

REVIEW ARTICLE

Molecular advances in high-grade glioma: From diagnosis to the hope of precision therapy

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Abstract

High-grade gliomas, particularly glioblastoma, remain among the deadliest primary brain tumors, with a median survival under 18 months despite aggressive multimodal therapy. The 2021 World Health Organization classification integrated molecular markers with histology, transforming diagnostic and therapeutic paradigms. *IDH* mutation status, *MGMT* methylation, and comprehensive genomic profiling now guide treatment decisions and provide critical prognostic information. Despite targeted therapy successes in other cancers, glioblastoma's profound intratumoral heterogeneity, redundant signaling pathways, impenetrable blood–brain barrier, and immunosuppressive microenvironment have limited single-agent efficacy. However, recent immunotherapy breakthroughs, particularly chimeric antigen receptor (CAR)-T cells with dramatic early responses, combined with novel delivery systems and rational combinations, offer renewed hope. This review synthesizes current molecular diagnostics, targeted therapies, emerging immunotherapeutic approaches, and technological innovations, emphasizing that future progress requires precision medicine integrating comprehensive profiling with multimodal treatments tailored to individual tumor biology.

Keywords: Glioblastoma; High-grade glioma; Precision medicine; Biomarker; Targeted therapy; Immunotherapy; Chimeric antigen receptor-T cells

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1. Introduction

1.1. Epidemiology and clinical burden

Glioblastoma accounts for 54% of malignant gliomas and 16% of primary brain tumors, affecting approximately 10,000 Americans annually with an incidence of 3.19 per 100,000 persons.^{1,2} The median age at diagnosis is 64 years, with a peak incidence at 75–84 years.¹ Despite maximal therapy—surgical resection, radiotherapy, and temozolomide—median survival remains 15–17 months with 5-year survival under 10%.^{2,3} This dismal prognosis reflects fundamental biological challenges distinguishing glioblastoma from other solid tumors.

1.2. Biological complexity

Glioblastoma exhibits rapid proliferation, diffuse brain infiltration, robust angiogenesis, and profound genetic heterogeneity.^{3,4} Tumor cells migrate extensively along white matter

tracts and blood vessels, rendering complete surgical resection impossible.⁴ Intratumoral heterogeneity—with multiple genetically distinct subclones within a single tumor—drives therapeutic resistance, as treatments that eliminate one clone leave behind resistant populations that drive recurrence.⁵ The tumor microenvironment is profoundly immunosuppressive, characterized by sparse T-cell infiltration, abundant immunosuppressive myeloid cells, and high expression of checkpoint molecules.⁶ This hostile immune landscape, combined with the blood–brain barrier (BBB)’s limitations on drug delivery, creates formidable therapeutic obstacles.

1.3. The 2021 World Health Organization classification

The 2021 World Health Organization (WHO) classification has revolutionized glioma diagnosis by integrating molecular markers with histology.⁷ Adult diffuse gliomas are now classified as *IDH*-mutant astrocytoma (grades 2–4), *IDH*-mutant and 1p/19q-codeleted oligodendroglioma (grades 2–3), or *IDH*-wild-type glioblastoma (grade 4). Critical markers include *IDH1/2* mutations, 1p/19q codeletion, *EGFR* amplification, +7/–10 copy number changes, *CDKN2A/B* deletion, *TERT* promoter mutations, *ATRX* loss, *TP53* mutations, and histone H3 alterations. This molecular classification provides superior prognostic stratification compared to histology alone.^{2,7} Importantly, the recognition that pediatric-type molecular signatures, such as diffuse pediatric-type high-grade glioma subtypes (receptor tyrosine kinase 1 [RTK1], RTK2, and N-myc proto-oncogene protein [MYCN]), can occur across age groups continues to expand our understanding of glioma molecular diversity. Recent reports of novel fusions, such as *SYN2::PPARG* in RTK1 subclass C tumors presenting in older adults, underscore the need for comprehensive

molecular profiling regardless of patient age.⁸ Similarly, integrated histological, molecular, and positron emission tomography (PET) imaging analysis has revealed cases of non-enhancing diffuse pediatric-type high-grade glioma with germline *ATM* mutations demonstrating remarkable radiosensitivity, highlighting the clinical importance of identifying such molecular subsets for treatment planning.⁹

2. Methods

For this narrative review, we conducted a comprehensive literature search of PubMed/MEDLINE, Embase, Cochrane Library, and Web of Science databases through February 2026. Search terms included combinations of “glioblastoma,” “high-grade glioma,” “molecular profiling,” “targeted therapy,” “immunotherapy,” “CAR-T,” “biomarker,” and “precision medicine.” We included randomized controlled trials, prospective and retrospective cohort studies, systematic reviews, meta-analyses, landmark case series, and Food and Drug Administration (FDA) regulatory documents published in English. Preclinical studies were included when they provided essential mechanistic context for clinical findings. Data were extracted and synthesized thematically across the sections of this review. The quality and level of evidence of the included studies were evaluated, with preference given to the highest available level of evidence for each topic.

3. Molecular profiling

Comprehensive molecular testing has become the standard of care following the 2021 WHO classification. The key biomarkers, their detection methods, and clinical significance are outlined in Table 1. Higher-grade gliomas characteristically show *IDH1/2* wild-type status, and *MGMT* methylation predicts response to alkylating agents.^{10,11}

Table 1. Molecular biomarkers in high-grade glioma^{1,2,7,11–18}

Biomarker/Predictive value	Frequency in GBM	Detection method	Clinical significance	Prognostic value
<i>IDH1/2</i> mutation	5–10% primary GBM; >80% secondary GBM	IHC (R132H), DNA sequencing	Defines glioblastoma vs. <i>IDH</i> -mutant astrocytoma	Favorable (higher median survival)
<i>MGMT</i> methylation/ predicts temozolomide benefit	45–50%	Methylation-specific PCR, pyrosequencing	Predicts temozolomide response	Improved OS (21 vs. 15 months)

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Table 1. (Continued)

Biomarker/Predictive value	Frequency in GBM	Detection method	Clinical significance	Prognostic value
<i>EGFR</i> amplification	40–50%	FISH, DNA sequencing, IHC	Common in primary GBM; <i>IDH</i> -wild-type	Neutral to slightly unfavorable
<i>EGFRvIII</i> mutation	20–30% of <i>EGFR</i> + tumors	IHC, RT-PCR, sequencing	Specific to GBM; heterogeneous	Unclear; conflicting data
<i>TERT</i> promoter mutations	70–80%	DNA sequencing (C228T, C250T)	Older age, <i>IDH</i> -wild-type	Unfavorable with +7/–10
<i>CDKN2A/B</i> deletion; <i>CDK4/6</i> inhibitor target	50–60%	FISH, sequencing, array CGH	Defines glioblastoma grade 4	Unfavorable; aggressive
Chromosome 1p/19q codeletion	<1% GBM; >70% oligodendroglioma	FISH, SNP array	Defines oligodendroglioma	N/A in GBM
<i>TP53</i> mutations	30–40%	IHC, DNA sequencing	Common in <i>IDH</i> -mutant	Variable
<i>ATRX</i> loss	<5% GBM; >70% <i>IDH</i> -mutant	IHC, DNA sequencing	With an <i>IDH</i> mutation, younger patients	Favorable with <i>IDH</i>
<i>PTEN</i> loss/mutation	30–40%	IHC, FISH, sequencing	Activates PI3K/AKT	Unfavorable; resistance
<i>BRAF</i> V600E; <i>BRAF</i> /MEK inhibitors	1–2% adult; 5–15% pediatric	IHC, sequencing	Pediatric, epithelioid GBM	Variable
Chromosome +7/–10	80%	Karyotype, SNP array	Classic GBM signature	Unfavorable

Abbreviations: CGH: Comparative genomic hybridization; FISH: Fluorescence in situ hybridization; GBM: Glioblastoma; IHC: Immunohistochemistry; OS: Overall survival; PCR: Polymerase chain reaction; PI3K: Phosphoinositide 3-kinase; RT-PCR: Reverse transcription PCR; SNP: Single-nucleotide polymorphism.

