Dual-targeting therapeutic strategies for glioblastoma (Review)

YUYU ZHAO[1,2], LEI XIAO[2], QIANG DONG[2] and LEI DUAN[2]

[1]The Second Clinical Medical College, Lanzhou University, Lanzhou, Gansu 730000, P.R. China;
[2]Department of Neurosurgery, Lanzhou University Second Hospital, Lanzhou, Gansu 730000, P.R. China

Received July 18, 2026; Accepted October 16, 2026

DOI: 10.3892/or.2026.9217

Abstract. Glioblastoma (GBM) is the most aggressive primary malignant tumour, owing to intratumoural heterogeneity, adaptive resistance, and an immunosuppressive microenvironment. Dual-targeting strategies aim to exploit complementary therapeutic vulnerabilities; however, their mechanisms of action and levels of supporting evidence vary considerably. The present narrative review evaluates single-molecule inhibitors, dual-antigen cellular therapies, oncolytic virus platforms, nanocarrier systems, and combination approaches directed against glioma stem-like and differentiated cellular states or spatially distinct tumour regions. Distinctions are made between molecular co-inhibition, dual-antigen recognition, coordinated drug delivery, and multimodal therapeutic strategies. Evidence derived from isocitrate dehydrogenase (IDH)-mutant glioma and other malignancies is considered separately from evidence in IDH-wildtype GBM. Vorasidenib (AG-881) inhibits both mutant IDH1 and mutant IDH2 within the same metabolic pathway; however, its clinical benefit in grade 2 IDH-mutant glioma does not demonstrate efficacy in GBM. Most alternative approaches remain at the preclinical stage, whereas early cellular therapy approaches have shown biological activity alongside significant limitations in response durability and neurological tolerability. Key challenges examined include clonal evolution, lineage plasticity, epigenetic and metabolic adaptation, patient stratification, and integration with radiotherapy. Translational priorities include confirming adequate drug exposure and engagement of both targets, identifying biologically appropriate patient populations, establishing tolerable treatment schedules, and streamlining manufacturing processes. Limiting tumour adaptation remains a testable therapeutic hypothesis rather than an established clinical outcome of dual targeting. Future progress will depend on research that links mechanistic complementarity to durable disease control and acceptable toxicity.

Correspondence to: Professor Lei Duan, Department of Neurosurgery, Lanzhou University Second Hospital, 82 Cuiyingmen, Chengguan, Lanzhou, Gansu 730000, P.R. China
E-mail: duanlll@163.com

Key words: dual targeting, glioblastoma, nanoparticles, chimeric antigen receptor T cell, oncolytic virus

Contents

1. Introduction
2. Therapeutic platforms
3. Complementary cellular and spatial dependencies
4. Integration with radiotherapy and radiosensitizers
5. Patient stratification and a precision framework
6. Resistance to dual-target interventions
7. Current challenges and prospects
8. Discussion and summary

1. Introduction

Glioblastoma (GBM) is the most aggressive form of glioma and remains associated with a dismal prognosis, with a median overall survival of ~14.6 months (1). Although the current standard of care comprises maximal safe surgical resection followed by radiotherapy and temozolomide-based chemotherapy, clinical outcomes remain unsatisfactory (2,3). A fundamental challenge is the marked heterogeneity of GBM, including intertumoural and intratumoural diversity, ongoing clonal evolution, microenvironmental heterogeneity, and dynamic epigenetic remodelling, all of which directly influence treatment response and patient survival. Molecular subtype differences, including isocitrate dehydrogenase (IDH)-mutant astrocytoma, oligodendroglioma with 1p/19q codeletion, and IDH-wildtype GBM, also contribute to variable therapeutic outcomes (4,5). Another major obstacle is the blood-brain barrier (BBB), which restricts the delivery of most therapeutic agents to the brain through tight junctions and active efflux transporters. Collectively, these features explain why durable disease control remains difficult to achieve using conventional therapeutic approaches (6,7).

Conventional treatment modalities each provide only limited benefit. Surgical resection can prolong survival when maximal safe resection is achieved; however, the infiltrative nature of GBM prevents complete tumour removal in most patients and increases the risk of postoperative neurological deficits (8). Radiotherapy, particularly when combined with temozolomide, improves local disease control and survival, although its efficacy is constrained by radioresistance and an immunosuppressive tumour microenvironment. Temozolomide remains the backbone chemotherapeutic agent for GBM; nevertheless, several patients develop intrinsic or acquired resistance, and therapeutic options following

recurrence are extremely limited (9). These challenges highlight the urgent need for more effective treatment strategies that can overcome both tumour-intrinsic resistance mechanisms and microenvironment-mediated adaptation (7,10,11).

Targeted therapy has transformed treatment paradigms across several malignancies, and GBM presents multiple biologically relevant targets associated with stemness maintenance, immune evasion, extracellular matrix remodelling, and metabolic reprogramming. In this context, dual-target therapy has emerged as a promising strategy because it allows simultaneous disruption of two critical, functionally related vulnerabilities. Rather than relying on single-pathway blockade, dual-target approaches aim to improve efficacy, delay resistance, and enhance treatment precision (12,13).

The present review summarises key dual-targeting strategies in GBM, including single-molecule dual-target inhibitors, dual-target chimeric antigen receptor T-cell (CAR-T) therapies, oncolytic virus (OV)-based dual-intervention platforms, functionalised nanocarrier systems, and therapeutic approaches that concurrently target glioma stem-like cells and differentiated tumour cells. This is a narrative, mechanism-oriented review. Selected primary studies illustrate therapeutic design principles and their associated limitations. Clinical evidence in GBM, preclinical evidence in GBM, and contextual findings from other diseases are considered separately and are not interpreted as validated treatments for GBM. Comparisons across studies are descriptive because experimental models, treatment regimens, endpoints, and follow-up durations differ substantially. Most dual-targeting approaches remain preclinical, while early cellular therapy studies have shown biological activity but also important limitations in response durability and neurological tolerability. Key issues discussed include clonal evolution, lineage plasticity, epigenetic and metabolic adaptation, patient stratification, and integration with radiotherapy. Translational priorities include confirming adequate drug exposure and engagement of both targets, identifying biologically appropriate patient populations, establishing tolerable treatment schedules, and simplifying manufacturing processes. Restricting tumour adaptation remains a testable therapeutic hypothesis, not an established clinical consequence of dual targeting. Progress will depend on studies that link mechanistic complementarity with durable disease control and acceptable toxicity (Fig. 1).

2. Therapeutic platforms

Single-molecule approaches. Combining two biological activities within a single molecule may help coordinate systemic exposure and reduce some of the pharmacokinetic interactions associated with separately administered agents. However, a fixed molecular architecture also imposes a fixed ratio of activity against both targets, which may not correspond to the levels of target engagement required across different tumour compartments. Consequently, target engagement, brain penetration, and selectivity must be demonstrated independently. Furthermore, mechanistic complementarity does not necessarily imply synergy; claims of synergistic activity require appropriate interaction analyses together with relevant single-agent controls (14-16).

Mechanistic target of rapamycin (mTOR) and G_1 to S phase transition 1 (GSPT1). YB-3-17 combines mTOR inhibition with cereblon (CRBN)-dependent degradation of GSPT1 within a single molecule, thereby producing a dual-target, dual-mechanism antitumour effect. Mechanistically, YB-3-17 has been shown to suppress mTORC2 signalling, activate tumour-suppressive genes such as DNA damage-inducible transcript 4 (*DDIT4*), and disrupt amino acid biosynthesis and p53-mediated apoptotic pathways. The degradation effect was found to be abolished following CRBN knockout, confirming the dependence of this activity on the CRBN adaptor protein. This example demonstrates how dual-target molecular design can simultaneously disrupt oncogenic signalling and proteostatic adaptation (17). However, these findings provide only proof of concept for integrating inhibition and targeted protein degradation within a single compound. They do not establish clinically effective brain exposure, an acceptable therapeutic window in humans, or prevention of acquired resistance.

IDH1 and IDH2. Mutations in *IDH1* and *IDH2* confer neomorphic enzymatic activity on IDH, resulting in the conversion of α-ketoglutarate to the oncometabolite 2-hydroxyglutarate. The accumulation of this metabolite promotes epigenetic dysregulation and impaired cellular differentiation, both of which contribute to gliomagenesis (18). Vorasidenib and AG-881 refer to the same compound, with AG-881 representing the developmental code name. Vorasidenib inhibits both mutant IDH1 and mutant IDH2, reducing D-2-hydroxyglutarate production while showing favourable BBB penetration (19,20). The designation 'dual mutant-IDH inhibitor' refers to activity against two closely related enzyme isoforms within a shared metabolic pathway rather than inhibition of two independent oncogenic pathways. Consequently, its medicinal chemistry rationale differs fundamentally from dual-targeting strategies such as mTOR/GSPT1 co-targeting and should not be interpreted as a distinct successor to AG-881.

In the randomised INDIGO trial, patients with grade 2 IDH-mutant glioma treated with surgery alone experienced significantly longer imaging-based progression-free survival with vorasidenib than with placebo, with median values of 27.7 and 11.1 months, respectively, corresponding to a hazard ratio of 0.39 (21,22). In August 2024, the FDA approved vorasidenib for patients aged 12 years or older with grade 2 astrocytoma or oligodendroglioma harbouring a susceptible *IDH1* or *IDH2* mutation following surgery. However, these findings do not demonstrate efficacy in IDH-wildtype GBM. Accordingly, vorasidenib is included here as a contextual example of successful brain-penetrant drug design rather than as a treatment recommendation for GBM (23-25).

