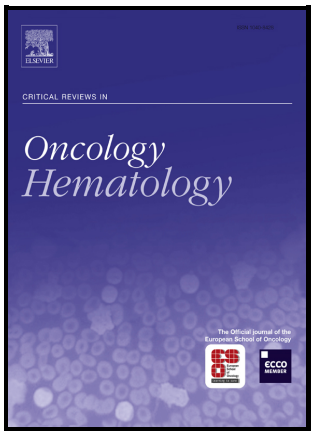

Revisiting Diffuse Gliomas in the Era of Precision Medicine: Molecular Insights and Therapeutic Implications

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Revisiting Diffuse Gliomas in the Era of Precision Medicine: Molecular Insights and Therapeutic Implications

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Abstract

Diffuse gliomas are aggressive and lethal tumors of the Central Nervous System characterized by high molecular complexity and heterogeneity, infiltrative growth and resistance to therapy. The advent of Next-Generation Sequencing integrated molecular profiling strongly redesigned glioma classification, diagnosis and clinical management following the 2021 World Health Organization guidelines.

Key molecular alterations, including *IDH* mutations, 1p/19q co-deletion, ATRX loss, *EGFR* amplification, *TERT* promoter mutations and chromosomal abnormalities define distinct oncotypes with prognostic and therapeutic relevance. Despite these advances, clinical outcomes remain often poor due to intrinsic tumor plasticity, persistence of glioma stem-cells and resistance mechanisms driven by the tumor microenvironment, DNA damage response and epigenetic reprogramming.

This review provides a comprehensive overview of diffuse gliomas in the era of precision medicine, focusing on molecular reclassification, pathogenesis, therapeutic strategies and resistance mechanisms. Emphasis is placed on advanced diagnostic methodologies, including Next-generation Sequencing, liquid biopsy and new interventions to overcome the restrictive nature of BBB. These approaches represent advancing tools for molecular profiling, disease monitoring, therapy efficacy improvement and early detection of recurrence and enable more personalized management of patients with glioma.

Keywords:

Diffuse Glioma, NGS, Diagnostic algorithm, Therapy Resistance, Liquid Biopsy

1. Introduction

Diffuse gliomas represent a group of infiltrative Central Nervous System (CNS) tumors characterized by high heterogeneity at histological, molecular and clinical levels. Although relatively rare, diffuse gliomas account for approximately 80% of all malignant CNS neoplasms and are responsible for the highest rate of CNS tumor-related deaths (Weller et al., 2024).

The incidence of diffuse gliomas increases with age, and the midline and hemispheric high-grade glioma mainly affect children and young adults. The global annual incidence of gliomas is approximately 6 per 100.000 individuals, with a 1.6-fold higher incidence in men compared with women (Ostrom et al., 2023). Although most gliomas are sporadic, both hereditary factors and irradiation exposure seem to be associated with an increased risk of gliomagenesis. Childhood cancer survivors who have received irradiation for a primary tumor showed higher risk of developing secondary gliomas and several hereditary cancer predisposition syndromes including type I neurofibromatosis, Li–Fraumeni syndrome, Turcot and Lynch syndromes have also been associated with an increased risk of glioma development. (Weller et al., 2024).

Historically, the diagnosis and classification of gliomas were based on histopathological evaluation alone followed by the discovery of IDH1/2 mutations that strongly impacted on glioma classification (Parsons et al., 2008; Yan et al., 2009). Subsequently, the advent of DNA-

and RNA-based Next-Generation Sequencing (NGS) reshaped diagnostic approaches by introducing the molecular landscape that led to a paradigm shift in the precise tumor classification through the integration of genomic profiling for several tumors (Massimino et al., 2025; Stella et al., 2024; Tirro et al., 2022a). The integration of molecular parameters into the diagnostic workup of these tumors was first codified in the historically important 2016 WHO classification (Reifenberger et al., 2017), which established the foundation for molecularly informed diagnosis. This framework was further refined in the 2021 WHO edition and is now being continuously updated through cIMPACT-NOW guidelines to enhance molecular classification and identify actionable targets (Weller et al., 2021; Wesseling et al., 2026). Currently, NGS-based analyses have become critical not only for tumor classification but also for the detection of actionable genetic alterations. These include *MGMT* promoter methylation, alterations in growth factor receptor pathways (e.g., *EGFR*, *PI3K*, *AKT*) and gene rearrangements involving *ALK*, *NTRK* or *RET*, which are useful for identifying patients who may be eligible for targeted therapies. However, while these approaches have significantly improved our understanding of the molecular mechanisms underlying gliomagenesis, their impact on therapeutic outcomes remains limited, particularly in glioblastoma (GBM). The reasons are linked to the multifactorial aspects that include high tumor heterogeneity, therapeutic resistance strongly dependent on their molecular complexity, glioma stem-cells (GSCs) and adaptative metabolic reprogramming. Resistance mechanisms involve a complex interplay of dysregulated DNA damage response (DDR) pathways, epigenetic plasticity and the immunomodulation influenced by the tumor microenvironment (TME) (Leelatian et al., 2025; Riyas Mohamed and Yaqinuddin, 2025; Sharma, P. et al., 2023). Finally, a crucial role is played by blood-brain barrier (BBB) that, caused by its restrictive nature negatively limit the drugs delivery in tumor (Durga and Pusapati, 2026).

To date, tumor tissue obtained by surgical biopsy has been the primary source for histological and molecular analyses and, although with several limitations, liquid biopsy has emerging as a minimally invasive alternative for tumor diagnosis, prognostic assessment and early detection of recurrence in glioma patients (Dwarshuis et al., 2025).

With this review we provide an overview about the evolving molecular and clinical landscape by a comprehensive synthesis of current knowledge on diffuse gliomas. Focus is given on molecular classification, pathogenesis, mechanisms of resistance, diagnostic methodologies and therapeutic advancements, highlight also, the need for innovative diagnostic strategies such as liquid biopsy as potential tool in the contest of precision oncology. This narrative literature review based on peer-reviewed evidence retrieved from PubMed and Web of Science up to June 2026. The search strategy prioritized systematic reviews, selected meta-analyses, clinical practice guidelines and key original research articles addressing diffuse glioma biology, molecular classification, liquid biopsy approaches, NGS and therapeutic resistance. Emphasis was placed on international consensus guidelines and recent translational studies exploring future directions in tumor heterogeneity, targeted therapy and minimally invasive biomarkers. Only English-language, peer-reviewed publications were

included and diffuse glioma, molecular classification, IDH mutations, 1p/19q co-deletion, GBM, astrocytoma, liquid biopsy, NGS, therapy resistance, precision medicine, precision diagnostic, diagnostic algorithms, guidelines and targeted therapy were used as keywords.

2. Rationale for this review

Since the introduction of the 2021 WHO Classification of CNS tumors, major advances in molecular diagnostics, integrated classification, and precision medicine have substantially reshaped the management of diffuse gliomas, while novel diagnostic and therapeutic strategies have rapidly emerged. Although several high-quality reviews have addressed specific topics, including molecular diagnostics, liquid biopsy, and targeted therapies, the rapid pace of these developments resulted in a literature that remains partially fragmented into disease- or topic-specific reviews. This review provides a timeline by an integrated overview of diffuse gliomas, following the evolution of the field from molecular classification to emerging diagnostic methodologies, mechanisms of therapeutic resistance, novel treatment strategies, and future perspectives. By bringing these interconnected developments together within a single translational framework, we aim to offer clinicians and researchers a comprehensive and up-to-date perspective on this rapidly evolving field.

