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REVIEW ARTICLE

The Molecular Landscape of Glioblastoma: Implications for Diagnosis and Therapy

Ozlem Yildirim[1], Evren Onay-Ucar[2]

[1]Istanbul University, Institute of Science, Department of Molecular Biyology and Genetics, Istanbul, Türkiye
[2]Istanbul University, Faculty of Science, Department of Molecular Biyology and Genetics , Istanbul, Türkiye

ABSTRACT

Glioblastoma, classified as grade IV astrocytoma by the World Health Organisation, is the most common and malignant primary brain tumour in adults, with a high mortality rate. It accounts for 14.5% of central nervous system tumours and 45.6% of primary malignant brain tumours, with an annual incidence of 3.19 per 100,000 people. Despite advances in our understanding of its molecular biology, patient outcomes remain poor, with a median survival of approximately 1 year. Glioblastoma is categorised into four subtypes: IDH wild-type, IDH mutant, not otherwise specified (NOS), and not elsewhere classified (NEC), each affecting prognosis and treatment. Key molecular alterations include *IDH1/2, ATRX, TERT, TP53, B-RAF, EGFR, MGMT*, and *PTEN* mutations, which contribute to tumour behaviour and therapeutic targets. Current diagnostic methods, including magnetic resonance imaging and advanced molecular imaging, aid in accurate diagnosis and treatment planning. Although existing therapies offer limited survival benefits, novel treatments like immunotherapy, oncolytic viral therapy, and targeted molecular therapies, are currently being investigated. These emerging therapies overcome challenges such as the blood-brain barrier and tumour heterogeneity, providing hope for improved outcomes. Future perspectives emphasise the importance of integrating molecular biomarkers, optimising treatment strategies, and enhancing clinical trial designs to develop more effective therapies for patients with glioblastoma.This review aims to delve into the intricate facets of glioblastoma, including its classification, histopathology, interactions with the microenvironment, molecular pathogenesis, diagnostic imaging techniques, clinical progression, current therapeutic approaches, challenges in treatment, identifiable risk factors, and exploration of emerging therapies and prospects in glioblastoma management.

Keywords: Glioblastoma, Temozolomide, IDH mutations, Diagnostic imaging, Blood-brain barrier

INTRODUCTION

Glioblastoma, classified as grade IV astrocytoma by the World Health Organisation (WHO), is the most prevalent and aggressive primary brain tumour in the adult population. It is the leading cause of death among patients with primary brain tumours. This disease accounts for 14.5% of central nervous system (CNS) tumors and 45.6% of primary malignant brain neoplasms.[1,2] Its annual incidence is 3.19 per 100,000 people, with the age-specific annual incidence reaching 0.15 per 100,000 in children and reaching a peak incidence of 15 per 100,000 among patients aged 75-84 years.[3] Newly diagnosed patients with glioblastoma typically have a median survival of approximately 1 year and often exhibit poor responses to all therapeutic modalities. Survival rates also decline with increasing age. Only 5% of all patients diagnosed with glioblastoma survive for five years, and this rate drops to 2% among patients aged 65 years and older.[4]

This review comprehensively explores the multifaceted aspects of glioblastoma, including its classification, histopathology, interactions within the microenvironment, molecular pathogenesis, diagnostic imaging modalities, clinical progression, existing therapeutic strategies, treatment challenges, and identifiable risk factors, as well as exploring emerging therapies and prospects in glioblastoma management.

CLASSIFICATION OF GLIOBLASTOMA

The WHO classification is the international standard for glioma nomenclature. According to this classification, glioblastoma is classified as a grade IV malignant tumor. The 2016 fourth edition of the WHO glioma classification predicts the degree of

Corresponding Author: Ozlem Yildirim **E-mail:** ozlm-yildirim@hotmail.com
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malignancy based on histopathological criteria. Histopathological features of glioblastoma include necrosis and endothelial proliferation, and four subtypes are defined in this classification[5]:

1. Isocitrate Dehydrogenase (IDH) Wild-Type Glioblastoma: This type typically develops *de novo* around the age of 60. The subgroup lacking IDH mutation has a worse prognosis and comprises approximately 90% of glioblastomas.[6]

2. IDH-Mutant Glioblastoma: This type generally develops in younger patients with more highly differentiated gliomas, known as "secondary glioblastoma," evolving from prior WHO grade II or III glioma diagnoses. Instead, for such tumours, it proposes the term "astrocytoma", IDH-mutant, WHO grade IV. These conditions account for approximately 10% of the cases. Tumours carrying specific point mutations in *IDH 1* or *2* genes are associated with younger age and better prognosis.[7] However, the Consortium to Inform Molecular and Practical Approaches to central nervous system Tumour Taxonomy (cIMPACT-NOW), which focuses on IDH mutant grade IV gliomas, emphasises that IDH mutant gliomas have distinct biology and clinical behaviours from IDHwt glioblastomas. Consequently, the authors recommend that the term "glioblastoma" should no longer be applied to IDH-mutant tumours. cIMPACT-NOW was established to inform molecular and practical approaches to the classification of CNS tumours. Although not officially recognised by the WHO, this consortium provides significant updates and recommendations for the classification and diagnosis of CNS tumours.

3. Not Otherwise Specified Glioblastoma: This subtype describes tumours for which the presence of an IDH mutation cannot be determined owing to the absence of requisite histological or molecular material for testing.

4. Not-Elsewhere-Classified Glioblastoma: This category refers to tumours that have undergone the necessary examinations for classification but cannot be matched with any of the categories in the 2016 WHO classification based on the results. This can occur due to inconsistencies between the clinical, histological, immunohistochemical, and genetic characteristics. Additionally, there is a possibility of the existence of glioblastoma subtypes exhibiting an unidentified combination of characteristics that have not yet been incorporated into the WHO classification.