Venetoclax-derived B-cell lymphoma 2 (BCL-2) and histone deacetylase (HDAC) inhibitors. Venetoclax is a selective BCL-2 inhibitor and does not inhibit HDAC1 or HDAC6. To address multiple resistance mechanisms simultaneously, researchers have developed chemically modified venetoclax-derived compounds that combine BCL-2 inhibition with HDAC1 or HDAC6 inhibitory activity; however, these molecules are novel experimental entities rather than modified applications of venetoclax itself. The cited study evaluated their anti-leukaemic activity. Mechanistically, HDAC1 inhibition may suppress myeloid cell leukemia 1 (*MCL-1*) expression, whereas HDAC6 inhibition can increase

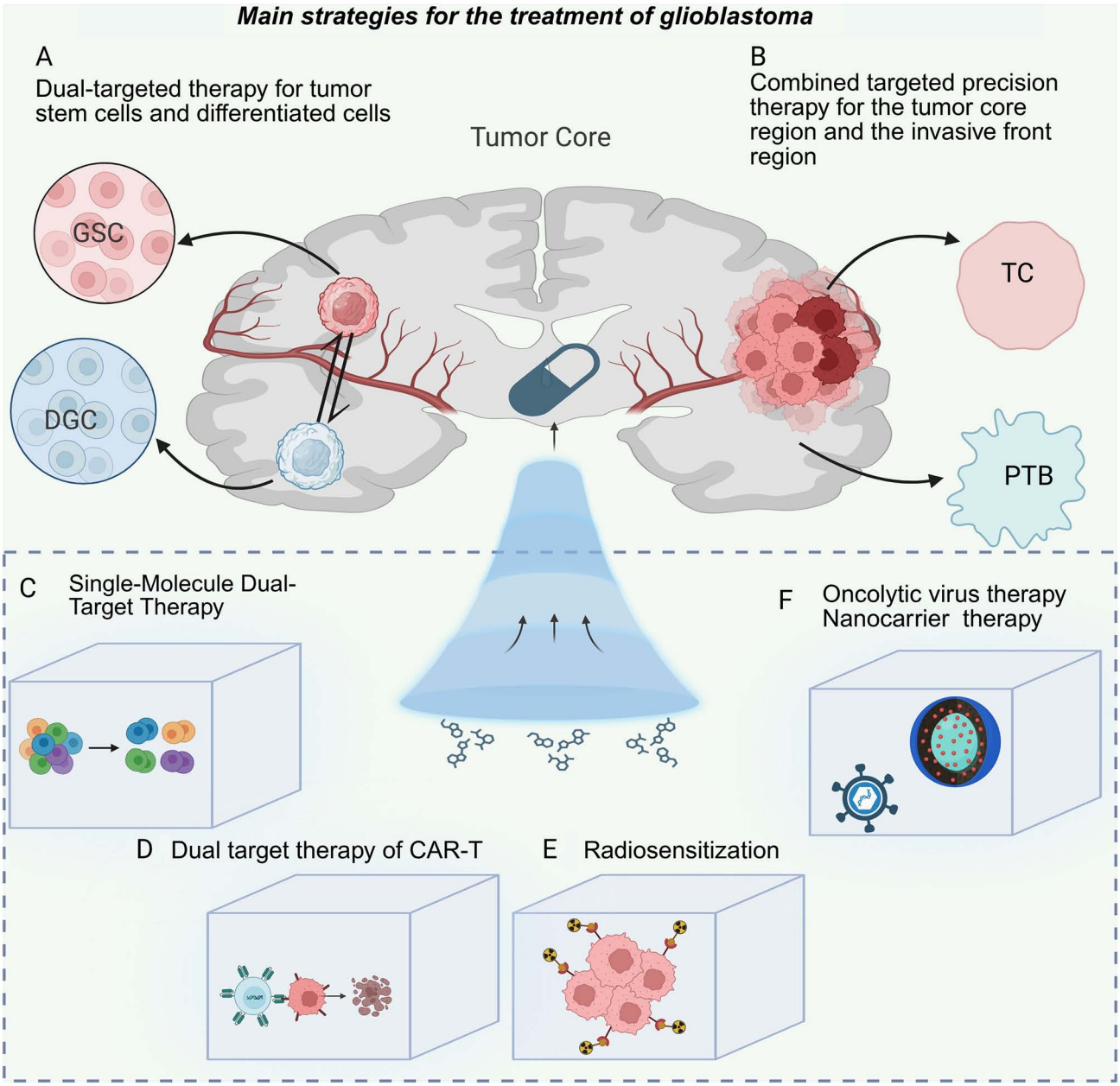


Figure 1. Conceptual framework of dual-target therapeutic strategies for glioblastoma. (A) Dual targeting of GSCs and DGCs, whose functional division of labor and dynamic plasticity drive recurrence and drug resistance. (B) Spatially coordinated targeting of the TC and invasive PTB to reduce residual disease and recurrence. (C) Single-molecule dual-target strategies designed to block driver pathways and compensatory signaling while improving BBB penetration. (D) Dual-target CAR-T strategies aimed at overcoming antigen heterogeneity and immune escape. (E) Integration with radiotherapy and radiosensitizers can enhance therapeutic efficacy. (F) Oncolytic virus- and nanocarrier-based strategies that remodel the local immune microenvironment and improve targeted delivery. GSCs, glioma stem cells; DGCs, differentiated glioma cells; TC, tumor core; PTB, peritumoral border; BBB, blood-brain barrier; CAR-T, chimeric antigen receptor T cell.

expression of the pro-apoptotic protein BCL-2-interacting mediator of cell death (BIM). Incorporation of HDAC1- or HDAC6-selective pharmacophores into a venetoclax-derived scaffold has therefore generated dual-target compounds that combine BCL-2 inhibition with HDAC isoform selectivity, illustrating the potential of polypharmacological drug design in cancer therapy (26).

In glioma-related studies, HDAC1 primarily regulates gene networks involved in DNA replication and chromosome segregation, whereas HDAC6 is more closely associated with extracellular matrix organisation and cell adhesion pathways. These observations support a mechanistic role for HDAC inhibition in epigenetic reprogramming and glioma biology, particularly in IDH1-mutant settings. Separate studies have implicated both HDAC1 and HDAC6 in glioma pathogenesis (27,28). Nevertheless, these findings do not validate venetoclax-derived BCL-2/HDAC inhibitors as therapeutic agents for GBM. Critical issues, including central nervous system (CNS) exposure, tumour penetration, and potential neurotoxicity, remain unresolved for this class of compounds.

Small molecules combining checkpoint modulation with a second target. Programmed cell death protein 1 (PD-1) blockade plays a central role in glioma immunotherapy.

Preoperative administration of PD-1 inhibitors can enhance the activation and clonal expansion of tumour-infiltrating T cells while promoting the recruitment and antigen-presenting activity of conventional type 1 dendritic cells (cDC1s). In addition, PD-1 inhibition may reverse T-cell exhaustion and stimulate adaptive immune responses (29,30). However, immune activation should not be equated with clinical benefit. In a randomised phase III trial involving patients with newly diagnosed GBM and an unmethylated O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter, nivolumab combined with radiotherapy failed to improve survival compared with temozolomide plus radiotherapy (9).

A major limitation of PD-1 monotherapy in glioma is the tumour-associated macrophage (TAM)-dominated immunosuppressive microenvironment. Even when T-cell and cDC1 activation is achieved, TAMs can continue to suppress effector T-cell cytotoxicity through immunosuppressive cytokine production, metabolic competition, and physical or biochemical barriers within the tumour microenvironment. As a result, immune activation may become dissociated from meaningful clinical benefit, reflecting the limited capacity of PD-1 blockade alone to overcome tumour-intrinsic signalling pathways and the myeloid-derived suppressive axis (31,32). These observations support investigating complementary therapeutic strategies while highlighting the need for caution when extrapolating immune biomarker responses to patient outcomes.

EP26. EP26 incorporates structural features of gefitinib and the programmed death-ligand 1 (PD-L1) inhibitor BMS-202, thereby combining inhibition of epidermal growth factor receptor (EGFR) and PD-L1-associated signalling within a single molecule. The original study evaluated its antitumour and immunomodulatory activity in GBM-related preclinical models (33). Compared with the non-glioma examples discussed below, this work is more directly relevant to GBM; however, the evidence remains entirely preclinical. Reported permeability characteristics and systemic pharmacokinetic findings do not establish uniform drug distribution within infiltrative human tumours. Clinical translation will require demonstrating that free drug concentrations in the brain reach levels sufficient for both pharmacological activities, assessing efflux transport and metabolite formation, and confirming that antitumour effects depend on engagement of both targets (34,35).

HP29 and PH3. HP29 is an experimental dual PD-L1/HDAC6 inhibitor. In the discovery study, HP29 exhibited an oral bioavailability of 15.3% and achieved 65.5% tumour growth inhibition in a melanoma model, compared with 53.9% in the tested combination-control group (36). Although these findings support further investigation, they do not demonstrate effective brain penetration or therapeutic efficacy in GBM.

PH3 is a dual PD-L1/HDAC3 inhibitor. The primary study reported dose-dependent antitumour activity in B16-F10 melanoma-bearing mice (37,38). However, based on these early experimental data, neither HP29 nor PH3 should be considered clinically effective therapies or less toxic alternatives to combination treatment. Further evaluation is required to determine isoform selectivity, long-term immunological effects, and tolerability in normal neural tissues.

CC-5. CC-5 simultaneously targets PD-L1 and CD73, potentially linking immune checkpoint modulation with reduction of adenosine-mediated immunosuppression. The compound was generated by optimising the PD-L1 inhibitor BMS1233 scaffold and incorporating a CD73-targeting pharmacophore. Relative to CD73-targeted monotherapy or PD-L1/CD73 combination treatment, CC-5 increased infiltration of $CD8^+$ T cells and promoted remodelling of the tumour immune microenvironment. In addition, it has favourable oral bioavailability. In preclinical studies, CC-5 achieved tumour growth inhibition rates of 64.3% in CT26 colorectal tumours and 39.6% in B16-F10 melanoma tumours. Nevertheless, the available data do not establish favourable BBB permeability or therapeutic potential in GBM (39,40).

SMC18. SMC18 targets both signal regulatory protein-α (SIRPα) and PD-L1, thereby disrupting the CD47/SIRPα and PD-1/PD-L1 signalling axes. Research in colorectal cancer and melanoma models showed enhanced macrophage-mediated phagocytosis alongside increased T-cell activity. SMC18 also promoted $CD8^+$ T-cell infiltration and M1-like macrophage activation (41). These findings illustrate how small-molecule dual-targeting approaches may extend beyond simple checkpoint blockade by simultaneously modulating tumour-promoting signalling pathways, epigenetic regulators, or tumour-associated macrophage-related mechanisms.

