Key genes altered in glioblastoma based on bioinformatics (Review)

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Abstract. Glioblastoma multiforme (GBM) is an aggressive brain tumor with poor prognosis. Recent advancements in bioinformatics have contributed to uncovering the genetic alterations that underlie the development and progression of GBM. Analysis of extensive genomic data led to the identification of significant pathways involved in GBM, such as the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK signaling pathways, alongside key genes such as EGFR, TP53 and TERT. These findings have enhanced our understanding of GBM biology and led to the identification of new therapeutic targets. Bioinformatics has become an indispensable tool in pinpointing the genetic modifications that drive GBM, paving the way for innovative treatment strategies. This approach not only aids in comprehending the complexities of GBM but also holds promise for improving outcomes in patients suffering from this devastating disease. The ongoing integration of bioinformatics in GBM research continues to be vital for advancing therapeutic options.

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1. Introduction

Gliomas are the most prevalent malignant tumors of the central nervous system (CNS), particularly the brain. The histological appearance of these cells resembles that of normal glial cells whose origin remains uncertain (1). Traditionally, gliomas were classified based on tumor cellular structure with tumor histology and location guiding treatment strategies and predicting clinical outcomes (2). The fifth edition of the World Health Organization's (WHO) CNS classification in 2021 (3) implemented significant updates based on the molecular characteristics of gliomas. As such, histological features are combined with molecular and genetic variations that, together, define glioma molecular pathology and aid in patient stratification, as well as targeted therapies (4).

Over time, the classification of gliomas has evolved, employing tools such as immunohistochemistry and electron microscopy for lineage identification. The histologically based classification has numerous limitations, one of which is the 'interobserver variability' or the concordance among multiple independent observers in clinical environments (5). This results in a decreased accuracy in patient prognoses under the conventional classification system. Diagnostic differences remained unaddressed, even when considering variables such as patient age, response to treatment and surgical resection. Consequently, a comprehensive understanding of gliomas at the molecular level became necessary. Such an understanding is vital for improving diagnostic criteria and identifying prognostic biomarkers, which are key to the development of effective targeted therapeutics (6).

The recent incorporation of molecular surrogates has enabled bioinformatics tools to offer valuable prognostic, diagnostic and predictive assistance in classifying gliomas. In adult patients, the majority of gliomas are of the diffuse type. Diffuse gliomas can be classified as astrocytic, oligodendroglial or a combination of both cell types. The categorization of these subtypes is determined by comparing normal, non-cancerous glial cells and tumor cells (7,8).

Gliomas are now categorized based on genetic etiology and prognostic factors, according to the WHO CNS5 2021. Diffuse gliomas in adults are now classified into three categories: Astrocytoma [with mutations in isocitrate dehydrogenase (IDH), classified as grade II, III or IV], oligodendroglioma (characterized by IDH mutation and co-deletion of 1p/19q,

either grade II or III) and glioblastoma multiforme (GBM) [IDH wild-type (wt), grade IV] (3). Several studies describe the chromosomal and molecular heterogeneity of glioma, which opens the door for a new classification system for this disease (8-10). Genetic heterogeneity in GBM promotes treatment resistance and disease progression by enabling cellular plasticity and diverse expression states. Despite this diversity, malignant cells converge into neural development- and cell cycle-related states driven by alterations in genes such as EGFR and PDGFRA, enhancing adaptability and limiting single-target therapies (9,11). Personalized treatments integrating genetic, epigenetic and microenvironmental data are essential (12).

Since the mapping of the human genome sequence, advances in bioinformatics and sequencing technology have substantially improved the investigation of genomic sequences in human malignancies. Single-cell RNA sequencing has revealed distinct cell lineages in GBM, including astrocytic, neuronal, oligodendrocytic, mesenchymal and GBM stem cells (GSCs), which drive tumor growth and heterogeneity. It has uncovered a neurodevelopmental hierarchy led by progenitor-like GSCs and has highlighted tumorigenic, rapidly cycling GSCs as key drivers of growth. Notably, neuronal lineages lack HLA expression, suggesting immunotherapy resistance (13,14). Microsatellite instability is observed in 5.5-25% of GBM cases, particularly in recurrent tumors, due to mismatch repair defects. Its clinical implications remain elusive, with studies showing mixed outcomes in terms of prognosis and potential as a predictor of immune checkpoint inhibitor efficacy (15,16).

One key feature driving the rapid proliferation of GBM cells is altered glucose metabolism, characterized by significant metabolic reprogramming. Altered glucose metabolism supports GBM growth by increasing glucose uptake via upregulated glucose transporter 1 (GLUT1) and GLUT3 and enhancing glycolysis through enzymes such as phosphofructokinase platelet-type (PFKP) and pyruvate kinase M2 (PKM2). This metabolic shift, known as the Warburg effect, prioritizes glycolysis for ATP and biosynthetic precursor production, even in oxygen-rich conditions, fueling rapid proliferation (17,18). Also, GBM exhibits altered lipid biosynthesis, including increased fatty acid synthesis via fatty acid synthase (FASN) and ATP citrate lyase (ACLY), lipid droplet accumulation for energy storage and reliance on exogenous cholesterol through low-density lipoprotein (LDL) uptake. Abnormal sphingolipid metabolism, with reduced ceramide and elevated sphingosine-1-phosphate (S1P) levels, promotes tumor growth, survival and therapy resistance (19,20).

Tumor-associated macrophages and microglia (TAMs) and hypoxia significantly contribute to GBM progression and therapeutic resistance. M2-like TAMs secrete factors like epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), promoting tumor growth, invasion, angiogenesis and immune evasion, while tumor cells polarize TAMs into a pro-tumor phenotype, amplifying malignancy (21). Hypoxia activates the hypoxia-inducible factor pathway, upregulating genes such as VEGF, ZEB1 and CXCR4, which drive metabolism, angiogenesis and invasion. It further promotes glioma stem cells, enhances glycolysis, suppresses immune responses and contributes to therapy resistance, highlighting key therapeutic challenges (22).

In the present study, the bioinformatic analysis of key genes that are altered in GBM according to the new WHO classification were reviewed. Such understanding is crucial for analyzing genes and pathways involved in the development and progression of GBM, allowing for the development of targeted therapies.

2. Overview of the genetic alterations in GBM

GBM, a grade IV tumor, resembles the most prevalent and deadliest form of brain cancer. Key factors influencing the prognosis of GBM include the extent of tumor removal, the patient's age at the time of diagnosis and their Karnofsky performance status (1,23). Survival rates for patients differ based on the glioma type, with pilocytic astrocytoma (grade I) showing the highest survival rates and GBM having the lowest. The five-year survival rate for individuals diagnosed with GBM ranges from 0.05 to 4.7% (1).

GBM is defined by a wide range of genetic or epigenetic changes. The extensive range of modifications results in various mutation subgroups, each necessitating distinct therapeutics and each associated with unique patient survival outcomes. Therefore, the designation 'multiforme' was adopted to reflect the genotypic and phenotypic diversity of this tumor type (24). In 2008, The Cancer Genome Atlas (TCGA) released a classification for GBM that was centered around genetic mutations and molecular indicators, and consisting of the classical, mesenchymal, neural and pro-neural categories (25,26). EGFR amplification, cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion and p53 mutations are characteristics of the classical subtype. The mesenchymal subtype displays changes in neurofibromatosis type 1 (NF1) and phosphatase and tensin homolog (PTEN), as well as expression of theMET and CD44 mesenchymal genes, while the pro-neural subtype is characterized by mutations in the p53, platelet-derived growth factor receptor alpha, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and IDH1 genes, along with heightened expression of the homeobox protein NKX2-2 and the oligodendrocyte transcription factor 2 (OLIG2) genes. Finally, the neural subtype, which accounts for 16% of GBM cases, is characterized by its expression of neural markers (27). Chromosomal gains (e.g., EGFR on chromosome 7) and losses (e.g., PTEN on chromosome 10, CDKN2A/B on chromosome 9 disrupt pathways such as RTK/ PI3K, p53 and retinoblastoma protein (RB), driving GBM growth, survival and therapy resistance (28).

In GBM, micro (mi)RNA dysregulation impacts tumor suppressors and oncogenes. Overexpressed miRNAs, such as miR-21 and the miR-17-92 cluster, act as oncogenes by promoting proliferation, invasion and therapy resistance, while downregulated miRNAs, such as miR-7, miR-34a and miR-137, suppress tumor growth. Altered miRNAs also regulate metabolism, angiogenesis and immune responses, highlighting their role in GBM progression and potential as therapeutic targets (29-31).

LncRNAs regulate GBM cell proliferation and invasion by acting as oncogenes or tumor suppressors. Pro-tumorigenic long non-coding (lnc)RNAs such as H19 and MIR31HG promote proliferation and invasion via Wnt/β-catenin and NF-κB signaling, while tumor-suppressive lncRNAs such as GAS5 and LINC-PINTinhibit these processes by targeting pathways such as STAT5. These lncRNAs hold potential as biomarkers and therapeutic targets (32-34).

A new stratification for GBM was established by the 2021 WHO CNS5. In adult cases of IDH-wt diffuse astrocytic gliomas, the presence of any one of three genetic factors is sufficient to identify the most severe grade of glioma. These parameters are: i) Telomerase reverse transcriptase (TERT) promoter mutation, ii) EGFR gene amplification and iii) combined gain of the entire chromosome 7 and loss of the entire chromosome 10 (+7/-10 chromosome copy number changes) (35,36). Consequently, these three genetic parameters have been incorporated into the diagnostic criteria for GBM. Histone acetylation and methylation regulate gene expression in GBM as well, promoting tumor progression. Acetylation by histone acetyltransferases such as p300/CBP enhances gene activity but drives gliomagenesis, while methylation by lysine methyltransferases and protein arginine methyltransferase 2 is linked to aggression and oncogenesis. The H3K27M mutation and epigenetic modifications in GSCs offer potential therapeutic targets (37-39).

ATP-binding cassette (ABC) transporters, such as ABCB1, ABCC1 and ABCG2, contribute to chemotherapy resistance in GBM by actively effluxing chemotherapeutic drugs like temozolomide, reducing their intracellular concentration and rendering them less effective. Genetic variations, including single nucleotide polymorphisms, further influence the chemotherapy response, complicating the prediction of treatment outcomes, as tumors with higher expression of ABC transporters may be less responsive to treatment (40,41).

Studying the molecular and genetic changes underlying the development of GBM is crucial for gaining a comprehensive understanding of this disease and developing targeted treatment plans for patients. Given the above, a one-size-fits-all approach does not exist for treating all cases of GBM. While surgery, radiation and chemotherapy remain the cornerstone therapeutic approaches for cancer, progress in understanding the molecular processes driving tumor development is leading to novel targeted strategies for the diagnosis and treatment of cancer. Over the last decade, there has been a surge in biological data on genes, proteins, CpG islands and a variety of other topics, which led to the introduction of numerous bioinformatics tools for analyzing biological data. Eventually, new databases emerged, such as The Cancer Genome Atlas (TCGA); https://www.cancer.gov/tcga), Therapeutically Applicable Research to Generate Effective Treatments (TARGET; https://www.cancer.gov/ccg/research/genome-sequencing/target) and the Cancer Genome Characterization Initiative (CGCI) (https://www.cancer.gov/ccg/research/genome-sequencing/cgci), leading to groundbreaking changes in the cancer field (42). In this review, a genomic evaluation of key genes involved in GBM tumorigenesis and progression was carried out, combining results of laboratory research and bioinformatics tools.

3. Overview of bioinformatics tools used to identify genetic alterations

cBioPortal. Given the nature of GBM and the multitude of genes implicated in its tumorigenesis, it is essential to employ a tool for the comprehensive analysis and comparison of the genetic alterations associated with this specific type of cancer. cBioPortal, an open-access web resource used in cancer research and molecular profiling, effectively meets this need and provides a platform for cancer researchers to explore, visualize and analyze multidimensional cancer genomics data compiled from extensive cancer genomics projects such as the International Cancer Genome Consortium and TCGA. This portal facilitates access to DNA copy number alterations, somatic mutations, DNA methylation, mRNA and miRNA expression, as well as protein and phosphoprotein abundance data (43). Scientists can then analyze the datasets to uncover patterns and biomarkers associated with cancer progression, or novel therapeutic targets that aid in the development of personalized GBM treatment strategies.

cBioportal offers an oncoprint feature that allows users to generate graphical representations of genomic alterations across specific genes within a sample where every row corresponds to a gene and every column represents a sample. This representation uses colors and symbols to depict amplifications, mutations, deletions and alterations in gene expression (43,44). This allows researchers to easily identify patterns and relationships between genetic alterations and specific cancer types.

cBioPortal also provides an analysis of mutual exclusivity, using Fisher's exact test to ascertain whether certain genetic alterations occur together or are mutually exclusive within the same tumor type. In mutual exclusivity, one genetic alteration replaces another within a tumor, suggesting that each tumor may harbor only one of these alterations. Alternatively, Co-occurring genetic alterations can arise concomitantly in the same tumor type (43,44).