HISTOPATHOLOGY

The pathological features of glioblastoma include a diffusely infiltrative tumour with an astrocytic morphology, high mitotic rates, microvascular proliferation, and/or pseudopalisading necrosis.[8] Additionally, immunohistochemical markers commonly used to diagnose glioblastoma include the expression of glial fibrillary acidic protein. The MIB-1/Ki-67 index is also examined to help determine the extent of proliferation.[9] Sometimes, a tumour sample may not exhibit the typical histopathological characteristics of glioblastoma. Before the integration of molecular classification with histopathology, these tumours would have been categorised with a lower WHO grades. Nevertheless, if a tumour is shown to carry the molecular profile of glioblastoma, it is anticipated to behave like a glioblastoma, and a treatment plan is recommended accordingly. For these types of tumours, cIMPACT-NOW has proposed the diagnostic criteria of "diffuse astrocytic gliomas, IDH-wild type, with molecular features of glioblastoma, WHO grade IV".[10] The recently defined CNS Tumour Methylation Classifier identifies specific glioblastoma subclasses, representing a significant advancement in the diagnostic accuracy of brain tumors.[11] Although the clinical significance of these glioblastoma variants has not been demonstrated, using a classifier to verify glioblastoma diagnosis can be advantageous in unusual clinical scenarios, such as patients with a long patient survival history or atypical tumour histopathology.

INTERACTION OF GLIOBLASTOMA WITH MICROENVIRONMENTS

The close interaction of glioblastoma with its microenvironment in the central nervous system is crucial for tumour development, particularly given the specificity of brain cell populations and the extracellular space.[12] Glioblastoma exhibits significant cellular heterogeneity and includes the tumour perivascular niche, which is the primary site of glioblastoma stem cell-like (GSC) populations.[13] This niche consists of stromal cells, such as microglia, astrocytes, pericytes, fibroblasts, and endothelial cells, which support tumour progression.[14] Interaction with the extracellular matrix of the brain is critical for glioblastoma cell survival and invasion.[15] Glioblastoma growth is associated with neo-angiogenesis, and it secretes proangiogenic factors like VEGF-A.[16] Antiangiogenic therapies offer limited benefits.[17] Moreover, endothelial cells and microglia secrete mediators that promote GSC renewal and activate MMP2 and MMP9, which support tumour invasion.[18,19] The impact of astrocytes on GBM is not fully understood, but research in this area is increasing.[20,21]

MOLECULAR PATHOGENESIS

Comprehensive large-scale analyses of genetic, epigenetic, and expression data significantly contribute to understanding the biological mechanisms of glioblastoma. These insights facilitate the continuous development of subclassification of glioblastomas beyond traditional histological grading. In a study conducted in 2006, three gene expression subtypes (proneural, mesenchymal, and proliferative) were identified, each characterised by specific somatic alterations among 35 genes strongly associated with survival.[22] Among these subtypes, the proneural subtype did not exhibit changes in the phosphatase and

tensin homologue (PTEN) or epidermal growth factor receptor (EGFR) expression profiles and was associated with younger age, longer survival, and anaplastic histology. The proliferative subtype was associated with genes related to proliferation. The mesenchymal subtype expressed genes associated with angiogenesis. Both proliferative and mesenchymal subtypes were linked to poor survival. These analyses were later conducted using broader datasets and mutation analyses for comprehensive evaluation. In 2008, The Cancer Genome Atlas Research (2008) identified three major signalling pathways: Receptor tyrosine kinase (RTK)/rat sarcoma (RAS)/phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma protein (RB). In the same year, *IDH-1/2* mutations were identified as molecular markers closely linked to secondary glioblastoma, younger age, and improved survival. In 2010, the initially proposed gene expression-based glioblastoma subclassification was updated to four subtypes (proneural, neural, classical, and mesenchymal) based on similar gene expression profiles.[23] In 2012, the epigenetic profiling results were also evaluated, leading to the classification of six subtypes. These six subtypes were correlated with different prognoses, tumour locations, and age distributions.[24] In addition to the data obtained from these genetic, epigenetic, and expression studies, next-generation sequencing technologies currently provide an additional layer of detail for understanding intra-tumour diversity and tumour progression in glioblastoma. Table 1 summarises the principal molecular changes identified in glioblastoma.

IDH1/2 Mutations

In adults, *IDH1/2* mutations in widespread diffuse gliomas predict long-term patient outcomes. IDH mutations are found in approximately 5-10% of all glioblastomas and are associated with younger age and more favourable prognosis.[25] They are rarely observed in patients aged 65 years and older. Mutant IDH generates the oncometabolite 2-hydroxyglutarate[26], which is associated with a unique epigenetic pattern called the glioma CpG island methylator phenotype (G-CIMP).[27] In the first stage of screening for IDH mutations, the main focus is on IDH1-R132H, which accounts for 90% of IDH mutations found in glioblastoma.[28,29] This analysis was performed using IDH1-R132H-specific immunohistochemistry, which is a rapid and cost-effective method. Targeted sequencing was performed for *IDH2* (codon 172) and *IDH1* R132C and R132S mutations. These can now be sequenced simultaneously as part of a larger next-generation sequencing (NGS) panel. Generally, IDH mutations are very rarely observed in older patients (>55 years).[30] On the other hand, IDH wild-type glioblastoma is typically observed in older patients and is associated with a poorer prognosis; the *TERT* promoter mutation, in particular, indicates a poor outcome in these patients.[31]