More broadly, combining PD-1/PD-L1 blockade with inhibition of pathways such as signal transducer and activator of transcription 3 (STAT3), HDAC, mitogen-activated protein kinase (MAPK), or tumour metabolism may reinforce adaptive immune activation while reshaping the immunosuppressive microenvironment, potentially improving the durability and efficacy of glioma immunotherapy (42-44). However, translation of these approaches requires careful assessment of brain exposure, the consequences of simultaneous activation of myeloid and lymphoid cell populations within the confined intracranial environment, and the potential for normal-tissue toxicity associated with checkpoint pathway interference.

For all five compounds discussed in this section, scalable synthesis, impurity control, formulation stability, and maintenance of reproducible dual-target potency remain developmental requirements rather than established clinical advantages (Table I).

Dual-antigen cellular therapies and complementary combinations. Single-target CAR-T cell therapies depend on stable and relatively homogeneous antigen expression. By contrast, glioma cells exhibit marked spatial and temporal heterogeneity in antigen presentation, allowing antigen loss or downregulation to promote rapid immune escape. CAR-T cells are genetically engineered lymphocytes that express CARs directed against tumour-associated antigens such as disialoganglioside GD2 (GD2), B7 homolog 3 (B7-H3), EGFR variant III (EGFRvIII), and interleukin-13 receptor subunit α2 (IL-13Rα2). Following antigen recognition, CAR-T cells activate intracellular signalling pathways, induce tumour-cell apoptosis, and secrete pro-inflammatory cytokines that modulate the tumour microenvironment. Local administration may enhance intratumoural accumulation while reducing systemic toxicity, whereas cytokine support and dual-target receptor designs may help mitigate antigen escape and T-cell exhaustion (45-49).

Table I. Summary of dual-targeting compounds for glioma.

Agent or class	Activities	Disease context	Interpretive limitation	(Refs.)
YB-3-17	mTOR inhibition; GSPT1 degradation	GBM-related cell and mouse experiments	Intracranial exposure and clinical therapeutic window unresolved	(17)
Vorasidenib (AG-881)	Mutant IDH1 and mutant IDH2 inhibition	Phase III grade 2 IDH-mutant glioma; approved indication	Same metabolic pathway	(19,20)
Venetoclax-derived experimental compounds	BCL-2/HDAC;BCL-2/HDAC6	Anti-leukemic cellular studies	Not venetoclax itself	(26-28)
EP26	PD-L1 and EGFR	GBM-related preclinical study	Regional unbound exposure and dual engagement need validation	(33-35)
HP29	PD-L1 and HDAC6	Melanoma model	Systemic pharmacokinetics do not establish CNS delivery	(36)
CC-5	PD-L1 and CD73	B16-F10 melanoma model	CNS exposure and chronic safety unresolved	(39,40)
PH3	PD-L1 and HDAC3	CT26 colorectal and B16-F10 melanoma models	Favorable brain permeability not established here	(37,38)

GBM, glioblastoma; mTOR, mechanistic target of rapamycin; GSPT1, G_1 to S phase transition 1; IDH, isocitrate dehydrogenase; BCL-2, B-cell lymphoma 2; HDAC, histone deacetylase; HDAC3, histone deacetylase 3; HDAC6, histone deacetylase 6; PD-L1, programmed death-ligand 1; EGFR, epidermal growth factor receptor; CNS, central nervous system.

EGFR-family and IL-13Rα2 targeting. Locoregional IL-13Rα2-directed CAR-T cell therapy has progressed to phase I clinical evaluation in recurrent high-grade glioma (50,51). EGFRvIII represents another therapeutically relevant GBM-associated antigen, and EGFRvIII-targeted CAR-T cells have demonstrated cytotoxic activity accompanied by increased interferon (IFN)-γ production (34,52,53). However, antigen loss remains a major limitation of single-target approaches.

Dual targeting of EGFRvIII and IL-13Rα2 has been shown to activate both $CD8^+$ and $CD4^+$ T cells and promote the production of multifunctional cytokines, including IFN-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2 (48). In dual-gated CAR-T designs, two antigen-recognition modules are co-expressed. Binding of either antigen can initiate CD3-mediated signalling, whereas simultaneous engagement of both antigens enhances co-stimulatory signalling, Ca^{2+} flux, and nuclear factor of activated T cells (NFAT)/activator protein 1 (AP-1)-dependent transcriptional activation. This strategy may reduce antigen escape and improve tumour-cell killing, thereby supporting more sustained antitumour activity (54). In addition, a trispecific T-cell engager targeting EGFRvIII, IL-13Rα2, and CD3 has shown preclinical activity in heterogeneous GBM models (55). Although encouraging, these findings remain preclinical and do not establish clinical benefit or improved tolerability.

Endothelial phosphoglycerate dehydrogenase (PHGDH) modulation combined with EGFRvIII-directed CAR-T cells. PHGDH-driven serine metabolism within tumour endothelial cells contributes to abnormal vascularisation, hypoxia, and resistance to CAR-T-cell therapy. In GBM models, genetic deletion or pharmacological inhibition of endothelial PHGDH improved vascular function and the immune microenvironment while enhancing EGFRvIII-directed CAR-T cell activity (55). These findings suggest that targeting PHGDH alongside EGFRvIII-directed CAR-T therapy may help overcome vascular barriers and microenvironmental mechanisms that limit cellular immunotherapy. However, this approach remains experimental, and the relative contribution of endothelial remodelling vs. direct immune effects requires further clarification.

GD2 and B7-H3. GD2-targeted CAR-T cells have been evaluated in H3K27M-mutant diffuse midline glioma and in a limited number of GBM investigations. Similarly, B7-H3-directed CAR-T and CAR-natural killer (NK) cells have demonstrated antitumour activity in preclinical GBM models (56,57). B7-H3 is an attractive glioma-associated target because it contributes to tumour-cell survival, proliferation, metabolic adaptation, and immune evasion through signalling pathways that include phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT)-mTOR and Janus kinase (JAK)-STAT3 (58,59).

B7-H3-targeted CAR-T and CAR-NK approaches have demonstrated tumour-eradicating activity and durable immune memory in preclinical glioma systems (60,61). These studies provide proof of principle for conditional antigen-recognition strategies but do not show reduced neurotoxicity or

Table II. Cellular therapies and complementary combinations.

Product or strategy	Architecture and intended function	Disease context	Primary limitations	(Refs.)
CAR-T-EGFR-IL13Rα2	Bivalent product targeting EGFR epitope 806 and IL-13Rα2	Phase I recurrent EGFR-amplified GBM	Antigen heterogeneity; persistence	(50,52)
EGFRvIII/IL-13Rα2/ CD3 engager	Tri-specific soluble engager	Preclinical GBM	Complex receptor design	(53,54)
PHGDH modulation plus EGFRvIII CAR-T cells	Endothelial metabolic interventionplus antigen-directed therapy	Preclinical GBM	Validation still limited	(55)
GD2-B7-H3 SynNotch CAR-T cells	Conditional antigen sensing and CAR expression	Preclinical neuroblastoma	Potential on-target toxicity	(56,57, 62-64)
PSMA CAR-T cells plus GD2 CAR-T cells	Combination of separately directed cell products	Exploratory refractory/ relapsed glioma report	Target selection and safety	(65,66)

CAR-T, chimeric antigen receptor T cell; EGFR, epidermal growth factor receptor; IL-13Rα2, interleukin-13 receptor subunit α2; GBM, glioblastoma; EGFRvIII, epidermal growth factor receptor variant III; PHGDH, phosphoglycerate dehydrogenase; GD2, disialoganglioside GD2; SynNotch, synthetic Notch; PSMA, prostate-specific membrane antigen.

improved survival in patients with GBM. Furthermore, evidence from diffuse midline glioma, neuroblastoma, and adult-type GBM should be considered separately because of substantial biological and clinical differences between these diseases (62-64).

Prostate-specific membrane antigen (PSMA) and GD2. PSMA is highly expressed within the neovasculature of high-grade gliomas. CAR-T cells co-targeting PSMA and GD2 have therefore been proposed to reduce antigen escape while enhancing antitumour activity (65,66). Similarly, dual-target CAR-T cells directed against CD44 and CD133 may provide broader coverage across GBM subtypes. Preclinical studies suggest that such designs may enhance anti-apoptotic signalling through the STAT5-BCL2 axis and support CAR-T-cell persistence within hypoxic tumour microenvironments by promoting expression of glycolytic enzymes (67,68) (Table II).

Conceptually, the principal advantage of dual-target CAR-T therapy may extend beyond increased cytotoxicity. By reducing opportunities for tumour escape through antigen modulation, these approaches may restrict the adaptive trajectories available to GBM under immune-selective pressure. Nevertheless, whether dual-target CAR-T therapies meaningfully constrain tumour evolution in patients remains a testable hypothesis rather than an established clinical outcome. Future progress will depend on optimisation of antigen selection, receptor signalling architecture, trafficking, persistence, and microenvironmental modulation within integrated therapeutic platforms. Ultimately, durable clinical control will require demonstration that enhanced antigen coverage translates into sustained efficacy without unacceptable neurological toxicity.

Oncolytic viral platforms. OVs combine tumour-cell lysis with inflammatory signalling and can be engineered to deliver immune-modulatory payloads. However, combining direct oncolysis with immune activation does not inherently constitute dual-target inhibition at the molecular level. Dual-target OV strategies are designed to exploit tumour-intrinsic vulnerabilities while remodelling the surrounding immune microenvironment. Accordingly, engineered OVs function not only as cytolytic agents but also as multifunctional therapeutic platforms that integrate tumour debulking with immune activation. During oncolysis, OVs release tumour-associated antigens and danger signals, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), thereby stimulating innate and adaptive immune responses and generating an *in situ* tumour-vaccine effect (69-72).

Reprogramming the tumour-immune interface through dual-target design. Bispecific antibodies are engineered molecules capable of simultaneously recognising two antigens or two distinct epitopes. They can bridge immune effector cells, such as T cells or NK cells, with tumour cells, promote immune synapse formation, activate cytotoxic responses, block paired immune checkpoints, or inhibit multiple tumour-signalling pathways. Combining bispecific antibodies or bispecific T-cell engagers (BiTEs) with OVs therefore represents a promising strategy for enhancing local antitumour immunity (73-75). Precise incorporation of BiTEs or other immunomodulatory payloads into herplex simplex virus type 1 (HSV-1) or adenoviral vectors may also permit spatially and temporally controlled transgene expression within the tumour microenvironment (76-78).