3.1. Next-generation sequencing panels

Comprehensive genomic profiling using targeted panels (Foundation Medicine, Tempus, UCSF500) or whole exome/genome sequencing has become standard in neuro-oncology, providing identification of clinically relevant mutations, copy number alterations, gene fusions, tumor mutational burden, microsatellite instability status, and actionable targets for clinical trials.^{19,20} These panels enable precision medicine approaches by matching patients to

targeted therapies or immunotherapies based on specific molecular alterations. While routine in other cancers, next-generation sequencing adoption in glioblastoma accelerated following the 2021 WHO classification's emphasis on molecular diagnosis. Panels reveal the complex mutational landscape underlying glioblastoma's therapeutic resistance, identifying multiple concurrent driver and resistance mutations that inform combination therapy strategies.²⁰

3.2. Complementary molecular technologies

DNA methylation profiling using EPIC or 450K arrays enables tumor classification independent of histology, molecular subgroup prediction, *MGMT* promoter methylation assessment, CpG island methylator phenotype identification, and copy number variation estimation.²¹ The Heidelberg Brain Tumor Classifier uses methylation patterns to refine diagnosis with high accuracy, particularly valuable in histologically ambiguous cases. *MGMT* methylation is critical for predicting temozolomide response, occurring in 45–50% of glioblastomas.^{21,22} RNA sequencing provides gene expression profiling, fusion transcript identification, pathway activation assessment, and classification into molecular subtypes (proneural, neural, classical, and mesenchymal), though it remains investigational rather than standard practice.²³ Liquid biopsy using circulating tumor DNA in blood or cerebrospinal fluid enables non-invasive monitoring of tumor burden, resistance mutation detection, treatment response assessment, and minimal residual disease identification, with cerebrospinal fluid analysis often more sensitive than plasma for brain tumors, though clinical adoption remains limited.²⁴

4. Driver mutations and impact on outcome

4.1. *IDH* mutations

IDH1/2 mutations occur in most grade 2–3 gliomas and secondary glioblastomas, but under 10% of primary glioblastomas.¹² *IDH*-mutant astrocytomas demonstrate markedly superior progression-free and overall survival (OS) compared to *IDH*-wild-type tumors, with a median OS of 67 months versus 17 months for *IDH*-wild-type glioblastoma.^{2,12,13} Indeed, in 2024, vorasidenib (Vorango®, Servier Pharmaceuticals), a brain-penetrant dual isocitrate dehydrogenase 1 (*IDH1*)/*IDH2* inhibitor, acquired FDA approval for grade 2 *IDH*-mutant astrocytoma or oligodendroglioma, representing the first targeted therapy showing clinical benefit in glioma and validating precision medicine approaches in primary brain tumors.²⁵ The approval was based on the phase 3 INDIGO trial (NCT04164901), a randomized, double-blind, placebo-controlled study of 331 patients, which demonstrated a progression-free survival (PFS) hazard ratio of 0.39 (95% CI: 0.27–0.56; $p < 0.0001$), with a median PFS of 27.7 months versus 11.1 months for placebo. Vorasidenib also significantly delayed time to next intervention and was well tolerated, with no decline in quality of life compared with placebo. This breakthrough demonstrates that molecularly selected brain tumor populations can benefit from targeted therapies, offering a template for future drug development. Vorasidenib provides proof

of concept that when the therapeutic target is a true oncogenic driver, and the agent achieves adequate central nervous system (CNS) penetration, targeted therapy can succeed in brain tumors—a principle that should guide future glioblastoma-directed drug development. To date, however, no molecular phenotypes have been associated with specific drug-induced disease amelioration based on biologic markers.

4.2. *MGMT* promoter methylation

MGMT promoter methylation occurs in 45–50% of glioblastomas and predicts a response to alkylating chemotherapy.^{11,26} Methylated tumors show significantly improved PFS with temozolomide, with a median OS of 17–22 months, compared with 12–14 months in unmethylated tumors.^{20,26} *MGMT* promoter methylation status has become an essential component of the diagnostic and prognostic workup for high-grade gliomas, with ongoing debate about optimal assay methodology (pyrosequencing vs. methylation-specific polymerase chain reaction vs. genome-wide methylation arrays) and the thresholds used to define methylation positivity. Importantly, *MGMT* methylation primarily predicts benefit in *IDH*-wild-type glioblastoma but not *IDH*-mutant lower-grade gliomas, suggesting these biomarkers have distinct biological implications.²⁶ Combined *IDH* mutation and *MGMT* methylation confers optimal prognosis, with median OS reaching 36 months in some series.^{13,26}

4.3. *EGFR*, *BRAF*, and other targetable alterations

EGFR amplification occurs in 40–50% of glioblastomas, with *EGFRvIII* deletions in 20–30% of these cases.¹⁴ Despite extensive investigation, epidermal growth factor receptor (*EGFR*)-targeted therapies, including tyrosine kinase inhibitors (erlotinib and gefitinib) and vaccines (rindopepimut), have failed in phase 3 trials.²⁷ Resistance mechanisms include alternative pathway activation, particularly *PI3K/AKT* and *PTEN* loss.¹⁴ This contrasts sharply with *EGFR*-mutant lung cancer successes, highlighting glioblastoma's redundant signaling pathways.¹⁹ *BRAF* V600E mutations occur in 1–2% of adult glioblastomas but 5–20% of pediatric gliomas.^{28,29} B-Raf proto-oncogene (*BRAF*)/mitogen-activated protein kinase (MEK) inhibitor combinations show promising activity in *BRAF*-mutant pediatric low-grade gliomas (80% response rates),²⁸ but more modest responses in adult glioblastoma (33% response rates).²⁹ Table 2 summarizes the contrasting outcomes of targeted therapies in glioblastoma compared with other cancers, illustrating the unique challenges posed by glioblastoma biology.

Table 2. Comparison of targeted therapy outcomes: GBM vs. other cancers

Target	Cancer type	Drug(s)	Response rate	Median PFS	Key success factors	References
<i>EGFR</i> mutation	NSCLC	Osimertinib, erlotinib	70–80%	18–20 months	Single mutation; homogeneous; <i>EGFR</i> -dependent; good BBB penetration	14,27
<i>EGFR</i> amplification/ <i>EGFRvIII</i>	GBM	Erlotinib, gefitinib	<10%	2–3 months	Failed trials; not oncogene-addicted; <i>PTEN</i> loss; poor penetration	14,27
<i>BRAF</i> V600E	Melanoma	Dabrafenib + trametinib	64–69%	11–15 months	Single mutation; good penetration; combination therapy	28
<i>BRAF</i> V600E	GBM	Dabrafenib + trametinib	33%	3.8 months	Limited success; concurrent drivers; cold TME	29
<i>BRAF</i> V600E	Pediatric LGG	Dabrafenib + trametinib	80%	3 years	Different biology; fewer mutations; better BBB penetration	28
<i>IDH1/2</i> mutation	AML	Ivosidenib, enasidenib	42%	8.2 months	Hematologic; systemic delivery	25
<i>IDH1/2</i> mutation	Glioma grade 2–3	Vorasidenib (dual <i>IDH1/2</i>)	61% PFS benefit	27.7 months	FDA approved in 2024; CNS penetrant; oral	25
PD-L1 checkpoint	Melanoma, NSCLC	Pembrolizumab, nivolumab	30–50%	6–24+ months	Durable responses; high TMB; inflammatory TME	30-32
PD-L1 checkpoint	GBM	Nivolumab, pembrolizumab	<10%	2–3 months	Failed uniformly; low TMB; immune cold; BBB limits immune cells	30-32

Abbreviations: AML: Acute myeloid leukemia; BBB: Blood–brain barrier; CNS: Central nervous system; FDA: Food and Drug Administration; GBM: Glioblastoma; IDH: Isocitrate dehydrogenase; LGG: Low-grade glioma; NSCLC: Non-small cell lung cancer; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; TMB: Tumor mutational burden; TME: Tumor microenvironment.

5. Treatment

Standard therapy comprises maximal safe surgical resection followed by concurrent radiation (60 Gy over 6 weeks) with temozolomide, then adjuvant temozolomide for 6–12 months (“Stupp protocol”).³³ Extent of resection correlates with survival, though complete resection is rarely achievable due to infiltrative growth.^{34,35} Recent guidelines emphasize balancing aggressive resection with functional preservation using intraoperative mapping and monitoring.^{35–37} Tumor treating fields (TTFields), 200 kHz alternating electric fields delivered via scalp arrays, combined with temozolomide, improved median OS from 16 to 20.9 months in newly diagnosed glioblastoma, representing the first survival improvement since temozolomide.^{38,39} Despite initial compliance challenges, second-generation devices show improved patient satisfaction.³⁹ However, even with maximal therapy, virtually all glioblastomas recur, necessitating novel approaches.