3. Molecular Classification and pathogenesis

The first integration of both histopathological grading and molecular profiling was performed by 2016 WHO guidelines that used specific biomarkers for the classification of diffuse gliomas according to *IDH1/IDH2* mutational status, 1p/19q co-deletion and ATRX loss. The combination of these alterations generated a diagnostic algorithm to classify diffuse gliomas into different oncological subtypes including astrocytoma, oligoastrocytoma, oligodendroglioma and GBM (Louis et al., 2016). Next, with 2021 WHO guidelines, the 2016 WHO old classification was refined and the consortium to Inform Molecular and Practical Approaches to CNS Tumour Taxonomy - Not Officially WHO (cIMPACT-NOW) provided an updated criteria for glioma diagnosis (Weller et al., 2021). To align with these new emerging criteria, the European Association of Neuro-Oncology (EANO) reorganized its guidelines for the management of patients with gliomas. Diffuse gliomas are now reorganized into three main clinical and molecular oncotypes based on isocitrate dehydrogenase (*IDH)* enzymes mutational status: I) Oligodendroglioma (*IDH*-mutant CNS WHO grades 2, 3) associated to 1p/19q-codeletion, II) Astrocytoma, (*IDH*-mutant, CNS WHO grades 2, 3 and 4) presenting *ATRX* loss; and III) GBM (GBM, *IDH*-wildtype, CNS WHO grade 4) defined also by hallmark alterations such as *EGFR* amplification, *TERT* promoter mutation and +7/−10 chromosomal

signature. In addition, pediatric-type diffuse gliomas have been reclassified as distinct biological entities based on recurrent histone alterations including H3K27-mutant (middle glioma) or H3G34-mutant (hemispheric glioma) (Louis et al., 2021). **Figure 1** shows the difference between the 2016 and 2021 WHO guidelines.

The molecular biomarkers used for gliomas classification have a pivotal role in the pathogenesis of these diseases. The *IDH* enzymes, comprising three isoforms (*IDH1*, *IDH2*, *IDH3*), are involved in the Krebs cycle and cellular metabolism. For glioma patients affected by oligodendroglioma and astrocytoma *IDH1* and *IDH2* isoforms are generally altered, and cancer-associated mutations affect the arginine residues ($IDH1^{R132}$; $IDH2^{R140/172}$) transforming these in oncometabolites (Sharma, N. et al., 2023). *IDH* mutations are associated to WHO grade 2 and 3 tumors and their presence correlates with improved prognosis, even though these may progress to higher-grade lesions during disease evolution resulting in adverse outcomes (astrocytoma grade 4). Furthermore, *IDH* mutations trigger two additional key biological processes: i) epigenetic reprogramming (hypermethylation phenotype leading to the accumulation of 5-methylcytosine) and ii) redox imbalance by disruption of cellular redox homeostasis by catalyzing the conversion of NADPH to $NADP^+$ that impairs the scavenger process for reactive oxygen species (ROS) (Han et al., 2020). For alterations such as 1p/19q co-deletion, *ATRX* loss, *EGFR* amplification, *TERT* (telomerase reverse transcriptase) mutations and chromosomal 7+/10− signature, several studies have reported differing impacts on tumorigenesis, reflecting the complex role of these alterations in glioma biology. The presence of the 1p/19q co-deletion identify patients affected by oligodendroglioma an oncotype associated with improved patient survival (Yao et al., 2020). *ATRX*, a commonly mutated tumor suppressor gene in astrocytoma, plays essential roles in chromatin remodeling, transcriptional regulation, DNA-repair and cell-cycle control, thereby maintaining genomic stability. Interestingly, the loss of function of *ATRX* exhibits a dual biological role, hence, while the ATRX loss disrupts these crucial cellular pathways contributing to the tumorigenesis, it also has been associated with enhanced radiosensitivity (Qin et al., 2022). The *EGFR* gene encodes a transmembrane receptor tyrosine kinase (RTK) that regulates cell proliferation, differentiation and survival, playing a pivotal role in glioma tumorigenesis, particularly in GBM. Aberrations such as *EGFR* amplification and activating mutations represent a key molecular driver in GBM development through the activation of *RAS*, *PI3K*/*AKT*-mediated tumorigenic signaling. Moreover, *EGFR* gene rearrangements can give rise to the *EGFRvIII* variant, resulting from intragenic deletions that produce a constitutively active ligand-independent receptor form commonly associated to GBM (Wang, H. et al., 2024). *TERT* gene is essential for telomere maintenance and genomic stability. The C250T (-146 C>T) and C228T (-124 C>T) upstream mutations in the promoter regions of *TERT* gene generate two new ETS (E26 transformation-specific) binding sites activated by several transcription proteins including GABPA, ETV1/4/5, ETS1/2 leading to cell immortalization and promoting the tumorigenesis process. However, although this improper activation is a hallmark of malignant transformation in gliomas, the role of these alterations presents a bivalent prognostic significance according to the specific

oncotype. Hence, *TERT* promoter alterations represent a fundamental mechanism of cellular immortality and therapeutic resistance with negative impact on prognosis for GBM patients (*IDH*-wild type). On the other hand, the same alterations seem to confer a favorable prognosis in *IDH*-mutant gliomas (Arita et al., 2020; Murugan et al., 2025). Lastly, the co-occurrence of 7+/10− signature is the most frequent aneuploidy in GBM, though its underlying mechanism remains to be fully defined. Studies performed on a large tumor datasets suggest that the loss of chromosome 10 precedes chromosome 7 gain indicating that the latter provides compensatory functions to reduce the deleterious impact of chromosome 10 loss (Nair et al., 2024). **Figure 2** depicts the association between biomarkers and tumorigenesis mechanisms.

Finally, a crucial role is emerging concerning the Alternative Lengthening of Telomeres (ALT), a telomerase-independent mechanism that maintains telomere length through homologous recombination, supporting unlimited tumor cell proliferation. ALT is frequently associated with ATRX mutations, particularly in IDH-mutant astrocytomas and some pediatric glioblastomas even if was observed in a subgroup of oligodendroglioma that typically are not associated to *ATRX* alterations (Ho Keung Ng, 2023; Simone Minasi, 2021). The ALT is associated with a poor prognosis.

4. Diagnostic approaches

Given the high molecular complexity of glioma cancer cells, the use of advanced diagnostic techniques for accurate and timely definition of prognosis and treatment is needed.

Several molecular techniques are employed in the diagnostic work-up of gliomas essentially to first identify the key biomarkers relevant for classification:

- *Oligodendroglioma:* Oligodendrogliomas (WHO grade 2 and 3) are defined by a characteristic molecular profile including IDH1/2 mutations, 1p/19q co-deletion and frequently *TERT* promoter mutations.
- *Astrocytoma:* Astrocytoma (WHO grade 2 and 3) typically show IDH1/2 mutations, associated with ATRX loss. The coexistence of homozygous deletion of *CDKN2A* represents a key marker defining astrocytoma grade 4.
- *IDH-wildtype GBM:* GBM (WHO grade 4) are defined by the absence of IDH mutations and the presence of at least one of the following alterations: *EGFR* amplification, *TERT* promoter mutation or the chromosomal signature +7/−10.
- *Diffuse gliomas with H3 alterations:* In these oncotype the ATRX in retained and associated to H3.3 G34R/V mutations, defining diffuse hemispheric glioma H3 G34-mutant, or H3 K27M mutation (with loss of H3K27me3) classifying diffuse midline glioma, H3 K27-altered (Weller et al., 2021).

Additionally, the cIMPACT-NOW Update 11 (Wesseling et al., 2026) highlights the need to refine diagnostic criteria for diffuse high-grade gliomas that are both IDH-wildtype and H3-wildtype. It recognizes that a subset of these tumors lacks the canonical molecular features of glioblastoma (*EGFR* amplification, *TERT* promoter mutations and combined chromosomal signature +7/−10) and therefore should not be automatically classified as such. This strategy aims to reduce overdiagnosis of GBM and improve biological and prognostic stratification within this heterogeneous group of tumors.

To concern the diagnostic techniques specific approaches can be used:

- *Immunohistochemistry* is a method used for detecting of IDH1 R132H, ATRX loss, TP53 overexpression, BRAF V600E, Ki-67 (Park et al., 2017).
- *Fluorescence in situ hybridization* (FISH) is used to assess chromosomal abnormalities and is commonly utilized to detect *EGFR* amplification, +7/-10q, *PTEN* and *CDKN2A* deletion, *BRAF*, *NTRK* fusions and 1p/19q co-deletion (Bourhis et al., 2022).

For single nucleotide polymorphisms (SNPs) or specific hot spot mutations, including *IDH1* R132, *IDH2* R172, *BRAF* V600, *H3* K27M or *H3* G34R/V different approaches can be employed including Sanger sequencing, Allele-specific qPCR, HRM and droplet digital PCR (Catteau et al., 2014; Saito et al., 2021; Wolter et al., 2022; Zhang et al., 2016).