Dual-target OV platforms are specifically designed to remodel the tumour-immune interface. One approach involves arming OVs with BiTEs directed against the tumour-associated antigen B7-H3 and the T-cell marker CD3. These OV-BiTE systems circumvent limitations in antigen presentation by directly linking tumour cells with cytotoxic T lymphocytes, thereby enhancing immune synapse formation and tumour-specific cytotoxicity. For example, researchers incorporated the B7H3nb/CD3 bispecific antibody (BsAb) gene into an oHSV-1 genome to combine localised oncolysis with immune activation. This platform selectively lysed B7-H3-positive tumour cells, increased T-cell infiltration, reduced immunosuppressive cell populations, and limited systemic toxicity through local BsAb expression (79,80). Another strategy combines an

oncolytic adenovirus carrying an early region 1B 55-kilodalton protein (E1B-55kD) deletion with a replication-defective adenovirus encoding an ephrin type-A receptor 2 (EphA2)-BiTE transgene. This system enhances intratumoural BiTE production, redirects T cells towards EphA2-positive glioma cells, increases apoptosis, upregulates activation markers including CD25 and Ki67, reduces tumour burden, and prolongs survival without evident neurotoxicity (81).

OVs can also be engineered to augment innate and adaptive immune-signalling pathways. Expression of NK group 2 member D (NKG2D) ligands or NKG2D-based adaptor systems exploits stress-associated signals that are frequently upregulated in tumour cells, thereby enhancing NK-cell and T-cell activation. The combined effects of viral oncolysis and immune receptor engagement establish a positive-feedback loop that promotes immune-mediated tumour clearance. NKG2D BiTEs can bridge GBM cells and T cells while increasing expression of CD25, CD69, granzyme B, perforin, and IFN-γ, thereby enhancing antitumour activity (82).

Cytokine and checkpoint combinations. Advances in viral engineering have improved intratumoural dissemination and targeting specificity. Modifying viral tropism, incorporating tumour-targeting ligands, and combining viruses with pharmaceutical or nanoparticle delivery platforms can enhance viral distribution throughout tumour parenchyma. Modified adenovirus (D24-RGD) and reovirus (R124) have been investigated for their capacity to lyse tumour cells and stimulate immune responses in paediatric diffuse midline glioma. Furthermore, combining engineered T cells with OV therapy may further enhance antitumour immunity (83).

OV-IL15C, an HSV-1-derived OV expressing the IL-15/IL-15Rα complex, integrates tumour lysis with immune stimulation. When combined with EGFR-CAR NK cells, the OV eliminates EGFR-negative tumour cells, whereas CAR-NK cells selectively target EGFR-positive cells. This complementary activity results in synergistic antitumour efficacy and reduced cytokine-associated toxicity compared with systemic IL-15 administration (84).

Genetic engineering of oHSV-1 has also enabled the development of OV-cetuximab-C-C motif chemokine ligand 5 (CCL5), which expresses a cetuximab (anti-EGFR)-CCL5 fusion protein to promote tumour-targeted delivery of CCL5 and enhance immune activation. Through signalling via the C-C motif chemokine receptor (CCR)1/CCR5 axis, CCL5 activates NK cells, macrophages, and T cells, thereby improving the cytotoxic activity directed against GBM cells (85). Another engineered platform, PD-1/Al@OV, combines an OV with anti-PD-1 therapy and alendronate to address both T-cell dysfunction and tumour-associated macrophage-mediated immunosuppression (86).

δ-24-ACT is an oncolytic adenovirus developed to improve immunotherapeutic efficacy through targeting of the CD137/PD-L1 axis. Infected tumour cells express functional 4-1BB ligand (4-1BBL) and remodel the tumour microenvironment by increasing immune-cell infiltration. Combination with PD-L1 blockade further enhances antitumour immunity while avoiding severe hepatotoxicity (87).

Local delivery of oncolytic HSV can also reverse tumour-associated immunosuppression. The oHSV C5252 construct co-expresses IL-12 and an anti-PD-1 antibody fragment, activating dendritic cells, NK cells, and $CD8^+$ T cells; promoting polarisation of Th1 cells and M1 macrophages; suppressing Treg activity; increasing IFN-γ production; and inhibiting the PD-1/PD-L1 pathway. Collectively, these effects reverse T-cell exhaustion while minimising systemic toxicity (88,89) (Table III).

Overall, dual-target and multifunctional OV platforms represent a promising strategy for overcoming intrinsic GBM resistance. Future OV studies should report viral dose, replication competence, payload expression, viral shedding, and biological activity in both immunocompetent and patient-derived models. The absence of overt toxicity in short-term murine studies does not establish an adequate therapeutic window for human application. Although payload incorporation may enhance local antitumour activity, it can also reduce viral fitness, complicate potency assessment, and increase manufacturing variability. Consequently, durable clinical benefit from the engineered combinations reviewed here remains to be established (Table III).

Nanocarriers and coordinated delivery. Functionalised nanocarriers extend dual-target therapy beyond combinations of molecular targets by addressing two major barriers simultaneously: Inefficient drug delivery and tumour spatial heterogeneity. Through multi-ligand surface modification, stimulus-responsive drug release, and multimodal payload incorporation, nanodelivery systems can achieve differential drug distribution across distinct cell populations and tumour regions, enabling dual-target intervention at both the delivery and functional levels (90,91). The progression from conventional nanocarriers to engineered cell-based and biomimetic delivery systems has further improved the precision and translational potential of GBM therapy. Nanocarriers offer an effective approach to enhance brain penetration, tumour accumulation, and controlled drug release. Optimisation of particle size, surface properties, and stimulus-responsive behaviour can improve tumour targeting and tissue penetration while reducing systemic toxicity (92,93).

Ligand-mediated and dual-ligand targeting. Researchers have investigated angiopep-2/trans-activator of transcription (TAT)-functionalised nanomicelles for docetaxel delivery in orthotopic glioma models. Angiopep-2 facilitates receptor-mediated targeting, whereas TAT enhances cellular uptake despite limited intrinsic tumour specificity. Researchers have also evaluated dual-functionalised extracellular vesicles in glioma models. These approaches have demonstrated improved delivery in the systems examined; however, receptor expression patterns, endothelial transport mechanisms, and non-specific uptake may vary substantially between species and across tumour regions (94).

Integration of angiopep-2 targeting with guanidine-fluoride-mediated stabilisation has been reported to improve CRISPR/Cas9 delivery efficiency in GBM. However, gene-editing strategies and immune modulation represent distinct therapeutic functions, and factors such as off-target editing, durability of gene modification, and exposure within healthy brain tissue require careful evaluation. Hyaluronic-acid (HA)-based drug-loaded nanoparticles have also been studied for their ability to induce immunogenic cell death and enhance uptake in patient-derived

Table III. OV strategies.

Platform	Payload or complementary treatment	Evidence and disease context	Primary limitations	(Refs.)
HSV-1 expressing B7H3nb/CD3	Local expression of a bispecific T-cell engager	Preclinical cancer-model study	Clinical GBM benefit not established	(79,80)
Oncolytic adenovirus plus EphA2-BiTE vector	OV plus a separate replication-defective adenovirus	Preclinical high-grade glioma	Not a single-vector product	(81)
NKG2D-ligand engager plus virotherapy	Soluble immune engager combined with oncolysis	Preclinical GBM	Ligand heterogeneity; timing and immune-cell access	(82)
OV-IL15C plus EGFR CAR NK cells	IL-15/IL-15Rα expression plus EGFR-directed cell therapy	Preclinical GBM	Coordinated delivery; cytokine exposure and neuroinflammation	(84)
OV-cetuximab-CCL5	Cetuximab-CCL5 fusion protein	Preclinical GBM	Chemokine distribution; immune effects and viral fitness	(85)
PD-1/Al@OV	Anti-PD-1 and alendronate with an OV	Preclinical GBM	Multicomponent manufacturing; myeloid effects and safety	(86)
δ-24-ACT plus PD-L1 blockade	Virus-encoded 4-1BB ligand plus separate checkpoint blockade	Preclinical GBM	Not intrinsic viral PD-L1 inhibition; scheduling and immune safety	(87)

HSV-1, herpes simplex virus type 1; nb, nanobody; OV, oncolytic virus; EphA2, Ephrin type-A receptor 2; BiTE, bispecific T-cell engager; NKG2D, natural killer group 2 member D; GBM, glioblastoma; IL-15, interleukin-15; IL-15Rα, interleukin-15 receptor subunit α; EGFR, epidermal growth factor receptor; CAR, chimeric antigen receptor; NK, natural killer; CCL5, C-C motif chemokine ligand 5; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

GBM specimens (95,96). Additionally, targeting the immune-evasion factor growth differentiation factor 15 (GDF15) promotes M1 macrophage polarisation and $CD8^+$ T-cell activation, suppresses M2 macrophages and exhausted T cells, reverses immune suppression, and enhances the efficacy of immune-checkpoint therapies (97).

HA- and polyarginine (PA)-based nanoparticles loaded with DACHPt enable dual uptake by tumour cells and myeloid cells through CD44-mediated recognition. This dual-targeting mechanism enhances tumour-specific accumulation, induces immunogenic cell death, promotes DAMP release, and remodels the immunosuppressive tumour microenvironment (98-100). HA-modified gold nanorods have also been used in photothermal therapy, where CD44-mediated targeting facilitates localised tumour-cell ablation and inhibits glioma progression (101).

Multifunctional nanoplatforms. Ti@FeAu core-shell nanoparticles integrate targeting, imaging, and therapeutic capabilities within a single platform. Angiopep-2 modification improves BBB penetration and tumour targeting, while the nanoparticle simultaneously supports magnetic resonance imaging (MRI) and magnetothermal therapy, establishing a dual-target theranostic strategy (102). In addition, NP1-temozolomide nanoparticles produced by nanoprecipitation increase temozolomide encapsulation efficiency and stability, extend drug-release duration, enhance brain-targeting capability, and reduce systemic toxicity (103).