ATRX (a-thalassaemia/mental-retardation X-linked Gene) Mutations

A protein encoded by *ATRX* is responsible for chromatin remodelling and the incorporation of histone H3.3 into heterochromatin.[32] Mutations in *ATRX* are found in approximately 57% of secondary glioblastomas. Within glioblastoma cells, *ATRX* mutations are more common in IDH-mutant tumours than in wild-type tumors.[5] *ATRX* mutations are associated with better prognosis.[33]

TERT (Telomerase Reverse Transcriptase Gene) Promoter Mutations

Mutations in the *TERT* gene promoter, which encodes telomerase, elongates telomeres by adding the missing 3' end during DNA replication, result in increased telomerase activity.[34,35] The most prevalent mutations in the *TERT* promoter are C228T and C250T[36], which cause a substantial increase in TERT expression, almost quadrupling it.[34,37] These mutations are present in nearly 80% of glioblastoma cases.[34,38,39] *TERT* promoter mutations are more common in IDH wild-type glioblastoma than in IDH mutant glioblastoma.[5] However, the prognostic role of *TERT* promoter mutations has not been definitively established due to the presence of several confounding factors. To independently evaluate the prognostic influence of *TERT* promoter mutations, further prospective studies on large homogeneous patient populations are needed.[40]

TP53 (Tumour Protein P53 Gene) Mutations

The p53 protein is critical for regulating proliferation, survival, genomic integrity, and other cellular functions. *TP53* mutations contribute to the advancement of glioblastoma.[41] These mutations are more frequent in IDH-mutant glioblastoma than in IDH-wild type glioblastoma.[5] Gain-of-function (GOF) *TP53* mutations endow the protein with a new function or alter its expression, leading to increased cellular malignancy.[41,42] GOF mutations increase *MGMT* expression, thereby reducing the sensitivity of glioblastoma to temozolomide and decreasing overall survival.[43]

BRAF (B-Rapidly Accelerated Fibrosarcoma Gene) V600E Mutations

As part of the RAS-RAF-MEK-ERK MAP kinase pathway governing cell growth, *B-RAF* can exhibit constitutive kinase activity due to mutations in its gene, fostering unregulated cell proliferation and tumour genesis. The V600E mutation, one of the most common *B-RAF* mutations, produces a constitutively active serine/threonine kinase *B-RAF*. This mutation activates ERK1/2 and MAP kinases, disrupting the tightly regulated control of this crucial pathway. The frequency of all *B-RAF* mutations in glioblastoma is estimated to be around 2-5%.[44] This mutation is considered suitable for personalised cancer therapy with kinase inhibitors.[45]

Table 1. Key molecular alterations in glioblastoma.

Gene	Mutation/Alteration	Impact on Glioblastoma	Prognostic Value
IDH1/2	R132H, R132C, R132S, and	Associated with younger age and better prognosis	Positive
ATRX	Mutations	Involvement in chromatin remodelling	Positive
TERT	C228T, C250T	Increased telomerase activity	Variable requires further study
TP53	Gain of Function (GOF)	Increased proliferation and drug resistance	Negative
B-RAF	V600E	Constitutive kinase activity and cell proliferation	Suitable for targeted therapy
EGFR	Amplification, EGFRvIII	Promotes cell proliferation	Mixed, not definitively established

EGFR Mutations

EGFR functions as a receptor with tyrosine kinase activity, promoting cell proliferation via the activation of the mitogen-activated protein kinase (MAPK) and PI3K-Akt pathways.[46] Approximately 40% of glioblastoma cases exhibit *EGFR* amplification.[47] While some studies have reported an association between *EGFR* amplification and poor prognosis, this relationship has not been definitively established.[48,49] *EGFR* amplification is more common in IDH-wild-type glioblastomas than in IDH-mutant glioblastoma.[5] The most common *EGFR* mutation is a large deletion spanning exons 2 to 7, known as EGFRvIII.[50]

MGMT (O6-methylguanine DNA Methyltransferase Gene) Mutations

The protein encoded by the *MGMT* gene is responsible for repairing DNA by removing an alkyl group located at the O6 position of guanine, a critical site for DNA alkylation. *MGMT* promoter methylation predicts the efficacy of temozolomide chemotherapy in both newly diagnosed and potentially recurrent glioblastomas.[51,52] The role of *MGMT* in resistance to alkylating chemotherapy was idehas beenfied, leadingand it has the most impactful biomarker in clinical decision-making, particularly for older patients glioblastoma patients.[53]

PTEN Mutations

The loss of heterozygosity or methylation mutations in *PTEN* results in the loss of functional *PTEN*, which disrupts the PI3K/Akt pathway and affects cell survival, growth, and proliferation regulation.[54] This disruption affects pathways involved in PI3K. *PTEN* mutations are found in at least 60% of glioblastoma cases.[55] The loss of *PTEN* function is associated with poor prognosis in glioblastoma.[56]

DIAGNOSIS AND IMAGING

Most glioblastomas are diagnosed symptomatically because of their rapid growth, which leads to the development of seizures or neurological deficits. Symptoms can include new-onset epilepsy, headache, altered mental status, and signs of increased intracranial pressure. Contrast-enhanced magnetic resonance imaging (MRI) is the diagnostic method of choice for glioblastoma diagnosis. These tumours typically appear as contrast-enhancing necrotic mass with surrounding oedema and infiltrative tumour tissue. Contrast-enhanced computed tomography is less sensitive in detecting the typical features of glioblastoma and is reserved for acute situations, such as suspected haemorrhage or when MRI is not possible because of the presence of a pacemaker or other metallic implants. Amino acid positron emission tomography (PET) is increasingly performed before biopsy to direct the biopsy site to metabolic hot spots that might represent higher tumour grades.[57] However, PET imaging is excluded from the conventional treatment regimen for individuals with glioblastoma. MRI offers crucial anatomical information about the tumour and surrounding brain structures, thereby aiding in surgical planning. Functional MRI is particularly valuable for tumours near critical areas, helping to plan the best surgical approach and ensuring the safe maximal resection of contrast-enhancing tumours to enhance patient survival. Additionally, MRI facilitates the differentiation of glioblastomas from other contrast-enhancing lesions like abscesses, primary central nervous system lymphomas, and metastases from non-primary brain tumors.[58,59] Despite this, the imaging characteristics of glioblastoma can vary greatly, necessitating a tissue-based diagnosis.[53] The tissue required to confirm the diagnosis of glioblastoma can be acquired through stereotactic or open biopsy or microsurgical resection of the tumour.

