chloroethyl)-1-nitrosourea or Carmustine

BiTEs - Bispecific T Cell Engagers

BV - Bevacizumab

CAR-T - Chimeric Antigen Receptor Loaded on Autologous T-cell

CDK - Cyclin Dependent Kinase

CDK2A - Cyclin Dependent Kinase Inhibitor 2A

CH13L1- Chitinase-3-like Protein 1

ClpP - Mitochondrial Caseinolytic Protease P

CMV - Cytomegalovirus

CNS - Central Nervous System

COVID-19 - Coronavirus Disease 2019

CT - Computed Tomography

CTLA-4 - Cytotoxic-associated Lymphocyte Antigen-4

CW - Carmustine Wafer

DC - Dendritic Cell

DTI - Diffusion Tensor Imaging

EBRT - External Beam Radiotherapy

EGFR - Epidermal Growth Factor Receptor

EMA - European Medicines Agency

EOR - Extent of Resection

ESMO - European Society for Medical Oncology

FDA - Food and Drug Administration

GABRA1- Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1

GBM - Glioblastoma

HSP - Heat Shock Protein

ICG - Indocyanine Green

IDH - Isocitrate Dehydrogenase

IL-10 - Interleukin-10

KPS – Karnofsky Performance Status

LOH – Loss of Heterozygosity

M1- Macrophage Type-1

mAb - Monoclonal Antibody

MAPK - Mitogen-activated Protein Kinase

MERTK - Tyrosine-protein Kinase MER

MET - Mesenchymal-epithelial Transition

MGMT – O6-Methylguanine-DNA Methyltransferase

mOS – Median Overall Survival

MRI - Magnetic Resonance Imaging

MRSI - Magnetic Resonance Spectroscopy Imaging

NCCN – National Comprehensive Cancer Network

NEFL - Neurofilament Light Chain

NF1 - Neurofibromatosis type 1

NF-κB - Nuclear Factor Kappa B

nGBM - Newly-Diagnosed Glioblastoma

NICE - National Institute for Health and Care Excellence

NK - Natural Killer

OS - Overall Survival

PARP - Poly-(ADP-Ribose)-DNA Polymerase

PD-1 - Programmed Death-1

PDGFR- α - Platelet-derived Growth Factor Receptor-α

PD-L1 - Programmed Death-Ligand 1

PFS - Progression-free Survival

PI3K - Phosphoinositide 3-Kinase

PK - Protein Kinase

PpIX - Protoporphyrin IX

PTEN - Phosphatase and Tensin Homolog

rGBM – Recurrent Glioblastoma

RT - Radiotherapy

RTK - Receptor Tyrosine Kinase

S1P - Spingosine-1-phosphate

SARS-CoV-2 - Acute Respiratory Syndrome Coronavirus 2

SCL12A5 - Potassium-chloride Transporter Member 5

siRNA - Small Interfering RNA

SYT1 - Synaptotagmin 1

TAM - Tumor-associated Macrophages

TGF- β - Transforming Growth Factor-β

TIL - Tumor Infiltrative Lymphocyte

TKI - Tyrosine Kinase Inhibitor

TMB - Tumor Mutation Burden

TME - Tumor Microenvironment

TMZ – Temozolomide

TTFields - Tumor-Treating Fields

VEGF - Vascular Endothelial Growth Factor

WHO – World Health Organization

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# 1. Introduction

## 1.1 Gliomas and Glioblastoma

Gliomas are the most common primary Central Nervous System (CNS) tumors (1). Histologically, they display features of glial cells (i.e. astrocytes, oligodendrocytes and ependymal cells) (2) and are classified according to their presumed cell of origin (3). However, whether gliomas originate from normal glial cells, glial or neural precursors, stem cells, or other cell types remains a topic of investigation (4).

Glioblastoma (GBM), also known as glioblastoma multiforme, is the most malignant and frequently occurring type of primary astrocytomas, representing approximately 60% of all gliomas (5) and 48% of all primary malignant CNS tumors (6). Common symptoms include seizures, headaches, nausea and vomiting, memory loss, changes of personality, mood or concentration, and localized neurological problems (7). GBM is extremely aggressive and has very poor prognosis, which makes this tumor a crucial public health issue (8).

### 1.1.1 Epidemiology

Although CNS tumors are rare, they are a significant cause of cancer morbidity and mortality (9). With little global incidence, about 3.19 cases per 100.000 people reported each year (10), it presents a median overall survival (mOS) of 14-15 months and a 5-year survival rate of 5% after diagnosis (11). The average age of diagnosis is 65 years but most patients are diagnosed between 75 and 84 years. About 1.5 times more common in men than in women (12), its incidence is higher in North America, Australia, Northern and Western Europe (13), which may be due to underreporting of cases, limited access to healthcare and differences in diagnostic practices in less developed countries (8).

### 1.1.2 Pathogenesis

The World Health Organization (WHO) grades GBM as a grade IV astrocytoma - a high-grade glioma with predominantly astrocytic differentiation, featuring nuclear atypia, cellular pleomorphism as well as microvascular proliferation and/or necrosis (14).

The 2016 CNS WHO classification breaks with the principle of diagnosis based entirely on microscopy by incorporating molecular parameters into the classification of CNS tumor entities (15). Based on information about isocitrate dehydrogenase (IDH) status, GBMs are divided into GBM IDH-wildtype, clinically defined as primary or *de novo* GBM, GBM IDH-mutant, corresponding to secondary GBM, and GBM NOS, a diagnosis that is reserved for those tumors in which full IDH evaluation cannot be performed (16).

#### 1.1.2.1 Types, Subtypes and Molecular Alterations in GBM

Primary GBM accounts for the vast majority of cases (90%) in adults over 50 years. These tumors manifest *de novo* (i.e. without clinical or histopathologic evidence of a preexisting, less-malignant precursor lesion) and within 4 months after a short clinical history (17).

Secondary GBMs (40%) normally develop in younger patients (less than 45 years) through malignant progression from a low-grade astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III), which can go from less than 1 year to more than 10 years. Several studies indicate that primary and secondary GBMs are two different disease entities, evolving through different genetic pathways, affecting patients at different ages, and showing different responses to present therapies. Of all the astrocytic tumors, GBMs present the greatest number of genetic changes, which in most cases, results from the accumulation of multiple mutations (18).

Some of the most common genetic abnormalities are described below:

- **Loss of heterozygosity (LOH):** LOH is a cross chromosomal event that results in the loss of the entire gene and the surrounding chromosomal region. It is a common occurrence in cancer development and LOH on chromosome arm 10q is the most observed genetic alteration in both primary and secondary GBM (60-90% of cases) (19). This mutation appears to be specific for GBM as is rarely found in other tumor grades. It is usually associated with poor survival. (20).

- **Epidermal growth factor receptor (EGFR) gene:** The EGFR gene is involved in the control of cell proliferation (20). Mutations that lead to EGFR overexpression are more common in primary glioblastoma (50% of cases). A specific mutation,

EGFRvIII, is often observed and has been studied as a promising target for kinase inhibitors, immunotoxins and peptide vaccines (21).

- **TP53:** Mutations in TP53, a tumor suppressor gene, were among the first genetic alterations identified in astrocytic brain tumors. The TP53 gene appears to be deleted or altered in approximately 25-40% of all GBMs, more commonly in secondary GBM (18).

- **IDH mutations:** IDH is as enzyme encoded by the IDH1 genes in chromosome 2. As an isocitrate dehydrogenase, its primary function is to catalyze the oxidative decarboxylation process within the Krebs cycle. In contrast to the wild-type enzymatic function, IDH mutants have an alternative responsibility to catalyze the production of 2-hydroxyglutarate, an oncometabolite that activates enzymes which support DNA demethylation, yielding a hypermethylation in the tumor cells and eventual tumorigenesis (22). Mutant IDH expression has shown to give a significantly favorable prognosis for GBM patients undergoing temozolomide (TMZ) and radiation therapy (23).

- **O6-methylguanine-DNA methyltransferase (MGMT) promotor methylation:** Located on chromosome 10q26, the MGMT gene encodes a DNA-repair protein that removes alkyl groups from the O-6 position of guanine, an important site of DNA alkylation. DNA renovation consumes the MGMT protein, which the cell must restore. When not repaired, chemotherapy-induced lesions, especially O6-methylguanine, trigger cytotoxicity and apoptosis. High levels of MGMT activity in cancer cells create a resistant phenotype by blunting the therapeutic effect of alkylating agents and thereby determinate treatment success. Epigenetic silencing of the MGMT gene by promoter methylation is associated with loss of MGMT expression and diminished DNA-repair activity (24).

- **Phosphatase and tensin homolog (PTEN):** PTEN is a tyrosine phosphatase that acts as a tumor suppressor gene by turning off signaling pathways through the action of its phosphatase protein product. Mutations in this gene results in the loss of phosphatase activity and may lead to the development of many cancers, specifically GBM, lung, breast cancer, and prostate cancer. PTEN mutations have been found in 20% of GBMs, more commonly in primary GBM (25).

Additional important genetic alterations include amplification of the platelet-derived growth factor receptor-α (PDGFR-α) gene (60% of overexpression in the pathway leading to secondary GBM) and p16INK4a deletion (30-40%) (18). In review, differences between primary and secondary GBM are described in Table 1.