Biomimetic delivery systems. Engineered bacterial outer membrane vesicles (OMVs) co-delivering doxorubicin and CD47-small interfering (si)RNA have been developed to overcome immune tolerance in GBM. Doxorubicin promotes immunogenic cell death through increased calreticulin (CRT) exposure and high-mobility group box 1 (HMGB1) release, thereby enhancing dendritic-cell maturation, whereas CD47 silencing increases macrophage-mediated phagocytosis. Together, these effects generate a synergistic chemo-immunomodulatory response (104).

Protein-mediated targeting further broadens nanodelivery strategies. The lipid nanoparticle Nb-C8Pt-Ls forms an low-density lipoprotein receptor-related protein 1 (LRP1)-targeting protein corona through interaction with plasma fibronectin, thereby facilitating both BBB penetration and tumour targeting while maintaining favourable scalability (105). Similarly, the BIP-2-methacryloyloxyethyl phoshorylcholine (MPC) nanoparticle incorporates dual-targeting peptides directed against EGFR and MET and simultaneously inhibits the Ras/MAPK and PI3K/AKT pathways, reducing compensatory signalling and improving sensitivity to temozolomide (106).

Table IV. Nanocarriers and coordinated delivery.

Platform	Delivery or therapeutic functions	Evidence and disease context	Primary limitations	(Refs.)
Angiopep-2/TAT nanoparticles	Receptor-mediated targeting plus enhanced cellular uptake	Preclinical GBM	Off-target uptake	(94)
CRISPR/Cas9 nanoparticles	Brain-directed editing; GDF15-directed immune modulation in a separate platform	Preclinical GBM	Editing safety	(95,96)
HA/CD44 nanoparticles	Tumor/myeloid uptake and immunogenic cell death	Patient-derived GBM material	Biodistribution control	(98-100)
Ti@FeAu nanoparticles	Imaging and thermal-treatment functions	Preclinical nanotheranostic study	Long-term toxicity	(102,103)
OMVs + Doxorubicin/ CD47-siRNA	Chemotherapy-associated immune activation plus phagocytosis	Preclinical GBM	Manufacturing and translation	(104)
ABT/A12 nanoparticles	Complementary pathway targeting	Preclinical temozolomide-resistant glioma	Toxicity and release control	(109)
EGFR/MET nanoparticles	Co-delivery of complementary anti-apoptotic inhibitors	Orthotopic GBM models	Complex design	(110,111)

GBM, glioblastoma; TAT, trans-activator of transcription; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; GDF15, growth differentiation factor 15; HA, hyaluronic acid; OMVs, outer membrane vesicles; Doxorubicin, DOX; siRNA, small interfering RNA; EGFR, epidermal growth factor receptor; MET, MET proto-oncogene, receptor tyrosine kinase; TMZ, temozolomide.

Surface modification can also exploit receptors frequently overexpressed in GBM, including members of the low-density lipoprotein receptor (LDLR) family, transferrin receptor (TfR), neuropilin-1 (NRP-1), and IL-13Rα2, to improve delivery precision. Dual-ligand systems such as angiopoietin-2 (ANG2)/TAT cell-penetrating peptide (CPP) and angiopep-2/TAT further improve BBB penetration and tumour specificity (107,108). To address anti-apoptotic resistance, the biomimetic AM@NP (ABT/A12) platform co-delivers the Bcl-2/B-cell lymphoma-extra large (Bcl-xL) inhibitor ABT and the Mcl-1 inhibitor A12. The system releases its payload in the acidic tumour microenvironment and benefits from LDLR-mediated uptake, thereby overcoming dual anti-apoptotic resistance mechanisms in GBM (109).

Stimulus-responsive peptide systems offer additional advantages. The cyclic arginine-glycine-aspartic acid (cRGD) peptide targets αvβ3 integrin, which is frequently overexpressed in GBM, whereas the pH-responsive peptide (LLHH)3 promotes endosomal escape. Co-delivery of siEGFR and siP65 enables simultaneous inhibition of the EGFR and NF-κB pathways, resulting in markedly enhanced radiosensitivity (110). The dual-responsive PC/M nanosystem further improves uptake in the acidic tumour microenvironment through a glutathione-responsive core that releases paclitaxel and an MPC shell that mimics choline, supporting delivery of both small-molecule drugs and siRNAs (111).

In summary, functionalised nanocarriers expand dual-target therapy beyond conventional molecular-target combinations toward integrated, spatiotemporally coordinated therapeutic strategies. By precisely regulating delivery pathways, release kinetics, and intratumoural distribution, these systems offer a more comprehensive and potentially translatable therapeutic approach for GBM (Table IV).

3. Complementary cellular and spatial dependencies

Systemic basis of dual targeting of glioma stem cells (GSCs) and differentiated tumour cells (DGCs). GSCs and DGCs represent useful experimental classifications; however, they do not constitute permanently distinct cellular populations. Stem-like states support self-renewal and therapeutic tolerance, whereas differentiated states contribute to tumour growth and microenvironmental regulation. Single-cell sequencing and lineage-tracing studies have demonstrated substantial bidirectional plasticity between these cellular states (112-115). Consequently, combining agents with preferential activity against different cellular phenotypes may broaden antitumour efficacy, although reductions in stem-cell marker expression alone do not eliminate tumour-propagating capacity. Reciprocal signalling between cellular compartments further limits strategies targeting a single state. For example, DGC-derived brain-derived neurotrophic factor supports GSC maintenance through neurotrophic receptor tyrosine kinase 2 (NTRK2) signalling, whereas cellular communication network factor 1 (CCN1) produced by differentiated cells promotes macrophage recruitment and tumour progression. In addition, exosome-mediated transfer of ATP-binding cassette subfamily B member 4 (ABCB4) provides a mechanism

for sharing resistant phenotypes between cellular compartments (116-118). GSCs depend on signalling pathways including Notch, Hedgehog, and PI3K/AKT/mTOR to maintain stemness, self-renewal, and therapeutic resistance, thereby driving tumour initiation, progression, and recurrence (119-121). Collectively, these findings support therapeutic strategies that disrupt intercellular support networks in addition to directly eliminating tumour cells.

Representative preclinical combinations. Function-selective drug combinations can synergistically target distinct GBM cell populations. The HDAC inhibitor belinostat shows greater activity against GSCs by disrupting the G_2/M checkpoint, whereas the tyrosine kinase inhibitor dasatinib primarily targets DGCs by inhibiting PI3K/AKT/mTOR signalling. Their combination suppresses key oncogenic programmes, impairs both GSC and DGC functions, reduces tumour volume and Ki-67 expression, and partially overcomes heterogeneity-associated therapeutic resistance (122).

In brain tumour stem-cell models, EGFR inhibition alone can trigger compensatory STAT3 activation, limiting therapeutic efficacy. Combined treatment with the EGFR inhibitor afatinib and the STAT3 inhibitor pacritinib significantly reduces brain tumor stem cell (BTSC) survival and spheroid formation while more effectively suppressing GSC self-renewal and stemness maintenance. In EGFRvIII-mutant GBM, this combination produces greater tumour reduction than either monotherapy or blocks STAT3-mediated adaptive compensation, illustrating a characteristic dual-target synergistic mechanism (123).

Nanodelivery-based combination strategies have also demonstrated therapeutic potential. In co-delivery systems containing the EGFR inhibitor osimertinib and the proteasome/NF-κB pathway inhibitor bortezomib, osimertinib primarily suppresses GSC proliferation and stem-like maintenance, whereas bortezomib targets differentiated and treatment-induced drug-resistant DGC populations. This dual-drug platform is more effective than monotherapy in suppressing tumour growth, reducing expression of stem-associated markers, and prolonging survival, further supporting the rationale for coordinated GSC-DGC targeting (124-126).

At the epigenetic level, HDAC activity is closely associated with stemness-related transcriptional programmes and chromatin accessibility. Although HDAC inhibitors can reduce GSC self-renewal and downregulate stem-cell markers such as SRY-box transcription factor 2 (SOX2) and oligodendrocyte transcription factor 2 (OLIG2), inhibition of stem-like populations alone is insufficient to halt tumour progression. Because Src/PI3K signalling contributes to adaptive survival in both GSCs and DGCs, combining belinostat with the Src/receptor tyrosine kinase (RTK) inhibitor dasatinib targets epigenetic dependencies in GSCs and growth-promoting signalling in DGCs, resulting in broader antitumour activity (127).

In differentiated tumour cells, the PI3K/AKT/mTOR and MAPK/MEK/ERK pathways form a central regulatory network governing proliferation and survival. Inhibiting one pathway often induces compensatory activation of the other. Consequently, combined inhibition using agents such as buparlisib plus trametinib or dactolisib plus trametinib more effectively suppresses DGC proliferation programmes, reduces tumour burden, and prolongs survival (128,129).

Localised drug-delivery strategies also exhibit dual-targeting potential. Although the lauroyl gemcitabine lipid nanocapsule (GemC12-LNC) hydrogel can delay tumour recurrence, its activity against GSCs is limited. Combining this platform with anti-GSC agents such as salinomycin enables simultaneous targeting of both GSCs and DGCs. Salinomycin selectively eliminates GSCs by inducing reactive oxygen species generation and endoplasmic reticulum stress, whereas curcumin suppresses tumour spheroid formation but may increase the proportion of GSCs. Accordingly, dual-drug delivery systems can reduce GSC spheroid-forming capacity and inhibit tumour parenchymal proliferation more effectively than single-agent treatment (130,131).

GSCs and DGCs collectively sustain GBM survival, proliferation, invasion, and tissue destruction. Effective dual-target strategies should therefore extend beyond simply increasing the number of therapeutic targets and instead simultaneously disrupt the tolerance module responsible for long-term tumour persistence and the execution module that drives tumour expansion. This framework provides a unified rationale for the therapeutic activity of diverse dual-target platforms and supports the rational development of multidimensional combinatorial strategies.