Overall, the methods listed above are unable to provide a comprehensive molecular profile and need to be combined to obtain information that are often partial and fragmented. New -omic approaches, including microarray technology and DNA- RNA-based NGS, are highly versatile, allowing the simultaneous analysis of multiple nucleic acid regions improving diagnostic accuracy and the discovering of novel biomarkers useful in clinical research.

Microarray technology provides genome-wide detection of CNVs and quantification of gene expression and has been extensively used to identify 1p/19q co-deletion, 7+/10- signature, *CDKN2A* deletion and *PTEN* loss, all critical for diagnosis, prognosis, and therapeutic decision-making (Buckner et al., 2017). Multiple targeted DNA- and RNA-based NGS panels have been developed for glioma diagnosis, such as the Glio-panel (Tirro et al., 2022b), GliomaSCAN (Shin et al., 2020) or GlioSeq (Nikiforova et al., 2016). All panels were designed to simultaneously detect different key molecular alterations relevant for glioma subtyping, defining prognosis and therapeutic decision. To date, only two NGS panel are FDA-approved for glioma molecular analysis. Ion Torrent Oncomine Dx Target, approved in 2024, to detect *IDH* mutations for prescribing Vorasidenib in grade 2 *IDH*-mutant astrocytoma or oligodendroglioma and FoundationOne® CDx approved in 2025 for the identification of *BRAF* alterations in pediatric low-grade glioma patients eligible for tovorafenib. **Table 1** summarizes the methods and provides a comparative overview.

5. Liquid Biopsy and Emerging Diagnostic Approaches

Liquid biopsy represents a promising tool in precision medicine across all tumor types, including gliomas (de Lima et al., 2025). It provides minimally invasive access to tumor-derived material through circulating biomarkers such as cell free DNA (cfDNA), microRNAs (miRNAs), extracellular vescicles (EVs), circulating tumor cells (CTCs), tumor educated platelets (TEPs) and several metabolites (de Lima et al., 2025). Although there are currently no established clinical guidelines supporting the routine use of liquid biopsy in the management of gliomas, growing evidence suggests that it may represent a valuable complementary approach. CSF-derived ctDNA shows higher sensitivity and strong concordance with tumor tissue, supporting its role in capturing the clinically relevant alterations such as IDH mutations, *TERT* promoter mutations or H3K27M alterations (Friedman et al., 2022). Despite its promise in precision oncology, liquid biopsy is affected by several critical limitations for tumor analysis. Low levels of circulating tumor DNA, especially in early-stage cancers, reduce sensitivity and increase false negatives resulting in an incomplete genomic profiling. Furthermore, technical variability and the absence of standardized protocols could lead to incorrect distinguishing between tumor-derived mutations from clonal hematopoiesis and tumor, causing false positives (Coppola et al., 2025). Additional features need to be evaluated for glioma patients such as the restrictive nature of BBB limiting the detection of tumor-derived biomarkers. Although these limitations exist, the growing body of literature has provided an overview of the utility of liquid biopsy, with encouraging data that will be discussed below.

Blood is the most accessible fluid, yet only a fraction of tumor-derived molecules can cross the BBB, leading to low concentration and high fragmentation in plasma (Riviere-Cazaux et al., 2025). On the other hand, cerebrospinal fluid (CSF), represents a rich source of glioma biomarkers, particularly when obtained intraoperatively or from intracranial compartments near the tumor (Riviere-Cazaux et al., 2025). However, intracranial CSF evaluation is not feasible in clinical practice, especially for longitudinal monitoring of the disease while the lumbar puncture, the standard technique for CSF longitudinal collection, is feasible and enables biomarker studies. However, surgical resection has a lasting impact on CSF composition independent of tumor burden, affecting biomarker interpretation in the post-operative setting (Riviere-Cazaux et al., 2025). Despite these constraints, lumbar CSF remains clinically informative for biomarkers such as cell-free DNA, where total analyte yield may be more relevant than local concentration, even if, the BBB disruption and procedural blood contamination can introduce plasma-derived proteins into CSF, potentially confounding biomarker interpretation support the use of paired plasma-CSF sampling and tissue-based validation for disease monitoring (Riviere-Cazaux et al., 2025). A prospective multicenter study confirmed that using an eight-gene panel (*IDH1, IDH2, ATRX, TP53, PTEN, PIK3CA, EGFR, BRAF*), ctDNA was detected in 59% of CSF samples, with frequent mutations in *EGFR*, *PTEN*, *TP53*, *IDH1* and *PIK3CA*, achieving a tumor-CSF concordance of 84%, while plasma sensitivity remained limited (14%) (Cabezas-Camarero et al., 2025). Moreover, CSF ctDNA positivity, higher variant VAF, and preoperative cfDNA levels have consistently been associated with

shorter progression-free and overall survival, emphasizing their prognostic significance (Juratli et al., 2012). Imaging-guided techniques like magnetic resonance (MR)-guided focused ultrasound (MRgFUS) can transiently disrupt the BBB to increase plasma biomarker levels, improving liquid biopsy sensitivity (Meng, Y. et al., 2021).

Cell-free RNAs (cfRNAs) are being increasingly recognized as valuable biomarkers for capturing transcriptional signature and tumor heterogeneity. Several miR including miR-21, miR-222, miR-124-3p and miR-451a, have been associated with glioma progression, while others, such as miR-181d and miR-21, may predict TMZ resistance or prognosis (de Lima et al., 2025). In a recent study, ddPCR assays quantified circulating miR-21-5p, miR-23b-3p, and miR-34a-5p in GBM patients at diagnosis and during follow-up detecting levels increased at 1-3 months post-surgery and gradually decreased at 6-12 months (Donofrio et al., 2025). Notably, plasmatic miR-34a-5p was significantly elevated in GBM patients compared to healthy volunteers and lower miR-34a-5p or miR-21-5p levels at specific timepoints correlated with shorter OS and recurrence-free survival (RFS). However, reproducibility remains limited, as no universal glioma miRNA panel has been validated and larger prospective cohorts are required to confirm these results (Donofrio et al., 2025).

Extracellular vesicle (EVs), particularly exosomes, are lipid-bound nanoparticles capable of crossing the BBB and carrying tumor-specific molecules, including proteins, nucleic acids and mutant transcripts. Proteomic analyses have identified EV-associated proteins correlating with tumor aggressiveness and histological grade, while EV-based detection of *MGMT* promoter methylation correlates with TMZ response (de Lima et al., 2025). Despite these advantages, clinical translation lacks caused by standardized isolation and characterization methods and additional studies are required to validate their clinical utility.

In gliomas, CTCs are rare due to the limited by restrictive BBB and methods such as the CellSearch System are limited in gliomas because they target epithelial markers (Lessi et al., 2023). Advanced technologies, including CTCs chips, EPISPOT, microfluidic platforms and immunomagnetic enrichment using markers like GLAST, have enabled more reliable isolation of glioma-derived CTCs and the study of their molecular features (Adamczyk et al., 2015; Muller Bark et al., 2021). Although clinical applicability of CTCs in glioma is currently limited, emerging evidence demonstrates that CTCs can harbor molecular alterations and post-surgical CTCs counts correlate with RFS and longitudinal monitoring for assessing treatment response (Gao et al., 2016).

TEPs in the tumor microenvironment may influence cancer progression through release signaling molecules such as VEGFR-1 (Campanella et al., 2020). Leveraging these features, a multicenter study developed a TEP RNA panel for GBM, accurately distinguishing true progression from pseudoprogression with 85% accuracy (Sol et al., 2020).