**Table 1 -** Key characteristics of IDH-wildtype and IDH-mutant GBMs. Adapted from (16,18,26).

| | IDH-wildtype GBM | IDH-mutant GBM |
|---|---|---|
| **Synonym** | Primary glioblastoma, IDH-wildtype | Secondary glioblastoma, IDH-mutant |
| **Precursor lesion** | Not identified; develops *de novo* | Diffuse astrocytoma<br>Anaplastic astrocytoma |
| **Proportion of glioblastomas** | ~ 90% | ~ 10% |
| **Median age at diagnosis** | ~ 62 years | ~ 44 years |
| **Male-to-female ratio** | 1.42:1 | 1.05:1 |
| **Mean length of clinical history** | 4 months | 15 months |
| **Median overall survival**<br>Surgery + radiotherapy<br>Surgery + radiotherapy + chemotherapy | <br>9.9 months<br>15 months | <br>24 months<br>31 months |
| **Necrosis** | Extensive | Limited |
| **LOH 10q mutation** | 70% | 63% |
| **EGFR amplification** | 35% | 8% |
| **TP53 mutations** | 30% | 65% |
| **PTEN mutations** | 25% | 4% |
| **IDH mutations** | 5% | 80% |
| **MGMT promotor methylation** | 42% | 79% |

Over the years, advancements in molecular technology and analysis of gene clusters based on abnormalities in PDGFR-α, IDH1, EGFR, and neurofibromatosis type 1 (NF1), have permitted to characterize four molecular subtypes of GBM (according to Verhaak classification): classical, mesenchymal, proneural, and neural (27). EGFR amplification and LOH 10 are more common in classical subtype. Lower levels of expression of NF1 and high expression of mesenchymal epithelial transition (MET) genes were observed in mesenchymal subtype, while altered PDGFR-α and mutated IDH1 were abundant in proneural subtype. TP53 mutation, EGFR amplification, and cyclin dependent kinase inhibitor 2A (CKDN2A) deletion are strongly associated with neural subytype of GBM. Major characteristics and molecular connections of each molecular subtype are listed in Figure 1.

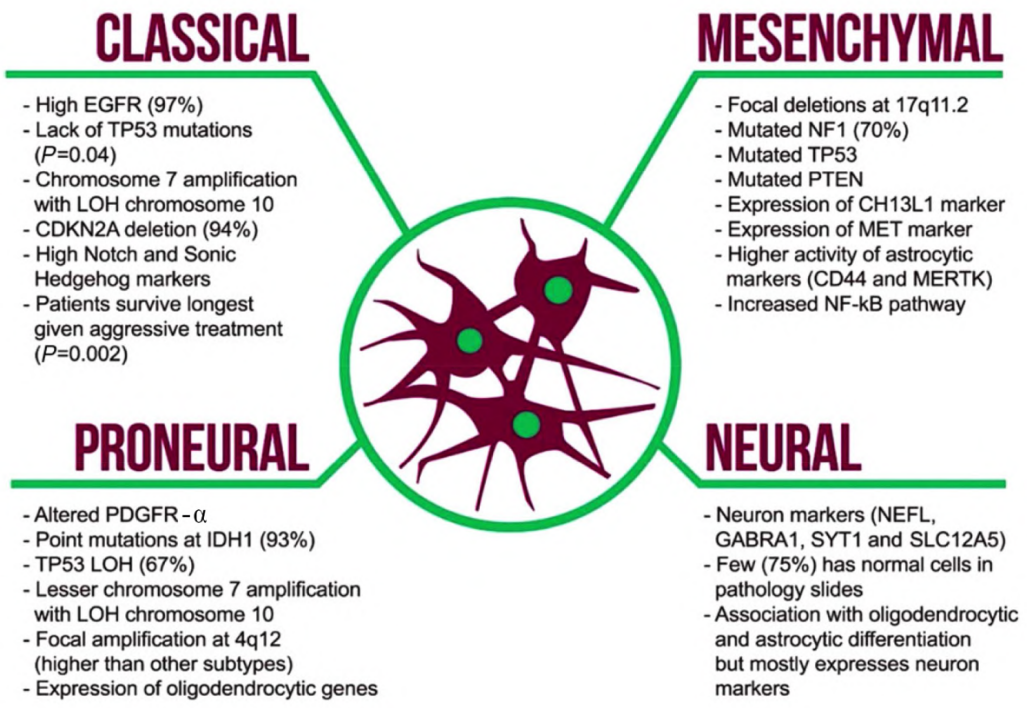



**Figure 1 -** Molecular biomarkers correlation with different Verhaak GBM subtypes. Cyclin Dependent Kinase Inhibitor 2A (CDK2A), Chitinase-3-like Protein 1 (CH13L1), Epidermal growth factor receptor (EGFR), Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1 (GABRA1), Isocitrate Dehydrogenase (IDH), Mesenchymal-epithelial Transition (MET), Tyrosine-protein Kinase MER (MERTK), Neurofilament Light Chain (NEFL), Neurofibromatosis type 1 (NF1), Nuclear Factor Kappa B (NF-κB), Loss of heterozygosity (LOH), Platelet-derived Growth Factor Receptor Alpha (PDGFR-α), Phosphatase and Tensin Homolog (PTEN), Potassium-chloride Transporter Member 5 (SCL12A5), Synaptotagmin 1 (SYT1). Adapted from (28).

### 1.1.3 Risk Factors

The vast majority of patients with high-grade glioma have no family history of brain tumors or identifiable risk factors for glioma. In rare cases, GBM and other high-grade gliomas may be a manifestation of tumor predisposition syndromes such as Li-Fraumeni Syndrome, Lynch Syndrome (or Hereditary Nonpolyposis Colorectal Cancer) or Constitutional Mismatch Repair-Deficiency Syndrome, which are related to mutations most in the TP53, MSH2 and PMS2 genes, respectively (29).

Aside from genetic factors, the only established risk factor is exposure to ionizing radiation, as a result of radiation therapy for childhood brain tumors or leukemia. The latency between irradiation and the development of a glioma like electromagnetic radiation, radiofrequency, radiation from cell phones and head trauma are inconclusive (29–31).

## 2. Objectives

This monograph provides an overview of the current therapeutic options in GBM, based on reliable international oncological organizations' guidelines and new approaches that are being explored to reverse the poor outcome of this tumor.

Furthermore, this review also mentions the presumable impact of COVID-19 in GBM treatment and diagnosis, clarifies some misconceptions regarding the immune desert in GBM, describes the present challenges in implementing effective immunotherapies and suggests that the characterization of patients' tumor molecular profiles can help identify subpopulations who could better benefit from currently available treatments. In addition, it also analyses the latest innovative approaches that under investigation

## 3. Methods

Several browsers for scientific publications were used, namely *PubMed* (pubmed.ncbi.nlm.nih.gov) generously provided by the National Library of Medicine (NLM), *ScienceDirect* (www.sciencedirect.com) managed by Elsevier and Google Scholar (scholar.google.com).

The criteria for the article selection went through filtering the search according to more recent publications, namely articles published between 2016 and 2021, with some of the selected articles having been published prior to that period. The research was done mostly in English and key terms such as the following were used: glioblastoma, chemotherapy, radiotherapy, immunotherapy, targeted therapies, and novel therapies.

Finally, in addition to the aforementioned research platforms, much of the information was also obtained from websites and documents provided by important health entities, namely the European Medicines Agency (EMA), the U.S. Food and Drug Administration (FDA), the World Health Organization (WHO), the National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO), the National Institute for Health and Care Excellence (NICE), and the Portuguese League Against Cancer. This was crucial to access the standards and scientific guidelines that regulate the subject under study, allowing this review to be built based on legal and current foundations and references.

# 4. Current Therapeutic Options in Glioblastoma

Despite several international efforts, GBM is still one of the most challenging assignments in clinical oncology (32). Over the last decade, a scope of different treatments was investigated but with very limited success.

The main challenges in GBM therapy are related with tumor location and its complex and heterogeneous biology (33). Advances in surgical approaches, radiotherapy (RT) and adjuvant chemotherapy have shown some improvements in survival and quality of life but the prognosis is still very reserved. However, more significant steps need to be made in order to achieve positive outcomes, when compared to those seen in certain cancers that can now be successfully treated (32).

## 4.1 Staging and Risk Assessment

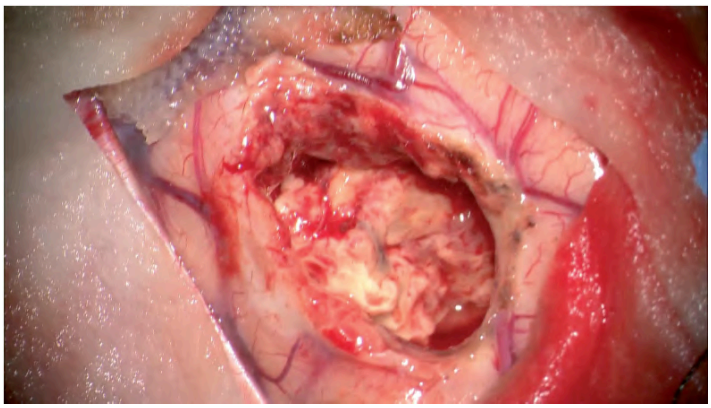
Although GBMs are invasive tumors with a strong tendency to migrate, tumor dissemination remains limited to the CNS and distant metastases are rare; thus, staging focuses on imaging of the brain by magnetic resonance imaging (MRI). The spine and cerebrospinal fluid are not normally assessed in the absence of clinical symptoms. The extent of tumor resection (EOR) and determination of residual disease should be assessed within 24 to 48 hours after surgery in order to distinguish post-surgical contrast enhancement from residual tumor. Lower tumor grade, radical tumor resection, younger age (<50 years), good performance status, and an intact neurological function are favorable prognostic factors. Determination of molecular markers (discussed above) will identify patients with a more favorable prognosis or better chance of response to alkylating agent chemotherapy (34).