5.1. Checkpoint inhibition: Limited success

Despite transforming melanoma and lung cancer treatment, checkpoint inhibitors (anti-programmed death-1 [PD-1] and anti-cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]) have largely failed in unselected glioblastoma patients.^{30,31} CheckMate-143 showed no survival benefit for nivolumab versus bevacizumab in recurrent glioblastoma.³² Multiple factors may explain this resistance: low tumor mutational burden, limiting neoantigen formation; profound immunosuppression in the tumor microenvironment; and systemic corticosteroid use, depleting effector T cells.^{6,40} However, rare hypermutated glioblastomas (often from mismatch repair deficiency or temozolomide-induced mutations) show dramatic checkpoint inhibitor responses, validating that appropriate patient selection is critical.^{5,31} Future trials should focus on biomarker-selected populations and combinations addressing immunosuppressive mechanisms.

5.2. Chimeric antigen receptor-T cell therapy: Breakthrough potential

Chimeric antigen receptor T (CAR-T)-cell therapy represents the most promising immunotherapy advance in glioblastoma. CAR-T cells genetically engineered to recognize tumor antigens (interleukin 13 receptor subunit alpha 2 [IL13Rα2], epidermal growth factor receptor variant III [EGFRvIII], B7 homolog 3 [B7-H3], human epidermal growth factor receptor 2 [HER2], and disialoganglioside GD2 [GD2]) have shown encouraging results in early trials.^{41–45} A landmark 2024 *NEJM* report described rapid, dramatic regression of recurrent glioblastoma using CARv3-TEAM-E cells, which

secrete T-cell-engaging bispecific antibodies recruiting endogenous T cells to attack tumors, overcoming tumor heterogeneity through bystander killing.⁴¹ The INCIPIENT trial (NCT05660369) was a first-in-human, phase 1 study in which three participants with recurrent EGFRvIII-positive glioblastoma received a single intraventricular infusion of 10×10^6 CAR-positive CARv3-TEAM-E T cells via an Ommaya reservoir. All three participants experienced dramatic and rapid radiographic tumor regression within days, with one patient achieving a near-complete response. Critically, the dual-targeting mechanism addresses a fundamental limitation of single-antigen CAR-T therapy: the CAR component targets EGFRvIII (expressed in 25–60% of glioblastomas), while the secreted T-cell-engaging antibody molecules (TEAMs) engage wild-type *EGFR*, which is expressed in >80% of glioblastomas but not in normal brain parenchyma. This creates a bystander-killing effect whereby CAR-T cells, upon engaging their cognate antigen, locally secrete bispecific molecules that redirect surrounding T cells—including regulatory T cells—against wild-type *EGFR*-expressing tumor cells. This mechanism effectively addresses intratumoral heterogeneity, a primary driver of therapeutic resistance in glioblastoma. No adverse events exceeded grade 3, and no dose-limiting toxicity was observed, underscoring the favorable safety profile of locoregional delivery. However, responses were transient in two of three participants, suggesting limited T-cell persistence, and future strategies may include preconditioning chemotherapy or scheduled repeated infusions to enhance durability.⁴¹

Regional delivery via intraventricular or intrathecal routes achieves higher concentrations at tumor sites with reduced systemic toxicity compared to intravenous administration.⁴² Bivalent CAR-T targeting both EGFR and IL13Rα2 showed promising interim phase 1 results with acceptable safety.⁴³ Bivalent and tandem CAR-T approaches that incorporate multiple antigen-recognition domains represent another strategy to mitigate antigen-loss escape, simultaneously engaging multiple tumor-associated antigens to reduce the probability of immune evasion through antigen downregulation. However, major challenges include CAR-T exhaustion in the immunosuppressive microenvironment, tumor heterogeneity with antigen-negative escape, and manufacturing complexity. Strategies to overcome exhaustion include checkpoint blockade combined with CAR-T, clustered regularly interspaced short palindromic repeats (CRISPR) deletion of inhibitory receptors, and metabolic reprogramming to enhance persistence.^{46,47} Armored CAR-T cells engineered to resist transforming growth factor-beta (TGF-β) suppression have shown enhanced efficacy in preclinical models.⁴⁶

5.3. Oncolytic viruses and vaccines

Oncolytic viruses selectively infect and lyse cancer cells while releasing tumor antigens that prime immune responses.^{48–51} G47Δ (modified herpes simplex virus type 1 [HSV-1]) gained approval in Japan in 2021 for recurrent glioblastoma.⁴³ DNX-2401 (modified adenovirus) demonstrated safety and activity in phase 2 pediatric diffuse intrinsic pontine glioma trials.⁵⁰ PVSRIPO (modified poliovirus) achieved 21% three-year survival in recurrent glioblastoma, with some durable responses.⁵¹ Combinations of oncolytic viruses with checkpoint inhibitors or CAR-T cells show synergistic immune activation in preclinical studies.^{48,51} Personalized neoantigen vaccines using whole-exome sequencing to identify patient-specific mutations have shown proof of concept in melanoma, with ongoing trials in glioblastoma.^{48,52} Off-the-shelf approaches targeting shared tumor-associated antigens (SurVaxM, HSPPC-96, IMA950) showed survival signals in phase 2 trials.⁵³ Messenger RNA (mRNA) vaccine technology, validated in COVID-19, is being adapted for cancer with rapid manufacturing enabling personalized or off-the-shelf approaches.⁵⁴

6. Tumor microenvironment modulation

Reversing immunosuppression requires targeting multiple mechanisms.^{55–59} Myeloid cell approaches include colony-stimulating factor 1 receptor (CSF-1R) inhibitors to deplete/repolarize tumor-associated macrophages, C–C chemokine receptor type 2 (CCR2) inhibitors to block monocyte recruitment, and CD40 agonists to activate macrophages to anti-tumor phenotypes. Adenosine pathway blockade using CD73 inhibitors or A2A receptor antagonists, combined with checkpoint inhibitors, has shown promise in trials.⁵⁶ Metabolic reprogramming strategies include glutaminase inhibitors to block glutamine metabolism, lactate dehydrogenase inhibitors to reduce immunosuppression, and arginase inhibitors to restore T-cell function.^{57,59} Vascular normalization using low-dose anti-vascular endothelial growth factor (VEGF) therapy improves immune cell infiltration and chemotherapy delivery, providing a rationale for immunotherapy combinations.⁵⁸

7. Emerging therapeutic strategies

The modest improvements in glioblastoma survival over recent decades underscore the critical need for innovative approaches. Multiple avenues are under investigation, spanning precision medicine, novel drug delivery, immunotherapy combinations, and technological advances. Table 3 provides an overview of ongoing therapeutic developments across eight major categories.

These approaches represent the breadth of current investigational efforts to improve outcomes in high-grade gliomas.

7.1. Novel delivery systems and surgical innovation

Penetrating the BBB represents a critical challenge in glioblastoma therapy. Focused ultrasound provides non-invasive, reversible BBB disruption under magnetic resonance guidance, enabling delivery of large molecules, including antibodies and nanoparticles, that normally cannot penetrate the CNS.^{60,61} Phase 1/2 trials have demonstrated the safety and feasibility of focused ultrasound, with ongoing studies combining it with chemotherapy, immunotherapy, and targeted agents.⁶¹ Convection-enhanced delivery achieves high local drug concentrations via direct catheter infusion, bypassing the BBB entirely, though technical challenges, including catheter placement, backflow, and distribution optimization, have limited clinical success.⁶⁰

Nanoparticle formulations using liposomes, polymeric carriers, or gold nanoparticles enable controlled drug release, active targeting via surface modifications, and potential imaging capabilities, with multiple platforms in early clinical development.⁶² Laser interstitial thermal therapy combines tumor ablation with enhanced drug delivery and has been FDA-approved for recurrent glioblastoma, with a median survival of 11–12 months post-treatment.⁶³ Skull remodeling to optimize TTF fields delivery represents a personalized approach that increases field intensity by 20–30%, with early phase 2 studies showing promise.⁶⁴

7.2. Intraoperative molecular and imaging adjuncts for margin assessment

Maximizing the extent of resection while preserving neurological function remains the cornerstone of glioblastoma surgery. Several intraoperative technologies now assist surgeons in real-time margin assessment. 5-Aminolevulinic acid (5-ALA) fluorescence-guided surgery has become a widely adopted standard: following oral administration, 5-ALA is metabolized via the heme biosynthesis pathway to protoporphyrin IX (PpIX), which selectively accumulates in malignant glioma cells and fluoresces red-pink under blue-violet excitation light (375–440 nm). Phase III trial data have demonstrated that 5-ALA increases gross total resection rates from 36% to 65% compared with white-light surgery alone, with sensitivity of 73.9–91.4% and specificity of 83.8–93.9% for intraoperative identification of high-grade glioma margins. Global adoption of 5-ALA fluorescence-guided surgery now exceeds 75% among surveyed neurosurgeons.^{65,66} Intraoperative magnetic resonance imaging (MRI)