The mutations tab is another feature that provides both a graphical and a tubular summary of the non-synonymous mutations detected within the queried gene. The 'lollipop model', or visual representation, illustrates the frequency and location of mutations within the protein domains as encoded by the standard gene isoform. Notably, all DNA mutations are adapted to the standard RefSeq isoform. The tabular summary offers additional details regarding every mutation within each queried gene, such as the sample case ID, amino acid change, annotations, type (missense, nonsense, splice site, frameshift insertion or deletion, in-frame insertion or deletion, nonstop, nonstart), copy number, number of mutations in a sample, number of mutations at a particular position in the Catalogue of Somatic Mutations in Cancer and frequency (43).

Additionally, the portal has a survival analysis section enabling researchers to compare overall survival and disease-free survival rates, represented in Kaplan-Meier plots, between tumor samples with and without alterations in the genes under investigation (43). These methods provided by cBioportal aid in unraveling the complex genetic dynamics underlying cancers such as GBM.

cBioPortal in GBM research enables the identification of key genomic alterations such as EGFR, PTEN and IDH1 mutations, as well as disrupted pathways such as PI3K/AKT

and p53. It stratifies patients with GBM into molecular subtypes, supports personalized treatment strategies and integrates clinical outcomes with genomic data to identify prognostic biomarkers and therapeutic responses. Tools such as oncoprints and survival plots facilitate the discovery of actionable targets and advancements in GBM diagnostics and treatment (45).

In a study published in *Nature*, cBioPortal was used for the analysis of large-scale genomic and transcriptomic datasets to uncover GBM-specific alterations such as TP53 mutations and EGFR amplifications. It revealed molecular drivers, therapeutic targets and distinct subtypes, correlating genomic changes with prognosis and treatment outcomes, making it crucial for translational GBM research (46).

cBioPortal also has several limitations. Its retrospective design introduces the possibility of convenience sampling and it lacks the ability to perform multivariate analyses to identify and adjust for confounding factors. However, metadata can be downloaded and analyzed using external statistical software like R or SPSS (47). While cBioPortal supports correlation analysis between query gene alterations, tools such as Regulome Explorer (https://www.regulomedb.org) and Oncomine (https://www.oncomine.com) are needed for more complex gene correlations, including mRNA expression (43). Currently, this tool is for research use only and not approved for patient diagnosis or treatment. Future developments aim to ensure compliance with Medical Diagnostic Regulation (https://eur-lex.europa.eu/eli/reg/2017/745/oj) and *In Vitro* Diagnostic Regulation (https://eur-lex.europa.eu/eli/reg/2017/746/oj) (48).

Cytoscape. The recent development of high-throughput 'omics' research areas, including transcriptomics, proteomics and metabolomics, has led to the generation of extensive datasets, which considerably increased the current understanding of biological processes at the molecular and physiological levels, particularly in the context of GBM. However, interpreting these large databases remains a challenge. As a result, there is an increased demand for computer-based support in visualizing and analyzing biological data, which plays an essential role in illustrating the numerous biological interactions as organized and logical pathways (49).

Cytoscape is an open-source software platform developed for visualizing molecular interaction networks and combining them with annotations, gene expression profiles and other high-throughput expression data (50). While it can be applied to various molecular systems, Cytoscape is most effective when utilized alongside large databases of molecular interactions, for both humans and model organisms, particularly those involving protein-protein and protein-DNA interactions as well as numerous genetic interactions (50,51). The core functionality of Cytoscape includes network layouts, data integration with molecular states and linking to functional annotation databases (50). The software's extensibility through plugins allows the rapid development of additional computational analyses. Overall, Cytoscape is an effective and adaptable framework for depicting and evaluating pathways at the biomolecular level (49).

Using the *Cytoscape* tool in GBM research facilitates the analysis and visualization of protein-protein interaction (PPI) networks and molecular pathways involved in the development of this disease. It enables researchers to identify key hub proteins and deregulated pathways, such as PI3K/AKT/mTOR and EGFR signaling, which are critical to GBM progression and resistance mechanisms (52). Integrating genomics, transcriptomics, proteomics, epigenomics and metabolomics provides a comprehensive view of GBM by identifying molecular interactions, tumor subtypes and biomarkers. Techniques like single-cell multi-omics and machine learning uncover cellular heterogeneity, disrupted pathways and therapeutic targets (53-55). By integrating multi-omics data, Cytoscape provides graphical representations of molecular interactions, aiding in the identification of therapeutic targets and enhancing the understanding of GBM's molecular biology.

In specific studies, Cytoscape was used to construct and analyze PPI networks for GBM using publicly available datasets. Plug-ins such as Molecular Complex Detection (MCODE; https://mcode.readthedocs.io) were employed to identify highly connected clusters or modules in the network that are crucial to GBM pathology. Topological analysis revealed hub genes and key nodes involved in tumor progression and cellular processes such as cell cycle regulation (56).

Additionally, pathway enrichment analysis using Cytoscape tools such as ClueGO and CluePedia mapped identified genes to biological pathways, uncovering disruptions in cell proliferation, apoptosis and DNA repair mechanisms (56). Enhanced DNA repair mechanisms, including homologous recombination, non-homologous end joining and base excision repair, enable GBM cells to resist radiotherapy-induced DNA damage. Key factors like ataxia telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), DNA-dependent protein kinase (DNA-PK) and RAD51, along with cancer stem cells' efficient repair capacity, further enhance resistance (57,58). In addition, Notch pathway activation sustains GBM stem cells by promoting self-renewal, inhibiting differentiation and driving tumor progression and therapy resistance (59,60). Targeting these pathways could improve radiotherapy outcomes.

In order to overcome the limitations of Cytoscape, it is important to connect directly with databases such as Database of Interacting Proteins (DIP) (http://dip.doe-mbi.ucla.edu), Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo) and Gene Ontology (GO; https://geneontology.org), eliminating the need for manual data parsing into annotations or attributes. A longer-term goal is to integrate high-level interaction networks with detailed physico-chemical models of biological processes, which are addressed by tools such as Ecell (https://www.e-cell.org), VirtualCell (https://vcell.org), Gepasi (http://www.gepasi.org) and Systems Biology (https://sbw.sourceforge.net) (50).

MutationTaster. As molecular biology progresses and its methodologies become more cost-effective, the use of DNA sequencing tools helps investigate potential biological markers and identify novel genetic alterations involved in the progression of GBM. MutationTaster is one of several web-based tools utilized for predicting the effects of DNA variants. It was developed in 2014 by Cardiff University and Universitätsmedizin Berlin to assess intronic, synonymous and short indel mutations using an annotation software on its website (mutationtaster.org).

To predict variant effect, the program uses pathogenic variants found in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) and the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk) (61), as well as, among others, single nucleotide polymorphisms and deletions from the 1,000 Genomes Project (62). Pathogenic variations in ClinVar are considered disease-causing, while variants that appear >4 times in 1,000 Genomes (https://www.internationalgenome.org)/HapMap (https://ftp.ncbi.nlm.nih.gov/hapmap) are considered neutral (63).

A significant advantage of MutationTaster2 (a new version of the tool) compared to other mutation-predicting tools is the ability to provide high-accuracy predictions, as evidenced by various benchmarking studies. In addition, MutationTaster2 is capable of processing large-scale datasets, which is particularly useful for analyzing data from high-throughput sequencing. However, a notable drawback of MutationTaster2 is its dependence on predefined rules and algorithms, which may not fully account for the intricate and situation-specific impacts of genetic variations (64).

The latest release of MutationTaster21, aimed at boosting whole-exome sequencing success rates by subjecting each variation to a battery of *in silico* tests and replacing Bayes classifier with Random Forest models, is suited for several kinds of variation. The models were built on balanced accuracy, ensuring equal predictive performance for both benign and malignant variations (64).

Several studies have demonstrated the advantages of using MutationTaster in GBM by predicting the functional impact of genetic mutations in key GBM-associated genes such as *TP53*, *EGFR*, *PTEN* and *IDH1*. MutationTaster analyzes these mutations to determine whether they are disease-causing by evaluating protein function, splice sites and regulatory elements. Hence, it helps prioritize mutations for further validation and identifies their role in tumor progression, therapy resistance and altered cellular processes, supporting the development of personalized therapies for GBM (65).

MutationTaster is limited by the fact that it can only predict the deleteriousness of variants within protein-coding genes and cannot assess variants in RNA genes or non-genic regions (64).

4. Analysis of genes involved in GBM survival pathways

EGFR. GBM is often associated with the amplification and overexpression and EGFR, a tyrosine kinase receptor. EGFR signaling is essential for GBM cell growth and survival, and targeting EGFR has been a major focus of GBM research. One of the most prevalent mutations in GBM is the EGFRvIII mutation, which is caused by an in-frame deletion of exons 2-7 of the EGFR gene. EGFRvIII has been associated with enhanced GBM cell proliferation, invasion and angiogenesis. It is expressed in ~30% of patients with GBM. Furthermore, EGFRvIII has been linked to radiation- and chemotherapy resistance (26,66). A cBioPortal analysis of 592 GBM samples, based on the TCGA database, indicated that EGFR shows the highest mutation percentage (47%) among the genes evaluated in this review (Figs. 1A and B and 2). Brennan *et al* (26) examined the genomic alterations in GBM and discovered that EGFR was amplified in ~45% of cases. Bioinformatics analysis of the 592 GBM samples (TCGA, Pan Atlas-GBM) for mutual exclusivity and co-occurrence using cBioPortal demonstrated that EGFR mutations co-occur with modification of AKT1 and MAP3K1 but are mutually exclusive with IDH1 and TP53 mutations ($P<0.01$) (Fig. 1C and D). Additional research confirmed this and showed that EGFR amplification is associated with both higher gene mRNA expression and increased activity of the PI3K/AKT/mTOR signaling pathway, an important regulator of cell growth and survival. The lollipop model developed by cBioPortal indicated that 26 out of 126 mutations are the highly oncogenic A289V/T/D and 16 mutations are G598 V/A (67). The pathogenicity of missense mutations may be predicted using the Rare Exome Variant Ensemble Learner (REVEL) for better understanding of their effect. Variants have scores that range between 0 and 1, and the greater their value, the more likely they are pathogenic. The A to V amino acid substitution is expected to have the highest REVEL score among the three A289 substitutions (A289V/T/D), suggesting that it is more oncogenic than the other two. Likewise, the amino acid shift from G to V in G598V/A is more oncogenic than the switch from G to A.