## 4.2 Surgical Resection

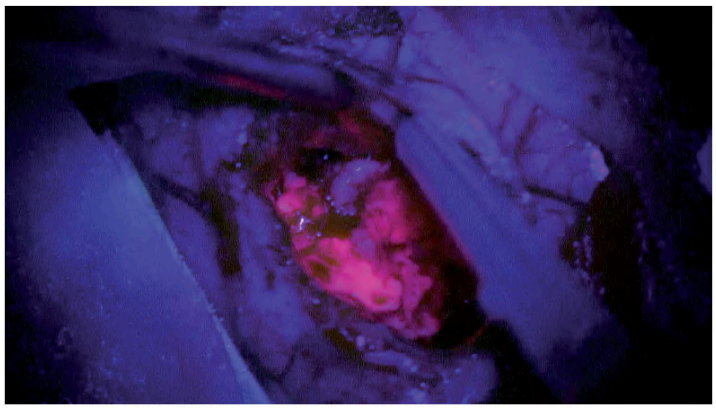
Surgery is the initial therapeutic approach in GBM and remains a hallmark in the treatment of malignant brain tumors (35). Depending on the tumor type, surgery can accomplish reduction of tumor burden, control seizures, reversal of neurological deficit, introduction of local therapeutic agents, and improve quality of life (36).

Medical condition of the patient, appropriate imaging and functional studies, neuropsychological evaluation, the use of corticosteroid, and antiepileptic drugs are some of the preoperative matters that must be taken into account (35). Although most predictors of good outcome are essentially patient-related, the most important one is EOR (37). A more extensive surgical resection results in a longer life expectancy, achieving the longest survival in patients who undergo gross total resection followed by RT and TMZ (37–39). One of the most important aspects is the fine balance between the aggressive removal and preservation of function; the goal is to achieve maximal safe surgical resection (40).

To increase EOR, some fluorescent agents have been used to enhance the visualization of tumor margins, namely 5-aminolevulinic acid (5-ALA). 5-ALA is a natural amino acid biosynthesized from glycine and succinyl CoA in the mitochondria. Following systemic administration, ALA is metabolized in tumor cells into protoporphyrin IX (PpIX), a photosensitizing porphyrin (41). The reason for the selective PpIX accumulation in malignant glioma is not fully understood but it is highly specific (98%) in areas of infiltrating tumor (42). Under blue light excitation (400–410 nm), the tumor tissue appears red, whereas normal tissue (including edema) does not show fluorescence (43) (Figure 2).



(A)



(B)

**Figure 2 -** Brain tumor resection using 5-ALA.
**(A)** Brain tumor resection using regular white light and **(B)** blue excitation light (400-410 nm) using 5-ALA; the tumor tissue appears red, whereas normal tissue shows no fluorescence. From (44)

Fluorescein can also be used but its biggest disadvantage is that fluorescence depends on the integrity of the blood–brain barrier (BBB), making it less specific. Since fluorescein concentration is higher in perfused tissues and vessels, if tissue is perturbed during surgery, there might be unspecific extravasation unrelated to tumor (45). (Figure 3).

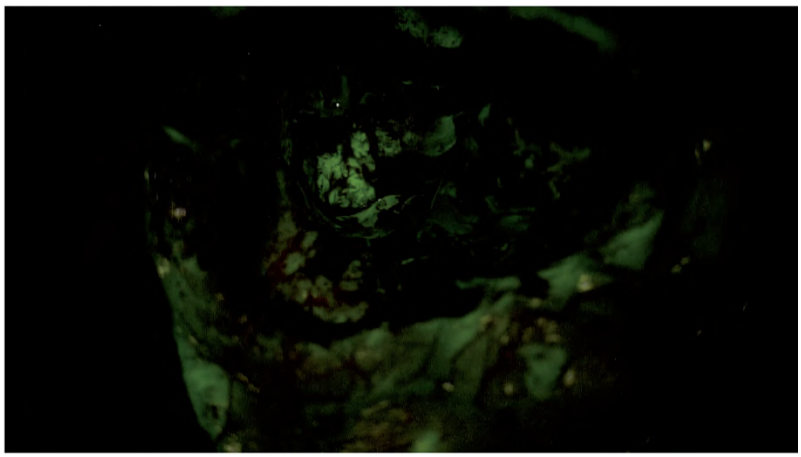


**Figure 3 -** Brain tumor resection using fluorescein.
From (44)

Through angiography with indocyanine green (ICG), several structures can be observed like neovascular architecture, alterations of the caliber, morphology, course of vessels, and hemodynamic patterns. The dye does not penetrate the membrane and therefore is unable to define the margins of the tumors (46). ICG helps to avoid injury by preserving small caliber vessels during brain tumor surgery (Figure 4).

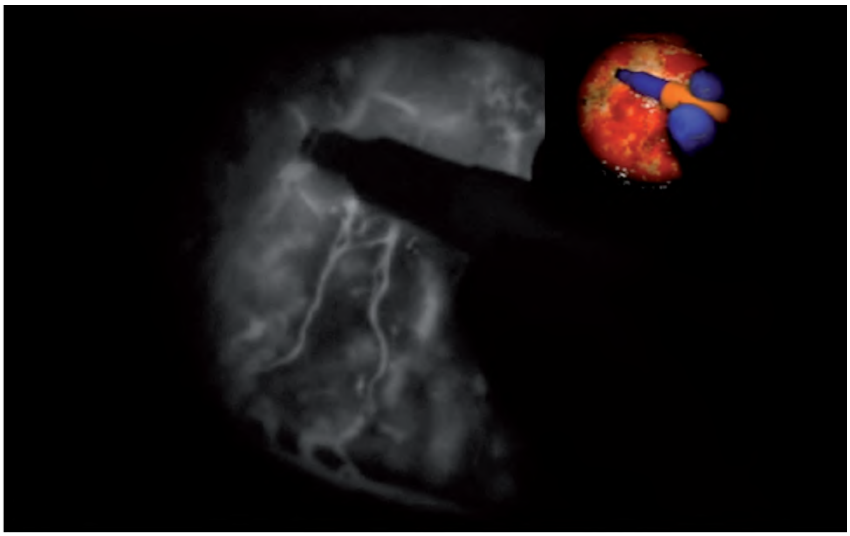


**Figure 4 -** Intraoperative videoangiography with ICG to localize major vasculature and avoid injury in smaller vessels.
From (44)

## 4.3 Radiotherapy

Subsequent to surgery, RT becomes the mainstay of treatment for people with GBM and has long played a crucial role.

RT is typically performed with giving TMZ. In 2004, a prospective randomized phase III trial by the European Organization for Research and Treatment of Cancer /National Cancer Institute of Canada Clinical Trials Group reported improved progression-free survival (PFS) and overall survival (OS) for patients with GBM with the addition of concomitant and adjuvant TMZ to RT following maximal safe resection (47).

For patients under 70 years with good performance status (Karnofsky Performance Status (KPS) ≥ 60), the optimal dose fractionation schedule for external beam radiotherapy (EBRT), following resection or biopsy, is 60 Gy in 2 Gy fractions

delivered over 6 weeks. Numerous other dose schedules have been explored without clear benefits but there is no clinical indication to give fractionated RT over 60 Gy (48).

RT delivery is largely limited by difficulties in target definition/delineation. MRI is preferred over computed tomography (CT) because of its superior spatial resolution but even this imaging modality cannot reliably indicate regions of active, non-enhancing, or microscopic tumor (49). Furthermore, the conventional method used to identify tumor-assessments of gadolinium enhancement on MRI is also a poor tumor indicator after anti-vascular endothelial growth factor (VEGF) therapy (i.e. bevacizumab). Anti-VEGF compromises interpretation of follow-up MRIs as it normalizes leaky tumor vasculature and decreases T1 gadolinium enhancement and peritumoral edema (50).

In order to provide better tumor definition, several promising and novel imaging techniques are being investigated such as magnetic resonance spectroscopy imaging (MRSI), diffusion-weighted MR imaging (DWI) and diffusion tensor imaging (DTI). Briefly, MRSI provides information about tumor activity based on the levels of cellular metabolites such as choline, creatine, N-acetylaspartate and lactate/lipid (51). By detecting alterations in these metabolite levels, MRSI can predict areas of occult disease and therefore decrease rates of local recurrence. DWI and DTI are both based on the Brownian motion of water molecules or their diffusion rate in the tissue (apparent diffusion coefficient (ADC) - water movement in $mm^2/s$). Different tissues have different ADCs and increased cellularity usually corresponds to reduced ADC values. Areas of glioma/tumor are hypothesized to have lower ADC values than areas of normal brain tissue (52). DTI is a more complex version of DWI that can determine the directionality and magnitude of water diffusion (53).

To improve outcomes, especially in cases of reirradiation, various strategies were employed to locally intensify RT. Such strategies include less traditional forms of RT (brachytherapy, radiolabeled antibodies, radiosurgery), alternative dosing schedules (hyofractionated and accelerated hyperfractionated RT), and the use of radiosensitizing agents (Table 2 and Table 3).