Table 3. Emerging and future therapeutic strategies for high-grade gliomas

Category	Specific approach	Mechanism/Target	Development stage	Key trials/evidence	Potential advantages
Precision medicine & molecular targeting	Vorasidenib	Dual IDH1/2 inhibitor	FDA approved in 2024	INDIGO trial; PFS 27.7 vs 11 months	CNS penetrant; oral; first targeted therapy
	Ivosidenib (AG-120)	IDH1 selective inhibitor	Phase 3 ongoing	COMBAT trial NCT04164901	Specific IDH1; combo potential
	PRMT/MTAP inhibitors	Synthetic lethality in <i>MTAP</i> -deleted	Phase 1/2	NCT05975320, NCT04089449	Novel mechanism; ~20% GBM have <i>MTAP</i> deletion
	PI3K/AKT/mTOR inhibitors	Cell cycle arrest in <i>CDKN2A/B</i> deleted	Phase 1/2	NCT03387020, NCT04238819	Oral agents; combo with TKIs
	HDAC inhibitors	Epigenetic modulation; immune activation	Phase 1/2	NCT02717455, NCT04266275	Upregulate MHC; synergy with IT
	PARP inhibitors	DNA repair deficiency; synthetic lethality	Phase 1/2	NCT03914742, NCT02974621	For DNA repair defects; combo with TMZ
Novel delivery systems	Focused ultrasound	MR-guided transient BBB disruption	Phase 1/2	NCT03616860, NCT04528680	Non-invasive; reversible; targeted
	Convection-enhanced delivery	Local high-dose catheter delivery	Phase 1/3	Multiple trials; mixed results	Bypass BBB; high local concentrations
	Nanoparticles	Liposomes, polymeric, gold NPs	Preclinical/Phase 1	Various early trials	Controlled release; targeting; imaging
	LITT	Thermal ablation; enhanced delivery	FDA-approved ablation	Median OS 11–12 months post-LITT	Ablation; deep lesions; real-time MRI
	Skull remodeling for TTFields	Optimize TTFields delivery intensity	Phase 2	Early studies promising	Increased field intensity 20–30%
Immunotherapy: checkpoint inhibitors	Neoadjuvant pembrolizumab	Pre-surgery immune priming	Phase 1/2 promising	Immune activation; survival signal	Intact BBB; T-cell priming
	Dual checkpoint blockade	Nivolumab + ipilimumab	Phase 1/2 negative	CheckMate 143; 40% toxicity	Greater T-cell activation; toxicity limits
	Novel checkpoints	LAG-3, TIGIT, TIM-3, CD137 inhibitors	Phase 1/2	NCT03493932, NCT04656535	Overcome PD-1 resistance; combinations
	Checkpoint + radiation	PD-1/PD-L1 + RT	Phase 1/2	NCT02336165, NCT03426891	Abscopal effect; antigen release
	Checkpoint + TTFields	PD-1/PD-L1 + TTFields	Phase 2	Multiple ongoing trials	Synergistic; enhanced antigen presentation

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Table 3. (Continued)

Category	Specific approach	Mechanism/Target	Development stage	Key trials/evidence	Potential advantages
Cellular immunotherapy	CAR-T (single target)	EGFRvIII, IL13Rα2, HER2, B7-H3	Phase 1/2	NCT05660369; dramatic responses	Direct killing; persistence; multi-target needed
	Dual-target CAR-T	EGFR + IL13Rα2	Phase 1	Bagley Nat Med 2024; 62% response	Address heterogeneity; prevent escape
	CAR-T + TEAM	CARv3-TEAM-E cells	Phase 1	Choi <i>et al.</i> [41] in <i>NEJM</i> in 2024; rapid regression	Bystander killing; amplify response
	TCR-engineered T-cells	Neoantigen-specific TCR	Phase 1	Early ongoing trials	Neoantigen-specific; MHC-dependent
	TIL therapy	Expand endogenous TILs ex vivo	Phase 1	NCT03347097	Endogenous immunity; personalized
Oncolytic viruses	HSV-based (G47Δ, G207, M032)	Tumor-selective replication	Phase 1/2; Japan approved in 2021	G47Δ approved in Japan; immune priming	Selective replication; safety shown
	Adenovirus (DNX-2401)	Selective replication; immune activation	Phase 1/2	NCT03896568	RGD-modified; intratumoral
	Poliovirus (PVSR1PO)	CD155 targeting	Phase 1/2	NCT01491893; 21% 3-year OS	Durable responses in the subset
	Reovirus (Pelareorep)	Ras-pathway targeting	Phase 1/2	Combinations with a checkpoint	Intravenous delivery; well-tolerated
	OV + checkpoint inhibitors	Multiple combinations	Phase 1/2	Multiple ongoing trials	Convert cold to hot TME; rational combo
	OV + CAR-T	OV + IL13Rα2 CAR-T	Preclinical/Phase 1	Mathematical modeling; optimal timing	Synergy demonstrated in vitro
Cancer vaccines	Dendritic cell vaccines	DCVax-L; autologous DC with lysate	Phase 3 controversial	331 patients; median OS 22.3 months	Personalized; safety shown
	Peptide vaccines	SurVaxM, rindopepimut, IMA950	Phase 2/3	NCT02455557, NCT03395587	Off-the-shelf; immune response noted
	Personalized neoantigen	NeoVax, GAPVAC, individualized	Phase 1/2	Immune responses noted	True neoantigens; expensive
	mRNA vaccines	Encoding tumor antigens	Preclinical/Phase 1	Early development	Rapid production; versatile

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Table 3. (Continued)

Category	Specific approach	Mechanism/Target	Development stage	Key trials/evidence	Potential advantages
Metabolic & TME targeting	Glutaminase inhibitors	CB-839; blocks glutamine metabolism	Phase 1/2	NCT03528642	Cancer metabolism; combo potential
	IDO inhibitors	Epacadostat, Indoximod	Phase 1/2; mostly negative	Limited efficacy as monotherapy	Immunosuppression reversal
	Adenosine pathway inhibitors	Ciforadenant; A2A antagonist	Phase 1/2	NCT03800095	TME modulation; reverse suppression
	Anti-angiogenic + IT	Bevacizumab + checkpoint inhibitors	Phase 1/2	Various trials	Vascular normalization; improve TIL infiltration
Technology & surgical innovation	Intraoperative technologies	5-ALA fluorescence, iMRI, iCT	FDA-approved	Improves EOR; unclear OS benefit	Real-time visualization; functional preservation
	Molecular imaging	DESI-MS, Raman spectroscopy	Development	Research phase	Intraoperative molecular diagnosis; margins
	Liquid biopsy	ctDNA, exosomes, miRNA	Development	Research phase	Monitoring; early detection; resistance ID
	Artificial intelligence	Radiomics, deep learning	Research	Multiple studies	Precision medicine; biomarker ID
	Gene editing	Prime editing, base editing	Preclinical	Research	Correct driver mutations; delivery hurdles

Abbreviations: B7-H3: B7 homolog 3; BBB: Blood–brain barrier; CAR-T: Chimeric antigen receptor T-cell; CNS: Central nervous system; ctDNA: Circulating tumor DNA; DC: Dendritic cell; DESI-MS: Desorption electrospray ionization mass spectrometry; EGFR: Epidermal growth factor receptor; EGFRvIII: Epidermal growth factor receptor variant III; EOR: Extent of resection; FDA: Food and Drug Administration; GBM: Glioblastoma; HDAC: Histone deacetylase; HER2: Human epidermal growth factor receptor 2; HSV: Herpes simplex virus; iCT: Intraoperative computed tomography; ID: Identifier; IDH: Isocitrate dehydrogenase; IDO: Indoleamine 2,3-dioxygenase; IL13Rα2: Interleukin 13 receptor subunit alpha 2; iMRI: Intraoperative magnetic resonance imaging; IT: Immunotherapy; LAG-3: Lymphocyte activation gene 3; LITT: Laser interstitial thermal therapy; MHC: Major histocompatibility complex; MR: Magnetic resonance; miRNA: MicroRNA; mRNA: Messenger RNA; MTAP: Methylthioadenosine phosphorylase; mTOR: Mammalian target of rapamycin; NP: Nanoparticle; OS: Overall survival; OV: Oncolytic virus; PARP: Poly(ADP-ribose) polymerase; PD-1: Programmed death 1; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; PI3K: Phosphoinositide 3-kinase; PRMT: Protein arginine methyltransferase; RGD: Arginine–glycine–aspartic acid motif; RT: Radiotherapy; TCR: T-cell receptor; TEAM: T-cell-engaging antibody molecules; TIGIT: T cell immunoreceptor with Ig and ITIM domains; TIL: Tumor-infiltrating lymphocytes; TIM-3: T cell immunoglobulin and mucin domain-containing protein 3; TKI: Tyrosine kinase inhibitor; TME: Tumor microenvironment; TMZ: Temozolomide; TTFields: Tumor treating fields.