CLINICAL COURSE

Glioblastoma clinical progression is dictated by the tumour's location and spread dynamics within the brain. Tissue destruction, oedema, and epilepsy exacerbate clinical symptoms, resulting in rapid deterioration in some patients. Although the prognosis for glioblastoma is inevitably fatal, standard treatments can temporarily stabilise or improve quality of life and cognitive functions, even in older and severely affected populations.[60-62] Approximately 20% of glioblastoma patients initially show sensorimotor deficits, whereas around 5% experience aphasia due to tumours in the speech-dominant hemisphere, usually the left side.[63] Epilepsy occurs initially in 24-68% of patients and develops in 19-38% as the disease progresses.[64,65] Early epilepsy symptoms are linked to longer survival, likely due to younger age, cortical tumour location, and smaller tumour size, indicating better surgical outcomes and earlier detection.[63,64] Less than one-third of patients report headache as the initial symptom.[63] Other symptoms like increased intracranial pressure, nausea, vomiting, dizziness, fatigue, and cognitive decline, may appear at diagnosis and may worsen over time. A few patients may have stable disease and remain largely neurologically asymptomatic for years. However, most patients experience a significant decline in quality of life after failure of first-line treatments.[60-62] In summary, glioblastoma lacks a typical clinical presentation. Compared with other gliomas, glioblastoma is characterised by faster dynamics and a slightly lower incidence of epilepsy.

CURRENT TREATMENTS

Despite recent advances in the understanding of the biology of glioblastoma, patient prognosis remains poor. Current treatment methods rely on a combination of surgery, radiotherapy, and chemotherapy. Even with this standard treatment, the median overall survival is approximately 15-18 months, and the 5-year survival rate is below 10%.[65,66]

Temozolomide, an alkylating agent used in chemotherapy, is a key component of treatment. The methylation status of the *MGMT* promoter predicts the benefit of alkylating chemotherapy with temozolomide and guides treatment choices. Phase III trials have consistently shown that glioblastoma patients with *MGMT* promoter methylation experience approximately 50% longer median survival when treated with Temozolomide. In glioblastomas without *MGMT* promoter methylation, temozolomide has little to no benefit.[51] The use of temozolomide in these patients, particularly within the context of clinical trials, is debated, but an increasing number of studies are exploring this approach. Currently, detecting specific gene mutations in the tumour provides valuable information about the clinical course of the disease and enables the development of targeted therapies. However, there are still many hurdles to overcome for treating this invariably fatal cancer.

Challenges in the treatment of glioblastomas

Due to their aggressive behaviour and resistance to treatment, glioblastomas have high mortality rates. This is partly attributed to the tumours' location within the central nervous system and the neurological toxicities associated with treatment.[67] Another critical issue is the blood-brain barrier. Glioblastomas utilise the blood-brain barrier, a natural defence mechanism of the brain against toxins. This barrier restricts the diffusion of compounds to small, uncharged, lipid-soluble molecules. Since most drugs do not possess these characteristics, they cannot significantly penetrate the blood-brain barrier.[68] Given that several recent clinical trials have failed to improve survival due to the inability to achieve therapeutic concentrations in the target area, brain penetration remains a major challenge in glioblastoma treatment. Approaches to tackle this challenge involve developing a greater number of substances that can effectively penetrate the blood-brain barrier, utilising endogenous entry transporters, and employing focused ultrasound to temporarily disrupt the blood–brain barrier.[69]

Another critical challenge in treating glioblastoma is the high degree of heterogeneity. Glioblastomas exhibit multiple genetic factors throughout tumour progression.[70] Intratumoral heterogeneity at both the molecular and functional levels heightens the complexity of glioblastomas. For example, various regions within the same tumour may possess distinct genetic compositions, transcriptional subtypes, or cells with varying proliferation kinetics.[71-75] This heterogeneity can impact treatment outcomes, as functionally diverse glioma cells within the tumour may respond differently to temozolomide or ionising radiation.[75,76]

RISK FACTORS

The vast majority of glioblastoma patients have no prior history of cancer, with approximately 5% of all gliomas being familial. However, there are multiple rare Mendelian inherited syndromes associated with adult glioma and glioblastoma. Less than 1% of glioblastomas are related to hereditary cancer syndromes, such as Li-Fraumeni Syndrome, Turcot Syndrome, Neurofibromatosis Types 1 and 2, tubrous Sclerosis Complex, and Cowden Syndrome. These glioblastomas are often diagnosed as secondary to WHO grade II or III gliomas.[77] Given family history data, the frequency of germline variants was higher than expected. Data indicate that 13% of patients with gliomas have at least one pathogenic or likely pathogenic variant.[78] Genome-wide association studies have identified 25 single-nucleotide polymorphisms (SNPs) associated with an increased risk of glioma, with 11 of these specifically linked to glioblastoma.[79] These studies have also identified loci involving critical glioma genes, such as *EGFR, TERT*, cyclin-dependent kinase inhibitor 2B (*CDKN2B*), and regulator of telomere elongation helicase 1 (*RTEL1*).[79]