**Table 2 -** Current and novel radiotherapy approaches in GBM.

| Modality | Mode of action | Examples | References |
|---|---|---|---|
| **Brachytherapy** | Sealed radiation source is placed inside or next to the area requiring treatment (i.e. I-125) | **GliaSite** An inflatable balloon catheter filled with an aqueous solution of I-125 is placed in a resection cavity following debulking or tumor resection | (54) |
| | | **GammaTile** Permanent placement of encapsulated radioactive Cesium-131 seeds in the surgical cavity | (55) |
| **Radioimmunotherapy** | Administration of radiolabelled monoclonal antibodies while tumor resection | **131-I-m81C6** Murine anti-tenascin monoclonal antibody labelled with I-131 | (56) |
| **Stereotactic Radiosurgery** | Precise delivery of high radiation dose in 1–5 treatments for tumors < 4cm | **Proton beam therapy** (proton beams) | (57) |
| | | **GammaKnife** (γ-rays) | (58) |
| | | **CyberKnife** (x-rays) | (59) |
| | | **Zap-X** (x-rays) Self-contained and self-shielded radiosurgery system | (60) |
| **Radiosensitizers** | Group of therapeutics that enhance the efficacy of radiation | **Poly-(ADP-Ribose)-DNA Polymerase (PARP) Inhibitors** Olaparib Veliparib Pamiparib | (61–63) |
| | | **DNA-dependent protein kinase (PK) Inhibitors** CC-115 (dual inhibitor of mammalian target of rapamycin (mTOR) kinase and DNA-PK inhibitor) | (64) |
| | | **Ataxia telangiectasia-mutated (ATM)/ Ataxia telangiectasia and Rad3-related (ATR) Inhibitors** AZD1390 | (65) |

**Table 3 -** Alternative radiotherapy dosing schedules in GBM.

| | Dosing schedule | References |
|---|---|---|
| **Hyprofractionated Radiotherapy** | Total dose of radiation is divided into large doses and treatments are given once a day or less often. Hypofractionated radiation therapy is given over a shorter period of time (fewer days or weeks) than standard radiation therapy. | (66) |
| **Accelerated Hyperfractionated Radiotherapy** | Total dose of radiation is divided into small doses and treatments are given more than once a day. Hyperfractionated radiation therapy is given over the same period of time (days or weeks) as standard radiation therapy | (67) |
| **FLASH Radiotherapy** | Delivery of ultra-high dose rates (>40 Gy/s) | (68) |

Although radiation is part of the standard of care for the treatment of GBM, there remain many areas of controversy and innovation. These include safe radiation regimens for the elderly or frail, and reirradiation options in previously treated patients (69).

## 4.4 Chemotherapy

Although the optimal chemotherapeutic regimen for GBM is yet not defined, several studies have suggested that more than 25% of patients obtain a significant survival benefit from adjuvant chemotherapy. Meta-analysis suggest that adjuvant chemotherapy results in a 6-10% increase in 1-year survival rate (70).

### 4.4.1 Temozolomide

TMZ is an alkylating agent derived from dacarbazine used as first-line treatment in GBM and in the treatment of anaplastic astrocytoma (71).

The therapeutic benefit of TMZ depends on its ability to alkylate/methylate DNA. This methylation often occurs at the N-7 or O-6 positions of guanine residues, which causes damage in the DNA and triggers pathways that lead to tumor cell death. However, some tumor cells have the ability to repair this type of damage and decrease

the therapeutic efficacy of TMZ by expressing a protein, O-6-alkylguanine DNA alkyltransferase (AGT), encoded by the MGMT gene (72). In some tumors, epigenetic silencing of the MGMT gene prevents the synthesis of this protein and in consequence, such tumors are more sensitive to TMZ (24). On the other hand, the presence of AGT protein in brain tumors predicts poor response to TMZ and these patients receive little benefit from chemotherapy with TMZ (73).

The current standard of care for patients with newly-diagnosed GBM (nGBM) is maximum safe surgical resection followed by concurrent TMZ (75 mg/m$^2$/day for 6 weeks) and RT (60 Gy in 30 fractions) and then six maintenance cycles of TMZ (150–200 mg/m$^2$/day for the first 5 days of a 28-day cycle— single-dose TMZ).

### 4.4.2 Bevacizumab

Bevacizumab (BV) is a recombinant humanized monoclonal antibody that blocks angiogenesis by VEGF-A. VEGF-A is a growth factor protein that stimulates angiogenesis in a variety of diseases, especially in cancer (74).

The use of BV in patients with recurrent GBM (rGBM) was first documented in 2005 in a phase I trial of 21 patients with relapsed malignant glioma in which BV was combined with irinotecan, showing a promising response rate of 42%. Improved 6-month PFS rates ranging from 30% to 46% in this and subsequent phase II trials well exceeded prior historical controls of 9% to 21% for radiation therapy and 4% to 9% for salvage chemotherapy (75). Encouraged by these results, in 2009 accelerated approval was granted by the Food and Drug Administration (FDA) for single-agent BV treatment of rGBM for patients who had failed prior treatment with another agent.

Many studies were further conducted to evaluate potential combination regimens of BV and cytotoxic agents in patients with rGBM but disappointingly, despite slight improvements in PFS, expected OS was not accomplished (76–78). Based on these results, there is no consensus on the best BV regimen in rGBM. Although BV is approved by the FDA for rGBM, it has not been similarly approved by the European Medicines Agency (EMA). Regardless, it is also approved in Switzerland and still used as off-label for rGBM in many European countries.

Two studies were carried out to evaluate the benefit of BV in patients with nGBM, RTOG 025 and AVAglio. However, neither of them revealed any difference in OS and therefore BV is not approved for use in nGBM (79,80).

### 4.4.3 Nitrosoureas

Nitrosoureas are DNA alkylating agents, namely Carmustine (BCNU), Lomustine, Nimustine and Fotemustine. They are characterized by high lipophilicity and thus can cross BBB, making them useful in the treatment of brain tumors such as GBM (81). Nitrosoureas, particularly BCNU, were the chemotherapeutic agents of choice for first-line treatment of GBM in the 1970s and 1980s, but have been relocated into second-line therapy since the approval of TMZ for recurrent high-grade gliomas (82,83).

#### 4.4.3.1 Carmustine Wafers

Carmustine is a nitrogen mustard β-chloro-nitrosourea compound used as an alkylating agent to treat several types of brain cancer such as glioma, GBM, medulloblastoma, astrocytoma, multiple myeloma and lymphoma (Hodgkin and Non-Hodgkin) (84). Approved for the first time in 1996 by the FDA, GLIADEL® Wafer (Arbor Pharmaceutics) is a biodegradable carmustine wafer (CW) indicated for the treatment of patients with newly-diagnosed high-grade glioma as an adjunct to surgery and radiation and rGBM as an adjunct to surgery (85). CW allows a controlled release of carmustine in the extracellular fluid of the brain, eliminating the need for the encapsulated drug to cross the BBB. Its implantation in GBM patients undergoing surgical resection aims to provide a therapeutic bridge during the period between surgical resection and RT, allowing higher concentration doses while minimizing systemic adverse effects (86). Combining CW implantation with surgical resection has been suggested to increase survival in nGBMs by 2 to 4 months (87), including when used in association with the standard combined chemoradiotherapy (88). Although the efficacy of CW implantation is established in seminal clinical trials, its safety remains a matter of debate, with varying results regarding postoperative infections, maintaining of the quality of life, and feasibility of adjuvant oncological treatments (89).

## 4.5 Non-Pharmacological Treatment Options

### 4.5.1 Tumor-Treating Fields

Tumor-Treating Fields (TTFields) has been called the “fourth cancer treatment modality” after surgery, RT, and pharmacotherapy.

TTFields consists in a locoregionally antimitotic treatment which delivers low-intensity, intermediate-frequency (200 kHz), alternating electric fields, through four transducer arrays, consisting of nine insulated electrodes applied to the shaved scalp and connected to a portable device. TTFields arrests cell division and kills tumor cells through multiple mechanisms, namely, misalignment of microtubule subunits during division, aberrant chromosomal segregation, and cytoplasmic blebbing during anaphase (90).

Two pivotal randomized trials studied TTFields in rGBM (EF-11) and nGBM (EF-14). In EF-11 trial, despite not meeting its primary endpoint of improving OS, similar mOS and PFS were observed and so TTFields was established as noninferior to chemotherapy. Moreover, the favorable quality of life and toxicity profile led to its approval in 2011 by the FDA as a therapeutic option in rGBM (91). The EF-14 trial evaluated the efficacy and safety of TTFields in combination with TMZ maintenance treatment, after RT on nGBM patients. The trial revealed an important improvement in PFS and OS, with 43% of patients alive after 2 years in the TTFields/TMZ group and 29% in the TMZ alone group (92). In October 2015, the FDA approved TTFields for nGBM patients and the National Comprehensive Cancer Network (NCCN) has further included TTFields in their recent updated guidelines (93).

TTFields are particularly safe since systemic toxic reactions were rarely observed. The most common side effects are mild to moderate skin reactions beneath the transducer arrays (44% of patients), and grade 3 skin reactions (1-2 % of patients) (94).

Although showing significant improvements in survival, the results still show that the majority of patients does not survive beyond 2 years, highlighting the need for additional improvements in GBM therapeutic strategies. Due to its unique and localized mechanism of action and general absence of systemic toxicity, TTFields seems to be well suited for combination therapies, such as immunotherapy and targeted therapies.


**Figure 5 -** TTFields device (2nd generation Optune).
The position of the transducer arrays is determined by the localization of the tumor using a mapping software (NovoTal™). From (90).