provides complementary structural information by updating neuronavigation in real time to compensate for brain shift during resection, though it adds approximately one hour of operative time and substantial cost.⁶⁶

Among emerging molecular imaging techniques, desorption electrospray ionization mass spectrometry (DESI-MS) represents a particularly promising approach for real-time intraoperative margin assessment.⁶⁷ DESI-MS analyzes tissue smears within three minutes during surgery, providing direct information on tissue type (glioma vs. normal white or gray matter), tumor cell percentage, and *IDH* mutation status via detection of the oncometabolite 2-hydroxyglutarate (2-HG). Studies evaluating DESI-MS on glioma surgical specimens have demonstrated 93% sensitivity and 83% specificity for detecting glioma at surgical margins.^{57,67} Notably, even in cases where postoperative MRI suggested gross total resection, DESI-MS detected residual tumor with >50% tumor cell percentage in approximately half of the margin biopsies, underscoring the limitations of conventional imaging for assessing surgical completeness. The combination of 5-ALA fluorescence with stimulated Raman histology and artificial intelligence-based real-time pathological diagnosis represents a further frontier, with recent data showing improved discrimination of fluorescent signal within tissue and between different cell types.^{68,69} These intraoperative adjuncts are increasingly enabling personalized, molecularly informed surgical strategies for glioblastoma.

7.3. Advanced imaging, gene editing, and functional testing

Advanced imaging, including 7T MRI, hyperpolarized MRI to assess tumor metabolism, and total-body PET scanners, combined with artificial intelligence, continues to improve diagnostic accuracy and outcome prediction.^{70,71} Gene editing technologies (CRISPR/Cas9, base editing, and prime editing) offer theoretical potential to correct driver mutations or disrupt oncogenes, though delivery challenges, off-target effects, and ethical considerations remain substantial hurdles.⁷² Patient-derived organoids and tumor-on-chip microfluidic devices enable rapid ex vivo drug screening on individual patient tumors before treatment, identifying effective agents and combinations, though turnaround time, cost, and validation against clinical outcomes require further study.⁷³

8. Prognostic factors and survival prediction

8.1. Molecular prognostic markers

Prognosis in high-grade gliomas depends on an intricate

interplay of clinical, molecular, and treatment factors, with molecular markers providing the most powerful prognostic stratification. *IDH* mutation status represents the single most important prognostic determinant, fundamentally dividing gliomas into distinct biological entities with markedly different natural histories.^{2,7,10,11} *IDH*-mutant tumors, regardless of histologic grade, have a median OS of 50–70 months, compared with 15–17 months for *IDH*-wild-type glioblastoma, reflecting fundamental differences in tumor biology, metabolism, and therapeutic vulnerabilities.¹¹ Among *IDH*-wild-type glioblastomas, *MGMT* promoter methylation provides critical prognostic and predictive information, with methylated tumors showing a median OS of 17–22 months versus 12–14 months in unmethylated cases when treated with standard temozolomide-based therapy.^{2,13,19} Importantly, recent large-scale analyses have demonstrated that *MGMT* methylation primarily predicts chemotherapy benefit in *IDH*-wild-type glioblastoma but not in *IDH*-mutant lower-grade gliomas, suggesting context-dependent biological effects.^{26,74}

Beyond *IDH* and *MGMT*, additional molecular markers refine prognostic stratification. *TERT* promoter mutations, present in 70–80% of primary glioblastomas, are associated with older age and *IDH*-wild-type status and, when combined with chromosome +7/–10 signatures, confer a particularly unfavorable prognosis.⁷ *CDKN2A/B* homozygous deletion, occurring in 50–60% of glioblastomas, defines WHO grade 4 behavior and predicts aggressive clinical course regardless of other molecular features.¹⁵ *EGFR* amplification and *EGFRvIII* mutations, while common in glioblastoma (40–50% and 20–30%, respectively), show variable and sometimes conflicting prognostic associations.¹⁶ *PTEN* loss, activating PI3K/AKT signaling, is consistently associated with unfavorable outcomes and therapeutic resistance.¹⁷ In pediatric gliomas, histone H3 K27M mutations, defining diffuse midline gliomas, predict devastating prognosis with a median survival of less than 12 months despite aggressive therapy.^{18,21}

8.2. Clinical and treatment-related factors

Traditional clinical factors remain important prognostic determinants. Age represents one of the strongest clinical predictors, with median survival declining progressively from approximately 20 months in patients under 50 years to less than 6 months in those over 75 years,⁷⁵ confirming earlier reports.⁷⁶ Performance status, typically assessed via Karnofsky Performance Status (KPS), powerfully predicts outcomes, with KPS ≥ 70 associated with significantly longer survival; extent of resection correlates with survival, with gross total resection (>98% tumor removal) associated

with a median OS of 15–16 months compared to 11–12 months for subtotal resection.⁷⁷ Tumor location, volume, and enhancement patterns on MRI provide additional prognostic information.⁷⁰

Treatment-related factors significantly impact prognosis. Completion of concurrent chemoradiation and adjuvant temozolomide according to the Stupp protocol is associated with superior outcomes.³³ The addition of TTFields in newly diagnosed glioblastoma improved median OS from 16.0 to 20.9 months.⁷⁸ Furthermore, time to recurrence after initial therapy provides important prognostic information, with early progression (within six months) predicting particularly poor outcomes.⁷⁹

8.3. Integrated prognostic models

Integrated models combining multiple variables improve individual risk stratification. Recursive partitioning analysis classifies patients into prognostic classes with median survival ranging from 4.6 months (Class VI) to 18 months (Class III).⁷⁵ More recent nomograms incorporate molecular markers, improving prognostic accuracy.⁸⁰

Advanced computational approaches promise further refinement. Deep learning models that integrate genomic, transcriptomic, radiomic, and clinical data have demonstrated superior predictive accuracy.⁷¹ Machine learning algorithms that analyze multiparametric MRI can predict molecular subtypes, *MGMT* status, and survival without requiring tissue.⁸¹ Liquid biopsy that detects circulating tumor DNA enables real-time monitoring of minimal residual disease and emerging resistance mutations.²⁴ Tumor mutational burden and microsatellite instability status predict immunotherapy responsiveness in a subset of hypermutated glioblastomas.⁸² Molecular subtyping based on gene expression profiling provides prognostic information, with the mesenchymal subtype predicting particularly aggressive behavior.²³ Immune profiling may identify patients who potentially benefit from immunotherapies.⁶ Epigenetic signatures reflecting tumor cell plasticity and stemness are associated with therapeutic resistance.⁸³

Despite these advances, 2–9% of glioblastoma patients survive beyond 5 years, often harboring favorable molecular profiles but sometimes lacking any identifiable favorable features.⁸⁴ This emphasizes the importance of individualized approaches and avoidance of therapeutic nihilism.

9. Future research directions

The future of glioblastoma treatment lies in rational combinations guided by molecular profiling rather than single breakthroughs.^{19,57} An integrated approach

would include maximal safe resection using advanced surgical technologies, comprehensive molecular profiling (genomics, transcriptomics, epigenomics, and immunoprofiling), targeted therapy based on driver mutations (*IDH*, *BRAF*, or other actionable alterations), combination chemoradiation optimized to molecular subtype, immunotherapy appropriate to immune profile (CAR-T for cold tumors, checkpoint inhibitors for tumor mutational burden-high cases), TTFields to enhance all modalities, novel delivery methods for BBB-impermeable agents, and real-time monitoring via liquid biopsy to enable adaptive adjustments.