Regarding emerging diagnostic approaches in neuro-oncology, precision medicine is increasingly shaped by the integration of advanced imaging, molecular profiling, and liquid

biopsy to overcome the challenges posed by glioma heterogeneity and the BBB. Advanced imaging modalities, including diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), novel PET radiotracers, and hybrid PET/MRI platforms, have improved tumor detection, grading, and characterization, particularly in differentiating true progression from pseudoprogression or radiation necrosis (Riviere-Cazaux et al., 2025). When combined with artificial intelligence (AI) approaches, these imaging data will be analyzed more rapidly, reducing interobserver variability, possibly automating tumor detection, and extracting radiomic features such as tumor heterogeneity, shape, and microenvironmental patterns. Studies using deep learning algorithms, such as SCAT, have demonstrated the ability to distinguish GBM from metastatic lesions with >92% accuracy, while transformer-based models have integrated multimodal MRI, histopathology, and molecular data to predict *IDH* mutation status, *MGMT* promoter methylation, and patients' survival (Gomaa et al., 2024; Jiang et al., 2024).

Translational studies using single-cell transcriptomics, particularly long noncoding RNA (lncRNA) profiling, have revealed the limitations of bulk tissue-based molecular classification in GBM, capturing intratumoral heterogeneity and identifying biologically distinct subtypes with prognostic relevance. These studies have suggested potential strategies for rational drug selection, including combinations targeting multiple subtypes to optimize efficacy while minimizing toxicity, although such approaches are not yet routinely applied or clinically validated (Meng, Q. et al., 2021).

Interesting evidence is emerging on the possible role for gut microbiome in GBM biology, which may influence not only tumor development and progression, but also therapeutic response (Mohamed et al., 2025). Specific microbial populations can modulate immune activity, inflammation and metabolic pathways, possibly contributing to oncogenesis and resistance to therapy. Strategies to manipulate gut microbiota, such as probiotics, dietary interventions, or microbiota-targeted therapeutics, hold potential to enhance treatment efficacy in the future. However, standardization, mechanistic studies, and clinical trials are still needed to build enough evidence to validate microbiome-based interventions as part of precision medicine approaches in gliomas.

Finally, the integration of AI with these domains can maximize the clinical utility of multi-omic data and may allow the design of predictive models, correlating with clinical outcomes, leading to a more personalized therapeutic decision-making. Transfer learning reduces the need for extensive training datasets, enabling application to rare tumor subtypes, while radiomics and transformer-based AI extract quantitative imaging features that can be combined with molecular and liquid biopsy data to generate a comprehensive, patient-specific profile.

6. Therapeutic Landscape

Despite the significant evolution in the understanding of gliomas biology, conventional therapeutic approaches such as surgery, radiotherapy and chemotherapy remain scarcely effective in this disease, with reduced chance of pursue a curative intent (Anwer et al., 2025). The biological and molecular heterogeneity among diffuse glioma subtypes necessitates distinct therapeutic strategies, as each entity is characterized by specific clinical behavior, prognosis and treatment response. Patients with newly diagnosed oligodendroglioma (IDH-mutant, 1p/19q-codeleted, CNS WHO grade 2 - 3) are typically treated with radiotherapy followed chemotherapy by PCV (procarbazine, lomustine, vincristine) (Cairncross et al., 2014; van den Bent et al., 2006), while temozolomide represents an alternative for patients unable to tolerate PCV, although clinical evidence supporting this approach are lacking. For astrocytoma (IDH-mutant, 1p/19q non-codeleted), treatment is dependent on grade, hence, grade 2 tumors are managed with radiotherapy plus adjuvant chemotherapy (TMZ or PCV), whereas grade 3 tumors receive radiotherapy followed by adjuvant temozolomide. Management of grade 4 IDH-mutant astrocytomas may align with either grade 3 astrocytoma protocols or glioblastoma regimens, while patients with newly diagnosed glioblastoma (IDH-wildtype, CNS WHO grade 4) should receive concurrent radiotherapy and temozolomide (Stupp et al., 2005).

Given the limited efficacy of current therapies, emerging therapeutic strategies and novel treatment approaches for gliomas are under active investigation, and an overview of these developments will be discussed.

Although the clinical benefit are controversial, targeted therapies are emerging using specific agents against IDH, EGFR and PI3K/AKT/mTOR pathway alterations, along with immunotherapy, chimeric antigen receptor (CAR)-T cells, oncolytic viruses, adaptative trials and nanotechnologies associated to genome editing (Peters, 2026; Sarfraz et al., 2025).

As pharmacological target, IDH enzyme is the substrate of IDH inhibitors showing encouraging results in *IDH*-mutant gliomas. FDA approved an IDH inhibitor vorasidenib, based on the results of the INvestigating VorasiDenib In Glioma (INDIGO) trial for patients aged ≥12 years with grade 2 astrocytoma or oligodendroglioma after surgery and Vorasidenib improved the progression-free survival (PFS) when compared with placebo (27.7 months vs 11.1 months). However, higher-grade or high-risk tumors were excluded due to limited efficacy driven by loss of dependence on IDH mutations making IDH inhibition less effective, especially at recurrence (Mellinghoff et al., 2023).

For *EGFR* and PI3K/AKT/mTOR pathway as target they related to glioblastoma. EGFR alterations, including amplification, mutations and rearrangement as EGFRvIII and its variants (EGFRvII, EGFRvIV and EGFRx), have prompted the development of pharmacological agents but without significant clinical benefit (Ezzati et al., 2024). For instance, Mohammad A.H. and co-authors presented a systematic review and meta-analysis evaluating the safety and efficacy of EGFR-targeting tyrosine kinase inhibitors (TKIs) in glioblastoma. Overall, TKIs showed no

significant improvement in overall survival and only modest or inconsistent effects on progression-free survival (Habibi et al., 2025). Similarly, the PI3K/AKT/mTOR pathway, frequently activated in glioblastoma, induces tumor proliferation and survival and has been targeted by dual PI3K/mTOR inhibitors although clinical efficacy to date has been modest (Pournajaf and Pourgholami, 2025).

Despite these limited results, efforts have been undertaken to define guidance on the identification and clinical exploitation of potential therapeutic targets in different gliomas oncotypes. In this context, EANO proposed a comprehensive overview to identify therapeutic biomarkers according to the ESCAT score *(ESMO Scale for Clinical Actionability of molecular Targets)* and rank molecular alterations based on the evidence supporting their clinical actionability (Capper et al., 2023). Briefly, this guideline summarizes current evidence for targeted therapies against alterations in the RAS/MAPK pathway, growth factor receptors, cell-cycle regulators, genomic stability defects and gene fusions. **Supplementary Table 2** summarizes the clinical actionability of targeted genes by ESCAT score associated to ongoing clinical trials.

However, given the controversial data regarding the clinical efficacy of the mentioned targeted therapies, clinical research is still ongoing, and novel strategies are under investigation across glioma subtypes, with particular emphasis on glioblastoma, which remains the most aggressive and lethal form to date.

The integration of targeted therapies with immunotherapy studies suggested that neoadjuvant PD-1 blockade followed by adjuvant therapy can enhance antitumor responses and improve survival when compared to adjuvant PD-1 inhibition alone (Schonfeld et al., 2024). This strategy was applied in glioblastoma in NRG-BN002 phase I study evaluating the safety and feasibility of ipilimumab, nivolumab and their combination. The treatments showed increased toxicity, particularly with the combination regimen and no improvement in clinical outcomes was observed (Sloan et al., 2024).

Recent biotechnological innovations have introduced strategies involving T-cells therapies and oncolytic viruses. CAR T-cells are engineered patient-derived T-cells that can target tumor-associated antigens such as EGFRvIII or HER2. Although, early trials have shown promising results, the clinical benefit was limited caused by T-cell exhaustion and the immunosuppressive microenvironment (Agosti et al., 2024; Sterner and Sterner, 2024). Another approach is represented using natural killer (NK) cells that, unlike T-cells, do not require antigen presentation and are able to escape from adaptive immunity events. Preclinical studies have demonstrated that NK cells can recognize and lyse GSCs, typically resistant to chemotherapy and radiotherapy, thanks to their capacity to cross the blood–brain barrier (Liu et al., 2025).

Another intricating study, involving the immune system, concerns the use of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination (DCVax-L) proposed by Liau M.L.

and colleagues (Liau et al., 2023). In this Phase 3 Prospective Externally Controlled Cohort Trial the authors report that DCVax-L is associated with improved overall survival in both newly diagnosed and recurrent GBM.