## 4.6 Supportive Care

Besides therapeutic management (i.e. anti-tumor therapy), the current standard of care for high-grade gliomas patients is also inclusive of providing effective supportive care. An effective supportive care requires management of the different signs and symptoms of the disease, such as cerebral edema, seizures, gastrointestinal tract disturbances, osteoporosis, venous thromboembolism, cognitive impairment, and mood disorders (95). Symptomatic relief of neurological symptoms is achieved by the administration of corticosteroids, however, due to its many side effects (i.e. myopathy, weakness, risk of infection, osteoporosis, and Cushing Syndrome), early tapering is recommended in the beginning of treatment. Dexamethasone (8-16 mg/day) is usually the corticosteroid of choice due to its low mineralocorticoid activity. Anti-epileptic therapy is indicated in patients presenting with seizures. Lamotrigine, Levetiracetam, Pregabalin or Valproic Acid are preferred to first generation anti-epileptic agents (Phenytoin, Carbamazepine, Phenobarbital, and their derivatives) since they may interfere with chemotherapy (but not with TMZ) as they are strong inducers of hepatic metabolism (96).

## 4.7 Guidelines Summary

There are many oncological societies, based on the best available evidence, that provide clinical practice guidelines detailing the sequential management decisions and interventions when it comes to cancer care. The goal is to assist in the decision making-process of all the individuals involved - including physicians, nurses, pharmacists, patients, and their relatives - and to ensure that the patient receives the best preventive, diagnostic, treatment, and supportive care that are most likely to lead to optimal outcomes (97,98).

However, recommendations from these organizations on both treatment and diagnostics can be different. This may due to lack of evidence-based medicine within that region for the particular management modality. Furthermore, we also have to consider that demographics, political policies, and different regulatory entities play an influential role. In order to consolidate information, a summary of the latest updated guidelines of NCCN and the European Society for Medical Oncology (ESMO) on the management of GBM is provided.

### 4.7.1 NCCN Guidelines

- **Diagnosis and Treatment**

**Table 4 -** GBM treatment options upon diagnosis and regarding surgery status. From (99).

| Surgery Status | Options |
|---|---|
| **Approved and agreed to removing most of tumor** | Maximal safe resection |
| **Approved and agreed to removing some of tumor** | Sterotactic biopy |
| | Open biopsy |
| | Subtotal resection |

- **Post-Surgery Treatment**

Performance status: KPS≥60

**Table 5 -** GBM treatment options in patients with a KPS≥60 and under 70 years, according to MGMT promoter status.
External beam radiotherapy (EBRT), Temozolomide (TMZ). From (99).

| Age | MGMT promoter status | Options |
|---|---|---|
| **≤70 years** | Methylated | TMZ during and after fractionated EBRT<br>• With alternating electric field therapy for upper brain tumors |
| | Unmethylated or unkown | TMZ during and after fractionated EBRT<br>• With alternating electric field therapy for upper brain tumors |
| | | Fractionated EBRT |

**Table 6 -** GBM treatment options in patients with a KPS≥60 and over 71 years, according to MGMT promoter status.
External beam radiotherapy (EBRT), Temozolomide (TMZ). From (99).

| Age | MGMT promoter status | Options |
|---|---|---|
| **≥71 years** | Methylated | Hypofractioned EBRT |
| | | TMZ during and after fractionated EBRT |
| | | TMZ during and after fractionated EBRT<br>• With alternating electric field therapy for upper brain tumors |
| | | TMZ |
| | Unmethylated or unknown | Hypofractioned EBRT |
| | | TMZ during and after fractionated EBRT<br>• With alternating electric field therapy for upper brain tumors |

Performance status: KPS≤59

**Table 7** - GBM treatment options in patients with a KPS≤59.

External beam radiotherapy (EBRT), O6-Methylguanine-DNA Methyltransferase (MGMT), Temozolomide (TMZ). From (99).

| Options |
|---|
| **Fractionated EBRT if patient is 70 years or younger** |
| **Hypofractinated EBRT** |
| **TMZ if methylated MGMT promotor regions** |
| **Best supportive care** |

### 4.7.2 ESMO Guidelines

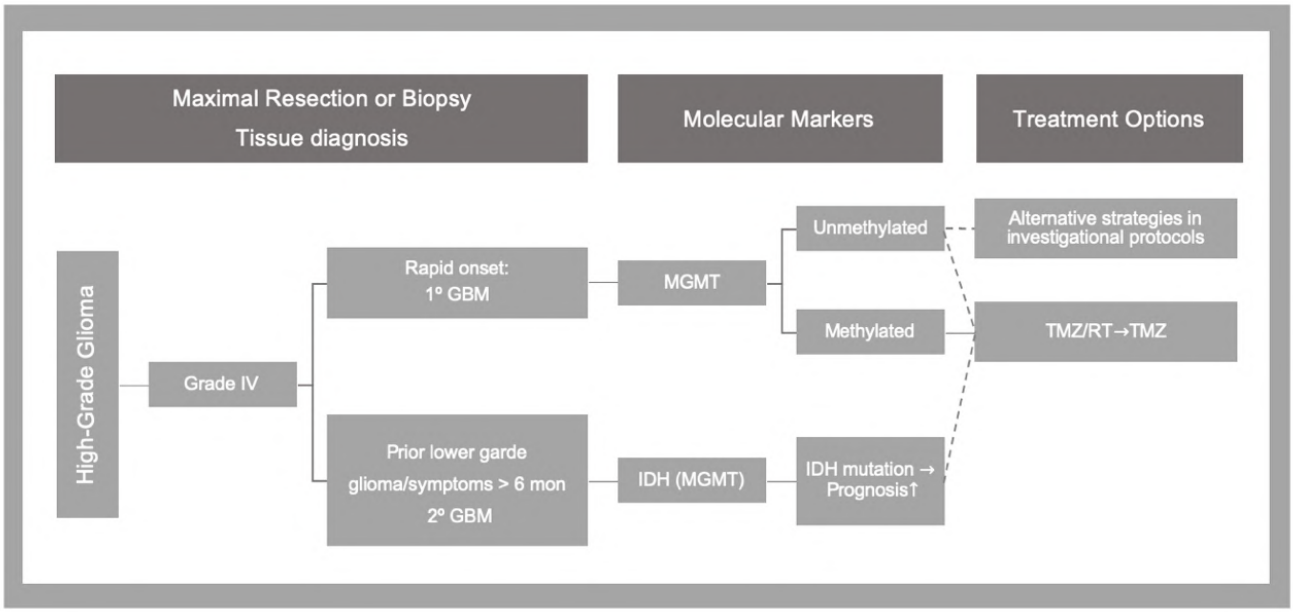



**Figure 6** - Treatment algorithm for high-grade glioma.

Primary (1º), Secondary (2º), Glioblastoma (GBM), Isocitrate Dehydrogenase (IDH), O6-Methylguanine-DNA Methyltransferase (MGMT), Radiotherapy (RT), Temozolomide (TMZ). Adapted from (34).

## 4.8 COVID-19's Impact in GBM Treatment and Diagnosis

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in December 2019 in Wuhan, China. The disease has since spread worldwide, leading to an ongoing pandemic that has caused millions of deaths (100).

Symptoms of COVID-19 are variable but often include fever, headache, fatigue, breathing difficulties, and loss of smell and taste. Although there might be infected people who do not develop noticeable symptoms, 5% of cases suffer critical symptoms such as respiratory failure, shock or multiorgan disfunction. Data from several sources suggest that severe illness and death from COVID-19 is higher among adult patients who present comorbidities like hypertension, diabetes, and cardiovascular and respiratory disease. Cancer is also considered as an established or probable risk factor for severe COVID-19 (101). However, it remains unsettled if all cancer patients, other than those with hematologic and lung malignancies, have increased risk of severe outcome from COVID-19 (102).

The treatment of cancer patients who test positive for SARS-CoV-2 presents a unique challenge, and relies on an ethical, consistent, and transparent decision-making process. Several oncological organizations (i.e. NCCN, NICE, ESMO and the American Society of Clinical Oncology (ASCO)) recommend that until more definitive information emerges, decisions about interrupting cancer treatment in patients with active COVID-19, should be based on a clinical benefit-risk assessment that considers the risk of interrupting cancer treatment versus the still poorly defined risk of adverse COVID-19 outcomes in patients receiving cancer treatment (103–105).

As such, COVID-19 has impacted cancer care delivery. Fewer patients are undergoing screenings, with many providers and patients choosing to postpone or completely forego screenings during the months of the pandemic, leading to fewer cancer diagnostics. A study conducted to more than 900 cancer patients/survivors and 300 relatives/providers by the Portuguese League Against Cancer, revealed that 13% of patients had their treatments suspended by medical indication, 2 out of 10 patients and 1 out of 10 providers pondered to suspend, by their own initiative, clinical acts, with fear of going to hospitals, and 57% of patients were afraid of getting infected by other patients or health care professionals (106). This is particularly important for cancers that rely on routine preventive screenings to detect a large portion of asymptomatic tumors. The

natural consequence of interruption in cancer screenings and delays in diagnosis and treatment is that cancers will be detected at a later stage and require a more complex approach, lowering the chances that patients will respond to therapy and, be cured of the disease. In the years after the pandemic, it is anticipated that the effects of COVID-19 on access to cancer treatment will result in a stage migration to higher stages of the disease and an overall increase in cancer mortality (107).

# 5. Novel Therapeutic Targets in Glioblastoma

## 5.1 Immunotherapy

### 5.1.1 Misconceptions and Challenges

The most important discovery that has changed the paradigm of cancer treatment and substantially led to improved outcomes in multiple cancer types is perhaps the discovery of the immune system role in preventing tumor growth and its power to kill cancer cells (108). For example, the development of monoclonal antibodies directed at checkpoint receptors such as cytotoxic-associated lymphocyte antigen-4 (CTLA-4) and programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) has demonstrated a significant prolonged PFS and OS in several tumors like melanoma, lung, liver, genitourinary, and breast cancers (109). The use of bioengineered chimeric antigen receptor loaded on autologous T-cells (CAR-T), which have shown improved outcomes in relapsed acute lymphoblastic leukemia, refractory diffuse large-B cell lymphoma, and relapsed follicular lymphoma (110). Furthermore, the efficacy of autologous dendritic vaccination therapy in prostate cancer (Sipuleucel-T) and oncolytic virus therapy such as talimogene laherparepvec in melanoma have also contributed for the extension of immunotherapy in cancer treatment (111,112).