Additional bioinformatics studies have been carried out to better elucidate the role of EGFR in GBM. Lu *et al* (68) used gene expression profiling to determine which gene expression patterns were different in GBM tumors with and without EGFR amplification. Various genes that regulate the cell cycle and DNA replication have been found to be upregulated in tumors with EGFR amplification (26,69). Computational modelling was used in another study by Jain *et al* (70) to find possible downstream effectors of the EGFR signaling pathway in GBM. They discovered that one of the major downstream pathways activated by EGFR in GBM was the RAS/RAF/MEK/ERK pathway. Small molecule tyrosine kinase inhibitors (TKIs) and monoclonal antibodies are two examples of targeted therapeutics that have been developed to block EGFR signaling in GBM (71,72). Clinical trials of these agents, however, have had mixed results in terms of improving the overall survival of patients with GBM (73). This can be the result of the complex signaling network involving EGFR and its downstream effectors, which can lead to other pathways being activated as a compensatory mechanism in response to EGFR inhibition. Recent studies have identified novel approaches to targeting EGFR in GBM, such as combination therapies that target both EGFR and other signaling pathways, or inhibitors that target specific downstream EGFR effectors (73). Overall, EGFR seems to be a potential therapeutic target in GBM. Traditional tyrosine kinase inhibitors (TKIs), such as erlotinib and gefitinib, are designed to target mutations in the intracellular tyrosine kinase domain. However, since GBM mutations primarily occur in the extracellular domain (ECD), these TKIs are less effective. By contrast, lapatinib has a higher affinity for the ECD, which likely accounts for its superior *in vivo* results (74). Additionally, GBM-associated EGFR mutations enable active signaling despite an inactive receptor conformation (75), further limiting the effectiveness of traditional TKIs in targeting EGFR. Moreover, erlotinib and gefitinib show limited efficacy in GBM clinical trials, with no significant survival improvements, even when used in combination therapies (73). Erlotinib's ineffectiveness is likely due to its inability to target the EGFR extracellular domain. While

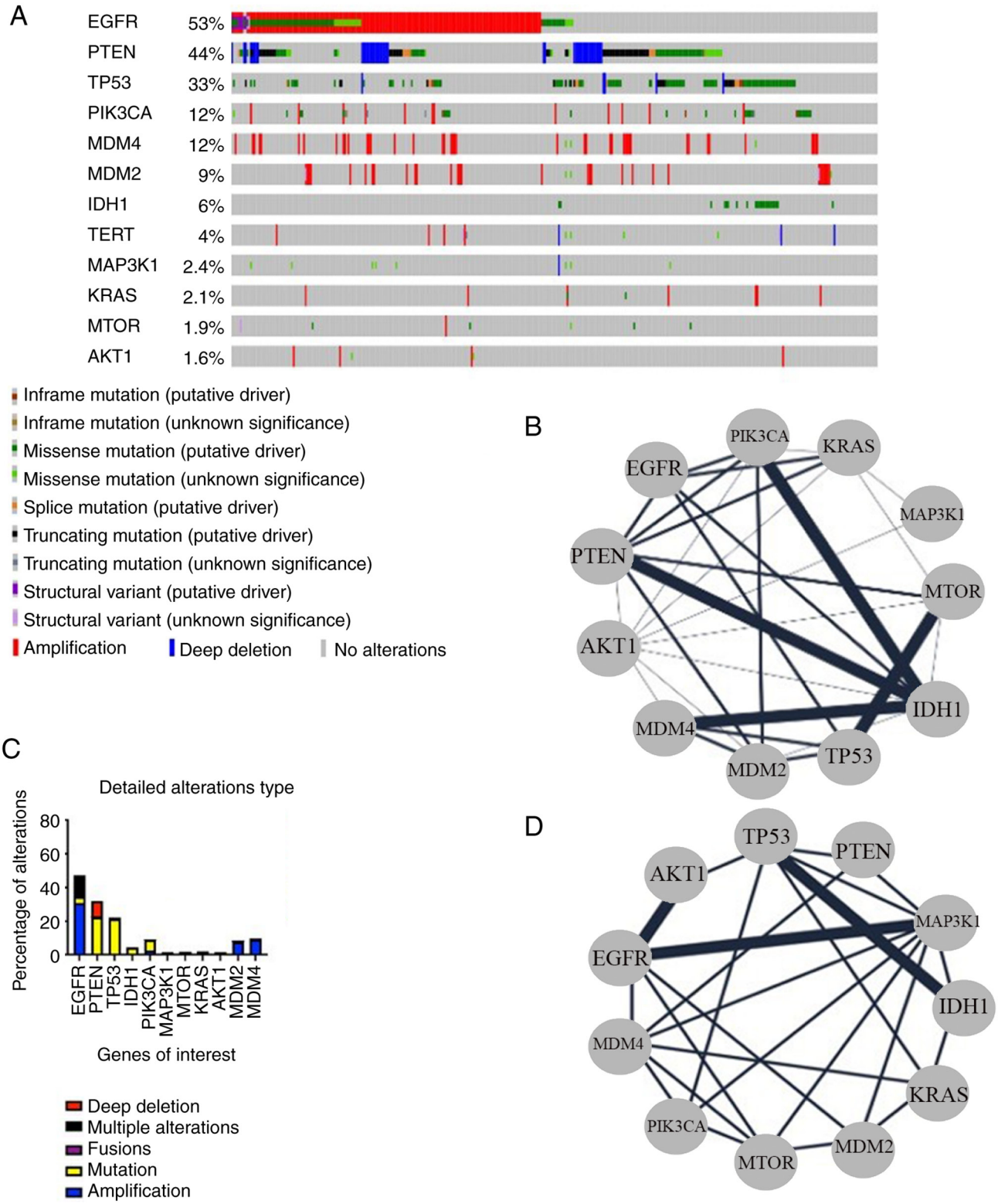

Figure 1. Bioinformatics analysis of genetic alterations in GBM. (A) Oncoprint of EGFR, PTEN, TP53, PIK3CA, MDM2, MDM4, IDH1, TERT, MAPK3K1, KRAS, mTOR and AKT1 in 592 samples of GBM showing the alteration type. (B) Network of the genes of interest showing their mutual exclusivity relationship. The network was generated by Cytoscape. (C) Percentage of alteration in each of the genes of interest according to the oncoprint. (D) Network of the genes of interest showing their co-occurrence relationship. The network was generated by cystoscape. GBM, glioblastoma.

gefitinib slightly improves survival and efficiently dephosphorylates EGFR, it fails to halt tumor growth signaling (74,76). These findings underscore the need for synergistic inhibition of downstream EGFR pathways and the development of new TKIs that specifically target ECD variants in GBM.

Monoclonal antibodies, which work by binding to EGFR overexpressed on tumor cells and blocking signaling required for cell growth, have also been investigated. However, antibodies targeting EGFR in GBM, such as cetuximab, panitumumab and nimotuzumab, show limited efficacy in clinical

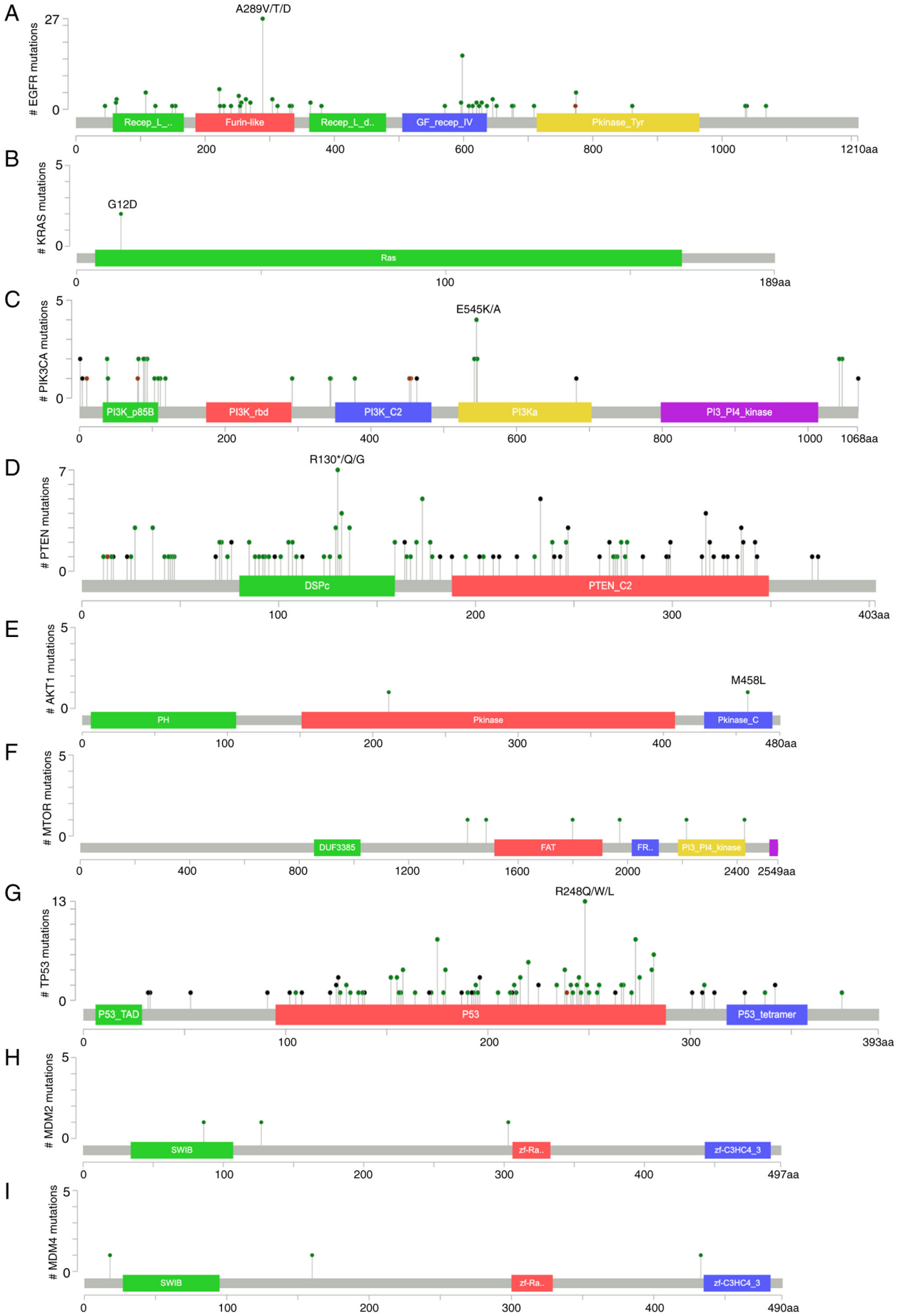

Figure 2. Lollipop models for gene alterations generated by cBioportal. (A) EGFR, (B) KRAS, (C) PIK3CA, (D) PTEN, (E) AKT1, (F) mTOR, (G) TP53, (H) MDM2 and (I) MDM4.

trials, with minimal improvements in progression-free survival (PFS) and overall survival (77-79). Cetuximab demonstrates potential in combination therapies but causes side effects like rash and gastrointestinal toxicity due to off-target effects on normal tissue (80,81). These side effects are worsened by the high doses required to penetrate the blood-brain barrier, emphasizing the need for synergistic inhibition to overcome resistance and the development of GBM-specific drugs or delivery mechanisms to minimize off-target antibody accumulation (82,83).

Sym004, a combination of two recombinant human-mouse chimeric monoclonal antibodies (futuximab and modotuximab), targets non-overlapping EGFR epitopes and demonstrates activity against various EGFR-expressing solid tumors in preclinical studies. It shows superior tumor growth inhibition in xenograft models compared to other anti-EGFR antibodies (84). However, in a phase II trial for recurrent GBM, Sym004 shows limited efficacy, with PFS reaching a maximum of 9.95 months (85). This may be due to the complex signaling network involving EGFR and its downstream effectors, which can activate compensatory pathways in response to EGFR inhibition.

Similarly, although novel anti-EGFR antibody GC1118 demonstrates promising preclinical data (86), it fails to improve PFS in a phase II trial for recurrent GBM patients with EGFR amplification, likely due to tumor evolution and inadequate pathway suppression (87). Depatuxizumab mafodotin, an anti-EGFR antibody-drug conjugate, also failed to improve overall survival in EGFR-amplified GBM patients in a phase III trial, despite promising preclinical data. While it improved PFS, particularly in patients with the EGFRvIII variant, tumor resistance via alternative pathways limits its efficacy (88).

Currently, novel approaches, including highly CNS-penetrant small molecule EGFR inhibitors like ERAS-801 and BDTX-1535, as well as dual-specific immunotoxins targeting both EGFRwt and EGFRvIII such as D2C7-IT, are under investigation, with certain drugs showing potential in early studies (85,89).