Three converging technological domains are poised to transform precision neuro-oncology. First, artificial intelligence applied to radiomics enables the extraction of quantitative imaging features from multiparametric MRI (T1-weighted, T2/fluid-attenuated inversion recovery, diffusion, and perfusion sequences) that predict molecular status (e.g., *IDH* mutation, *MGMT* methylation, *EGFRvIII* expression), delineate tumor heterogeneity subregions, and monitor treatment response non-invasively. Habitat imaging approaches that partition tumors into spatially distinct radiomic phenotypes correlating with underlying molecular subclones represent a particularly promising application, enabling longitudinal monitoring of clonal evolution without repeated biopsies.⁸⁵

Second, liquid biopsy technologies—including circulating tumor DNA, extracellular vesicle RNA (evRNA), and tumor-educated platelets analyzed from cerebrospinal fluid and peripheral blood—offer non-invasive molecular monitoring of tumor burden, resistance mutations, and treatment response. The clinical feasibility of these approaches was demonstrated in the INCIPIENT CAR-T trial, in which cerebrospinal fluid-derived evRNA was used to track *EGFRvIII* and *EGFR* copy numbers longitudinally as pharmacodynamic biomarkers of response.⁴¹ Integration of liquid biopsy with artificial intelligence-driven analytics could enable real-time, individualized treatment adaptation before clinical or radiographic progression becomes apparent.

Third, CRISPR/Cas9 gene editing technology offers multiple applications across the glioma research and treatment continuum: functional genomic screening to identify novel therapeutic vulnerabilities in patient-derived models, engineering next-generation CAR-T cells with enhanced persistence and resistance to T-cell exhaustion (e.g., through knockout of inhibitory receptors such as PD-1, lymphocyte activation gene 3 [LAG-3], or T cell immunoglobulin and mucin domain-containing protein 3 [TIM-3]), and potential future in vivo therapeutic applications targeting driver oncogenes directly. While

delivery challenges, off-target effects, and regulatory considerations remain substantial, the rapid maturation of CRISPR technology makes clinical translation increasingly feasible.⁷²

Critical research needs include better preclinical models that predict human responses, adaptive trial designs that allow rapid iteration, biomarker-selected populations, combinations rationally designed based on resistance mechanisms, early-phase trials in newly diagnosed rather than heavily pretreated patients, and public-private partnerships to fund expensive trials. Understanding epigenetic plasticity mechanisms that enable therapy resistance, and developing PARP inhibitors or other synthetic lethal approaches that exploit DNA repair deficiencies, represent active investigation areas. The regulatory environment must support innovative trial designs and early approval based on surrogate endpoints to accelerate progress.

10. Conclusion

High-grade gliomas remain among the most challenging cancers despite decades of research. Molecular marker integration into their classification has improved prognostic stratification, with *IDH* mutation and *MGMT* methylation providing critical prognostic and predictive information. However, unlike targeted therapy successes in other cancers, attempts to exploit these mutations have yielded limited success in glioblastoma, reflecting profound heterogeneity, redundant signaling pathways, BBB impermeability, and immunosuppression. Recent immunotherapy advances, particularly CAR-T cell therapy with dramatic early responses, combined with improved understanding of resistance mechanisms, suggest breakthroughs may be achievable through rational combination strategies and precision medicine approaches. Future progress requires integrating comprehensive molecular profiling to guide personalized treatment, combination approaches targeting multiple resistance mechanisms, improved BBB drug delivery, strategies to overcome immunosuppression, earlier intervention before the development of resistant clones, better patient selection using predictive biomarkers, and supportive regulatory environments. While current outcomes remain sobering, the convergence of advanced molecular techniques, novel immunotherapies, improved delivery methods, and rational combination strategies provides cautious optimism for meaningful progress. Our patients deserve no less.

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References

1. Ostrom QT, Price M, Neff C, *et al.* CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015-2019. *Neuro Oncol.* 2022;24:v1-v95.
doi: 10.1093/neuonc/noac202
2. Li D, Chen Y, Wong TF, *et al.* Management and survival trends for diffuse gliomas diagnosed at a single neurooncology center in China during 2000 to 2020. *Sci Rep.* 2025;15:12574.
doi: 10.1038/s41598-025-95693-5
3. Alexander BM, Cloughesy TF. Adult Glioblastoma. *J Clin Oncol.* 2017;35:2402-2409.
doi: 10.1200/JCO.2017.73.0119
4. Liang BC, Thornton AF, Sandler H, Greenberg H. Malignant Astrocytoma: Focal tumor recurrence after focal external beam radiation therapy. *J Neurosurg.* 1991;75:559-563.
doi: 10.3171/jns.1991.75.4.0559
5. Touat M, Li YY, Boynton AN, *et al.* Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature.* 2020;580(7804):517-523.
doi: 10.1038/s41586-020-2209-9
6. Badani A, Ozair A, Khasraw M, *et al.* Immune checkpoint inhibitors for glioblastoma: emerging science, clinical advances, and future directions. *J Neurooncol.* 2025;171(3):531-547.
doi: 10.1007/s11060-024-04881-2

7. Louis DN, Perry A, Wesseling P, *et al.* The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol.* 2021;23:1231-1251.
doi: 10.1093/neuonc/noab106
8. Brentlinger MN, Patel J, Khan A, *et al.* Diffuse pediatric-type high grade glioma, RTK1 subtype, subclass C with SYN2::PPARG fusion in an older adult. *CNS Oncol.* 2026;15(1):2641996.
doi: 10.1080/20450907.2026.2641996
9. Cheaney B, Wood MD, Szidonya L, *et al.* Remarkable response to radiation in a non-enhancing diffuse pediatric-type high-grade glioma with germline ATM mutation: The role of PET imaging and integrated histological and molecular analysis. *Neuro Oncol Adv.* 2026;8(1):vdag059.
doi: 10.1093/nojnl/vdag059
10. Hegi ME, Diserens AC, Gorlia T, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;352(10):997-1003.
doi: 10.1056/NEJMoa043331
11. Binabaj MM, Bahrami A, ShahidSales S, *et al.* The prognostic value of MGMT promoter methylation in glioblastoma: a meta-analysis of clinical trials. *J Cell Physiol.* 2018;233(1):378-386.
doi: 10.1002/jcp.25896
12. Cohen AL, Holmen SL, Colman H. IDH1 and IDH2 Mutations in Gliomas. *Curr Neurol Neurosci Rep.* 2013;13(5):345.
doi: 10.1007/s11910-013-0345-4
13. Eid M, Alset D, Timoshkina N, *et al.* IDH mutation and MGMT methylation status in glioblastoma and other gliomas patients: a Russian retrospective cohort study. *J Egypt Natl Cancer Inst.* 2025;37(1):36.
doi: 10.1186/s43046-025-00296-w
14. An Z, Aksoy O, Zheng T, Fan QW, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. *Oncogene.* 2018;37(12):1561-1575.
doi: 10.1038/s41388-017-0045-7
15. Fortin Ensign SP, Jenkins RB, Giannini C, Sarkaria JN, Galanis E, Kizilbash SH. Translational significance of CDKN2A/B homozygous deletion in isocitrate dehydrogenase-mutant astrocytoma. *Neuro Oncol.* 2023;25(1):28-36.
doi: 10.1093/neuonc/noac205
16. Yuan F, Wang Y, Yuan L, *et al.* EGFRvIII-positive glioblastoma contributes to immune escape and malignant progression via the c-Fos-MDK-LRP1 axis. *Cell Death Dis.* 2025;16(1):453.
doi: 10.1038/s41419-025-07771-1
17. Tang S, Zhou Q, Bergholz JS, *et al.* PTEN Loss Promotes PI3K β Phosphorylation and EPHA2/SRC/p-PI3K β Y962 Complex Assembly to Drive Tumorigenesis. *Cancer Discov.* 2026;16:521-540.
doi: 10.1158/2159-8290.CD-25-1126
18. Solomon DA, Wood MD, Tihan T, Bollen AW, Gupta N, Phillips JJ, Perry A. Diffuse Midline Gliomas with Histone H3-K27M Mutation: A Series of 47 Cases Assessing the Spectrum of Morphologic Variation and Associated Genetic Alterations. *Brain Pathol.* 2016;26(5):569-580.
doi: 10.1111/bpa.12336
19. Aldape K, Brindle KM, Chesler L, *et al.* Challenges to curing primary brain tumours. *Nat Rev Clin Oncol.* 2019;16(8):509-520.
doi: 10.1038/s41571-019-0177-5
20. Gong G, Jiang L, Zhou J, Su Y. Advancements in targeted and immunotherapy strategies for glioma: toward precision treatment. *Front Immunol.* 2025;15:1537013.
doi: 10.3389/fimmu.2024.1537013
21. Al Sharie S, Sawaftah K, Qasim H, Al-Hussaini M. Methylation profiling in neuropathological tumors diagnosis: a comprehensive review. *Front Oncol.* 2025;15:1720458.
doi: 10.3389/fonc.2025.1720458
22. Moosa E, Alanany R, Sherif S, *et al.* Enhancing the accuracy of molecular classification of pediatric CNS tumors: a dual-classifier approach using DNA methylation profiling. *Front Oncol.* 2026;15:1701113.
doi: 10.3389/fonc.2025.1701113
23. Guo S, Sidhu R, Ramar V, Guo AA, Wang G, Liu M. RNA Sequencing Identifies Novel Signaling Pathways and Potential Drug Target Genes Induced by FOSL1 in Glioma Progression and Stemness. *Biologics.* 2025;19:157-176.
doi: 10.2147/BTT.S509774
24. Eibl RH, Schneemann M. Liquid biopsy and glioblastoma. *Explor Target Anti-Tumor Ther.* 2023;4(1):28-41.
doi: 10.37349/etat.2023.00121
25. Mellinghoff IK, van den Bent MJ, Blumenthal DT, *et al.* Vorasidenib in IDH1- or IDH2-Mutant Low-Grade Glioma. *N Engl J Med.* 2023;389(7):589-601.
doi: 10.1056/NEJMoa2304194
26. Pham J, Cote DJ, Kang K, *et al.* Treatment practices and survival outcomes for IDH-wildtype glioblastoma patients according to MGMT promoter methylation status: insights from the U.S. National Cancer Database. *J Neurooncol.* 2025;172(3):655-665.
doi: 10.1007/s11060-025-04952-y
27. Kizilbash SH. Why has targeting EGFR aberrations in