GBM has always been regarded as an isolated tumor, encased by the BBB, and with an immunosuppressive environment that downregulates antigen presentation. Nevertheless, the notion of the immune desert within GBM has changed in the last few years after several preclinical and clinical studies demonstrated the role of the immune system in the tumor microenvironment (TME) of intracranial tumors including GBM. To better explain the current challenges in implementing effective immunotherapies in GBM, we have to understand the several factors that rule the interaction between malignant cells of GBM and immune cells and the immunosuppressive nature of the tumor.

#### 5.1.1.1 Factors related to the BBB

BBB is a highly specialized barrier that regulates the transport of metabolically important molecules between systemic circulation and the brain. This system forms a dynamic interaction of endothelial cells, microglia, astrocytes and pericytes, and is composed by tight junctions and enzymatic–metabolic pathways that control the delivery

of water, ions, immune cells, and nutrients. BBB is a major barrier to the passage of therapeutic drugs into the brain as it is estimated that 98% of small molecules and 100% of large molecules do not cross it (113). In GBM, the integrity of the BBB is disrupted, its aggressive nature leads to the invasion of endothelial cells and breakage of tight junctions which results in a breach in the BBB. Moreover, GBM invasion into the surrounding structures is associated with angiogenesis because of hypoxia-induced VEGF formation, which although could potentially increase drug availability, it also leads to pathological alterations in the BBB and suboptimal drug concentration (114,115). Such disruption seems not to be sufficient to allow increased concentration of therapeutic agents and so, several methods are under ongoing investigation to overcome the BBB and achieve sufficient delivery of therapeutic compounds.

Nanoparticle delivery is one promising method. Nano-immunoconjugates (anti-CTLA-4 and anti-PD-1) on natural biopolymer scaffold were able to induce immune response and lead to a significant prolonged survival in mice with intracranial GL261 GBM (116). Similarly, another group of investigators showed that small interfering RNAs (siRNA) against both EGFR and PD-L1 through solid lipid nanoparticles in mouse models, were able to decrease the growth of GBM and prolong mouse survival (117). Convection-enhanced delivery is another encouraging method that has shown promising preclinical and clinical results (101). By establishing a pressure gradient during interstitial infusion, distribution of the pharmaceutical compound is augmented into the intracranial tumor, increasing local drug delivery.

#### 5.1.1.2 Factors Related to GBM Immunosuppressive Microenvironment

GBM employs an immunosuppressive effect even though the abundance of different immune cells. Despite the presence of a functional lymphatic system in the CNS, GBM inhibits the normal function of T cells through several mechanisms (118). Furthermore, GBM can disrupt the non-resident T cells functionality beyond the tumor bed and affect potential effective peripheral T-cell populations by trapping them in the bone marrow due to the loss of spingosine-1-phosphate (S1P) receptor, an event commonly observed in intracranial tumors (119). Moreover, GBM patients presents higher levels of T-regulatory cells, responsible for inducing immune tolerance and promoting tumor growth (120). In addition, natural killer (NK) cells, known for their potent cytotoxic activity against tumor cells, are suppressed in GBM (121). Tumor-associated

macrophages (TAMs) and microglia constitute the majority of cellular mass in GBM (30–50%). Regarded as antigen presenting cells (APCs), they are responsible for the presentation of tumor-associated antigens to be recognized by effector T cells. Both TAMs and microglia are involved in gliomagenesis through several mechanisms including immune suppression and secretion of several factors such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) which can further exacerbate immunosuppression (122).

In this way, the presence of a TME with dysfunctional and deregulated immune cell types in GBM makes it difficult to implement efficient immunotherapies. Current efforts are being made to exploit vulnerabilities in order to reactivate the immune system against tumor cells. TAMs have the ability to switch their phenotype that leads to a change in their function. This plasticity could be exploited in order to select a phenotype that favors macrophage type-1 (M1), a pro-inflammatory cell that can boost host immunity unlike macrophage type-2 which is considered pro-tumorigenic (123).

#### 5.1.1.3 Factors Related to Tumor Heterogeneity and GBM Subtypes

As described above, several subtypes can be found within GBM (classical mesenchymal, proneural and neural) and they all display its own predominant molecular alterations. Tumor heterogeneity seems to reflect distinct GBM immune subtypes based on the molecular signature. For example, GBM wild-type is associated with a higher tumor infiltrative lymphocyte (TIL) and higher PD-L1 expression compared to GBM IDH-mutant. There is a PD-L1 overexpression on both resident T cells and circulating monocytes in GBM presenting PTEN loss. Moreover, GBM mesenchymal subtype, associated with NF-1 mutations, can have significantly increased TIL (124).

Tumor mutation burden (TMB) status estimates the frequency of mutations per megabase of malignant cell DNA and correlates it with responses observed in several solid malignancies during treatment with immune checkpoints inhibitors (ICI), as a result of neoantigen formation and presence of new mutant proteins that are immunogenic and can induce anti-tumor response. Therefore, it is expected that GBM patients with high TBM will respond better to immunotherapies like ICI when compared to patients with low TMB (125).

To this end, the characterization of immune abundance based on gene expression of GBM can help identify patients who could better respond to certain immunotherapy approaches. However, evidence on clinical correlation is still lacking and a deeper understanding is necessary to evaluate the effect of molecular variations on tumor cell survival and sensitivity or resistance to immunotherapy.

### 5.1.2 Immune Checkpoints Inhibitors

In the last decade, ICI have revolutionized cancer treatment since the approval of Ipilimumab (CTLA-4 inhibitor) in 2011, which was followed by the approval of other ICI (PD-1/PD-L1 inhibitors). After successful implementation in several solid malignancies, further research was conducted to assess ICI viability in other tumor types including GBM.

However, results were not as exciting as anticipated. A pivotal phase III randomized controlled trial (CheckMate 143), evaluated safety and efficacy of Nivolumab (PD-1 inhibitor) versus BV in patients with rGBM previously treated with TMZ and RT (126). This study failed to demonstrate survival benefit, with a lower objective response rate, lower PFS, similar mOS, and higher treatment-related adverse events in patients who received nivolumab. A separate phase III trial evaluating the addition of nivolumab to concurrent TMZ and RT in newly-diagnosed MGMT-unmethylated GBM failed to demonstrate improved outcomes (127). Similarly, another phase II study evaluating Pembrolizumab (PD-1 inhibitor) did not achieve improved PFS at 6 months or improved OS when comparing pembrolizumab alone to pembrolizumab plus BV in rGBM (128).

Several factors may contribute to this lack of response. One is related to the inability of ICI to reach tumor cells. While BBB allows the passage of compounds less than 400 Da, nivolumab and pembrolizumab have a molecular weight of 146 kDa and 149 kDa, respectively. In that way, investigation groups are focusing their efforts on nanoparticle delivery systems or RT-combined ICI to increase the disruption of BBB. Another factor that appears to be fundamental for the efficacy of ICI in GBM treatment is the timing of administration. Most of the trials to date have investigated ICI response either in rGBM or in nGBM patients that have received RT after surgical resection. Thus, it was tempting to evaluate the neo-adjuvant administration of ICI after surgical resection since it has demonstrated positive results in breast cancer and melanoma (129). Furthermore, it is hypothesized that neo-adjuvant administration of ICI can increase T-cell expansion because of the presence potential high load of immunogenic antigens that

would be unavailable after surgical resection. Cloughesy et al. randomized 35 patients with rGBM to receive either neo-adjuvant pembrolizumab (one single dose intravenously 2 weeks prior to surgical resection) and/or adjuvant ICI (130). A mOS of approximately 14 months was observed in patients who received neo-adjuvant pembrolizumab while those who did not receive ICI prior to surgery showed an mOS of 7.5 months. Similarly, another phase II trial replicated similar findings in patients with rGBM (131). The administration of nivolumab before surgery was associated with a higher immune cell activity and higher TIL. This suggests that timing of ICI administration seems to be imperative in the enhancement of tumor response.

The ongoing research also focuses on combining ICI with different treatment modalities and with potential new targets. Proposed approaches for the improvement of GBM treatment with ICI are summarized in Figure 7.