Spatially coordinated targeting of the tumour core (TC) and invasive frontier. Spatial heterogeneity provides an additional rationale for dual-target precision therapy. Tumour cells located within the TC and the invasive peritumoural border (PTB) differ in functional state, microenvironmental context, and signalling dependencies. Consequently, the coordinated targeting of heterogeneous cell populations across these spatially distinct regions may improve eradication of residual disease and reduce the likelihood of recurrence. Advances in single-cell and spatial multi-omics technologies have enabled identification of region-specific molecular signatures and critical biological dependencies. For example, PTB-associated cells may rely more heavily on AP-1-driven transcriptional programmes involved in migration and stromal interactions, whereas TC-associated cells may preferentially depend on BTB domain and CNC homolog 1 (BACH1)-mediated oxidative stress responses (132).

AP-1 and BACH1 activities exhibit distinct spatial distributions and are negatively correlated across tumour regions. Simultaneous inhibition of AP-1 and BACH1 has been reported to suppress tumour growth, reduce invasion, and prolong survival more effectively than single-target approaches (133). In the same study, ulixertinib, an ERK1/2 inhibitor used to suppress AP-1-associated signalling, was combined with either BACH1 knockdown or hemin-induced BACH1 degradation. This complementary strategy improved tumour control and survival in murine models (134).

However, several limitations remain. Ulixertinib is not a direct or selective AP-1 inhibitor, and no selective BACH1-targeted agent has been approved for GBM treatment. Although both genetic and pharmacological interventions provide convergent evidence supporting this therapeutic concept, the specificity of the available agents, their spatial distribution within tumours, and their potential clinical benefit remain to be established (135) (Table V).

Table V. Dual-target strategies based on cellular hierarchy and spatial precision in GBM.

Strategy category	Target dimension	Key targets/pathways	Therapeutic effect	Representative agents	(Refs.)
EGFR + STAT3 inhibition	Compensatory signalling	EGFR/STAT3	Suppress GSC survival	Afatinib/Pacritinib	(123)
EGFR+NF-κB inhibition	GSC, resistant DGC	EGFR/NF-κB	Prolong survival	Osimertinib/Bortezomib	(124,126)
HDAC + Src/ PTK inhibition	Epigenetic growth	HDAC/Src	Enhanced antitumour effect	Belinostat/Dasatinib	(127)
AKT + MEK inhibition	Proliferation signalling	PI3K/AKT + MAPK	Reduce tumour burden	Buparlisib, Trametinib/ Dactolisib, Trametinib	(128,129)
AP-1 + BACH1 inhibition	Region-specific	AP-1/BACH1	Extend survival	Ulixertinib	(133-135)

EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; NF-κB, nuclear factor-κB; GSC, glioma stem cell; DGC, differentiated glioma cell; HDAC, histone deacetylase; Src, SRC proto-oncogene, non-receptor tyrosine kinase; PTK, protein tyrosine kinase; AKT, protein kinase B; MEK, mitogen-activated protein kinase kinase; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; AP-1, activator protein 1; BACH1, BTB domain and CNC homolog 1.

4. Integration with radiotherapy and radiosensitizers

Radiotherapy remains a cornerstone of malignant tumour treatment; however, its therapeutic efficacy is frequently limited by intrinsic tumour radioresistance and an immunosuppressive tumour microenvironment. Clearance of apoptotic cells can promote immunosuppressive phenotypes, while the tumour microenvironment further restricts effective antitumour immune responses, creating two major barriers to durable treatment efficacy. Consequently, an ideal radiosensitising strategy should not only enhance radiation-induced tumour-cell death but also disrupt these immunological barriers, thereby promoting robust systemic antitumour immunity (136-138).

Synergistic combination of hypoxia-targeted strategies and radiotherapy sensitisation. Improving the radiation response under hypoxic conditions represents an effective approach for enhancing radiosensitivity in hypoxic tumours (139,140). Under severe hypoxia, ferredoxin 1 (FDX1) regulates the phosphorylation status of ataxia-telangiectasia mutated (ATM), DNA-dependent protein kinase, catalytic subunit (DNA-PKcs), AKT, and EGFR, thereby influencing GBM cell radioresistance. These findings indicate that hypoxia is not merely a microenvironmental characteristic but also a functional regulator that can directly modulate the DNA damage response network (141).

In addition, collagen type I α1 has been shown to enhance radiation tolerance in hypoxic glioma cells by promoting autophagy, suggesting that hypoxia-associated extracellular matrix remodelling contributes to radioresistance (142). From a translational perspective, tumour-depolymerisation strategies have demonstrated the potential to improve radiotherapy responses in low rectal tumours by modifying local structural barriers and enhancing treatment accessibility. These observations support the therapeutic concept of combining microenvironmental remodelling with radiotherapy sensitisation in hypoxic tumours (143).

Combination of nanomedicine-enhanced radiotherapy and conventional radiotherapy. The radiosensitising effects of nanomedicine arise primarily from its ability to integrate targeted delivery, microenvironmental modulation, and amplification of radiation responses within a single platform. Through these combined functions, nanomedicine can enhance both the local efficacy and biological precision of radiotherapy-based combination strategies. Compared with conventional radiosensitisers, nanoplatforms improve tumour accumulation and cellular uptake while enhancing therapeutic stability and activity through surface engineering, size optimisation, and incorporation of functional modules.

Polyoxometalate-based nanosystems are a notable example of radiotherapy-sensitising nanoplatforms that can alleviate hypoxia-associated radioresistance and enhance radiation responses (144). Similarly, surface modification of ultra-small palladium nanoparticles with polyethylene glycol (PEG) and glucose significantly improves cellular uptake and radiosensitising efficacy, demonstrating that nanoparticle interfacial chemistry can strongly influence biological activity (145).

In neuro-oncology, intracranial delivery of peptide-based nano-photosensitisers using a magnetic continuum robot has emerged as an innovative strategy for precise local treatment of brain tumours. This approach highlights the advantages of integrating advanced delivery technologies with multifunctional nanotherapeutic platforms (146).

Overall, nanomedicine has evolved from a simple drug-delivery system into a multifunctional platform that integrates therapeutic, regulatory, and delivery functions. In the context of radiotherapy sensitisation, these technologies provide opportunities to improve treatment precision, enhance local tumour control, and facilitate the development of more effective combination-based radiotherapeutic strategies.

Combination therapy of ferroptosis-inhibitory sensitizers with radiotherapy. Ferroptosis has emerged as a critical determinant of tumour-cell responses to radiotherapy, and increasing evidence implicates dysregulated ferroptosis in

the development of glioma radioresistance. Tumour cells can evade radiotherapy-induced ferroptotic cell death through metabolic reprogramming, suppression of lipid peroxidation, and activation of antioxidant defence systems, thereby maintaining a survival advantage during treatment. For example, adenylate kinase 4 (AK4) has been reported to promote radioresistance in GBM by inhibiting radiation-induced ferroptosis through metabolic regulation, highlighting a close association between metabolic homeostasis and ferroptosis suppression (147). Similarly, GDF15 suppresses ferroptosis while simultaneously remodelling the immune microenvironment, thereby enhancing GBM tolerance to radiotherapy. These findings suggest that ferroptosis evasion not only supports intrinsic tumour-cell survival but may also reinforce therapeutic resistance through immune-mediated mechanisms (148).

Conversely, nanotherapeutic approaches that enhance oxidative stress and lipid peroxidation have shown potential to improve radiotherapy efficacy by promoting ferroptosis. H_2S-generating semiconducting polymer nanoparticles, for example, have been shown to enhance radiodynamic-ferroptosis therapy and exhibit favourable synergistic antitumour activity in orthotopic GBM models (149). Collectively, these observations indicate that ferroptosis-centred radiosensitisation strategies are evolving beyond modulating a single cell-death pathway toward an integrated therapeutic framework encompassing metabolic regulation, redox homeostasis, and immune microenvironment remodelling.

Transcriptional-epigenetic circuits in radiotherapy sensitisation. Radioresistance develops not only through enhanced DNA damage repair but also through extensive alterations in transcriptional regulation, chromatin organisation, and epigenetic state. Recent studies show that interconnected transcriptional-epigenetic networks govern GBM radioresistance and influence cellular state transitions, DNA repair efficiency, and invasive behaviour. The Fos-related anitgen 1 (FOSL1)-protein arginine methyltransferase 1 (PRMT1) transcriptional-epigenetic circuit, for example, promotes radioresistance through calyphosine-dependent regulation of DNA repair and tumour invasion, indicating that coordinated interactions between transcription factors and methyltransferases can establish resistance-associated epigenetic programmes (150).

Conversely, bromodomain-containing protein 4 (BRD4) inhibition enhances GBM radiosensitivity by suppressing super-enhancer-driven collagen type I α1 chain (COL1A1) expression, showing that chromatin-dependent transcriptional regulation is amenable to therapeutic intervention (151). Additional studies have identified other mechanisms linking transcriptional and post-transcriptional regulation to radiotherapy response. N-acylneuraminate-9-phosphatase (NANP) targeting promotes radiosensitisation by suppressing mesenchymal transition mediated through tumor necrosis factor receptor 1 (TNFR1) sialylation, whereas prolactin-releasing hormone receptor (PRLHR) enhances accumulation of radiation-induced DNA damage by inhibiting Y-box-binding protein-1 (YBX1) nuclear translocation. These findings indicate that glycosylation-dependent regulation, control of nuclear trafficking, and integration of post-transcriptional signalling collectively determine radiotherapy sensitivity (152,153).

Other signalling pathways also contribute to radiosensitisation. α-Sulfoquinovosyl-acylpropanediol (SQAP) exerts synergistic antitumour and radiosensitising effects by inhibiting PI3K/AKT signalling and impairing DNA repair capacity, whereas mamalian ste-20-like kinase 1 (MST1) enhances radiosensitivity by suppressing autophagy and promoting apoptosis. Together, these observations highlight the extensive crosstalk between signalling pathways and epigenetic regulatory networks in shaping radiotherapy responses (154,155).

Overall, precise therapeutic intervention directed at transcriptional-epigenetic circuits has emerged as a promising strategy for improving glioma radiosensitivity. These findings provide a mechanistic foundation for developing rational, biology-driven combination therapies designed to enhance radiotherapeutic efficacy (156,157).