Among long-term survivors who received high-dose radiation for primary brain tumours in childhood, an increased risk of glioblastomas has been demonstrated.[80] The findings indicate that patients who received high-dose radiation therapy were significantly more likely to develop glioblastoma than those who did not receive such treatment. These findings suggest that high-dose radiation can cause DNA damage and malignant transformation, thereby increasing the risk of glioblastoma. However, radiation doses during diagnostic imaging are not considered a risk factor.[81,82]

The potential risk factor for cell phone use (non-ionising radiation exposure) for brain tumours has been extensively studied, but no definitive link has been established.[4,83] No association with smoking or other carcinogens. The expression of cytomegalovirus genes and their interaction with key pathways that drive the malignant phenotype of glioblastoma suggest a potential oncomodulatory role for cytomegalovirus, although its role as an initiating agent for glioma has not been definitively confirmed.[84,85]

Ionising radiation to the brain is the only external risk factor for the development of glioblastoma.[86-88]

There is no strong link between specific ethnic groups and glioblastoma risk although one study found higher incidence rates among Caucasians than among Asians and African Americans.[2] Another study showed high molecular similarity in glioblastoma between Japanese and Swiss patients despite their different genetic backgrounds.[89,90]

Conducting more extensive epidemiological studies, genome-wide association studies (GWAS), and molecular and biomarker studies will contribute significantly to the better identification of potential risk factors. These efforts will be crucial in future research into the risk factors associated with glioblastoma.

NOVEL THERAPIES AND FUTURE PERSPECTIVES OF GLIOBLASTOMA TREATMENT

Advancements in the molecular characterisation of glioblastoma have paved the way for the development of new therapeutic strategies. The traditional WHO classification based on histology is complemented by evaluating molecular markers. A more personalised approach to glioblastoma treatment is necessary. However, the molecular profile of glioblastoma exhibits both intratumoral and temporal heterogeneity, thereby complicating treatment strategies.[71,74] Despite these challenges, emerging technologies and increased knowledge continue to facilitate the discovery and clinical testing of promising novel treatment concepts. Completed and ongoing clinical trials related to glioblastoma are shown in Table 2.[91-94]

Immunotherapy

The application of immunotherapy in glioblastoma treatment has been investigated for many years, with limited success so far.[95] However, the discovery of promising targets, technological advancements, and success in early-stage clinical trials have reignited interest in this approach. Recurrence of glioblastoma is a common issue that limits patient recovery, with approximately 50% of patients unable to access second-line treatments.[96,97] Research has shown that glioblastoma tumour cells create an immunosuppressive microenvironment by increasing factors such as FASL, PD-1, indoleamine 2,3-dioxygenase (IDO), and STAT3.[98] Microglial cells promote systemic immunosuppression by producing IL-1 and TGF-β.[99] Immunosuppressive markers like PD-1 increase, altering CTL phenotypes. Vaccination and anti-PD-1/CTLA-4 treatment targets glioblastoma-associated antigens (e.g., EGFRvIII). Oncolytic viral therapy uses viruses to trigger an immune response against the tumour, exploiting viral defence weaknesses to infect tumour cells.[100] U.S Food and Drug Administration (FDA) has approved monoclonal antibodies targeting PD-1, CTLA-4, and PD-L1 for cancer treatment.[101,102]

In recent years, the therapeutic successes achieved with immune checkpoint blockade and CAR-T cells in immunotherapy aimed at utilising the immune system to treat cancer have laid the foundation for the clinical development of immunotherapy, significantly improving treatment outcomes for many cancer patients.[103-105] Using this method, T cells are genetically engineered to express Chimeric Antigen Receptors (CAR) that are specifically directed against antigens on tumour cells.[106] The clinical potential of CAR-T cell therapy has been best demonstrated in haematologic malignancies.[107,108] Various clinical studies have tested CAR-T cell therapies targeting epidermal growth factor receptor variant III (EGFRvIII), interleukin (IL)13Rα2 (IL-13Ra2), and ephrin-A2 (Her2) for glioblastoma, showing clinical benefits in progressive glioblastoma patients.[109-111] However, antigen escape mechanisms can negatively impact the durability of responses to CAR-T cell therapy.[104]

Oncolytic Viral Therapy

For treating glioblastoma, oncolytic viruses are used as vectors for somatic gene therapy by targeting the molecular pathways that drive malignant tumour. These viruses exert their effects by either provoking an inflammatory host response or directly destroying glioma cells through extensive replication (Figure 1).[112] Oncolytic virotherapy, a promising immunotherapy for glioblastoma, includes replication-competent viruses that destroy cancer cells and replication-deficient viral vectors that deliver therapeutic genes.[113,114] The first group of oncolytic viruses includes Newcastle disease viruses, reoviruses, and parvoviruses. The second group consists of adenoviruses, herpes simplex viruses, vaccinia viruses, vesicular stomatitis viruses,

Table 2. Clinical trials for glioblastoma.