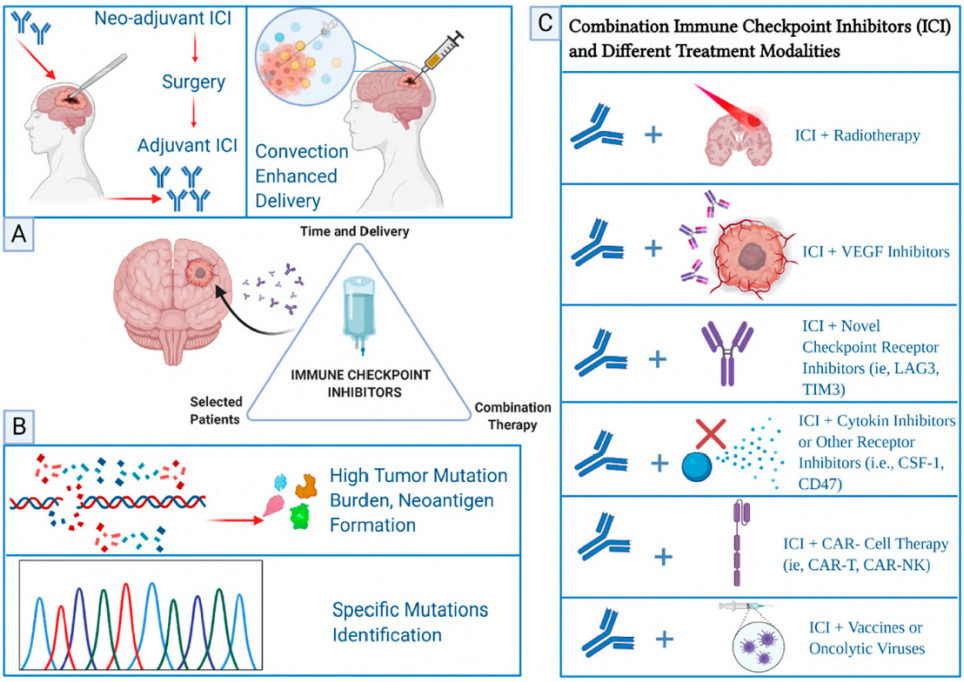



**Figure 7 -** Proposed approaches for the improvement of GBM treatment with ICI. **Panel (A)** shows the promising approach of neo-adjuvant ICI administration before surgical resection and followed by adjuvant ICI. Convection enhanced delivery of nanoparticle ICI is another method that could improve the availability and action of ICI in GBM. **Panel (B)** shows that proper patient selection for treatment with ICI could be beneficial for patients with high TMB and with specific mutations that could attenuate the immunosuppressive effect in TME. **Panel (C)** illustrates ongoing investigational combination of ICI with other novel therapies. From (132).

### 5.1.3 Adoptive Cell Therapy

Adoptive cell therapy relies on bioengineered CAR-T cells and their interaction with specific tumor antigens. Upon binding to their respective surface exposed tumor antigen, CAR-T cells proliferate and activate an immunostimulatory cascade, resulting in a cytotoxic attack on the antigen-bearing tumor cell (110). This approach has shown promising results in hematological malignancies and signs of response in CNS lymphoma (133), and so there was a great enthusiasm in applying this new therapy in GBM. Early studies targeting a variety of tumor antigens by Brown et al. have demonstrated the overall feasibility and safety of CAR-T therapy (134,135). Other GBM-related tumor cell receptors were also explored such as EGFRvIII. In a phase I trial accessing EGFRvIII–directed CAR-T, posterior tissue analyses found that most subjects had specific loss or decreased expression of EGFRvIII but with limited durability of response due to antigen escape mechanisms. Recently, bispecific T cell engagers (BiTEs) have been proposed as a solution against antigen escape (136). CAR-T BiTEs target not only EGFRvIII but also wildtype EGFR, that is amplified in over 80% of GBM. These bispecific antibodies have demonstrated minimal toxicities and antitumor activity against heterogeneous tumors, highlighting a promising avenue for CAR-T therapy. Another approach that has just entered in early clinical investigation is the administration of bioengineered NK cells due to their high cytotoxic effect against tumor cell (137). Nevertheless, the immature results of CAR-cell-based therapy in GBM require further evidence and investigation to support safety and efficacy.

### 5.1.4 Vaccine Therapy

Like ordinary vaccines, therapeutic cancer vaccination consists in inducing an immune response through the exposure of selected tumor-associated antigens. In other to achieve a significant anti-tumor response, these antigens can be enhanced *ex vivo* or incubated in APCs such as dendritic cells that are then administered into the patient (138). There are several approaches in order to accomplish such immune effect like tumor-associated antigens vaccines, vaccines that use heat shock proteins and, dendritic cell (DC) vaccines.

As mentioned above, mutations in EGFRvIII are frequently observed in GBM (around 20%) and so there was a great enthusiasm in vaccine-based therapies using

EGFRvIII. Rindopepimut, the inaugural EGFRvIII peptide vaccine, showed impressive responses in nGBM patients, however, the follow phase-III clinical trial failed to demonstrate clinical benefit (139,140). A recent phase II trial tried to evaluate the response in the concomitant administration of EGFRvIII vaccine and BV in rGBM and patients demonstrated improved PFS (141). In this way, we can expect that the future of EGFRvIII vaccines will pass through the identification of specific subpopulations with enhanced EGFRvIII mutations and the combination with other treatment modalities. However, further clinicals trials are necessary to confirm these assumptions. In addition, SurVaxM is another peptide vaccine that targets survivin, an anti-apoptotic protein responsible for the survival of cancer cells, expressed in 95% of GBM cases. A phase II study found benefits in both PFS and OS (142). VBI-1901 is a novel cancer vaccine composed of enveloped virus-like particles that targets two highly immunogenic cytomegalovirus (CMV) antigens, gB and pp65. Scientific literature suggests that CMV infection is prevalent in multiple solid tumors, including GBM. In June 2021, FDA granted fast track designation for VBI-1901 for the treatment of rGBM due to positive outcomes presented in the phase II study (143,144).

Heat Shock proteins (HSPs), also known as chaperon proteins, are produced when cells are exposed to stressful events. They stimulate both innate and adaptive immune systems, and induce an anti-tumor immune response by forming HSP-antigens complexes that are able to interact with APCs. Since tumor cells have an increased expression of HSPs, these proteins make a useful instrument in cancer vaccine development to fight GBM (145). HSP peptide complex-96 is being studied as a patient specific vaccine (Prophage) against GBM (146). The phase II clinical trial showed promising results due to a safe profile and significant immune response to treatment. In a subsequent phase II clinical trial, it was evaluated the combination with TMZ and the results ensured security and efficacy when using both. More studies are currently on going, exploring new combinations (i.e. pembrolizumab) (147).

DC-based cancer vaccines use autologous tumor lysates or tumor antigens to sensitize naïve T cells and induce anti-tumor immune response. In a phase III clinical trial, DCVax® showed encouraging results in nGBM patients, with a mOS of 34.7 months from surgery, with a 3-year survival of 46.4%. Nowadays it is approved in Switzerland for the treatment of GBM (148) . AV-GBM-1 and ITI-1000 are novel DC vaccines currently in phase II of clinical trials who also demonstrated improved PFS in nGBM patients (149,150).

## 5.2 Oncolytic Virus Therapy

Oncolytic virotherapy relies on the ability of using genetically modified viruses that have neural tropism to target and replicate in cancer cells. By intratumoral administration, herpes simplex virus, adenovirus, poliovirus, gamma-retrovirus, and zika virus are some of the tumor-selective lytic viruses that have the shown the ability to target glioma cells (151–153). Although the majority of the results still remain in the early phases of clinical trials, there are already some results that have demonstrated safety and efficacy.

In example, Ji et al. reported acceptable safety on the intra-arterial cerebral infusion of adenovirus mutant thymidine kinase (Delta-24-RGD) in patients with rGBM (154). Similarly, another phase I clinical trial tried to demonstrate tolerability in rGBM patients. The administration of Toca 51, a retroviral replicating virus who delivers a yeast of cytosine deaminase (suicide gene) which then converts the also administrated prodrug Toca FC (extended-release 5-fluorocytosine) into the antimetabolite 5-fluorouracil, managed to show safety and OS benefit (155). PVSRIPO, a recombinant oncolytic polio-rhinovirus, has demonstrated safety and significant OS improvement in patients with rGBM (21% OS rate at 36 months) (156). These positive results led to FDA approval in 2016. Another clinical trial managed to replicate the OS rate at 36 months by using oncolytic-replicating adenovirus (DNX-2401) (157). Interestingly, it was observed an infiltration of CD8+ T cells after the treatment and so a different clinical trial is on the run to evaluate the combination of this vaccine with PD-1 inhibitor (158). Ofranergene obadenovec (VB-111), a non-replicating adenovirus carrying a Fas-chimera transgene, is another viral therapy that demonstrated in a phase II study survival benefit for patients with rGBM who were primed with VB-111 monotherapy that was continued after progression with concomitant BV. Unfortunately, the phase III trial failed to increase OS and PFS in rGBM patients who received concomitant VB-111 and BV. However, change of treatment regimen, with the lack of VB-111 monotherapy priming, may explain the differences from the favourable phase II results and VB-111 is being further studied in GBM and other indications in primed treatment regimens (159).

Despite the fact that this cumulative evidence shows promise for the immunotherapies in GBM, further investigation is required.

## 5.3 Targeted Therapies

GBM presents a vast heterogeneity of genetic and epigenetic alterations. However, there are three main pathways that are commonly dysregulated and represent possible targets: receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma (160). These pathways may be targeted by using small molecule inhibitors, monoclonal antibodies or antibody drug conjugates (Figure 8).

### 5.2.1 Small Molecule Inhibitors

Tyrosine kinase receptors (RTKs) are high-affinity cell surface receptors for many growth factors, cytokines, and hormones. Despite regulating normal cellular processes, they also play a key role in cancer development and progression. Most of the targeted therapies are tyrosine kinase inhibitors (TKIs) that can prevent kinase phosphorylation and therefore signal transduction (161). Since its success has already been demonstrated in multiple solid and hematological malignancies, several TKIs and other small molecule inhibitors that target upstream factors have been investigated to evaluate their effect in GBM patients. Although Figure 8 illustrates some of the pharmaceuticals that have been tested for GBM, unfortunately most of them has not demonstrated clinical benefit and so, other alternatives had to be considered. Regorafinib is a multikinase inhibitor that targets angiogenic, stromal and oncogenic RTKs (162). A phase II clinical trial comparing regorafenib to lomustine showed increased OS in rGBM (163). Nowadays, regorafenib is being evaluated in both nGBM and rGBM (161). Osimertinib, approved for the treatment of non-small cell lung cancer, was thought to have activity against EGFRvIII but a recent study revealed efficacy in EGFRvIII-negative patients, suggesting that this molecule acts further downstream in the MAPK-signaling pathway and might be used in patients that do not present such mutation (164).