5. Patient stratification and a precision framework

Establish the integrated diagnosis. Patient selection for dual-target therapies should begin with an integrated histological and molecular diagnosis. IDH status distinguishes adult-type IDH-wildtype GBM from IDH-mutant astrocytoma and oligodendroglioma, while specific histone alterations define additional diffuse glioma entities (4,158). DNA methylation profiling can further resolve diagnostically ambiguous cases and help identify biologically coherent cohorts for clinical trials (159). However, a methylation class alone should not be regarded as a validated predictor of response to a specific dual-target therapeutic strategy. Studies that enrol patients with multiple glioma diagnoses should therefore report outcomes separately for each molecularly defined subgroup.

Distinguish diagnostic biomarkers from predictive biomarkers. IDH-mutant gliomas are predominantly observed in low-grade gliomas and secondary high-grade gliomas. Mutations most commonly involve the IDH1 and IDH2 genes, with IDH1 alterations occurring more frequently. The most prevalent IDH1 mutation affects codon R132. Co-occurrence of IDH mutations and 1p/19q codeletion, particularly in frontal-lobe oligodendrogliomas, is associated with a favourable prognosis. Moreover, 1p/19q codeletion was shown to be strongly associated with IDH1/2 mutations, especially in oligodendroglial tumours (158).

Homozygous cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion is a strong adverse prognostic factor across the major categories of IDH-mutant gliomas, highlighting the need to complement histological grading with molecular biomarkers to improve risk stratification and clinical decision-making (160).

At the molecular level, IDH-wildtype diffuse gliomas can be classified as GBM when they harbour any one of three defining molecular features: EGFR amplification, combined chromosome 7 gain and chromosome 10 loss (+7/-10), or telomerase reverse transcriptase (TERT) promoter mutation (160,162). MGMT promoter methylation remains an important biomarker for predicting responsiveness to temozolomide and for stratification in clinical trial design (163).

Match therapeutic interventions to measurable biological dependencies. Select dual-target therapies based on demonstrable tumour dependencies. For example, clinical trials

evaluating EGFR-directed cellular therapies should require appropriate assessment of target-antigen expression; a study investigating a bispecific EGFR/IL-13Rα2-targeted strategy specifically enrolled patients with EGFR-amplified recurrent GBM (164).

Similarly, evidence should support combination therapies directed at signalling pathways, including pathway activation and biologically plausible compensatory mechanisms. EGFR-associated activation of STAT3 provides one such rationale for dual-pathway inhibition (123). Myeloid-directed immunotherapies should incorporate detailed characterisation of the immune microenvironment rather than relying solely on tumour PD-L1 expression. Likewise, spatially informed therapeutic approaches should demonstrate the relevant regional biological dependencies and confirm that adequate drug delivery can be achieved within the individual tumour. At present, AP-1/BACH1-guided therapeutic strategies remain experimental and require further validation (135).

Reassess treatment feasibility and biological response over time. Functional testing using patient-derived cell cultures or organoid models may help prioritise treatment candidates; however, culture-induced selection pressures and the absence of native immune interactions limit their predictive accuracy. Clinical factors, including performance status, corticosteroid exposure, prior radiotherapy, lymphocyte fitness, and the manufacturing time required for cellular therapies, should be incorporated into patient selection and outcome analyses.

Because tumour biology evolves during treatment, the molecular dependencies initially identified may not remain stable. Serial radiological assessment, neurological evaluation, and molecular monitoring should therefore be incorporated to determine whether selected therapeutic targets persist over time and remain relevant vulnerabilities for intervention.

6. Resistance to dual-target interventions

Clonal evolution and genomic instability. Dual targeting does not eliminate the capacity of tumour cells to adapt genetically during treatment. Therapeutic pressure may select for pre-existing clones that lack one targeted vulnerability, retain alternative oncogenic drivers, or possess mechanisms that permit tolerance to both interventions. Longitudinal analyses of diffuse gliomas have revealed highly heterogeneous evolutionary trajectories rather than a single, recurrent mechanism of therapeutic escape (133). Furthermore, copy-number alterations and chromosomal instability can modify target dosage and generate new biological dependencies. In GBM, reversible loss of extrachromosomal EGFRvIII DNA during exposure to EGFR inhibitors represents a well-characterised example of rapid target adaptation (165). Although these observations illustrate broader principles of treatment resistance, they provide a rationale rather than direct evidence for resistance mechanisms across all dual-target therapeutic platforms.

Lineage plasticity and epigenetic adaptation. Tumours can evade therapy without acquiring additional driver mutations. GBM cells exist along a spectrum of neural progenitor-like, oligodendrocyte progenitor-like, astrocyte-like, and mesenchymal-like cellular states and can transition between these phenotypes (112). Epigenetic remodelling can alter receptor expression, stress-response pathways, and differentiation programmes, enabling tumour cells to survive despite inhibition of their original dependencies. As a result, therapies selected to target the dominant baseline cellular states may fail to eliminate newly induced treatment-adapted populations.

To distinguish true depletion of specific tumour-cell states from reversible phenotypic switching, therapeutic evaluation should incorporate serial chromatin-based and transcriptomic analyses. Functional tumour-regrowth assays are also required to determine whether residual tumour cells retain tumour-propagating capacity despite apparent molecular responses.

Signalling and metabolic rewiring. Tumour adaptation may also occur through alterations in signalling networks, nutrient utilisation, and redox regulation. Notably, resistance mechanisms are not restricted to malignant cells. For example, endothelial PHGDH-dependent metabolism demonstrates that metabolically mediated treatment resistance can originate within the tumour microenvironment rather than the tumour cells themselves (57).

Even when two therapeutic nodes are inhibited simultaneously, downstream signalling pathways or alternative metabolic routes may remain available to sustain tumour survival. Consequently, select the most effective dual-target combinations based on experimentally verified biological dependencies and treatment-induced adaptive responses rather than solely on the presence or expression of two therapeutic targets. This approach recognises the dynamic nature of tumour adaptation and increases the likelihood of achieving durable therapeutic benefit.

7. Current challenges and prospects

Research into dual-target strategies for GBM has advanced rapidly; however, the field remains at a pivotal stage between mechanistic discovery and the development of clinically translatable therapeutic approaches. Despite substantial progress, significant challenges remain in heterogeneous BBB/blood-tumour barrier (BTB) transport, unbound regional exposure, engagement of both targets, fixed vs. adjustable dose ratios, neurological and systemic toxicity, to treatment.

Each additional mechanism may increase on-target normal-tissue toxicity or off-target effects. Small molecules may have mismatched target potencies; cell therapies may cause neuroinflammation; viruses and bacterial vesicles may trigger innate immune toxicity. A fixed-ratio molecule or formulation simplifies some exposure relationships but reduces dosing flexibility. Separate agents permit adjustment of dose and timing but introduce interactions and unequal tissue distribution. Dose escalation and schedule optimization must assess the combined biological effect rather than simply combining individually tolerated doses.

Autologous CAR-T products require reliable collection, transduction, expansion, release testing and delivery within a clinically usable interval. OVs require control of identity, replication, genetic stability and payload expression. Nanocarriers require consistent size, ligand density, loading

ratio, release profile and sterility. For two-component products, separate potency assays should be complemented by an assay of the intended combined function. These requirements become harder to satisfy as more components are added. Regulatory development should link each component to a defined contribution to benefit and characterize risks of the final product, including interactions that are absent when components are tested alone. Manufacturing changes require comparability assessment. Safety follow-up should be appropriate to persistence of the vector, cell product or material. Cost and access are also practical barriers: Centralized manufacturing, cold-chain transport, repeated intracranial delivery and specialist monitoring can exclude patients or delay treatment.

The reviewed evidence cannot support a platform-wide claim of superior efficacy. A non-glioma flank model cannot validate the BBB, a xenograft may not reproduce an intact immune system, and a short experiment may not reveal adaptive recurrence. Positive studies should be reproduced across molecularly characterized patient-derived models, with monotherapy and standard-treatment controls. Formal synergy analysis should use a prespecified reference model and dose matrix where applicable; improved activity over one comparator alone is not sufficient to establish synergy. Clinical trials should distinguish feasibility, target engagement, radiographic response and survival as different outcomes. The bivalent CAR-T cell experience shows that tumor regression can coexist with short median progression-free survival and substantial neurotoxicity. Molecularly stratified trials, longitudinal tissue or liquid-biopsy studies where feasible, and validated neurological and quality-of-life endpoints are needed to connect target selection with patient benefit. Treatment sequencing may eventually exploit transient sensitivity states, but such schedules remain hypotheses requiring prospective testing.

In summary, future dual-target strategies should consider GBM as a dynamic, hierarchically organised biological system characterised by evolving functional dependencies, spatially distinct microenvironmental niches, and extensive cellular plasticity.

8. Discussion and summary

Dual-targeting strategies offer multiple approaches to address complementary biological dependencies in GBM, including compensatory signalling, antigen heterogeneity, cellular plasticity, and limitations in drug delivery. However, their shared conceptual framework should not obscure important differences in therapeutic architecture, mechanism of action, and supporting evidence. Most of the approaches reviewed remain at the preclinical stage, and evidence of clinical activity in selected glioma populations or observations of early tumour regression do not, in themselves, establish durable therapeutic benefit in adult-type IDH-wildtype GBM.

Further progress will require rigorous molecular classification of patients, verification that both therapeutic components reach and effectively engage their intended cellular or microenvironmental targets, development of tolerable treatment schedules, and establishment of robust and reproducible manufacturing processes. Addressing these challenges will be essential for translating dual-target strategies from promising experimental concepts into clinically effective therapies for GBM.

Acknowledgements

Not applicable.

Funding

The present review was funded by the National Natural Science Foundation of China (grant no. 82560469), the Natural Science Foundation of Gansu Province (grant no. 24JRRA1088), the Project of Health and Family Planning Commission of Gansu (grant no. GSWSKY2024-41), the Key Incubation Project Funds of the Second Hospital and Clinical Medical School, Lanzhou University (grant no. 2025-25-zdfy-020) and the Cuiying Scientific and Technological Innovation Program of Lanzhou University Second Hospital (grant no. CY2022-YB-A05).

Availability of data and materials

Not applicable.