Nanotechnology enables the design of advanced drug delivery systems have been explored in glioblastoma (Rizwani et al., 2025). CRISPR–Cas9–based nanocapsules and exosome-based are most recent and promising innovative targeted therapy (Zou et al., 2022). A recent study demonstrated the delivery of doxorubicin using exosome-based nanocarriers was able to BBB penetration delivering doxorubicine (Wang, Y. et al., 2024). Lastly, oncolytic viruses are viruses genetically modified to selectively infect and lyse tumor cells. Adenovirus, herpes simplex virus or poliovirus derivatives showed signals of clinical activity in early phase clinical trials, particularly when combined with immunotherapy (Jiang et al., 2023).

Finally, a new methodology concerns the Adaptive Trials, such as AGILE (Adaptive Global Innovative Learning Environment) and INSIGhT (Individualized Screening Trial of Innovative Glioblastoma Therapy) for GBM. AGILE is a global, multi-arm, biomarker-driven adaptive Phase 2/3 platform trial for newly diagnosed and recurrent glioblastoma. The trial has screened over 1.000 patients representing a rapid and adaptable model for testing novel glioblastoma therapies on a global scale (Rizwani et al., 2025). INSIGhT is a phase II platform trial that uses response adaptive randomization and genomic profiling to efficiently identify novel therapies for phase III testing (Rahman et al., 2023). Both adaptive trials aim to identify the most effective therapeutic strategies for glioblastoma patients; however, no significant overall survival benefit has been observed, underscoring that optimal treatment for gliomas, particularly glioblastoma, remains an unmet need.

Overall, future challenges are represented by the optimization of these treatments, clarifying the aspects related to safety and resistance, and further studies are needed before any of them could be implemented in the practice (Sarfraz et al., 2025).

7. Multimodal mechanisms underlying therapy resistance

Different mechanisms leading to resistance have been proposed defining the therapy resistance in gliomas as a multifactorial event influenced by several biological compartment and events including GSCs, tumor microenvironment (TME), alterations in DDR and epigenetic mechanisms and BBB. Their dynamic interplay results in resistance (Yalamarty et al., 2023).

7.1 Glioma stem cells (GSCs)

The primary tumor harbors glioma stem cells (GSCs), which are key drivers of tumor initiation and heterogeneity. While initial therapy often reduces the bulk tumor, a subset of recurrence-initiating stem-like cancer (RISC) cells persists evading from treatment, surviving

in the tumor microenvironment by intrinsic and adaptive mechanisms (Osuka and Van Meir, 2017) **(Figure 3A)**

7.2 Tumor microenvironment (TME)

TME is a highly heterogeneous and adaptive ecosystem composed of glioma cells, vascular and stromal components, immune cells, and extracellular matrix (ECM), collectively sustaining tumor growth and invasion (Jayaram and Phillips, 2024) (**Figure 3B**). Glioma cells further interact with the neural compartment through synaptic signaling, paracrine factors and ECM remodeling, contributing to a pro-invasive and immunosuppressive niche (Sharma, P. et al., 2023; White et al., 2024). Within the TME, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) are actively reprogrammed by tumor cells to promote immune evasion via cytokine secretion and immune checkpoint activation (Jang and Park, 2025). Specialized microanatomical niches further support tumor persistence. Perivascular niches (PVNs) sustain glioma stem cells (GSCs), preserving slow-cycling, self-renewal and therapy-resistant states, while tumor microtube (TM) networks enable intercellular connectivity and rapid repair after therapeutic injury (Hillen et al., 2023; Jung et al., 2021). Finally, a crucial role is determined by NOTCH1 signaling that acts as a key regulator of these processes, supporting both PVN maintenance and TM formation (Jung et al., 2021). Overall, these results have led different authors to propose the disruption of TME as a promising approach to overcome treatment resistance (Bayona et al., 2026).

Finally, in line with these concepts, a recent spatial transcriptomic study (Sonpatki et al., 2026) has provided further evidence of spatially organized tumor-microenvironment interactions. In this study the authors identified distinct functional niches associated with specific molecular programs refining a framework to understand how niche-specific interactions contribute to glioma progression, immune evasion and therapeutic resistance.

7.3 DNA damage response (DDR)

DDR pathways, including ATM, ATR, DNA-PK, Chk1/2, and PARP, coordinate DNA repair and cell cycle control, representing both a mechanism of resistance and a therapeutic vulnerability when inhibited in combination with DNA-damaging agents such as TMZ and radiotherapy (Drew et al., 2025; Ferri et al., 2020) (**Figure 4**).

7.4 Epigenetic mechanisms of resistance

Epigenetic plasticity further contributes to glioma progression and therapy resistance by promoting reversible transcriptional reprogramming of GSCs through DNA methylation and histone modifications (Shahani et al., 2025). Methylation profiling studies have linked

aberrant epigenetic states with prognosis and tumor progression, suggesting that targeting epigenetic plasticity may impair GSC maintenance (Johnson et al., 2021). Histone acetylation/deacetylation (HAT/HDAC), as well as histone methylation/demethylation (HMT/HDM), regulate stemness-associated transcriptional programs (SOX2, OCT4, NANOG), with HDAC inhibitors emerging as more promising therapeutic agents due to the dual role of HATs (Everix et al., 2023; Lopez-Bertoni et al., 2015).

7.5 BBB and drug delivery limitations

BBB remains a strong limitation for drug delivery in glioma, with heterogeneous permeability that protects infiltrative tumor regions. BBB integrity is regulated by pathways including Wnt and Sonic Hedgehog (Shh), and its disruption is a key determinant of therapeutic response. Strategies to overcome BBB-mediated resistance include chemical modification, nanocarriers, transporter inhibition and physical disruption approaches such as focused ultrasound (Durga and Pusapati, 2026; Ter Linden et al., 2024).

8. Future Directions

The management of gliomas is undergoing a profound transformation, driven by rapid advances in molecular biology, artificial intelligence and innovative therapeutic strategies. Critical progress concerning in liquid biopsy, AI-assisted integrated diagnostics, epigenetic therapies, adaptive clinical trial designs and BBB disruption technologies may significantly improve both patient stratification and therapeutic outcomes. The following sections outline these key developments and discuss their potential impact on the future landscape of glioma diagnosis and management.

8.1 Liquid biopsy replacing tissue biopsy

Looking ahead, continued advances in CSF-based liquid biopsy may provide a reliable alternative to tissue-based molecular profiling in selected patients. In this context, the detection of H3K27 alterations in diffuse midline gliomas is an area of active investigation. As assay sensitivity improves and prospective clinical validation accumulates, CSF molecular profiling may become a first-line diagnostic approach, reserving tissue biopsy for diagnostically challenging cases or when histopathological confirmation remains necessary (10.1093/neuonc/noad229).

8.2 *AI-integrated diagnostics*

AI is expected to play an increasingly important role in integrated CNS tumor diagnostics. By combining radiological, histopathological, and molecular data, AI-assisted multimodal to support real-time clinical decision-making (Redlich et al., 2024). Hollon et al., achieved a mean molecular classification accuracy (93.3 ± 1.6%) in a prospective multicenter study using multimodal data by DeepGlioma algorithm (Hollon et al., 2023).

8.3 Epigenetic therapies

Epigenetic therapies target reversible alterations in DNA methylation and histone modifications that contribute to glioma progression and therapeutic resistance. Enzymes involved in DNA methylation (methyltransferase - DNMT) and HDAC define distinct epigenomic and chromatin modifications landscapes across glioblastoma subtypes. Agents targeting aberrant DNA methylation, such as DNMT inhibitors (decitabine) and HDAC inhibitors (vorinostat), can modulate gene expression programs involved in cellular differentiation and antitumor immunity through epigenomic reprogramming, thereby resensitizing tumor cells to therapy. Although clinical efficacy as monotherapy remains limited, these agents show increasing promise in combination with chemotherapy or immune checkpoint blockade, where they may overcome resistance by enhancing immune responses (Riyas Mohamed and Yaqinuddin, 2025; Zang et al., 2018). Collectively, their capacity to reprogram resistant tumor states supports their development as therapeutic sensitizers in combination strategies potentially entering in clinical practice as reported by several clinical trials (NCT01378481, NCT01324635, NCT00848523, NCT00302159, NCT03426891).