ONC201 is a highly selective antagonist of dopamine receptor D2 and mitochondrial caseinolytic protease P (ClpP) activator that induces selective cancer cell death (165). The phase II clinical trialed was conducted in rGBM patients who were not previously treated with BV. ONC201 was well-tolerated with a durable objective response in one patient presenting a H3K27M mutation (166). In this way, there are ongoing studies to evaluate the efficacy of ONC201 in patients with progressive H3K27M mutant gliomas (167,168).

As mentioned above, the retinoblastoma pathway is one of the many dysregulated routes in GBM. The cyclin dependent kinase (CDK) family is one of many components involved in this pathway, especially CDK4 and CDK6. Considering this, pre-clinical trials were enrolled to inhibit CDK4/6. Palbociclib, Ribociclib, and Abemaciclib are some of many molecules that were trialled to inhibit CD4/6 but only abemaciclib managed to demonstrate antitumor activity (169–171). Abemaciclib is undergoing multiple trials to evaluate its efficacy in GBM (158).

Other molecules of interest are Val-083 and Paxalisib. VAL-083 (Dianhydrogalactitol) is a first-in-class small molecule bifunctional alkylating agent (inhibits both HUVEC endothelial and U251 glioma cells) that has the ability to cross BBB (172). VAL-083 was previously granted orphan drug designation for GBM by the FDA and EMA as well as a fast track designation by the FDA for the treatment of patients with rGBM (173,174). The ongoing phase-II study aims to determine OS improvement in unmethylated MGMT BV-naïve nGBM and rGBM patients (172,175). Paxalisib is a potent inhibitor of the PI3K/AKT/mTOR who demonstrated an improved OS and PFS on the ongoing phase-II clinical trial (176). In August 2020, the FDA granted fast track designation for the treatment of nGBM patients with unmethylated MGMT promoter status who have completed initial radiation with concomitant TMZ (177).

### 5.2.2 Monoclonal Antibodies

Monoclonal antibodies (mAbs) are another strategy that has been studied to inhibit tumor pathways (i.e. BV). Cetuximab (Figure 8) is anoher mAb developed against EGFR but failed to demonstrate survival benefit in the phase II trial (178). As mentioned above, a factor that seems to limit the efficacy of mAbs is their inability to penetrate BBB due to their large size.

### 5.2.3 Antibody Drug Conjugates

Antibody drug conjugates (AbDCs) result in the combination of an antibody with a cytotoxic compound. An example is Depatuxizumab mafodotin (Depatux-M, ABT-414) (Figure 8), an EGFR-directed mAb, depatuxizumab, linked to the potent tubulin inhibitor, monomethyl auristatin F. The phase II trial suggested improved survival in rGBM when combined with TMZ but failed to show survival benefit in the phase III study (179,180).

ABBV-221 (monomethyl auristatin E AbDC with EGFR-targeting antibody) was also evaluated but the phase I study had to be terminated due to safety concerns (181). A third-generation AbDC, ABBV-321 (anti-EGFR mAb conjugated to a pyrrolobenzodiazepine), completed successfully the phase I trial (182).

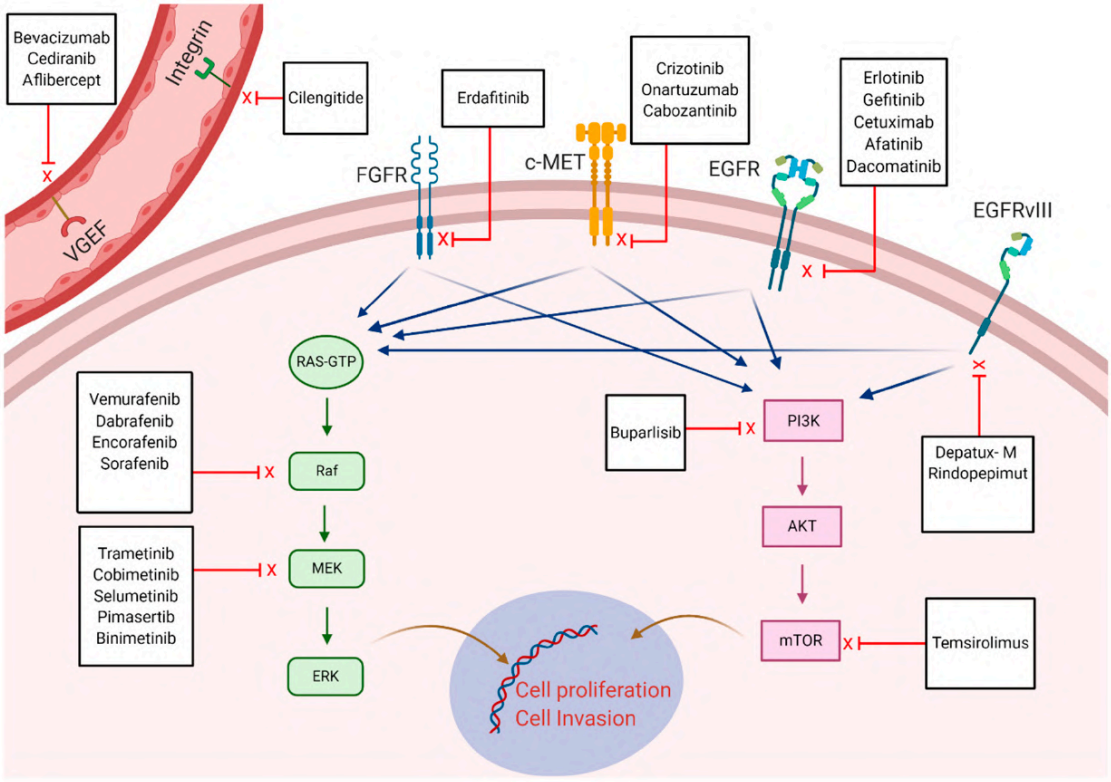



**Figure 8 -** Pharmaceutical that have been trialed to target pathways and receptors involved in cell proliferation, aggressiveness, invasion and angiogenesis in GBM. From (132).

## 6. Future Perspectives

Despite the increased number of effective cancer therapies, GBM still remains a major oncological challenge yet to be overcome. The high number of ongoing clinical trials (470 clinical trials registered at clinicaltrials.gov as October 2020) reflects the need for further research. Even though the successes observed in early-phase clinical trials, phase III randomized studies are essential for the evaluation of new effective medication.

The exploitation of the immune system and the benefit of immunotherapy seems to be high priority given its successful implementation in several solid and hematological malignancies and the observed positive outcomes in GBM. However, this promising avenue can only be validated by large prospective randomized trials. Table 8 summarizes some of several factors that contribute to the immunosuppressive TME and the proposed strategies that are under investigation.

**Table 8 -** Factors implicated in TME and possible solutions to enhance response. From (132).

| Factors Related to GBM | Possible Solutions | References |
|---|---|---|
| **Restricted drug passage through BBB** | Convection enhanced delivery and nanoparticles<br>BBB disruption with RT combined with ICI<br>Adoptive cell therapy | (101,113)<br>(132)<br>(135–137) |
| **Effector T-cell suppression and peripheral T-cell entrapment** | Use of ICI, vaccines and adoptive cell therapy | (118,119) |
| **Increased expression of checkpoint receptors** | ICI targeting several receptors | (132) |
| **Suppression of NK cells** | Use of ICI and NK cell adoptive transfer | (137) |
| **Abundance of TAMs (protumorigenic phenotype)** | Boosting host immunity trough exploiting plasticity in TAM to express inflammatory phenotype M1 | (123) |
| **Increased secretion of immune suppressors (i.e TGF- β)** | Use ICI with TGF- β inhibitors | (132) |

As immunotherapy continues the be the main focus in GBM research, it would be important the selection of patients according to their previous corticoid use due to their immunosuppressive effect that can diminish the efficacy of agents targeting the immune system.

# 7. Conclusion

Glioblastoma is a very complex tumor with no effective therapies and poor prognosis. Despite ongoing efforts, new targeted therapies fail to demonstrate clinical benefit due to many factors such as the presence of a BBB that restricts the passage of therapeutic agents, a tumor microenvironment that downregulates antigen presentation and a tumor heterogeneity that shows different treatment responses due to its unique features. The key to solve these issues starts with strategies to enhance drug delivery, methods to overcome the immunosuppressive state of glioblastoma, and a better patient stratification in future clinical trials based on molecular tumor subtypes and identification of predictors of response.

However, the future for glioblastoma therapy looks promising. The evolution of glioblastoma standard therapies, with improvements in surgical and imaging methods, and novel radiotherapy approaches are signs of the constant knowledge that is attained from scientific research and progress in the technological field.

Further progress is being made with innovative therapies. The vaccines SurVax and DCVax®, and virotherapy with PVSRIPO are already approved for the treatment of glioblastoma. Convection enhanced and nanoparticle delivery, CAR-T cell therapy and small molecule inhibitors like Osimertinib, Val-083, and Paxalisib are examples of the newest strategies that have demonstrated encouraging results and conducted to tumor regression and increased OS and PFS.

Since the future of Medicine lays on personalized treatments, the key to successful therapies for GBM will stand for the same principle and will pass through the combination of different treatment modalities.

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