The Ras/Raf/MEK/ERK pathway. Among the pathways that regulate cell division, proliferation and survival is the Ras/Raf/MEK/ERK pathway. Its dysregulation has been associated with the development of several types of cancer, including GBM. Aberrant activation of this pathway in GBM has been associated with poor prognosis and resistance to therapy. Several bioinformatic analyses have been performed to investigate the prevalence and functional significance of mutations in the genes of this pathway. One study examined GBM patient samples using bioinformatics to identify mutations and changes in the copy number of genes encoding components of this pathway. The most commonly mutated genes in GBM are EGFR, KRAS, NRAS and BRAF (Figs. 1A and B and 2B), and these mutations are associated with an increased activity of the Ras/Raf/MEK/ERK pathway (90,91). Inhibitors that block this pathway, such as sorafenib or U0126, reduced GBM cell proliferation while increasing apoptosis *in vitro*. Brennan *et al* (26) used a combination of bioinformatic and experimental approaches to investigate the functional significance of Ras/Raf/MEK/ERK pathway mutations in GBM. They discovered that mutations in the BRAF and KRAS genes were linked to pathway activation and increased cell proliferation. They also demonstrated that small-molecular-weight inhibitors selectively reduced the viability of GBM cells with BRAF or KRAS mutations (26). An additional study examined the role of this pathway in the mesenchymal subtype of GBM, which is associated with increased pathway activity. Inhibition of the pathway using the MEK1/2 inhibitor trametinib led to a decrease in the *in vitro* migration and invasion of mesenchymal GBM cells. Along with genetic alterations, epigenetic modifications, including DNA methylation, have been associated with the dysregulation of the Ras/Raf/MEK/ERK pathway in GBM. Specific DNA methylation changes in GBM regulate gene expression, influencing tumor progression and prognosis. Changes like EZH2 activation in the mesenchymal subtype, Wnt signaling demethylation and promoter methylation of genes like FNDC3B and SOX10 are linked to poor survival and treatment resistance. Methylation-regulated biomarkers offer therapeutic potential (92-94). One study found that increased pathway activity in GBM is linked to hypermethylation of the RASSF1A promoter region, causing lower expression of the RASSF1A tumor suppressor gene (95).

These results suggest that targeting the Ras/Raf/MEK/ERK pathway may be a potential strategy for treating GBM and that dysregulation of this pathway plays a significant role in the development of this type of cancer. Studies suggested that BRAF-targeted therapy is an effective treatment for patients with BRAF-mutated GBM. BRAF inhibitors such as vemurafenib, dabrafenib and encorafenib are generally well-tolerated and lead to rapid clinical improvement. These inhibitors inactivate Raf kinase through competitive occupation of the ATP binding pocket, disrupt the MAPK signaling cascade, cause G1 cell-cycle arrest and induce apoptosis. However, no BRAF inhibitor is currently Food and Drug Administration (FDA)-approved for use in patients with GBM (96). However, dabrafenib and encorafenib are tested in phase II clinical trials alongside the MEK inhibitors trametinib and binimetinib, respectively (97).

In addition, MEK1/2 inhibitors show promise as targeted treatments for GBM. Blocking MEK signaling in GBM results in antiproliferative effects, reducing cell division and Ki67-positive cells. Trametinib, a MEK1/2 inhibitor, inhibits the Ras-Raf-MEK-ERK pathway, limiting GBM proliferation, migration and invasion (96). Other MEK inhibitors, such as cobimetinib, are also considered potential treatments, demonstrating good tolerance and effectiveness, as evidenced by tumor size reduction (98).

On the other hand, atorvastatin, a Ras/MAPK inhibitor, enhances the efficacy of TMZ both *in vitro* and in GBM xenografts by inhibiting Ras signaling through a prenylation-dependent mechanism (99). However, when evaluated in combination with radiotherapy and TMZ in patients with GBM (phase II, NCT02029573), atorvastatin does not improve PFS (100).

The PI3K/AKT/mTOR pathway. The PI3K/AKT/mTOR pathway plays a pivotal role in several biological processes, such as cell survival, growth and proliferation. Dysregulation of this pathway has been associated with the onset and proliferation of various malignant tumors, including GBM.

In addition, aberrant activation of this pathway has also been associated with poor prognosis and resistance to radiation and chemotherapy (101).

Several studies examined GBM patient samples using bioinformatics to identify mutations and changes in copy number changes of genes implicated in the PI3K/AKT/mTOR cascade (Figs. 1A and B and 2C-F). Changes in genes such as PIK3CA, PTEN and AKT1 have been commonly found in GBM, and these changes have been associated with an elevated activity of the PI3K/AKT/mTOR pathway. A cBioPortal analysis of 592 GBM samples (TCGA, PanCancer Atlas-GBM) suggested that PIK3CA exhibits oncogenic E545K/A mutations, which are known to most frequently occur in the helical domain of the protein (102). Parsons *et al* (101) examined the genomic alterations in a large cohort of patients with GBM and discovered that PIK3CA is mutated in ~15% of cases. Further investigation revealed that these mutations are mostly missense and clustered in the kinase domain of its p110 subunit. In addition, the researchers discovered that PIK3CA mutations are associated with increased cell proliferation, PI3K/AKT/mTOR pathway activation and reduced responsiveness to chemotherapy (101). In another study, Liang and Slingerland (103) aimed to explore the functional significance of PIK3CA mutations in GBM using a combination of bioinformatics and experimental approaches. As a result, they were able to identify an association between PIK3CA mutations and an elevated activation of the PI3K/AKT/mTOR signaling cascade along with increased cell proliferation. Furthermore, they showed that inhibiting the PI3K/AKT/mTOR pathway using a small molecule inhibitor selectively reduced the viability of GBM cells with PIK3CA mutations (103). Dysregulated Wnt signaling interacts with the PI3K/Akt pathway to enhance GBM invasiveness and therapy resistance. Aberrant Wnt/β-catenin activation promotes epithelial-to-mesenchymal transition, increasing cell migration and survival. Cross-talk between the Wnt and PI3K/Akt pathways amplifies invasive behavior and supports tumor progression by creating a tumor-promoting microenvironment and enhancing angiogenesis (104,105).

Apart from genetic alterations, GBM has been associated with dysregulation of the PI3K/AKT/mTOR pathway due to epigenetic modifications, including DNA methylation. According to one study, increased activity of the PI3K/AKT/mTOR pathway in GBM is associated with hypermethylation of the PTEN promoter region, which results in decreased expression of the PTEN tumor suppressor gene (106). Based on the lollipop model produced by cBioPortal, five of the 132 PTEN mutations were R173H/C alterations, while six of the mutations were R130*/Q. Among the 6 R130 mutations, three were missense mutations (R130Q), involving the conversion of Arginine to Glutamine, while the remaining three were nonsense mutations (R130*). On the contrary, each of the 5 R173H/C mutations was of the missense type. Furthermore, a truncation occurring at residue 223 results in an alteration in the amino acid sequence, leading to a nonsense mutation (R223*). The pair of missense mutations, R130Q and R173H, are identified within the phosphatase domain of PTEN, impacting its phosphatase activity. On the other hand, R233 is located in the C2 domain, a crucial region responsible for PTEN's binding to the lipid plasma membrane (107). Earlier research indicated that mutations at R130Q and R173C/H result in the loss of PTEN function. Likewise, the truncations at R130* and R233* lead to functional loss (108).

These results imply that the dysregulation of the PI3K/AKT/mTOR pathway is crucial for the onset and advancement of GBM. Hence, targeting this pathway may be a promising approach for treating the disease. PI3K inhibitors are classified into pan-PI3K, isoform-selective and dual PI3K/mTOR inhibitors. Although >50 PI3K inhibitors have been developed for cancer treatment, only a few, such as BKM120, XL147 and XL765, have progressed to clinical trials for GBM (109).

First-generation pan-PI3K inhibitors, like wortmannin and LY294002, had limited clinical utility due to poor solubility, short half-life, off-target effects and high toxicity. In response, second-generation pan-PI3K inhibitors, including BKM120, XL147, PX-866, GDC-0941 and GDC-0032, were developed with improved safety, efficacy and pharmacokinetics, and are now undergoing clinical trials (109).

Among these, Buparlisib (BKM120) is the most frequently used PI3K inhibitor in GBM clinical trials, as it is well-tolerated and able to cross the blood-brain barrier (BBB) (109). Buparlisib induces G2/M cell cycle arrest and apoptosis in GBM cells through p53-dependent microtubule misalignment and mitotic dysfunction. It also enhances apoptosis induced by TNF-related apoptosis-inducing ligand (TRAIL) and B-cell lymphoma 2 (Bcl-2) inhibitors by increasing Noxa expression, sequestering Mcl-1 and releasing pro-apoptotic proteins Bim and Bak. However, a phase II study of BKM120 in recurrent GBM showed mild toxicities, including elevated liver enzymes, rash and hyperglycemia, but limited efficacy in patients with PTEN loss or PIK3CA mutations, despite its ability to inhibit Akt phosphorylation (NCT01339052) (110).

Similarly, Pilaralisib (XL147), an oral, reversible pan-PI3K inhibitor, has shown promise. A phase I study combining XL147 and XL765 (a dual PI3K/mTOR inhibitor) in recurrent GBM demonstrated moderate BBB penetration, with tumor-to-plasma ratios of 0.27-0.40. Both drugs reduced S6K1 phosphorylation and Ki67 expression, suggesting their potential for GBM growth inhibition (111).

On the mTOR inhibitor front, early agents like rapamycin faced challenges as cancer treatments due to immunosuppressive effects. Improved analogs, such as everolimus and temsirolimus, are FDA-approved for specific cancers but still encounter issues like persistent mTOR signaling and Akt activation through an mTORC1-mediated feedback loop. To address these limitations, second-generation mTOR inhibitors that target both mTORC1 and mTORC2 by binding the ATP-binding pocket have been developed. This category includes dual PI3K/mTOR inhibitors (e.g., NVP-BEZ235, XL765) and mTORC1/2 inhibitors (e.g., Torin1, AZD8055), which are currently being tested in GBM clinical trials (109,112).

However, resistance to first- and second-generation inhibitors has spurred the development of third-generation mTOR inhibitors. RapaLink-1, a BBB-penetrating compound, shows greater potency in GBM models compared to its predecessors. Another promising strategy involves selective mTORC2 inhibitors, such as CID613034 and JR-AB2-011, which avoid mTORC1-related feedback loops and exhibit strong anti-tumor effects in GBM studies. These advancements renew optimism

for the development of effective mTOR-targeted therapies for GBM (112).

5. Analysis of genes involved in cell cycle and apoptosis pathway

The cell cycle is a complex sequence of processes that govern cellular growth and division. Cancer is characterized by the unregulated growth and invasion of cancerous cells that result from dysregulations in cell cycle control. Over the years, several studies have looked into the cell cycle in GBM and discovered several key molecular pathways that are dysregulated (25).

TP53. GBM commonly exhibits mutations in the tumor suppressor gene TP53 (Figs. 1A and B and 2G). TP53 regulates DNA repair, cell cycle progression and apoptosis, and loss of TP53 function is linked to increased genomic instability and oncogenesis (113). Numerous studies have revealed a high prevalence of TP53 mutations in GBM, with mutation rates ranging from 30-60% in different cohorts (114). Furthermore, mutations in TP53 have been associated with tumor cell survival and invasion, as well as resistance to therapy and poor prognosis. For instance, Kim *et al* (115) utilized TCGA data to identify gene signatures associated with GBM prognosis. Their findings revealed a correlation between TP53 mutations and an unfavorable prognosis in this type of cancer (115). Data from the Ensmbl genome database for the p53 rs121912651 variant reveal that these mutations are considered pathogenic and are referred to as contact mutations. These mutations disrupt the normal binding of p53 to DNA, thereby contributing to the invasiveness of GBM cells (108). Familial GBM is linked to germline mutations in tumor suppressor and DNA repair genes. TP53 mutations in Li-Fraumeni syndrome, NF1/NF2 in neurofibromatosis, APC and mismatch repair genes in Turcot syndrome, and BRCA1/BRCA2 mutations are associated with a higher risk of glioblastoma. These mutations predispose individuals by disrupting cell-cycle regulation and DNA repair, causing the genetic basis of susceptibility in some families (116,117).

Research has been carried out to examine the potential of the TP53 pathway as a therapeutic avenue for treating GBM. One approach is to use gene therapy to restore TP53 function, either by delivering wt-TP53 or by using small molecules that activate TP53 signaling (118). Another strategy is to target TP53 downstream effectors, such as the p53-regulated gene mouse double minute 2/homolog (MDM2), which is commonly overexpressed in GBM (119,120). Research has also discovered possible interactions between TP53 and other signaling pathways in GBM. According to one study, co-deletion of TP53 and PTEN tumor suppressor gene were frequently encountered in GBM, increased AKT signaling and decreased apoptosis in GBM cells (121).