- glioblastoma therapy had limited success? *Expert Rev Anticancer Ther.* 2022;22(12):1261-1263.
doi: 10.1080/14737140.2022.2146581
28. Kaley T, Touat M, Subbiah V, *et al.* BRAF inhibition in BRAFV600E-mutant gliomas: results from the VE-BASKET study. *J Clin Oncol.* 2018;36(35):3477-3484.
doi: 10.1200/JCO.2018.78.9990
 29. Nobre L, Zapotocky M, Ramaswamy V, *et al.* Outcomes of BRAF V600E Pediatric Gliomas Treated With Targeted BRAF Inhibition. *JCO Precis Oncol.* 2020;4:PO.19.00298.
doi: 10.1200/PO.19.00298
 30. Omuro A, Vlahovic G, Lim M, *et al.* Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. *Neuro Oncol.* 2018;20(5):674-686.
doi: 10.1093/neuonc/nox208
 31. Schonfeld E, Choi J, Tran A, Kim LH, Lim M. The landscape of immune checkpoint inhibitor clinical trials in glioblastoma: A systematic review. *Neurooncol Adv.* 2024;6(1):vdae174.
doi: 10.1093/noajnl/vdae174
 32. Reardon DA, Brandes AA, Omuro A, *et al.* Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 Phase 3 Randomized Clinical Trial. *JAMA Oncol.* 2020;6(7):1003-1010.
doi: 10.1001/jamaoncol.2020.1024
 33. Stupp R, Mason WP, van den Bent MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987-996.
doi: 10.1056/NEJMoa043330
 34. Ringel F, Pape H, Sabel M, *et al.* Clinical benefit from resection of recurrent glioblastomas: results of a multicenter study including 503 patients with recurrent glioblastomas undergoing surgical resection. *Neuro Oncol.* 2016;18(1):96-104.
doi: 10.1093/neuonc/nov145
 35. Gerritsen JKW, Broekman MLD, De Vleeschouwer S, *et al.* Safe surgery for glioblastoma: Recent advances and modern challenges. *Neurooncol Pract.* 2022;9(5):364-379.
doi: 10.1093/nop/npac019
 36. American Society for Radiation Oncology. ASTRO updates guideline on radiation therapy for high-grade diffuse glioma, the most common primary brain tumor in adults. June 26, 2025. Available from: <https://www.astro.org/news-and-publications/news-and-media-center/news-releases/2025/astro-updates-guideline-on-radiation-therapy-for-high-grade-diffuse-glioma> [Last accessed on November 1, 2025].
 37. Fernández C, Ciérvide R, Díaz A, Garrido I, Couñago F. Radiotherapy in Glioblastoma Multiforme: Evolution, Limitations, and Molecularly Guided Future. *Biomedicines.* 2025;13(9):2136.
doi: 10.3390/biomedicines13092136
 38. Khagi S, Kotecha R, Gatson NTN, *et al.* Recent advances in Tumor Treating Fields (TTFields) therapy for glioblastoma. *Oncologist.* 2025;30(2):oyae227.
doi: 10.1093/oncolo/oyae227
 39. Kinzel A, Ambrogi M, Varshaver M, Kirson ED. Tumor Treating Fields for glioblastoma treatment: patient satisfaction and compliance with the second-generation Optune system. *Clin Med Insights Oncol.* 2019;13:1179554918825449.
doi: 10.1177/1179554918825449
 40. Stepanenko AA, Sosnovtseva AO, Valikhov MP, *et al.* The need for paradigm shift: prognostic significance and implications of standard therapy-related systemic immunosuppression in glioblastoma for immunotherapy and oncolytic virotherapy. *Front Immunol.* 2024;15:1326757.
doi: 10.3389/fimmu.2024.1326757
 41. Choi BD, Gerstner ER, Frigault MJ, *et al.* Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. *N Engl J Med.* 2024;390(14):1290-1298.
doi: 10.1056/NEJMoa2314390
 42. Begley SL, O'Rourke DM, Binder ZA. CAR T cell therapy for glioblastoma: A review of the first decade of clinical trials. *Mol Ther.* 2025;33(6):2454-2461.
doi: 10.1016/j.ymthe.2025.03.004
 43. Bagley SJ, Logun M, Fraietta JA, *et al.* Intrathecal bivalent CAR T cells targeting EGFR and IL13Ra2 in recurrent glioblastoma: phase 1 trial interim results. *Nat Med.* 2024;30(5):1320-1329.
doi: 10.1038/s41591-024-02893-z
 44. Czyżewski W, Kus-Budzyńska K, Sobstyl J, *et al.* CAR-T cell therapy for glioblastoma: advances, challenges, and future directions. *Ann Med Surg (Lond).* 2025;87(9):5743-5756.
doi: 10.1097/MS9.0000000000003607
 45. Park S, Maus MV, Choi BD. CAR-T cell therapy for the treatment of adult high-grade gliomas. *NPJ Precis Oncol.* 2024;8(1):279.
doi: 10.1038/s41698-024-00753-0
 46. Li N, Rodriguez JL, Yin Y, *et al.* Armored bicistronic CAR T cells with dominant-negative TGF- β receptor II to overcome resistance in glioblastoma. *Mol Ther.* 2024;32(10):3522-3538.
doi: 10.1016/j.ymthe.2024.07.020
 47. Su H, Peng Y, Wu Y, Zeng X. Overcoming immune evasion with innovative multi-target approaches for glioblastoma. *Front Immunol.* 2025;16:1541467.