8.4 Adaptive platform trials

Adaptive platform trials represent an emerging paradigm in glioblastoma clinical research, offering a more flexible and efficient alternative to traditional phase III trial designs. The current landscape of adaptive platform trials in glioblastoma is exemplified by studies such as GBM AGILE trial and INSIGhT trial. GBM AGILE trial is a global, Bayesian response–adaptive platform trial designed to evaluate multiple therapies simultaneously across recurrent and newly diagnosed GBM. A key feature is its ability to dynamically allocate patients to more promising treatment arms based on accumulating efficacy data, while discontinuing ineffective interventions early. Regorafenib, a multikinase inhibitor, has been one of the evaluated agents within this framework, reflecting the platform's capacity to rapidly assess repurposed targeted therapies in a heterogeneous disease setting (Wen et al., 2026). Similarly, INSIGhT trial applies response-adaptive randomization combined with genomic profiling to match therapeutic strategies to molecularly defined subgroups of newly diagnosed glioblastoma. Early reports from the initial treatment arms demonstrated the feasibility of integrating molecular stratification with adaptive allocation, while also highlighting the

operational complexity and the challenge of identifying robust efficacy signals in an immunologically and genetically heterogeneous tumor (Rahman et al., 2023). Overall, these trials highlight the growing role of adaptive platform designs in GBM, providing proof-of-concept for more efficient drug evaluation and supporting the transition toward trial structures capable of continuous learning, real-time adaptation and biomarker-driven patient stratification.

8.5 BBB disruption technologies

1 As well established, BBB represents a major limitation in glioblastoma as it significantly restricts the delivery of systemically administered drugs to the tumor site and contributing to therapeutic resistance and failure. To overcome this limitation BBB disruption technologies, such as Focused Ultrasound (FUS), have emerged as a promising strategy to enhance intracerebral drug penetration across a range of brain tumors, including GBM, brain metastases and diffuse intrinsic pontine glioma. Among FUS methods, Magnetic Resonance-guided Focused Ultrasound (MRgFUS) is an encouraging approach enables transient, localized and non-invasive BBB opening, able to improve the delivery of therapeutic agents and minimizing systemic toxicity (Nabavizadeh et al., 2026). Clinical experience showed that MRgFUS is expected to become an important adjunct to systemic therapies potentially to be integrated into routine neuro-oncological practice gliomas patients (Carpentier et al., 2024).

9. Conclusions

The management of diffuse gliomas underwent an intense transformation in the era of precision medicine, driven by the integration of molecular profiling into diagnostic classification and therapeutic decision-making. The 2021 WHO classification represented a pivotal milestone, redefining distinct glioma oncotypes based on histo-molecular criteria enabling more accurate prognostic stratification.

While traditional tissue-based molecular analyses remain crucial, they are constrained by sampling bias, invasiveness and limited ability to capture spatial and temporal tumor heterogeneity. Liquid biopsy offer a promising and minimally invasive methodology to identify circulating signatures in association with omics techniques. In this setting, CSF - based analyses have demonstrated superior sensitivity compared to plasma for detecting glioma-derived biomarkers. Clinical implementation of this approach remains limited by technical challenges, lack of standardization, context-dependent sensitivity, the need for large-scale prospective validation, as well as additional inherent drawbacks. First, many advanced technologies remain confined to specialized research settings, with limited regulatory approval and

accessibility in routine clinical practice. Second, the interpretation of *multi-omics* data requires robust bioinformatic infrastructure and interdisciplinary expertise, which are not uniformly available across institutions. Lastly, these advances in molecular diagnosis were not coupled with improvements in the clinical outcomes of patients with gliomas and future directions in glioma classification focus on incorporating emerging molecular, genetic, and clinical insights to improve diagnostic accuracy and prognostic stratification. Initiatives such as the cIMPACT-NOW updates exemplify these efforts, providing interim recommendations that refine criteria between WHO editions and guide the integration of novel biomarkers into clinical practice (Aldape et al., 2025; Wesseling et al., 2026).

In conclusion, the future of gliomas management lies in the convergence of molecular classification, liquid biopsy, single-cell and multi-omic profiling, advanced imaging and AI-driven data integration. Continued collaborative efforts, multi-institutional studies, and standardized workflows will be essential to translate these innovations into tangible clinical benefits.

10. Critical view section

Most medical treatments are traditionally developed for the "*average patient*" meaning they may be effective for some individuals but not for others. In contrast, personalized medicine represents an innovative approach aimed at identifying patients who are most likely to benefit from a specific targeted therapy, while guiding others toward alternative and more efficacy treatment strategies. This principle is particularly crucial in diffuse gliomas, where high molecular complexity and heterogeneity associated to variable clinical behavior strongly influence treatment response and patient outcomes, making tailored therapeutic approaches essential.

In this contest, this review provides an integrative and comprehensive perspective on diffuse gliomas in the era of precision medicine, distinguishing itself by linking molecular classification with functional and therapeutic implications. Beyond describing key genetic alterations, it emphasizes the complex interplay between genomic, epigenetic, and microenvironmental factors in driving tumor progression and resistance.

This work highlights the translational relevance of molecular insights, with particular focus on next-generation sequencing and liquid biopsy as tools for personalized disease monitoring and management. Moreover, this work provides an inclusive approach that offers to the readers a complete overview of the current state of the art in diffuse gliomas. It covers molecular classification according to the 2021 WHO criteria and cIMPACT-NOW up-date concerning the pathogenesis, therapeutic strategies and resistance mechanisms, providing a balanced depth of coverage that is both comprehensive and clinically oriented.

Overall, it aims to advance current knowledge by promoting a more integrated and application-driven understanding of diffuse gliomas, serving as a valuable resource for clinicians and researchers seeking a thorough synthesis of the field.

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Figures Legends

Figure 1. *Molecular classification for the integrated diagnosis of diffuse gliomas.* The figure illustrates the differences between the diagnostic algorithms for molecular classification of gliomas according to the WHO classifications of 2016 (A) and 2021 (B). (A) IDH status defines the main oncological subtypes of diffuse gliomas, including astrocytoma, oligoastrocytoma, oligodendroglioma, and glioblastoma, classified as IDH-mutant or IDH-wild type. (B) In the WHO 2021 classification, diffuse gliomas are divided into two groups based on IDH status: IDH-mutant gliomas include oligodendroglioma (grades 2–3) and astrocytoma (grades 2–4), whereas IDH-wild-type gliomas correspond to glioblastoma (grade 4). NOS: not otherwise specified.