The high prevalence of p53 mutations in GBM highlights their potential as key targets for precision medicine. Strategies to restore wt-p53 function in mutant p53 (mut-p53) tumors, inhibit Gain-of-function (GOF) mut-p53 and inhibit the MDM2/p53 complex to prevent wt-p53 degradation offer promising therapeutic avenues for GBM and other cancers (122,123).

GOF p53 mutations in GBM can be corrected by restoring wt-p53 function through additional point mutations that stabilize the p53 protein (124). Various compounds aimed at reactivating wt-p53 by altering the conformation of mut-p53 have been developed (123). Among these, PRIMA-1 {2,2-bis (hydroxymethyl)-1-azabicyclo[2.2.2]octan-3-one} stands out as a highly effective molecule identified for its ability to restore wt-p53 properties in select missense mutants (125). PRIMA-1 promotes p21 expression, cell cycle arrest and apoptosis by refolding mut-p53 to a wt-p53 conformation (125,126). Its analog, PRIMA-1MET (APR-246), has demonstrated efficacy in inhibiting cell growth, reducing stemness and inducing apoptosis in GBM, as well as suppressing tumor growth in mouse models (127-129). While PRIMA-1 has not been tested in patients with GBM, PRIMA-1MET has undergone phase I/IIa trials in hematological malignancies and prostate cancer (130).

Enhancing protein turnover using histone deacetylase (HDAC) inhibitors is another approach for p53-based therapies. Mut-p53 relies on the chaperone complex of Hsp70 and Hsp90, which requires HDAC6 for stability. HDAC6 inhibition disrupts this complex, promoting mut-p53 degradation (131). Several HDAC inhibitors, including vorinostat, SAHA, CUDC-907, CCNU and CUDC-101, have shown promise in GBM models (132-135). Vorinostat, combined with tranylcypromine, reduces GBM stem cell viability and alters apoptosis-regulatory genes (132). SAHA destabilizes mut-p53 by disrupting HDAC6-Hsp90 interactions, but it also downregulates wt-p53, limiting its use to homozygous mut-p53 tumors (131,136-139). CUDC-907, a dual HDAC and PI3K inhibitor, degrades mut-p53, radiosensitizing pediatric high-grade GBMs (135). CCNU sensitizes adult GBM to chemotherapy by degrading mut-p53 (134), while CUDC-101 enhances mut-p53 degradation, improving responses to EGFR inhibitors (133). These findings highlight HDAC inhibitors' potential in mut-p53-targeted GBM therapies.

Mouse double minute 2/4 homolog (MDM2/4). MDM2 and MDM4 are essential genes responsible for regulating p53, a commonly mutated or dysregulated tumor suppressor protein in GBM (Figs. 1A and B and 2H-I). In that context, MDM2 and MDM4 act as negative regulators and their overexpression has been associated with the progression of GBM (140,141). MDM2 functions as an E3 ubiquitin ligase that degrades p53, while MDM4 (also recognized as MDMX) serves as a suppressor of p53, inhibiting its transcriptional activity. In GBM, both genes are typically overexpressed, and this overexpression is associated with both therapeutic resistance and an unfavorable prognosis.

Biernat *et al* (142) illustrated that the MDM2 gene, which is implicated in PI3K/AKT/mTOR pathway regulation, is upregulated in nearly 10% of primary cases of GBM. Several studies have looked into MDM2 and MDM4's roles in GBM and identified potential therapeutic targets for their inhibition. For instance, researchers have examined the effect of small-molecule inhibitors of MDM2 and MDM4 in preclinical models of GBM. The results revealed a reduction in tumor growth and enhanced survival. Several other strategies, such as using RNA interference to target MDM2 and MDM4 expression, and developing immunotherapies targeting MDM2 and

MDM4, have also been investigated (143). Overall, TP53 and cell cycle regulators are critical targets for GBM research, and further research is needed to unravel the impact of their mutations on the development of GBM and to develop effective therapeutic strategies targeting these proteins and their downstream effectors.

MDM2 inhibitors have gained attention as potential therapies for GBM, as blocking the MDM2/p53 interaction to reactivate p53 function represents a promising approach for cancer and GBM treatment (123). Nutlins, identified through chemical library screenings, are MDM2 inhibitors, with RG7112 being the first in class (144). RG7112 restored p53 activity in MDM2-amplified, TP53 wt-GBM cell lines, crossed the BBB and blood-tumor barrier, reduced tumor growth and improved survival in xenograft models. Being the first MDM2 inhibitor to enter clinical trials, it demonstrated p53 activation and pro-apoptotic effects but was limited by significant toxicities at high doses. Its second-generation analogue, RG7388 (idasanutlin), addressed these limitations with improved potency, selectivity and pharmacokinetics and has been tested in the NOA-20 trial alongside radiotherapy for patients with GBM with unmethylated MGMT promoters (73,145).

Other MDM2 inhibitors, including MI77301, CGM097, MK8242 and AMG232, have been developed (146), with AMG232 showing high potency and selectivity for wt-p53 GBM stem cells. AMG232 effectively inhibited tumor spheroid growth and stemness-related factors (147). Nutlin3a demonstrated the ability to impair DNA repair and enhance temozolomide-induced cell death in GBM models (148). In addition, novel indolylglyoxylyldipeptides targeting both MDM2 and the translocator protein (TSPO) reactivated p53, disrupted the mitochondrial membrane potential and inhibited GBM-cell viability, suggesting dual TSPO/MDM2 targeting as a potential anti-GBM therapy (149).

6. Analysis of genes involved telomerase activity

The key gene responsible for telomerase activity in GBM is TERT, which encodes the enzyme's catalytic subunit. This enzyme is involved in the maintenance of telomere length and prevention of cellular senescence. Various malignant cancers, including GBM, have been shown to overexpress TERT (Fig. 1A). This overexpression has been implicated in the development of GBM and associated with lower overall survival and poor prognosis (150-152). By analyzing the genomic landscape of gliomas, Killela *et al* (153) showed that TERT promoter mutations are present in 83% of GBM cases. They also discovered that these mutations are linked to increased telomerase activity and decreased patient survival (153).

Telomerase, as a target for drug development, appears to be an attractive candidate due to its pivotal role in cancer progression. Various anti-telomerase strategies are under development, including small-molecule enzymatic inhibitors and indirect approaches. One such inhibitor, BIBR1532, a selective noncompetitive inhibitor targeting the telomerase thumb domain (154,155), has shown effectiveness *in vitro* by halting cancer cell proliferation and inducing telomere shortening at high doses (156). However, its poor pharmacokinetic properties, such as low cellular permeability, have hindered its clinical advancement (157).

In parallel, eribulin, a mitotic inhibitor, is undergoing clinical investigation for its potential against GBM. It has demonstrated potent activity, particularly in TERT promoter-mutant GBM cells, both *in vitro* and *in vivo*, with effective tumor tissue penetration (158). These promising findings have led to the initiation of clinical trials exploring its telomerase-inhibiting potential in GBM (159).

Telomerase-based vaccines, which aim to trigger anti-tumoral immune responses, have also been in clinical development for decades (160). These vaccines utilize telomerase-derived peptides to generate immune responses in CD8+ cytotoxic lymphocytes, with trials across various cancers. However, clinical efficacy has been limited, particularly for GBM, which is resistant to immune checkpoint blockade (161). For instance, the GV1001 peptide vaccine trial in advanced pancreatic cancer showed no survival benefit over chemotherapy alone, underscoring the challenges in achieving clinical success (162).

To address these challenges, newer generations of anti-telomerase vaccines, such as INO5401, have been developed. INO5401 incorporates a modified full-length TERT gene to reduce immune tolerance and enhance CD8+ T-cell responses (163). In a phase I/II clinical trial, this vaccine, combined with standard care, showed promising early results. Specifically, patients withO-6-methylguanine-DNA methyltransferase (MGMT)-unmethylated tumors had a 12-month overall survival rate of 84.4%, while those with MGMT-methylated tumors had a rate of 85%, suggesting potential clinical benefits (164).

In addition, the use of telomerase-specific oncolytic viruses, which selectively replicate in cancer cells and express therapeutic agents like TERT-specific siRNAs or immunostimulatory cytokines, is emerging as a novel approach. Preclinical studies have shown promising results for these viruses, and they are currently being assessed in clinical trials (NCT03491683 and NCT03548571) (73). This combination of strategies underscores the ongoing efforts to harness telomerase for cancer therapy, offering new hope for GBM and other cancers.

7. Conclusion

Over the years, numerous significant molecular mechanisms underlying the development of gliomas have been unraveled. These discoveries changed the classification of gliomas and offered insight into the initiation and progression of these tumors. GBM stands out as the predominant and highly aggressive primary brain tumor in adults with a median survival of merely 12-15 months despite treatment efforts. Progress in bioinformatics has enabled a more thorough comprehension of the genetic alterations underlying GBM, offering new perspectives of understanding the development of the disease and novel potential targets for therapeutic interventions. The analysis of large-scale genomic datasets, such as TCGA, has been used in several studies to identify recurrently altered genes in GBM, including EGFR, TP53, PTEN and IDH1, among others. Further bioinformatics analysis of these genes revealed information regarding the pathways and processes disrupted in GBM, including cell cycle control and the PI3K/AKT/mTOR pathway. Furthermore, the utilization of whole-genome

sequencing has been instrumental in pinpointing recurring mutations in GBM, such as those found in TP53 and PTEN, alongside less frequent mutations in other genes like RB1 and NF1. Validating bioinformatics algorithms in GBM research requires benchmarking against established datasets like TCGA, cross-validation to minimize overfitting and external cohort testing to ensure generalizability. Biological validation through wet-lab experiments confirms prediction relevance, while reproducibility is supported by open-source code and transparent workflows. Metrics such as accuracy, sensitivity, specificity and Area Under Curve are critical for evaluation, alongside testing on synthetic data and alignment with known biological pathways. Addressing scalability and computational efficiency ensures the practical application of these algorithms in large-scale GBM studies (165-167).

Additional research will continue to use bioinformatic tools to uncover new insights into GBM and improve patient outcomes. Bioinformatics is critical to further the understanding of GBM by allowing the analysis of large amounts of genomic data and to identify genetic changes that contribute to tumor development and progression. These discoveries have not only improved our understanding of GBM biology, but have also led to the recognition of novel therapeutic targets for treating this cancer. In conclusion, bioinformatics analysis is a critical tool for furthering the understanding of GBM and identifying new treatment strategies for this devastating disease.

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MAG designed the review article and wrote the majority of the article. NEJ, TM and MM researched references and contributed to the writing. RAH and MES wrote the final draft and edited the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

1. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, *et al:* The epidemiology of glioma in adults: A 'state of the science' review. Neuro Oncol 16: 896-913, 2014.
2. Bailey P and Cushing H: Microchemical color reactions as an aid to the identification and classification of brain tumors. Proc Natl Acad Sci USA 11: 82-84, 1925.
3. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, *et al*: The 2021 WHO classification of tumors of the central nervous system: A summary. Neuro Oncol 23: 1231-1251, 2021.
4. Masui K, Cloughesy TF and Mischel PS: Review: Molecular pathology in adult high-grade gliomas: From molecular diagnostics to target therapies. Neuropathol Appl Neurobiol 38: 271-291, 2012.
5. Van den Bent MJ: Interobserver variation of the histopathological diagnosis in clinical trials on glioma: A clinician's perspective. Acta Neuropathol 120: 297-304, 2010.
6. Chen R, Smith-Cohn M, Cohen AL and Colman H: Glioma subclassifications and their clinical significance. Neurotherapeutics 14: 284-297, 2017
7. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW and Kleihues P: The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114: 97-109, 2007.
8. Perry A and Wesseling P: Histologic classification of gliomas. Handb Clin Neurol 134: 71-95, 2016.
9. Cancer Genome Atlas Research Network; Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, *et al*: Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 372: 2481-2498, 2015.
10. Kleihues P, Burger PC and Scheithauer BW: The new WHO classification of brain tumours. Brain Pathol 3: 255-268, 1993.
11. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, *et al*: Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344: 1396-1401, 2014.
12. Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, *et al*: An integrative model of cellular states, plasticity, and genetics for glioblastoma. Cell 178: 835-849.e21, 2019.
13. Couturier CP, Ayyadhury S, Le PU, Nadaf J, Monlong J, Riva G, Allache R, Baig S, Yan X, Bourgey M, *et al*: Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. Nat Commun 11: 3406, 2020.
14. Martínez AH, Madurga R, García-Romero N and Ayuso-Sacido Á: Unraveling glioblastoma heterogeneity by means of single-cell RNA sequencing. Cancer Lett 527: 66-79, 2022.
15. Martinez R, Schackert HK, Plaschke J, Baretton G, Appelt H and Schackert G: Molecular mechanisms associated with chromosomal and microsatellite instability in sporadic glioblastoma multiforme. Oncology 66: 395-403, 2004.
16. Tepeoglu M, Borcek P, Ozen O and Altinors N: Microsatellite instability in glioblastoma: Is it really relevant in tumor prognosis? Turk Neurosurg 29: 778-784, 2019.
17. Agnihotri S and Zadeh G: Metabolic reprogramming in glioblastoma: The influence of cancer metabolism on epigenetics and unanswered questions. Neuro Oncol 18: 160-172, 2016.
18. Deshmukh R, Allega MF and Tardito S: A map of the altered glioma metabolism. Trends Mol Med 27: 1045-1059, 2021.
19. Virtuoso A, Giovannoni R, De Luca C, Gargano F, Cerasuolo M, Maggio N, Lavitrano M and Papa M: The glioblastoma microenvironment: Morphology, metabolism, and molecular signature of glial dynamics to discover metabolic rewiring sequence. Int J Mol Sci 22: 3301, 2021.
20. Lee H, Kim D and Youn B: Targeting oncogenic rewiring of lipid metabolism for glioblastoma treatment. Int J Mol Sci 23: 13818, 2022.
21. Andersen RS, Anand A, Harwood DSL and Kristensen BW: Tumor-associated microglia and macrophages in the glioblastoma microenvironment and their implications for therapy. Cancers (Basel) 13: 4255, 2021.
22. Park JH and Lee HK: Current understanding of hypoxia in glioblastoma multiforme and its response to immunotherapy. Cancers (Basel) 14: 1176, 2022.

23. Lamborn KR, Chang SM and Prados MD: Prognostic factors for survival of patients with glioblastoma: Recursive partitioning analysis. Neuro Oncol 6: 227-235, 2004.
24. Stoyanov GS, Dzhenkov D, Ghenev P, Iliev B, Enchev Y and Tonchev AB: Cell biology of glioblastoma multiforme: From basic science to diagnosis and treatment. Med Oncol 35: 27, 2018.
25. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455: 1061-1608, 2008.
26. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, *et al*: The somatic genomic landscape of glioblastoma. Cell 155: 462-477, 2013.
27. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, *et al*: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17: 98-110, 2010.
28. Godek KM, Venere M, Wu Q, Mills KD, Hickey WF, Rich JN and Compton DA: Chromosomal instability affects the tumorigenicity of glioblastoma tumor-initiating cells. Cancer Discov 6: 532-545, 2016.
29. Buruiană A, Florian ȘI, Florian AI, Timiș TL, Mihu CM, Miclăuș M, Oșan S, Hrapșa I, Cataniciu RC, Farcaș M, *et al*: The roles of miRNA in glioblastoma tumor cell communication: Diplomatic and aggressive negotiations. Int J Mol Sci 21: 1950, 2020.
30. Mafi A, Rahmati A, Aghdam ZB, Salami R, Salami M, Vakili O and Aghadavod E: Recent insights into the microRNA-dependent modulation of gliomas from pathogenesis to diagnosis and treatment. Cell Mol Biol Lett 27: 65, 2022.
31. Tluli O, Al-Maadhadi M, Al-Khulaifi AA, Akomolafe AF, Al-Kuwari SY, Al-Khayarin R, Maccalli C and Pedersen S: Exploring the role of microRNAs in glioma progression, prognosis, and therapeutic strategies. Cancers (Basel) 15: 4213, 2023.
32. Stackhouse CT, Gillespie GY and Willey CD: Exploring the roles of lncRNAs in GBM pathophysiology and their therapeutic potential. Cells 9: 2369, 2020.
33. Bagheri-Mohammadi S, Karamivandishi A, Mahdavi SA and Siahposht-Khachaki A: New sights on long non-coding RNAs in glioblastoma: A review of molecular mechanism. Heliyon 10: e39744, 2024.
34. Hashemi M, Roshanzamir SM, Orouei S, Daneii P, Raesi R, Zokaee H, Bikarannejad P, Salmani K, Khorrami R, Paskeh MDA, *et al*: Shedding light on function of long non-coding RNAs (lncRNAs) in glioblastoma. Noncoding RNA Res 9: 508-522, 2024.
35. Brat DJ, Aldape K, Colman H, Holland EC, Louis DN, Jenkins RB, Kleinschmidt-DeMasters BK, Perry A, Reifenberger G, Stupp R, *et al*: cIMPACT-NOW update 3: Recommended diagnostic criteria for 'Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV'. Acta Neuropathol 136: 805-810, 2018.
36. Tesileanu CMS, Dirven L, Wijnenga MMJ, Koekkoek JAF, Vincent AJPE, Dubbink HJ, Atmodimedjo PN, Kros JM, van Duinen SG, Smits M, *et al*: Survival of diffuse astrocytic glioma, IDH1/2 wildtype, with molecular features of glioblastoma, WHO grade IV: A confirmation of the cIMPACT-NOW criteria. Neuro Oncol 4: 515-523, 2020.
37. Zhang B, Gu X, Han X, Gao Q, Liu J, Guo T and Gao D: Crosstalk between DNA methylation and histone acetylation triggers GDNF high transcription in glioblastoma cells. Clin Epigenetics 12: 47, 2020.
38. Azab MA: The potential role of histone modifications in glioblastoma therapy: Review article. J Mol Pathol 4: Article 4, 2023.
39. McCornack C, Woodiwiss T, Hardi A, Yano H and Kim AH: The function of histone methylation and acetylation regulators in GBM pathophysiology. Front Oncol 13: 1144184, 2023.
40. Rama AR, Alvarez PJ, Madeddu R and Aranega A: ABC transporters as differentiation markers in glioblastoma cells. Mol Biol Rep 41: 4847-4851, 2014.
41. Ahmed M, Verreault M, Declèves X and Idbaih A: Role of multidrug resistance in glioblastoma chemoresistance: Focus on ABC transporters. In: Cancer sensitizing agents for chemotherapy, glioblastoma resistance to chemotherapy: Molecular mechanisms and innovative reversal strategies. Paulmurugan R and Massoud TF (eds). Academic Press 15: 243-261, 2021.
42. Canzoneri R, Lacunza E and Abba MC: Genomics and bioinformatics as pillars of precision medicine in oncology. Medicina (B Aires) 79: 587-592, 2019.
43. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al*: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6: pl1, 2013.
44. Wu P, Heins ZJ, Muller JT, Katsnelson L, de Bruijn I, Abeshouse AA, Schultz N, Fenyö D and Gao J: Integration and analysis of CPTAC proteomics data in the context of cancer genomics in the cBioPortal. Mol Cell Proteomics 18: 1893-1898, 2019.
45. Brlek P, Kafka A, Bukovac A and Pećina-Šlaus N: Integrative cBioPortal analysis revealed molecular mechanisms that regulate EGFR-PI3K-AKT-mTOR pathway in diffuse gliomas of the brain. Cancers (Basel) 13: 3247, 2021.
46. Ahsan H, Asghar M and Malik SI: Potential diagnostic and drug target markers in glioblastoma. Sci Rep 14: 7292, 2024.
47. Dhar C: Utilizing publicly available cancer clinicogenomic data on cBioPortal to compare epidermal growth factor receptor mutant and wildtype non-small cell lung cancer. Cureus 13: e14683, 2021.
48. Reimer N, Unberath P, Busch H, Börries M, Metzger P, Ustjanzew A, Renner C, Prokosch HU and Christoph J: Challenges and experiences extending the cBioPortal for cancer genomics to a molecular tumor board platform. Stud Health Technol Inform 18: 139-143, 2021.
49. Kohl M, Wiese S and Warscheid B: Cytoscape: Software for visualization and analysis of biological networks. Methods Mol Biol 696: 291-303, 2011.
50. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
51. Donaldson, IM: Protein interaction data resources. In: Handbook of cell signaling. 2nd Edition. Academic Press, San Diego, CA, pp1375-1385, 2010.
52. Jean-Quartier C, Jeanquartier F and Holzinger A: Open data for differential network analysis in glioma. Int J Mol Sci 21: 547, 2020.
53. Ma T and Zhang A: Integrate multi-omics data with biological interaction networks using multi-view factorization AutoEncoder (MAE). BMC Genomics 20 (Suppl 11): S944, 2019.
54. Picard M, Scott-Boyer MP, Bodein A, Périn O and Droit A: Integration strategies of multi-omics data for machine learning analysis. Comput Struct Biotechnol J 19: 3735-3746, 2021.
55. Sanches PHG, de Melo NC, Porcari AM and de Carvalho LM: Integrating molecular perspectives: Strategies for comprehensive multi-omics integrative data analysis and machine learning applications in transcriptomics, proteomics, and metabolomics. Biology (Basel) 13: 848, 2024.
56. Zhou Y, Yang L, Zhang X, Chen R, Chen X, Tang W and Zhang M: Identification of potential biomarkers in glioblastoma through bioinformatic analysis and evaluating their prognostic value. Biomed Res Int 2019: 6581576, 2019.
57. Erasimus H, Gobin M, Niclou S and Van Dyck E: DNA repair mechanisms and their clinical impact in glioblastoma. Mutat Res Rev Mutat Res 769: 19-35, 2016.
58. Wu Y, Song Y, Wang R and Wang T: Molecular mechanisms of tumor resistance to radiotherapy. Mol Cancer 22: 96, 2023.
59. Stockhausen MT, Kristoffersen K and Poulsen HS: The functional role of notch signaling in human gliomas. Neuro Oncol 12: 199-211, 2010.
60. Bazzoni R and Bentivegna A: Role of notch signaling pathway in glioblastoma pathogenesis. Cancers (Basel) 11: 292, 2019.
61. Stenson PD, Mort M, Ball EV, Shaw K, Phillips A and Cooper DN: The human gene mutation database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet 133: 1-9, 2014.
62. 1,000 Genomes Project Consortium; Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT and McVean GA: An integrated map of genetic variation from 1,092 human genomes. Nature 491: 56-65, 2012.
63. Garcia FAO, de Andrade ES and Palmero EI: Insights on variant analysis in silico tools for pathogenicity prediction. Front Genet 13: 1010327, 2022.
64. Steinhaus R, Proft S, Schuelke M, Cooper DN, Schwarz JM and Seelow D: MutationTaster2021. Nucleic Acids Res 49: W446-W451, 2021.
65. Crespo I, Vital AL, Gonzalez-Tablas M, Patino MDC, Otero A, Lopes MC, de Oliveira C, Domingues P, Orfao A and Tabernero MD: Molecular and genomic alterations in glioblastoma multiforme. Am J Pathol 185: 1820-1833, 2015.