Therapeutic Approach	Trial Name	Status	Outcome	Participants	Start Date	NCT Number
Oncolytic Viral Therapy	Phase II DNX-2401 for Recurrent Glioblastoma	Active, not recruiting	Median survival improvement	25	2017	NCT03152318
	DNX-2401 plus pembrolizumab	Active	Improved survival and safety rates	48	2018	NCT02798406
	Toca 511 and Toca FC	Completed	Increased overall survival	403	2015	NCT02414165
	PVSRIPO for Recurrent GBM	Active	Enhanced survival and safety	61	2017	NCT01491893
	Oncolytic DNX-2401 adenovirus	Recruiting	Tumour response and safety	170	2017	NCT03178032
	Adenoviral gene therapy vector carrying IL-12 (NCT03636477)	Recruiting	Safety and efficacy evaluation	35	2024	NCT03636477
	Oncolytic herpes simplex virus (oHSV) with IL-12 (NCT02062827)	Completed	Increased immune cell infiltration	25	2023	NCT02062827
	Adenovirus DNX-2401 with mesenchymal stem cells (NCT03896568)	Recruiting	Reduction in tumour size	50	2024	NCT03896568
Immunotherapy	PD-1 Blockade in Recurrent Glioblastoma	Active	Increased survival in some patients	30	2015	NCT02337686
	Nivolumab + Bevacizumab	Completed	Optimal dosing and safety	75	2017	NCT03452579
	Ipilimumab and Nivolumab	Active	Tumour response and survival	153	2016	NCT02311920
	Dual-target CAR T-cell therapy (NCT05168423)	Ongoing	Tumour size reduction	6	2024	NCT05168423
	CAR-TEAM cells (NCT02986178)	Ongoing	Tumour regression	3	2023	NCT02986178
	mRNA Vaccine Boost (NCT05101212)	Ongoing	Immune response boosting	4	2024	NCT05101212
Molecular Therapeutic Targets	BRAF V600E Mutation in Paediatric Glioblastoma	Completed	Tumour response to targeted therapy	30	2013	NCT01677741
	Targeting EGFRvIII with Rindopepimut	Completed	No significant benefit	745	2015	NCT01480479
	BRAF Inhibitor Vemurafenib for Recurrent GBM	Active	Efficacy and safety	25	2019	NCT03973918

Table 2. Continued

	CDK4/6 inhibitor abemaciclib/EGFR/HER2 inhibitor ceratinia (INSIGhT trial)	Ongoing	No improvement in overall survival	150	2023	NCT02977780
Anti-Angiogenic Therapy	Bevacizumab Plus Radiotherapy for Newly Diagnosed Glioblastoma	Completed	Progression-free survival	120	2011	NCT01390948
	Bevacizumab + Radiation + Temozolomide	Completed	No improvement in overall survival	978	2008	NCT00884741
	Blood-brain barrier opening using ultrasound for chemotherapy	Recruiting	Increased drug concentrations in the brain	20	2024	NCT04121455
Targeting DNA Damage Response	PARP Inhibitor along with Temozolomide for Glioblastoma	Completed	Enhanced DNA damage in tumour cells	40	2012	NCT01477489
	PARP Inhibitor Olaparib + Temozolomide	Recruiting	Safety and efficacy	60	2018	NCT03212742
	PARP inhibitor velitaris combined with temozolomide	Ongoing	Enhanced sensitivity to chemotherapy	38	2023	NCT03581292
	Veliparib and Radiation for MGMT-unmethylated GBM	Active	Increased survival and safety	150	2017	NCT02152982
Targeting Tumour Metabolism	Targeting Tumour Metabolism in Glioblastoma Cells using Ketoconazole	Terminated	No significant benefit was observed	15	2016	NCT02873416
	Metformin with Temozolomide and Radiation Therapy	Active	Tumour response and progression-free survival	30	2016	NCT02338516
	PTEN pathway inhibition with posaconazole	Recruiting	Tumour size reduction	40	2024	NCT03757805
	Ketogenic Diet for Recurrent Glioblastoma	Active	Feasibility and safety	18	2019	NCT05110918
Others	Mesenchymal Stem Cells in Recurrent GBM	Recruiting	Safety and preliminary efficacy	15	2017	NCT03072134
	Dose-escalated Photon Irradiation or Proton Beam Radiation Therapy	Active	Tumour response and safety	110	2017	NCT02179086
	Temozolomide vs. Radiotherapy for Elderly Patients	Completed	Survival benefit for specific patient populations	373	2011	NCT00786682

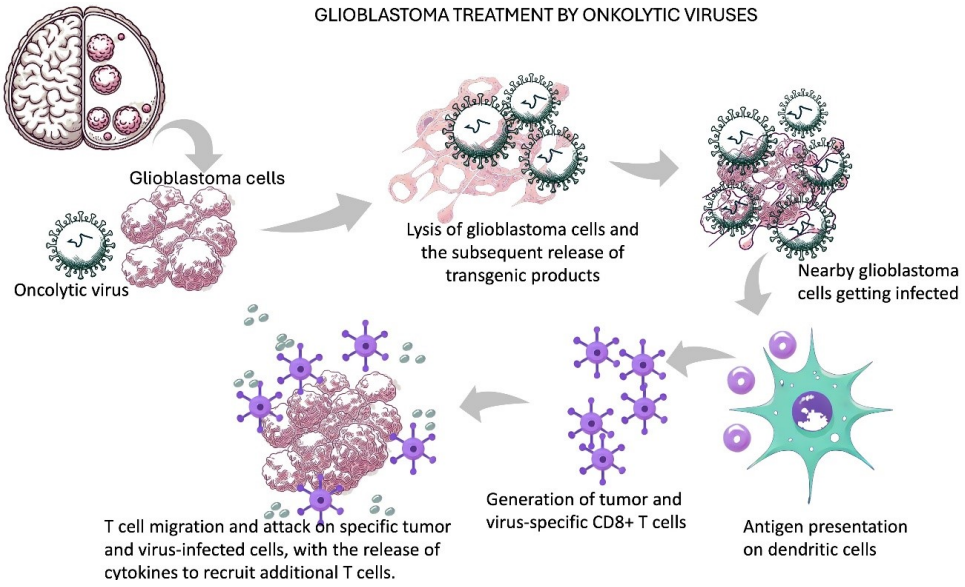

Figure 1. Mechanism of oncolytic virus-induced glioblastoma treatment.