- doi: 10.3389/fimmu.2025.1541467
48. Liao LM, Ashkan K, Brem S, *et al.* Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma: A Phase 3 Prospective Externally Controlled Cohort Trial. *JAMA Oncol.* 2023;9(1):112-121.
doi: 10.1001/jamaoncol.2022.5370
 49. Todo T, Ino Y, Ohtsu H, Shibahara J, Tanaka M. A phase I/II study of triple-mutated oncolytic herpes virus G47Δ in patients with progressive glioblastoma. *Nat Commun.* 2022;13(1):4119.
doi: 10.1038/s41467-022-31262-y
 50. Gállego Pérez-Larraya J, Garcia-Moure M, Labiano S, *et al.* Oncolytic DNX-2401 Virus for Pediatric Diffuse Intrinsic Pontine Glioma. *N Engl J Med.* 2022;386(26):2471-2481.
doi: 10.1056/NEJMoa2202028
 51. Russell L, Swanner J, Jaime-Ramirez AC, *et al.* PTEN expression by an oncolytic herpes simplex virus directs T-cell mediated tumor clearance. *Nat Commun.* 2018;9(1):5006.
doi: 10.1038/s41467-018-07344-1
 52. Ahluwalia MS, Reardon DA, Abad AP, *et al.* Phase IIa Study of SurVaxM Plus Adjuvant Temozolomide for Newly Diagnosed Glioblastoma. *J Clin Oncol.* 2023;41(7):1453-1465.
doi: 10.1200/JCO.22.00996
 53. Huang B, Li X, Li Y, Zhang J, Zong Z, Zhang H. Current Immunotherapies for Glioblastoma Multiforme. *Front Immunol.* 2021;11:603911.
doi: 10.3389/fimmu.2020.603911
 54. Mao M, Yang W, Zhang X. Current mRNA-based vaccine strategies for glioma treatment. *Crit Rev Oncol Hematol.* 2024;202:104459.
doi: 10.1016/j.critrevonc.2024.104459
 55. Tripathy DK, Panda LP, Biswal S, Barhwal K. Insights into the glioblastoma tumor microenvironment: current and emerging therapeutic approaches. *Front Pharmacol.* 2024;15:1355242.
doi: 10.3389/fphar.2024.1355242
 56. Bova V, Filippone A, Casili G, *et al.* Adenosine Targeting as a New Strategy to Decrease Glioblastoma Aggressiveness. *Cancers.* 2022;14(16):4032.
doi: 10.3390/cancers14164032
 57. Liang BC, Grootveld M. The Importance of Mitochondria in Tumorigenesis: Glioma as the Paradigm (review). *Int J Mol Med.* 2011;27(2):159-171.
doi: 10.3892/ijmm.2010.579
 58. Ho RLY, Ho IAW. Recent Advances in Glioma Therapy: Combining Vascular Normalization and Immune Checkpoint Blockade. *Cancers.* 2021;13(15):3686.
doi: 10.3390/cancers13153686
 59. Trejo-Solis C, Silva-Adaya D, Serrano-García N, *et al.* Role of Glycolytic and Glutamine Metabolism Reprogramming on the Proliferation, Invasion, and Apoptosis Resistance through Modulation of Signaling Pathways in Glioblastoma. *Int J Mol Sci.* 2023;24(24):17633.
doi: 10.3390/ijms242417633
 60. ter Linden E, Abels ER, van Solinge TS, Neefjes J, Broekman MLD. Overcoming barriers in glioblastoma – Advances in drug delivery strategies. *Cells.* 2024;13(12):998.
doi: 10.3390/cells13120998
 61. Timbie KF, Mead BP, Price RJ. Drug and gene delivery across the blood–brain barrier with focused ultrasound. *J Control Release.* 2015;219:61-75.
doi: 10.1016/j.jconrel.2015.08.059
 62. Islam S, Ahmed MMS, Islam MA, Hossain N, Chowdhury MA. Advances in nanoparticles in targeted drug delivery—A review. *Results Surf Interfaces.* 2025;19:100529.
doi: 10.1016/j.rsufi.2025.100529
 63. De Simone M, Conti V, Palermo G, De Maria L, Iaconetta G. Advancements in Glioma Care: Focus on Emerging Neurosurgical Techniques. *Biomedicines.* 2023;12(1):8.
doi: 10.3390/biomedicines12010008
 64. Korshoej AR, Lukacova S, Lassen-Ramshad Y, *et al.* OptimalTTF-1: Enhancing tumor treating fields therapy with skull remodeling surgery. A clinical phase I trial in adult recurrent glioblastoma. *Neurooncol Adv.* 2020;2(1):vdaa121.
doi: 10.1093/noajnl/vdaa121
 65. Eatz TA, Eichberg DG, Lu VM, Di L, Komotar RJ, Ivan ME. Intraoperative 5-ALA fluorescence-guided resection of high-grade glioma leads to greater extent of resection with better outcomes: a systematic review. *J Neurooncol.* 2022;156(2):233-256.
doi: 10.1007/s11060-021-03901-9
 66. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomized controlled multicentre phase III trial. *Lancet Oncol.* 2006;7(5):392-401.
doi: 10.1016/S1470-2045(06)70665-9
 67. Pirro V, Alfaro CM, Jarmusch AK, Hattab EM, Cohen-Gadol AA, Cooks RG. Intraoperative assessment of tumor margins during glioma resection by desorption electrospray ionization-mass spectrometry. *Proc Natl Acad Sci USA.* 2017;114(26):6700-6705.
doi: 10.1073/pnas.1706459114

68. Hollon TC, Pandian B, Adapa AR, *et al.* Near real-time intraoperative brain tumor diagnosis using stimulated Raman histology and deep neural networks. *Nat Med.* 2020;26(1):52-58.
doi: 10.1038/s41591-019-0715-9
69. Brown HM, Alfaro CM, Pirro V, *et al.* Intraoperative Mass Spectrometry Platform for IDH Mutation Status Prediction, Glioma Diagnosis, and Estimation of Tumor Cell Infiltration. *J Appl Lab Med.* 2021;6(4):902-916.
doi: 10.1093/jalm/jfaa233
70. Hu LS, Smits M, Kaufmann TJ, *et al.* Advanced imaging in the diagnosis and response assessment of high-grade glioma: AJR Expert Panel Narrative Review. *AJR Am J Roentgenol.* 2025;224(1):e2330612.
doi: 10.2214/AJR.23.30612
71. Patricoski-Chavez JA, Nagpal S, Singh R, Warner JL, Gamsiz Uzun ED. A deep learning model to predict glioma recurrence using integrated genomic and clinical data. *Commun Med.* 2025;5(1):359.
doi: 10.1038/s43856-025-01083-3
72. Anwer MS, Abdel-Rasol MA, El-Sayed WM. Emerging therapeutic strategies in glioblastoma: drug repurposing, mechanisms of resistance, precision medicine, and technological innovations. *Clin Exp Med.* 2025;25(1):117.
doi: 10.1007/s10238-025-01631-0
73. Xu C, Yuan X, Hou P, *et al.* Development of glioblastoma organoids and their applications in personalized therapy. *Cancer Biol Med.* 2023;20(5):353-368.
doi: 10.20892/j.issn.2095-3941.2023.0061
74. Miller JJ, Cahill DP. MGMT promoter methylation and hypermutant recurrence in IDH mutant lower-grade glioma. *Neuro Oncol.* 2020;22(11):1553-1554.
doi: 10.1093/neuonc/noaa212
75. Yang F, Yang P, Zhang C, *et al.* Stratification according to recursive partitioning analysis predicts outcome in newly diagnosed glioblastomas. *Oncotarget.* 2017;8(26):42974-42982.
doi: 10.18632/oncotarget.17322
76. Grant R, Liang BC, Page MA, Crane DL, Greenberg HS, Junck L. Age influences chemotherapy response in astrocytomas. *Neurology.* 1995;45(5):929-933.
doi: 10.1212/WNL.45.5.929
77. Han Q, Liang H, Cheng P, Yang H, Zhao P. Gross Total vs. Subtotal Resection on Survival Outcomes in Elderly Patients With High-Grade Glioma: A Systematic Review and Meta-Analysis. *Front Oncol.* 2020;10:151.
doi: 10.3389/fonc.2020.00151
78. Wick W. TTFIELDS: Where does all the skepticism come from? *Neuro Oncol.* 2016;18(3):303-305.
doi: 10.1093/neuonc/now012
79. Sipsos D, Raposa BL, Freihat O, *et al.* Glioblastoma: Clinical Presentation, Multidisciplinary Management, and Long-Term Outcomes. *Cancers.* 2025;17(1):146.
doi: 10.3390/cancers17010146
80. Wick W, Hartmann C, Engel C, *et al.* NOA-04 Randomized Phase III Trial of Sequential Radiochemotherapy of Anaplastic Glioma With Procarbazine, Lomustine, and Vincristine or Temozolomide. *J Clin Oncol.* 2009;27(35):5874-5880.
doi: 10.1200/JCO.2009.23.6497
81. Yu X, Zhou J, Wu Y, *et al.* Assessment of MGMT promoter methylation status in glioblastoma using deep learning features from multi-sequence MRI of intratumoral and peritumoral regions. *Cancer Imaging.* 2024;24(1):172.
doi: 10.1186/s40644-024-00817-1
82. McGrail DJ, Pilié PG, Rashid NU, *et al.* High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol.* 2021;32(5):661-672.
doi: 10.1016/j.annonc.2021.02.006
83. Amirmahani F, Kumar S, Muthukrishnan SD. Epigenetic mechanisms of plasticity and resistance in glioblastoma: therapeutic targets and implications. *Front Epigenetics Epigenomics.* 2025;3:1519449.
doi: 10.3389/freae.2025.1519449
84. Brown NE, Ottaviani D, Tazare J, *et al.* Survival Outcomes and Prognostic Factors in Glioblastoma. *Cancers.* 2022;14(13):3161.
doi: 10.3390/cancers14133161
85. Abdel Razek AAK, Alksas A, Shehata M, *et al.* Clinical applications of artificial intelligence and radiomics in neuro-oncology imaging. *Insights Imaging.* 2021;12(1):152.
doi: 10.1186/s13244-021-01102-6