66. Huang PH, Xu AM and White FM: Oncogenic EGFR signaling networks in glioma. Sci Signal 2: re6, 2009.
67. Binder ZA, Thorne AH, Bakas S, Wileyto EP, Bilello M, Akbari H, Rathore S, Ha SM, Zhang L, Ferguson CJ, *et al*: Epidermal growth factor receptor extracellular domain mutations in glioblastoma present opportunities for clinical imaging and therapeutic development. Cancer Cell 34: 163-177, 2018.
68. Lu KV, Zhu S, Cvrljevic A, Huang TT, Sarkaria S, Ahkavan D, Dang J, Dinca EB, Plaisier SB, Oderberg I, *et al*: Fyn and SRC are effectors of oncogenic epidermal growth factor receptor signaling in glioblastoma patients. Cancer Res 69: 6889-6898, 2009
69. Nagane M, Coufal F, Lin H, Bögler O, Cavenee WK and Huang HJ: A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. Cancer Res 56: 5079-5086, 1996.
70. Jain A, Penuel E, Mink S, Schmidt J, Hodge A, Favero K, Tindell C and Agus DB: HER kinase axis receptor dimer partner switching occurs in response to EGFR tyrosine kinase inhibition despite failure to block cellular proliferation. Cancer Res 70: 1989-1999, 2010.
71. Sathornsumetee S and Rich JN: Designer therapies for glioblastoma multiforme. Ann N Y Acad Sci 1142: 108-132, 2008.
72. Huang TT, Sarkaria SM, Cloughesy TF and Mischel PS: Targeted therapy for malignant glioma patients: Lessons learned and the road ahead. Neurotherapeutics 6: 500-512, 2009.
73. El Atat O, Naser R, Abdelkhalek M, Habib RA and El Sibai M: Molecular targeted therapy: A new avenue in glioblastoma treatment. Oncol Lett 25: 46, 2022.
74. Ezzati S, Salib S, Balasubramaniam M and Aboud O: Epidermal growth factor receptor inhibitors in glioblastoma: Current status and future possibilities. Int J Mol Sci 25: 2316, 2024.
75. Vivanco I, Robins HI, Rohle D, Campos C, Grommes C, Nghiemphu PL, Kubek S, Oldrini B, Chheda MG, Yannuzzi N, *et al*: Differential sensitivity of glioma-versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. Cancer Discov 2: 458-471, 2012.
76. Hegi ME, Diserens AC, Bady P, Kamoshima Y, Kouwenhoven MCM, Delorenzi M, Lambiv WL, Hamou MF, Matter MS, Koch A, *et al*: Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib-A phase II trial. Mol Cancer Ther 10: 1102-1112, 2011.
77. Neyns B, Sadones J, Joosens E, Bouttens F, Verbeke L, Baurain JF, D'Hondt L, Strauven T, Chaskis C, Veld PI, *et al*: Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. Ann Oncol 20: 1596-1603, 2009.
78. Westphal M, Heese O, Steinbach JP, Schnell O, Schackert G, Mehdorn M, Schulz D, Simon M, Schlegel U, Senft C, *et al*: A randomized, open-label phase III trial with nimotuzumab, an anti-epidermal growth factor receptor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma. Eur J Cancer 51: 522-532, 2015.
79. Nitta Y, Shimizu S, Shishido-Hara Y, Suzuki K, Shiokawa Y and Nagane M: Nimotuzumab enhances temozolomide-induced growth suppression of glioma cells expressing mutant EGFR in vivo. Cancer Med 5: 486-499, 2016.
80. Hasselbalch B, Lassen U, Hansen S, Holmberg M, Sorensen M, Kosteljanetz M, Broholm H, Stockhausen MT and Poulsen HS: Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: A phase II trial. Neuro Oncol 12: 508-516, 2010.
81. McCrea HJ, Ivanidze J, O'Connor A, Hersh EH, Boockvar JA, Gobin YP, Knopman J and Greenfield JP: Intraarterial delivery of bevacizumab and cetuximab utilizing blood-brain barrier disruption in children with high-grade glioma and diffuse intrinsic pontine glioma: Results of a phase I trial. J Neurosurg Pediatr 28: 371-379, 2021.
82. Pérez-Soler R, Delord JP, Halpern A, Kelly K, Krueger J, Sureda BM, von Pawel J, Temel J, Siena S, Soulières D, *et al*: HER1/EGFR inhibitor-associated rash: Future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. Oncologist 10: 345-356, 2005.
83. Thomas M: Cetuximab: Adverse event profile and recommendations for toxicity management. Clin J Oncol Nurs 9: 332-338, 2005.
84. Keir ST, Chandramohan V, Hemphill CD, Grandal MM, Melander MC, Pedersen MW, Horak ID, Kragh M, Desjardins A, Friedman HS and Bigner DD: Sym004-induced EGFR elimination is associated with profound anti-tumor activity in EGFRvIII patient-derived glioblastoma models. J Neurooncol 138: 489-498, 2018.
85. Shikalov A, Koman I and Kogan NM: Targeted glioma therapy-clinical trials and future directions. Pharmaceutics 16: 100, 2024.
86. Lim Y, Yoo J, Kim MS, Hur M, Lee EH, Hur HS, Lee JC, Lee SN, Park TW, Lee K, *et al*: GC1118, an anti-EGFR antibody with a distinct binding epitope and superior inhibitory activity against high-affinity EGFR ligands. Mol Cancer Ther 15: 251-263, 2016.
87. Choi SW, Jung HA, Cho H, Kim TM, Park CK, Nam DH and Lee SH: A multicenter, phase II trial of GC1118, a novel anti-EGFR antibody, for recurrent glioblastoma patients with EGFR amplification. Cancer Med 12: 15788-15796, 2023.
88. Lassman AB, van den Bent MJ, Gan HK, Reardon DA, Kumthekar P, Butowski N, Lwin Z, Mikkelsen T, Nabors LB, Papadopoulos KP, *et al*: Safety and efficacy of depatuxizumab mafodotin + temozolomide in patients with EGFR-amplified, recurrent glioblastoma: Results from an international Phase I multicenter trial. Neuro Oncol 21: 106-114, 2019.
89. Desjardins A, Chandramohan V, Landi DB, Johnson MO, Khasraw M, Peters KB, Low J, Herndon JE, Threatt S, Bullock CA, *et al*: A phase I trial of D2C7-IT in combination with an Fc-engineered anti-CD40 monoclonal antibody (2141-V11) administered intratumorally via convection-enhanced delivery for adult patients with recurrent malignant glioma (MG). J Clin Oncol 40 (Suppl): e14015, 2022.
90. Kowalewski A, Durślewicz J, Zdrenka M, Grzanka D and Szylberg Ł: Clinical relevance of BRAF V600E mutation status in brain tumors with a focus on a novel management algorithm. Target Oncol 15: 531-540, 2020.
91. Ohgaki H and Kleihues P: Genetic pathways to primary and secondary glioblastoma. Am J Pathol 170: 1445-1453, 2007.
92. Etcheverry A, Aubry M, de Tayrac M, Vauleon E, Boniface R, Guenot F, Saikali S, Hamlat A, Riffaud L, Menei P, *et al*: DNA methylation in glioblastoma: Impact on gene expression and clinical outcome. BMC Genomics 11: 701, 2010.
93. Klughammer J, Kiesel B, Roetzer T, Fortelny N, Kuchler A, Nenning KH, Furtner J, Sheffield NC, Datlinger P, Peter N, *et al*: The DNA methylation landscape of glioblastoma disease progression shows extensive heterogeneity in time and space. Nat Med 24: 1611-1624, 2018.
94. Mao YK, Liu ZB and Cai L: Identification of glioblastoma-specific prognostic biomarkers via an integrative analysis of DNA methylation and gene expression. Oncol Lett 20: 1619-1628, 2020.
95. Sun X, Yuan W, Hao F and Zhuang W: Promoter methylation of RASSF1A indicates prognosis for patients with stage II and III colorectal cancer treated with oxaliplatin-based chemotherapy. Med Sci Monit 23: 5389-5395, 2017.
96. Szklener K, Mazurek M, Wieteska M, Wacławska M, Bilski M and Mańdziuk S: New directions in the therapy of glioblastoma. Cancers (Basel) 14: 5377, 2022.
97. Bouchè V, Aldegheri G, Donofrio CA, Fioravanti A, Roberts-Thomson S, Fox SB, Schettini F and Generali D: BRAF signaling inhibition in glioblastoma: Which clinical perspectives? Front Oncol 11: 772052, 2021.
98. Shannon S, Jia D, Entersz I, Beelen P, Yu M, Carcione C, Carcione J, Mahtabfar A, Vaca C, Weaver M, *et al*: Inhibition of glioblastoma dispersal by the MEK inhibitor PD0325901. BMC Cancer 17: 121, 2017.
99. Peng P, Wei W, Long C and Li J: Atorvastatin augments temozolomide's efficacy in glioblastoma via prenylation-dependent inhibition of ras signaling. Biochem Biophys Res Commun 489: 293-298, 2017.
100. Altwairgi AK, Alghareeb WA, AlNajjar FH, Alhussain H, Alsaeed E, Balbaid AAO, Aldanan S, Orz Y and Alsharm AA: Atorvastatin in combination with radiotherapy and temozolomide for glioblastoma: A prospective phase II study. Invest New Drugs 39: 226-231, 2021.
101. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, *et al*: An integrated genomic analysis of human glioblastoma multiforme. Science 321: 1807-1812, 2008.
102. Ranjbar R, Mohammadpour S, Esfahani AT, Namazian S, Yaghob-Taleghani M, Baghaei K, Tabatabaei SA, Pasharavesh L and Nazemalhosseini-Mojarad E: Prevalence and prognostic role of PIK3CA E545K mutation in iranian colorectal cancer patients. Gastroenterol Hepatol Bed Bench 12: S22-S29, 2019.
103. Liang J and Slingerland JM: Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. Cell Cycle 2: 339-345, 2003.
104. Zuccarini M, Giuliani P, Ziberi S, Carluccio M, Iorio PD, Caciagli F and Ciccarelli R: The role of Wnt signal in glioblastoma development and progression: A possible new pharmacological target for the therapy of this tumor. Genes (Basel) 9: 105, 2018.