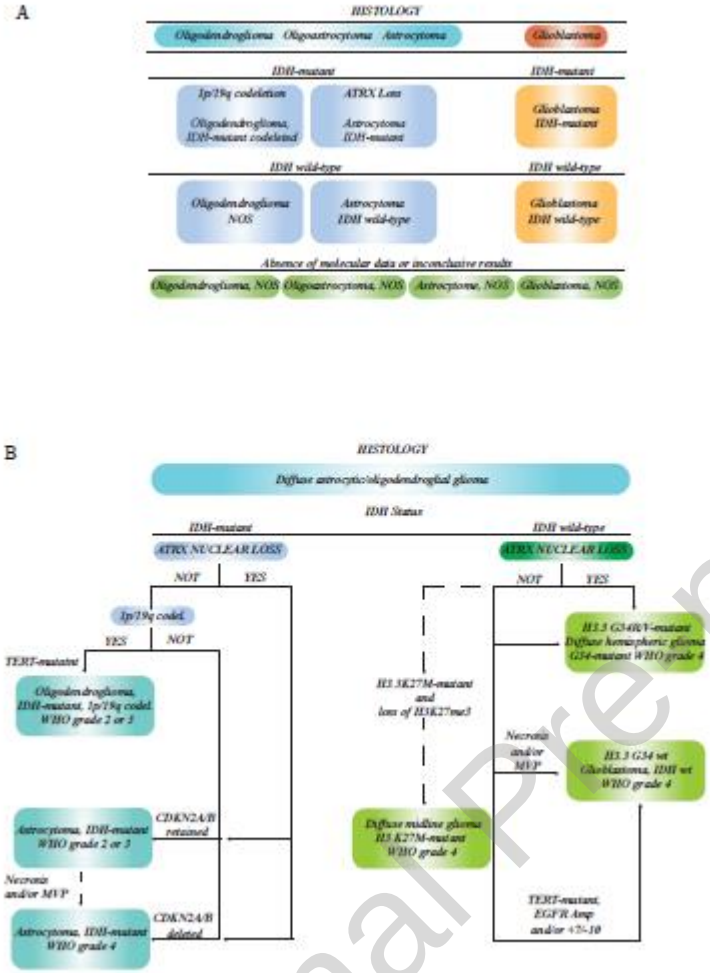


Figure 2. *Molecular pathogenesis of specific altered biomarkers for glioma classification.* (A) IDH mutation plays a central role in glioma pathogenesis working as oncometabolite involved different biological event. (B) ATRX loss is characteristic of astrocytic tumors and is associated to genomic instability causing tumor progression. (C) EGFR alterations, including amplification, rearrangement and activating mutations, are hallmarks of IDH-wild-type glioblastoma and drive oncogenic signaling pathways involved in cell proliferation and survival. (D) TERT promoter mutations lead to telomerase reactivation and cellular immortality and are frequently observed in oligodendrogliomas and IDH-wild-type glioblastomas, representing an early and critical event in gliomagenesis.

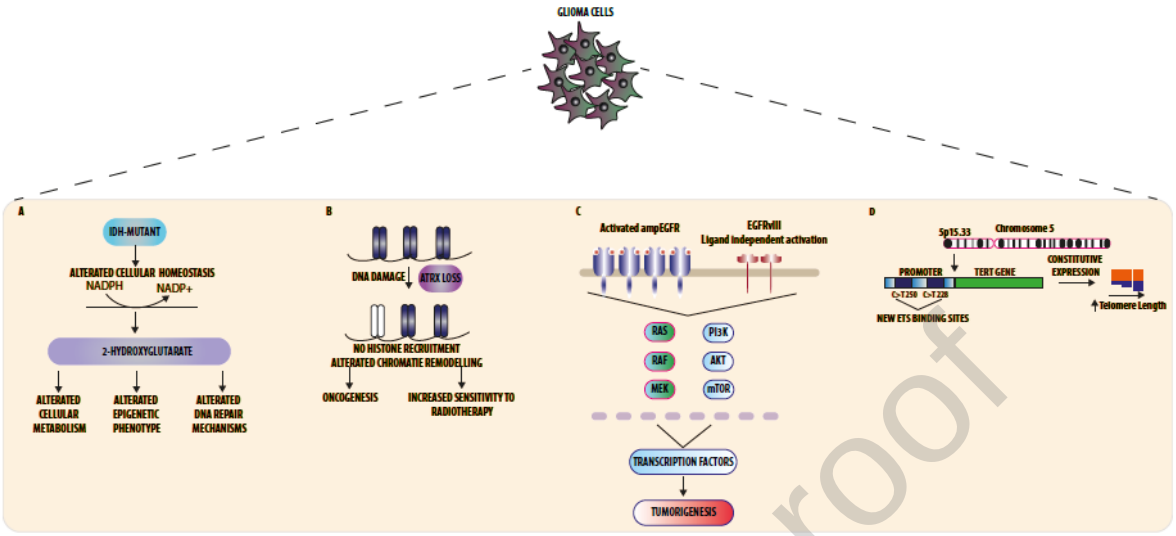


Figure 3. *Schematic overview of the glioma multimodal resistance mechanisms.* **A.** Tumor heterogeneity drives disease recurrence by therapy-resistant cells. The primary tumor contains GSCs. After initial therapy the tumor mass is reduced but RISC (recurrence-initiating stem-like cancer) cells survive driving disease recurrence by new tumor clonal evolution. **B** The image depicts the TME components of glioma. TME in gliomas represents a highly heterogeneous ecosystem that critically acts on tumor behavior, progression and therapeutic resistance. It is an integrated network of malignant glioma cells, vascular, stromal, and immune components, along with extracellular matrix remodeling, to support tumor survival, growth, and invasion. TAMs: Tumor-Associated Macrophages; TM: Tumor Microtube; PVN: Perivascular Niche.

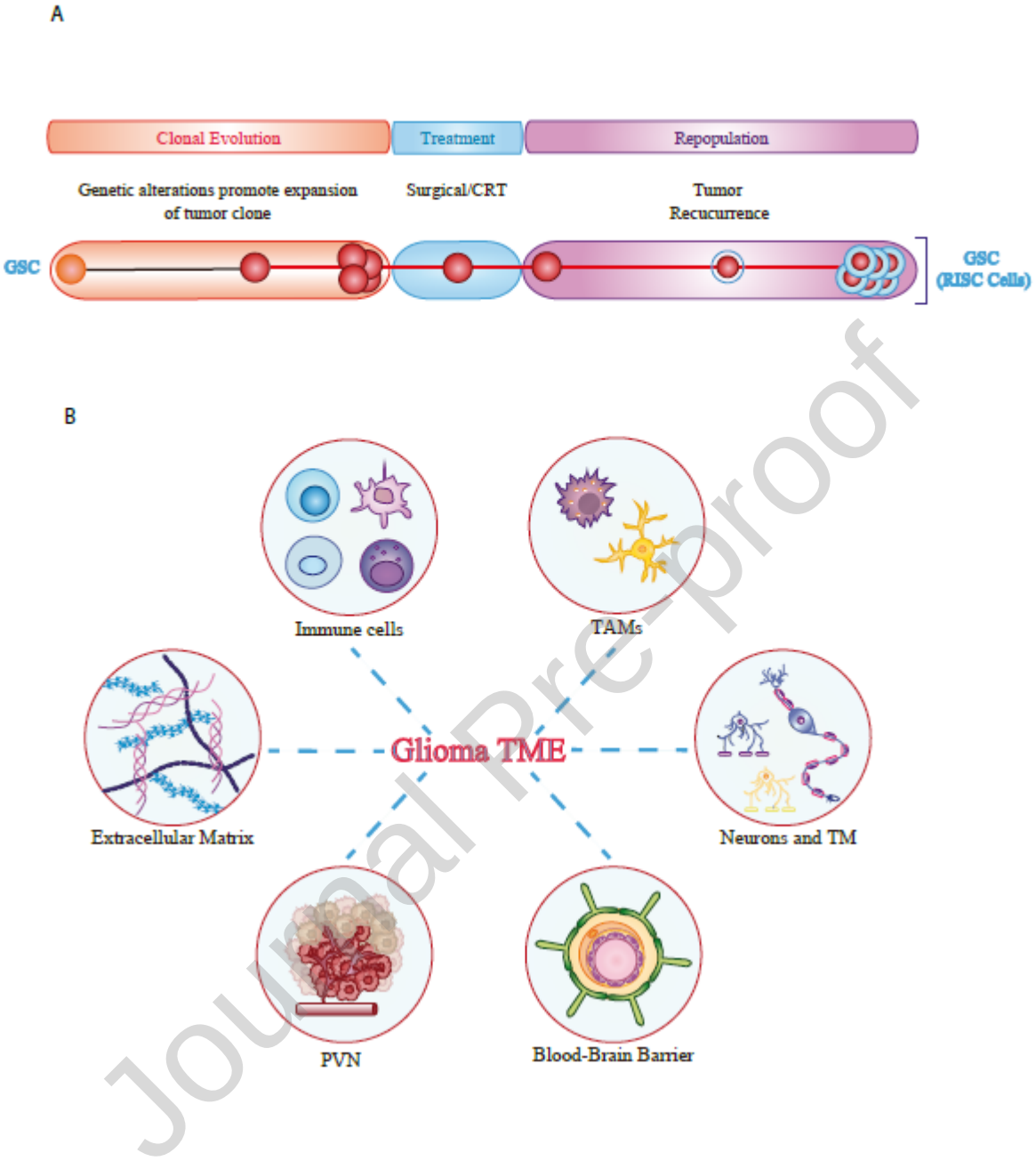
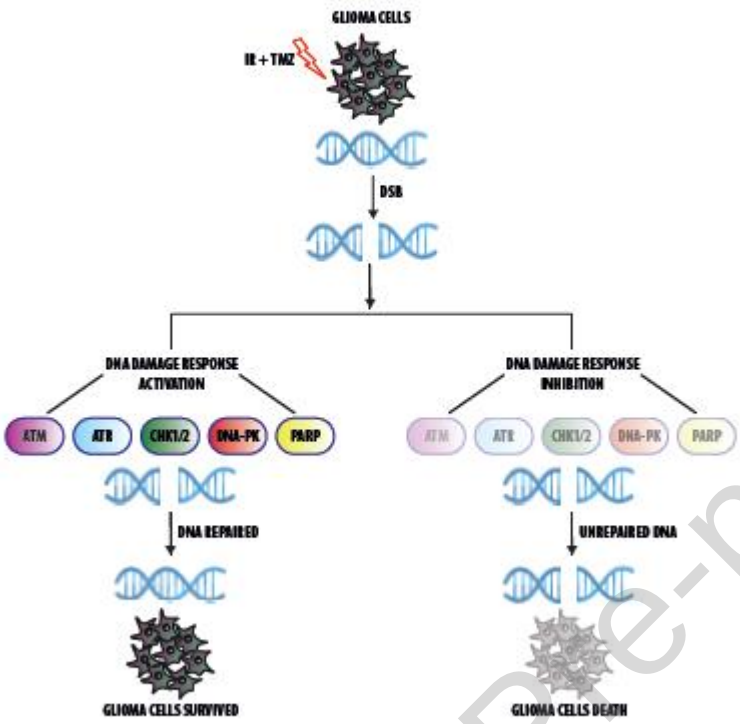