polioviruses, and measles viruses. These viruses are genetically modified to improve their ability to target tumours specifically and to reduce their pathogenic effects.[113-115] To date, over 20 oncolytic virus candidates have been tested in clinical trials for the treatment of glioblastoma.[116-119] Oncolytic viruses are effective in glioblastoma treatment because they replicate rapidly in fast-growing cells and adapt well to the brain environment, where distant metastases are absent.[120,121] They initiate an anti-cancer immune response by transforming "cold tumours" into "hot tumours," making them susceptible to immune attacks.[122,123] This process, called immunogenic cell death, involves the release of DAMPs, PAMPs, TAAs, and cytokines.[124-126] Oncolytic viruses also enhance antigen-presenting cell function, leading to the recruitment of cytotoxic $CD8^+$ T lymphocytes to the tumour site, resulting in tumour cell destruction.[127,128]

Numerous oncolytic viral therapies for glioblastoma recurrence are either in Phase I clinical trials or have completed this phase. Clinical research studies for the treatment of recurrent glioblastoma using an adenoviral gene therapy vector carrying IL-12 cDNA, which is activated by an orally administered agent (NCT03636477), are still ongoing. Recent updates indicate that the trial is actively recruiting patients and is in the phase of evaluating the results. This study aimed to investigate the efficacy and safety of the combination of adenoviral gene therapy and valacyclovir, particularly in patients who do not respond to immune checkpoint inhibitors. In this study (NCT03576612), participants taking the oral drug valacyclovir were administered an adenoviral vector carrying thymidine kinase cDNA, which causes cytotoxicity. This trial is investigating the safety and efficacy of adenoviral vectors combined with valacyclovir and chemoradiation in patients newly diagnosed with glioblastoma. The treatment involving the stereotactic injection of oncolytic herpes simplex virus type 1 carrying IL-12 cDNA (NCT02062827) aims to evaluate the safety and efficacy of a genetically engineered herpes simplex virus type 1 in patients with recurrent or progressive glioblastoma, anaplastic astrocytoma, or gliosarcoma.[100,118] The treatment involving the stereotactic injection of an oncolytic herpes simplex virus engineered to better replicate in glioblastoma cells and in cells expressing the stem cell marker nestin (NCT03152318) uses an oncolytic herpes simplex virus designed to replicate more efficiently in cells expressing nestin.[112] Another clinical trial (NCT03896568) is evaluating the intra-arterial delivery of the oncolytic adenovirus DNX-2401 loaded with allogeneic bone marrow-derived human mesenchymal stem cells.[124,128] The study (NCT03072134) involving the injection of neural stem cells carrying oncolytic adenovirus into newly diagnosed glioblastoma patients is being conducted to gather safety and efficacy data.[124,128] Based on the results, both oncolytic viral and gene therapy treatments have been observed to be well tolerated. Posttreatment tissue analysis revealed increased immune cell infiltration and changes in immune response, including the presence of cytotoxic T cells.

Advanced stage trials (Phase II and beyond), such as the recombinant nonpathogenic polio-rhinovirus chimaera PVSRIPO trial (NCT02986178) for patients with recurrent glioblastoma, also show promise. To overcome the limitations posed by the BBB, Desjardins et al. reported a new technique for convection-enhanced delivery of PVSRIPO.[129] convection-enhanced delivery is an innovative method that uses a pressure gradient in a catheter to deliver therapeutic agents to the CNS's interstitial areas.[130] For successful virotherapy, oncolytic viruses need safe and effective delivery. Given the challenges of transporting viruses to the central nervous system and the immune system's ability to neutralise them, intratumoral delivery has become

the primary method. This treatment is typically administered via injection during surgical procedures. PVSRIPO is a live attenuated poliovirus type 1 vaccine modified with the internal ribosome entry site of human rhinovirus type 2 to reduce neurovirulence. Targeting glioblastoma via CD155, which is commonly upregulated in malignant cells, PVSRIPO showed no neurovirulent potential in a Phase I trial (NCT01491893) using intratumoral convection-enhanced delivery in patients with recurrent glioblastoma. Additionally, preliminary data indicated that patients receiving PVSRIPO immunotherapy had higher 24- and 36-month survival rates compared with the control groups.[129] Based on the findings from Phase I, a Phase II randomised trial (NCT02986178) is ongoing, investigating PVSRIPO alone or in combination with lomustine in patients with recurrent glioblastoma. The FDA has granted PVSRIPO both breakthrough therapy designation and an orphan drug status, highlighting its potential for significant therapeutic advancement for patients with glioblastoma. The therapeutic efficacy of this novel treatment modality is eagerly anticipated in patients with glioblastoma.

Another viral therapy, foraneen obadenovec (VB-111), was evaluated in a phase III trial that revealed that the combination of VB-111 with bevacizumab did not offer a survival advantage compared with bevacizumab alone.[131] This failure suggests that the simultaneous administration of bevacizumab may have inhibited the viral therapy's effects. Despite this, optimism remains for developing new treatment strategies. This optimism is further supported by ongoing research efforts to identify molecular and immunological variables and targets.