105. Dreyer CA, VanderVorst K, Natwick D, Bell G, Sood P, Hernandez M, Angelastro JM, Collins SR and Carraway KL: A complex of Wnt/planar cell polarity signaling components Vangl1 and Fzd7 drives glioblastoma multiforme malignant properties. Cancer Lett 567: 216280, 2023.
106. Mueller S, Phillips J, Onar-Thomas A, Romero E, Zheng S, Wiencke JK, McBride SM, Cowdrey C, Prados MD, Weiss WA, *et al*: PTEN promoter methylation and activation of the PI3K/Akt/mTOR pathway in pediatric gliomas and influence on clinical outcome. Neuro Oncol 14: 1146-1152, 2012.
107. Chang H, Cai Z and Roberts TM: The Mechanisms underlying PTEN loss in human tumors suggest potential therapeutic opportunities. Biomolecules 9: 713, 2019.
108. Carico C, Nuño M, Mukherjee D, Elramsisy A, Dantis J, Hu J, Rudnick J, Yu JS, Black KL, Bannykh SI and Patil CG: Loss of PTEN is not associated with poor survival in newly diagnosed glioblastoma patients of the temozolomide era. PLoS One 7: e33684, 2012.
109. Zhao HF, Wang J, Shao W, Wu CP, Chen ZP, To ST and Li WP: Recent advances in the use of PI3K inhibitors for glioblastoma multiforme: Current preclinical and clinical development. Mol Cancer 16: 100, 2017
110. Wen PY, Wen PY, Yung WKA, Mellinghoff IK, Ramkissoon S, Alexander B, Rinne M, Colman H, Omuro AM, DeAngelis LM, *et al:* Phase II trial of the phosphatidyinositol-3 kinase (PI3K) inhibitor buparlisib (bkm120) in recurrent glioblastoma conducted by the ivy foundation early phase clinical trials consortium. Neuro Oncol 16(Suppl 3): iii47, 2014.
111. Cloughesy TF, Mischel PS, Omuro AMP, Prados M, Wen PY, Wu B, Rockich K, Xu Y, Lager JJ and Mellinghoff IK: Tumor pharmacokinetics (PK) and pharmacodynamics (PD) of SAR245409 (XL765) and SAR245408 (XL147) administered as single agents to patients with recurrent glioblastoma (GBM): An Ivy Foundation early-phase clinical trials consortium study. J Clin Oncol 31: 2012-2012, 2013.
112. Papavassiliou KA and Papavassiliou AG: The bumpy road towards mTOR inhibition in glioblastoma: Quo vadis? Biomedicines 9: 1809, 2021.
113. England B, Huang T and Karsy M: Current understanding of the role and targeting of tumor suppressor p53 in glioblastoma multiforme. Tumour Biol 34: 2063-2074, 2013.
114. Ohgaki H: Genetic pathways to glioblastomas. Neuropathology 25: 1-7, 2005.
115. Kim YW, Koul D, Kim SH, Lucio-Eterovic AK, Freire PR, Yao J, Wang J, Almeida JS, Aldape K and Yung WK: Identification of prognostic gene signatures of glioblastoma: A study based on TCGA data analysis. Neuro Oncol 15: 829-839, 2013.
116. Kyritsis AP, Bondy ML, Rao JS and Sioka C: Inherited predisposition to glioma. Neuro Oncol 12: 104-113, 2010.
117. Rice T, Lachance DH, Molinaro AM, Eckel-Passow JE, Walsh KM, Barnholtz-Sloan J, Ostrom QT, Francis SS, Wiemels J, Jenkins RB, *et al*: Understanding inherited genetic risk of adult glioma: A review. Neurooncol Pract 3: 10-16, 2016.
118. Mohyeldin A and Chiocca EA: Gene and viral therapy for glioblastoma: A review of clinical trials and future directions. Cancer J 18: 82-88, 2012.
119. Olafson LR, Gunawardena M, Nixdorf S, McDonald KL and Rapkins RW: The role of TP53 gain-of-function mutation in multifocal glioblastoma. J Neurooncol 147: 37-47, 2020.
120. Sherr CJ and McCormick F: The RB and p53 pathways in cancer. Cancer Cell 2: 103-112, 2002.
121. Chow LM, Endersby R, Zhu X, Rankin S, Qu C, Zhang J, Broniscer A, Ellison DW and Baker SJ: Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain. Cancer Cell 19: 305-316, 2011.
122. Muller PA and Vousden KH: Mutant p53 in cancer: New functions and therapeutic opportunities. Cancer Cell 25: 304-317, 2014.
123. Zhang Y, Dube C, Gibert M Jr, Cruickshanks N, Wang B, Coughlan M, Yang Y, Setiady I, Deveau C, Saoud K, *et al*: The p53 pathway in glioblastoma. Cancers (Basel) 10: 297, 2018.
124. Joerger AC and Fersht AR: Structural biology of the tumor suppressor p53. Annu Rev Biochem 77: 557-582, 2008.
125. Bykov VJ, Issaeva N, Shilov A, Hultcrantz M, Pugacheva E, Chumakov P, Bergman J, Wiman KG and Selivanova G: Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. Nat Med 8: 282-288, 2002.
126. Lambert JM, Gorzov P, Veprintsev DB, Soderqvist M, Segerback D, Bergman J, Fersht AR, Hainaut P, Wiman KG and Bykov VJ: PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. Cancer Cell 15: 376-388, 2009.
127. Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G and Wiman KG: PRIMA-1MET synergizes with cisplatin to induce tumor cell apoptosis. Oncogene 24: 3484-3491, 2005.
128. Zache N, Lambert JM, Wiman KG and Bykov VJ: PRIMA-1MET inhibits growth of mouse tumors carrying mutant p53. Cell Oncol 30: 411-418, 2008.
129. Patyka M, Sharifi Z, Petrecca K, Mansure J, Jean-Claude B and Sabri S: Sensitivity to PRIMA-1MET is associated with decreased MGMT in human glioblastoma cells and glioblastoma stem cells irrespective of p53 status. Oncotarget 7: 60245-60269, 2016.
130. Lehmann S, Bykov VJ, Ali D, Andren O, Cherif H, Tidefelt U, Uggla B, Yachnin J, Juliusson G, Moshfegh A, *et al*: Targeting p53 in vivo: A first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. J Clin Oncol 30: 3633-3639, 2012.
131. Li D, Marchenko ND, Schulz R, Fischer V, Velasco-Hernandez T, Talos F and Moll UM: Functional inactivation of endogenous MDM2 and CHIP by HSP90 causes aberrant stabilization of mutant p53 in human cancer cells. Mol Cancer Res 9: 577-588, 2011.
132. Singh MM, Johnson B, Venkatarayan A, Flores ER, Zhang J, Su X, Barton M, Lang F and Chandra J: Preclinical activity of combined HDAC and KDM1A inhibition in glioblastoma. Neuro Oncol 17: 1463-1473, 2015.
133. Liffers K, Kolbe K, Westphal M, Lamszus K and Schulte A: Histone deacetylase inhibitors resensitize EGFR/EGFRvIII-overexpressing, erlotinib-resistant glioblastoma cells to tyrosine kinase inhibition. Target Oncol 11: 29-40, 2016.
134. Staberg M, Michaelsen SR, Rasmussen RD, Villingshoj M, Poulsen HS and Hamerlik P: Inhibition of histone deacetylases sensitizes glioblastoma cells to lomustine. Cell Oncol (Dordr) 40: 21-32, 2017.
135. Pal S, Kozono D, Yang X, Fendler W, Fitts W, Ni J, Alberta JA, Zhao J, Liu KX, Bian J, *et al*: Dual HDAC and PI3K inhibition abrogates NFκB- and FOXM1-mediated DNA damage response to radiosensitize pediatric high-grade gliomas. Cancer Res 78: 4007-4021, 2018.
136. Kitange GJ, Mladek AC, Carlson BL, Schroeder MA, Pokorny JL, Cen L, Decker PA, Wu W, Lomberk GA, Gupta SK, *et al*: Inhibition of histone deacetylation potentiates the evolution of acquired temozolomide resistance linked to MGMT upregulation in glioblastoma xenografts. Clin Cancer Res 18: 4070-4079, 2012.
137. Rasmussen RD, Gajjar MK, Jensen KE and Hamerlik P: Enhanced efficacy of combined HDAC and PARP targeting in glioblastoma. Mol Oncol 10: 751-763, 2016.
138. Choi SA, Kwak PA, Park CK, Wang KC, Phi JH, Lee JY, Lee CS, Lee JH and Kim SK: A novel histone deacetylase inhibitor, CKD5, has potent anti-cancer effects in glioblastoma. Oncotarget 8: 9123-9133, 2017.
139. Yang XF, Zhao ZJ, Liu JJ, Yang XH, Gao Y, Zhao S, Shi S, Huang KQ and Zheng HC: SAHA and/or MG132 reverse the aggressive phenotypes of glioma cells: An in vitro and vivo study. Oncotarget 8: 3156-3169, 2017.
140. Reifenberger G, Liu L, Ichimura K, Schmidt EE and Collins VP: Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res 53: 2736-2739, 1993.
141. Riemenschneider MJ, Büschges R, Wolter M, Reifenberger J, Boström J, Kraus JA, Schlegel U and Reifenberger G: Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. Cancer Res 59: 6091-6096, 1999.
142. Biernat W, Kleihues P, Yonekawa Y and Ohgaki H: Amplification and overexpression of MDM2 in primary (de novo) glioblastomas. J Neuropathol Exp Neurol 56: 180-185, 1997.
143. Wade M, Li YC and Wahl GM: MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer 13: 83-96, 2013.
144. Vu B, Wovkulich P, Pizzolato G, Lovey A, Ding Q, Jiang N, Liu JJ, Zhao C, Glenn K, Wen Y, *et al*: Discovery of RG7112: A small-molecule MDM2 inhibitor in clinical development. ACS Med Chem Lett 4: 466-469, 2013.
145. Miles X, Vandevoorde C, Hunter A and Bolcaen J: MDM2/X inhibitors as radiosensitizers for glioblastoma targeted therapy. Front Oncol 11: 703442, 2021.
146. Canon J, Osgood T, Olson SH, Saiki AY, Robertson R, Yu D, Eksterowicz J, Ye Q, Jin L, Chen A, *et al*: The MDM2 inhibitor AMG 232 demonstrates robust antitumor efficacy and potentiates the activity of p53-inducing cytotoxic agents. Mol Cancer Ther 14: 649-658, 2015.

147. Her NG, Oh JW, Oh YJ, Han S, Cho HJ, Lee Y, Ryu GH and Nam DH: Potent effect of the MDM2 inhibitor AMG232 on suppression of glioblastoma stem cells. Cell Death Dis 9: 792, 2018.
148. Wang H, Cai S, Bailey BJ, Saadatzadeh MR, Ding J, Tonsing-Carter E, Georgiadis TM, Gunter TZ, Long EC, Minto RE, *et al*: Combination therapy in a xenograft model of glioblastoma: Enhancement of the antitumor activity of temozolomide by an MDM2 antagonist. J Neurosurg 126: 446-459, 2017.
149. Daniele S, Taliani S, Da Pozzo E, Giacomelli C, Costa B, Trincavelli ML, Rossi L, La Pietra V, Barresi E, Carotenuto A, *et al*: Apoptosis therapy in cancer: The first single-molecule co-activating p53 and the translocator protein in glioblastoma. Sci Rep 4: 4749, 2014.
150. Mosrati MA, Malmström A, Lysiak M, Krysztofiak A, Hallbeck M, Milos P, Hallbeck AL, Bratthäll C, Strandéus M, Stenmark-Askmalm M and Söderkvist P: TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. Oncotarget 6: 16663-16673, 2015.
151. Xu J, Xu FP, Liu ZH, Cui Q, Zhang KP and Li Z: The correlation analysis of TERT promoter mutations with IDH1/2 mutations and 1p/19q detected in human gliomas. Medicine (Baltimore) 101: e29668, 2022.
152. Yaltirik CK, Yilmaz SG, Ozdogan S, Bilgin EY, Barut Z, Ture U and Isbir T: Determination of IDH1, IDH2, MGMT, TERT and ATRX gene mutations in glial tumors. In Vivo 36: 1694-1702, 2022.
153. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr, Friedman AH, Friedman H, Gallia GL, Giovanella BC, *et al*: TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA 110: 6021-6026, 2013.
154. Pascolo E, Wenz C, Lingner J, Hauel N, Priepke H, Kauffmann I, Garin-Chesa P, Rettig WJ, Damm K and Schnapp A: Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem 277: 15566-15572, 2002.
155. Bryan C, Rice C, Hoffman H, Harkisheimer M, Sweeney M and Skordalakes E: Structural basis of telomerase inhibition by the highly specific BIBR1532. Structure 23: 1934-1942, 2015.
156. Nakashima M, Nandakumar J, Sullivan KD, Espinosa JM and Cech TR: Inhibition of telomerase recruitment and cancer cell death. J Biol Chem 288: 33171-33180, 2013.
157. Aquilanti E, Kageler L, Wen PY and Meyerson M: Telomerase as a therapeutic target in glioblastoma. Neuro Oncol 23: 2004-2013, 2021.
158. Takahashi M, Miki S, Fujimoto K, Fukuoka K, Matsushita Y, Maida Y, Yasukawa M, Hayashi M, Shinkyo R, Kikuchi K, *et al*: Eribulin penetrates brain tumor tissue and prolongs survival of mice harboring intracerebral glioblastoma xenografts. Cancer Sci 110: 2247-2257, 2019.
159. Takahashi M, Miki S, Fukuoka K, Maida Y, Hayashi M, Hamada A, Nishikawa R, Nagane M, Maruyama T, Mukasa A, *et al*: EXTH-50. Development of investigator initiated clinical trial of TERT-targeting therapy using eribulin mesylate in patients with recurrent glioblastoma. Neuro Oncol 19 (Suppl 6): vi83, 2017.
160. Zanetti M: A second chance for telomerase reverse transcriptase in anticancer immunotherapy. Nat Rev Clin Oncol 14: 115-128, 2017.
161. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, Baehring J, Ahluwalia MS, Roth P, Bähr O, *et al*: Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The CheckMate 143 phase 3 randomized clinical trial. JAMA Oncol 6: 1003-1010, 2020.
162. Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, *et al*: Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): An open-label, randomised, phase 3 trial. Lancet Oncol 15: 829-840, 2014.
163. Yan J, Pankhong P, Shin TH, Obeng-Adjei N, Morrow MP, Walters JN, Khan AS, Sardesai NY and Weiner DB: Highly optimized DNA vaccine targeting human telomerase reverse transcriptase stimulates potent antitumor immunity. Cancer Immunol Res 1: 179-189, 2013.
164. Reardon DA, Brem S, Desai AS, Bagley SJ, Kurz SC, De La Fuente MI, Nagpal S, Welch MR, Hormigo A, Forsyth P, *et al*: LTBK-01. INO-5401 AND INO-9012 delivered intramuscularly (IM) with electroporation (EP) in combination with cemiplimab (REGN2810) in newly diagnosed glioblastoma. Neuro Oncol 22: ii237, 2020.
165. Richters MM, Xia H, Campbell KM, Gillanders WE, Griffith OL and Griffith M: Best practices for bioinformatic characterization of neoantigens for clinical utility. Genome Med 11: 56, 2019.
166. You Y, Ru X, Lei W, Li T, Xiao M, Zheng H, Chen Y and Zhang L: Developing the novel bioinformatics algorithms to systematically investigate the connections among survival time, key genes and proteins for Glioblastoma multiforme. BMC Bioinformatics 21 (Suppl 13): S383, 2020.
167. Shi J: Machine learning and bioinformatics approaches for classification and clinical detection of bevacizumab responsive glioblastoma subtypes based on miRNA expression. Sci Rep 12: 8685, 2022.