Figure 4. *Diagram showing the dual role of DDR in promoting tumor survival and therapeutic vulnerability.* DDR (DNA Damage Response) is a complex intracellular network coordinating DNA repair and cell cycle control. DDR proteins include ATM, ATR, DNA-PK, Chk1/2, and PARP regulating genomic stability and tumorigenesis. The DDR inhibitors block the DNA repair favoring the IR+TMZ treatment efficacy. IR: Ionizing Radiation; TMZ: temozolomide.



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CRediT author statement

Conceptualization: MM, PV; Formal analysis: MM, SS; Investigation: MM, SS, GDG, CT, SRV, GP; Visualization: MM, SS, GDG, CT, SRV, FM, GP, GM, GB, LS, RC, LM, PV; Supervision: PV; Writing - Original Manuscript: MM, SS, GDG, CT, SRV, GP; Writing - Review & Editing: MM, SS, GDG, CT, SRV, FM, GP, GM, GB, LS, RC, LM, PV.

Declaration of Interest Statement

The authors declare no conflict of interest related to this work

Graphical abstract

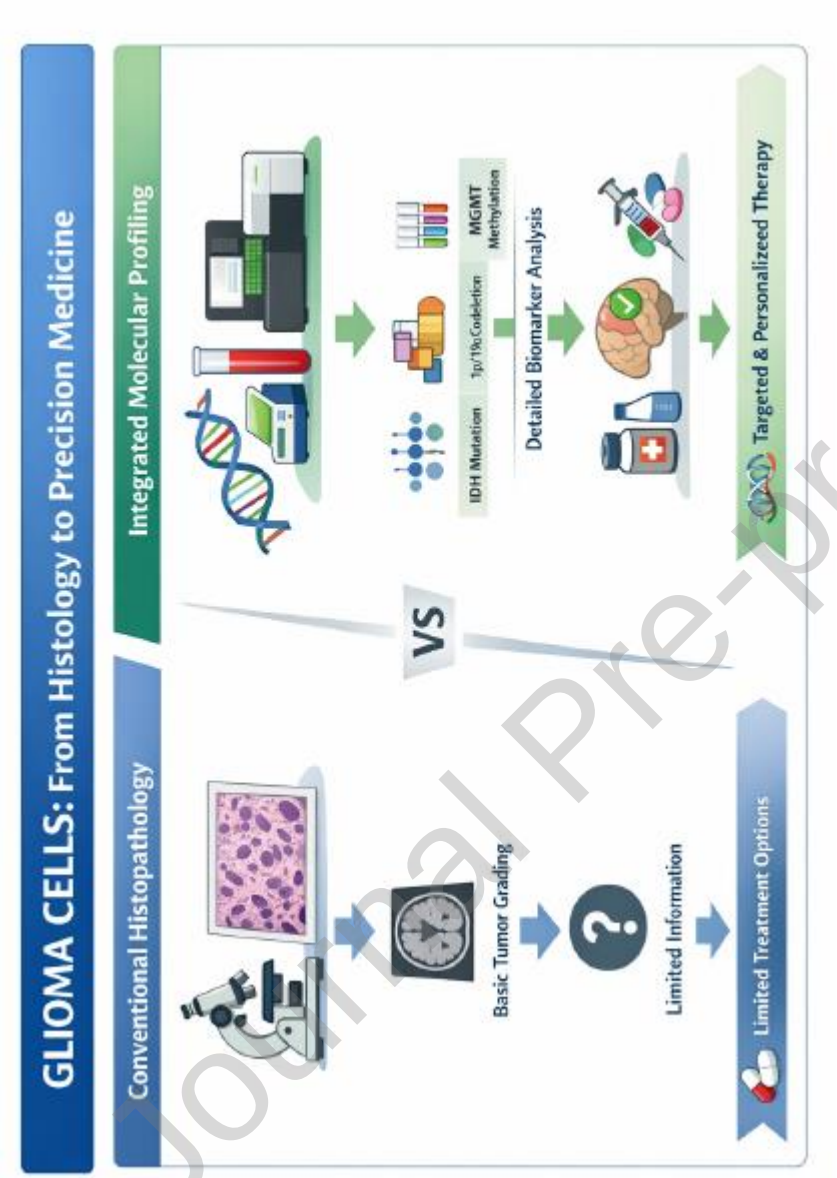

Table 1

Method	Applications	Advantage	Disadvantage
IHC	IDH1 (R132H), ATRX nuclear loss, TP53 overexpression, BRAF V600E, Ki-67, BCL7A, SRSF1 and TXNDC11 expression, specific surface antigens	Simpler and cost-effective	Only specific mutations can be assessed, antibody background may affect signal specificity, simultaneous analysis of multiple targets is not feasible
FISH	Chromosomal abnormalities i.e. *EGFR* amplification, 7+/10q loss signature, *PTEN* loss, *CDKN2A/B* deletion, gene rearrangement, 1p/19q co-deletion	No reference sample is required	High cost, high resolution microscope associated to correct interpretation
Sanger Sequencing	DNA alterations: SNPs, missense mutations, and small indels for different cancer type including glioma	High specificity	Low sensitivity, with reliable detection limited to variants with a VAF ≥ 15–20%, simultaneous analysis of multiple targets is not feasible
AS-qPCR	DNA alterations: SNPs, missense mutations, and small indels for different cancer type including glioma	High specificity, multiple samples	Primers and probes optimization, PCR cycle optimization, specific probe set for different mutations
ddPCR	DNA alterations: SNPs, missense mutations, small indels and CNVs for different cancer type including glioma	High specificity and sensitivity	Less effective for multiple hotspot identification, primers and probes optimization, specific probes set for different mutations
Microarray technology	Performing genome-wide for gene expression quantification and CNV analysis including 1p/19q co-deletion, 7+/10- signature, CDKN2A/B deletion and PTEN loss	Genome-wide, multiple samples	Predefined probes set missing novel alterations, data analysis challenges, complex bioinformatic pipelines

NGS	Performing genome-wide or specified targets regions for genetic and genomic alterations including SNVs, Indels, CNVs, LOH and fusion	Genome-wide, specific target regions and multiple samples	Incomplete target coverage with missing/false negative of hotspots not included in the designed amplicons. Data analysis challenges cause complex bioinformatic pipelines, clinical classification of variants (pathogenic, VUS, CIP, novel) remains challenging.

Highlights

- Diffuse gliomas are molecularly complex CNS tumors
- Key altera8ons define oncotypes with prognos8c and therapeu8c value
- Precision medicine integrates NGS and liquid biopsy for molecular profiling and personalized management