Molecular Therapeutic Targets

The discovery of small molecules that interfere with the molecular mechanisms of glioblastoma treatment is promising. However, significant challenges remain, such as the scarcity of agents capable of crossing the blood-brain barrier, recurrent signalling pathways, and tumour heterogeneity. The 2016 WHO classification includes molecular parameters for some brain tumours (e.g., *BRAF*V600E, *IDH1*-R132H). Biomarkers like EGFR amplification and EGFRvIII mutation are prominent in glioblastoma treatment.[132,133]

IDH mutations are observed in approximately 10% of glioblastomas and serve as significant therapeutic targets.[23] Mutant IDH inhibitors have shown promising preclinical findings, but they need to be validated through clinical trials.[133] EGFR amplification is mutually exclusive to IDH mutations and is present in approximately 50% of IDH wild-type glioblastomas.[134]

Receptor tyrosine kinase inhibitors like erlotinib and sorafenib are among the molecular targets used in glioblastoma treatment. It is crucial to verify the presence of molecular targets in tumours to evaluate the efficacy of these inhibitors. Accordingly, clinical trials on selected patients possessing specific molecular characteristics are ongoing. The NCT01975701 trial investigated the efficacy of targeted therapy in newly diagnosed patients with glioblastoma, focusing on fibroblast growth factor (FGF) fusion proteins and activation mutations.[135,136]

The NCT01349660 trial evaluated the use of a PI3K inhibitor in patients with recurrent glioblastoma. The completed NCT01339052 trial assessed the efficacy of PI3K inhibitors, alone or in combination with other anti-angiogenic agents, in patients with recurrent glioblastoma. The results indicated that while PI3K inhibitors were effective, they did not significantly improve overall survival. Similarly, the NCT01870726 trial also examined the impact of PI3K inhibitors in patients with recurrent glioblastoma.[137] The findings revealed that although PI3K inhibitors were safe and well-tolerated, they did not provide substantial clinical benefits.

These findings highlight the importance of molecular-based patient selection for targeted therapies against glioblastoma. Future studies should focus on optimising treatment strategies by investigating novel therapeutic targets.

Anti-Angiogenic Therapy

Angiogenesis is a hallmark of glioblastoma, and the anti-VEGF antibody bevacizumab is the most extensively studied drug targeting this process. While bevacizumab extends progression-free survival, it has not demonstrated a clear benefit in overall survival.[131,137] Ongoing research is focused on identifying molecular markers that could predict which patients would benefit from antiangiogenic therapies.[138]

Targeting DNA Damage Response Pathways

Among the most effective nonsurgical treatments for gliomas are radiotherapy and cytotoxic chemotherapy, both of which induce DNA damage.[65] Enhancing the effects of these agents on tumours while protecting normal tissue is particularly crucial for the treatment of glioblastoma. DNA damage response (DDR) inhibitors, when used with these therapies, can increase unrepaired double-strand breaks and single-strand breaks, thereby enhancing sensitivity to chemotherapy and radiotherapy.[139] However, DDR inhibitors can cause myelosuppression, limiting their combination with temozolomide.[140] The combination of DDR inhibitors with temozolomide could be an important biomarker.[140,141] Loss of MGMT protein expression predicts sensitivity to temozolomide, and PARP inhibitors, a type of DDR inhibitor, have proven to be effective biomarkers in combination with temozolomide.[140,141] Therefore, identifying molecular biomarkers associated with glioblastoma is critical for developing effective and safe drug combinations.

Targeting Tumour Metabolism

Targeting tumour metabolism is a significant strategy for glioblastoma treatment. The inhibition of metabolic regulators,

such as PTEN, HK2, and PINK1, has shown therapeutic benefits in glioblastoma.[142,143] Additionally, cholesterol metabolism presents a potential therapeutic target in some glioblastomas.[144]

Tumour-Treating Fields Therapy

Tumour-treating field therapy is an innovative approach for treating glioblastoma that employs low-intensity alternating electric fields to disrupt tumour cell division. This non-invasive therapy has been integrated into standard treatments for glioblastoma, recurrent glioblastoma, and mesothelioma, demonstrating significant survival benefits by exerting biophysical forces on charged molecules, thereby inhibiting cancer cell proliferation and affecting processes such as DNA repair and immunological responses.[145,146] Current research underscores tumour-treating fields therapy's efficacy when combined with conventional treatments like chemotherapy and radiation, leading to promising advancements in patient survival outcomes. Ongoing studies refine treatment protocols, elucidate their mechanistic impact on glioblastoma progression, and explore innovative applications to enhance therapeutic effectiveness. Future directions include investigating personalised approaches and integrating tumour-treating fields therapy into comprehensive treatment strategies to improve patient outcomes.

CONCLUSION

Despite substantial advancements in our understanding of the molecular pathogenesis and biology of glioblastoma, patient outcomes have not significantly improved. Glioblastoma remains a highly aggressive and lethal cancer with limited effective treatment options. The prognosis for patients with glioblastoma is generally poor, with standard treatments offering only temporary stabilisation and modest improvements in quality of life. To address these challenges, several promising therapeutic strategies are being explored and translated into clinical practise. The development of novel therapies grounded in robust scientific rationale is crucial. Moreover, enhancing the efficiency of clinical trial evaluations is essential for accelerating the discovery of effective treatments. Increasing the participation of patients with glioblastoma in phase I oncology trials can help identify potential new therapies earlier in the treatment pipeline. Conducting "window-of-opportunity" phase 0 surgical studies to assess blood-brain barrier penetration and pharmacodynamic effects can provide early insights into the potential efficacy of new treatments. Incorporating molecular imaging techniques and using blood and cerebrospinal fluid biomarkers more extensively can help monitor treatment responses and better understand the disease. Including a broad range of molecular biomarkers in clinical trial designs can help tailor treatments to individual patients and improve outcomes. Streamlining the design of clinical trials and increasing patient enrolment can accelerate the development of new therapies. By implementing these changes, the goal is to identify more effective treatments for patients with glioblastoma, ultimately improving survival and quality of life.

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ORCID IDs of the authors

Ozlem Yildirim 0000-0003-2674-4828
Evren Onay-Ucar 0000-0003-1152-4881

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