Authors' contributions

YZ conceived the topic of the review. QD and LX were involved in revising the manuscript. YZ and LD made final critical revisions. All authors have read and approved the final manuscript. Data authenticcation is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Reference

1. Yan D, Kowal J, Akkari L, Schuhmacher AJ, Huse JT, West BL and Joyce JA: Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. Oncogene 36: 6049-6058, 2017.
2. Becker AP, Sells BE, Haque SJ and Chakravarti A: Tumor heterogeneity in glioblastomas: From light microscopy to molecular pathology. Cancers (Basel) 13: 761, 2021.
3. Spinelli C, Adnani L, Meehan B, Montermini L, Huang S, Kim M, Nishimura T, Croul SE, Nakano I, Riazalhosseini Y and Rak J: Mesenchymal glioma stem cells trigger vasectasia-distinct neovascularization process stimulated by extracellular vesicles carrying EGFR. Nat Commun 15: 2865, 2024.
4. 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.
5. Ohgaki H and Kleihues P: The definition of primary and secondary glioblastoma. Clin Cancer Res 19: 764-772, 2013.

6. Wang D, Wang C, Wang L and Chen Y: A comprehensive review in improving delivery of small-molecule chemotherapeutic agents overcoming the blood-brain/brain tumor barriers for glioblastoma treatment. Drug Deliv 26: 551-565, 2019.
7. Singh S, Dey D, Barik D, Mohapatra I, Kim S, Sharma M, Prasad S, Wang P, Singh A and Singh G: Glioblastoma at the crossroads: Current understanding and future therapeutic horizons. Signal Transduct Target Ther 10: 213, 2025.
8. Roh TH and Kim SH: Supramaximal resection for glioblastoma: Redefining the extent of resection criteria and its impact on survival. Brain Tumor Res Treat 11: 166-172, 2023.
9. Omuro A, Brandes AA, Carpentier AF, Idbaih A, Reardon DA, Cloughesy T, Sumrall A, Baehring J, van den Bent M, Bähr O, *et al*: Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: An international randomized phase III trial. Neuro Oncol 25: 123-134, 2023.
10. Bao J, Sun R, Pan Z and Wei S: Current chemotherapy strategies for adults with IDH-wildtype glioblastoma. Front Oncol 14: 1438905, 2024.
11. Bai RY, Staedtke V and Riggins GJ: Molecular targeting of glioblastoma: Drug discovery and therapies. Trends Mol Med 17: 301-312, 2011.
12. Davare MA and Tognon CE: Detecting and targetting oncogenic fusion proteins in the genomic era. Biol Cell 107: 111-129, 2015.
13. Hassan A, Hafiz B, Alsinani T, Bokhari R, Mirdad D, Tayyib A, Alkhotani A, Fallata A, Mirza I, Faizo E, *et al*: RNA expression signatures in glioblastoma: A systematic review of tumour biology and therapeutic targets. Oncol Res 33: 3293-3325, 2025.
14. Cheng L, Wei T, Cui X, Chen HF and Yu Z: ProDualNet: Dual-target protein sequence design method based on protein language model and structure model. Brief Bioinform 26: bbaf391, 2025.
15. Qin C, Wang YL, Zheng J, Wan XB and Fan XJ: Current perspectives in drug targeting intrinsically disordered proteins and biomolecular condensates. BMC Biol 23: 118, 2025.
16. Zhou Q, Wang W and Deng C: Advancements in proteolysis targeting chimeras for targeted therapeutic strategies in Alzheimer's disease. Mol Neurobiol 62: 9686-9709, 2025.
17. Liu Y, Sun X, Liu Q, Han C and Rao Y: A dual-target and dual-mechanism design strategy by combining inhibition and degradation together. J Am Chem Soc 147: 3110-3118, 2025.
18. Ma X, Sun C, Ding X, Xu J, Zhang Y, Deng T, Wang Y, Yang H, Ding R, Li H, *et al*: Mechanism analysis and targeted therapy of IDH gene mutation in glioma. Am J Cancer Res 15: 248-270, 2025.
19. Konteatis Z, Artin E, Nicolay B, Straley K, Padyana AK, Jin L, Chen Y, Narayaraswamy R, Tong S, Wang F, *et al*: Vorasidenib (AG-881): A first-in-class, brain-penetrant dual inhibitor of mutant IDH1 and 2 for treatment of glioma. ACS Med Chem Lett 11: 101-107, 2020.
20. Ma R and Yun CH: Crystal structures of pan-IDH inhibitor AG-881 in complex with mutant human IDH1 and IDH2. Biochem Biophys Res Commun 503: 2912-2917, 2018.
21. Mellinghoff IK, van den Bent MJ, Blumenthal DT, Touat M, Peters KB, Clarke J, Mendez J, Yust-Katz S, Welsh L, Mason WP, *et al*: Vorasidenib in IDH1- or IDH2-mutant low-grade glioma. N Engl J Med 389: 589-601, 2023.
22. Lin MD, Tsai AC, Abdullah KG, McBrayer SK and Shi DD: Treatment of IDH-mutant glioma in the INDIGO era. NPJ Precis Oncol 8: 149, 2024.
23. US Food and Drug Administration. FDA approves vorasidenib for grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation. August 6, 2024. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-vorasidenib-grade-2-astrocytoma-or-oligodendroglioma-susceptible-idh1-or-idh2-mutation. Accessed September 8, 2026.
24. de la Fuente MI, Touat M, van den Bent MJ, Preusser M, Peters KB, Young RJ, Huang RY, Ellingson BM, Capper D, Phillips JJ, *et al*: The role of vorasidenib in the treatment of isocitrate dehydrogenase-mutant glioma. Neuro Oncol 27: 1135-1148, 2025.
25. Mellinghoff IK, Lu M, Wen PY, Taylor JW, Maher EA, Arrillaga-Romany I, Peters KB, Ellingson BM, Rosenblum MK, Chun S, *et al*: Vorasidenib and ivosidenib in IDH1-mutant low-grade glioma: A randomized, perioperative phase 1 trial. Nat Med 29: 615-622, 2023.
26. Chan AM, Eberly C, Drennen B, Goodis CC, Wuyts Z, Civin CI and Fletcher S: Augmenting the anti-leukemic activity of the BCL-2 inhibitor venetoclax through its transformation into polypharmacologic dual BCL-2/HDAC1 and dual BCL-2/HDAC6 inhibitors. Drug Dev Res 86: e70084, 2025.
27. Garrett MC, Albano R, Carnwath T, Elahi L, Behrmann CA, Pemberton M, Woo D, O'Brien E, VanCauwenbergh B, Perentesis J, *et al*: HDAC1 and HDAC6 are essential for driving growth in IDH1 mutant glioma. Sci Rep 13: 12433, 2023.
28. Wu AC, Yang WB, Chang KY, Lee JS, Liou JP, Su RY, Cheng SM, Hwang DY, Kikkawa U, Hsu TI, *et al*: HDAC6 involves in regulating the lncRNA-microRNA-mRNA network to promote the proliferation of glioblastoma cells. J Exp Clin Cancer Res 41: 47, 2022.
29. Lee AH, Sun L, Mochizuki AY, Reynoso JG, Orpilla J, Chow F, Kienzler JC, Everson RG, Nathanson DA, Bensinger SJ, *et al*: Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to overcome the immunosuppressive tumor associated macrophages in recurrent glioblastoma. Nat Commun 12: 6938, 2021.
30. Lee J, Nicosia M, Hong ES, Silver DJ, Li C, Bayik D, Watson DC, Lauko A, Kay KE, Wang SZ, *et al*: Sex-Biased T-cell exhaustion drives differential immune responses in glioblastoma. Cancer Discov 13: 2090-2105, 2023.
31. Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, Yi M and Xiang B: Regulatory mechanisms of PD-1/PD-L1 in cancers. Mol Cancer 23: 108, 2024.
32. Li T, Xu D, Ruan Z, Zhou J, Sun W, Rao B and Xu H: Metabolism/immunity dual-regulation thermogels potentiating immunotherapy of glioblastoma through lactate-excretion inhibition and PD-1/PD-L1 blockade. Adv Sci (Weinh) 11: e2310163, 2024.
33. Yang Z, Liu Z, Wan S, Xu J, Huang Y, He H, Liu T, Li L, Ren Y, Zhang J and Chen J: Discovery of novel small-molecule-based potential PD-L1/EGFR dual inhibitors with high druggability for glioblastoma immunotherapy. J Med Chem 67: 7995-8019, 2024.
34. An Z, Fan QW, Wang L, Yoda H, Barata MJ, Jimenez-Morales D, Phillips JJ, Swaney DL, Stevenson E, Lee E, *et al*: EGFR and EGFRvIII coopt host defense pathways promoting progression in glioblastoma. Neuro Oncol 27: 383-397, 2025.
35. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, Cachola KE, Murray JC, Tihan T, Jensen MC, *et al*: Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 13: 84-88, 2007.
36. Hu Z, Li S, He H, Pan W, Liu T, Liang H, Xu C, Lu B, Tao C, Qi Z, *et al*: Discovery of novel and highly potent dual PD-L1/histone deacetylase 6 inhibitors with favorable pharmacokinetics for cancer immunotherapy. J Med Chem 68: 5426-5454, 2025.
37. Wang Z, He H, Liao X, Yuan L, Sun S, Xu C, Yang X, Zang Q, Peng X, Chen J and Guo X: Discovery of dual PD-L1/HDAC3 inhibitors for tumor immunotherapy. J Med Chem 68: 8046-8064, 2025.
38. Li N, Liu B, Xiong R, Li G, Wang B and Geng Q: HDAC3 deficiency protects against acute lung injury by maintaining epithelial barrier integrity through preserving mitochondrial quality control. Redox Biol 63: 102746, 2023.
39. Wang S, Kong Z, Shi Y, Shao C, Wang W, Su Z, Liu J, Zhou Y, Fei X, Cheng B, *et al*: Discovery of small and bifunctional molecules targeting PD-L1/CD73 for cancer dual immunotherapy. J Med Chem 67: 9447-9464, 2024.
40. Gao H, Zhang T, Li K and Li X: CD73: A new immune checkpoint for leukemia treatment. Front Immunol 16: 1486868, 2025.
41. Jin S, Wang H, Li Y, Yang J, Li B, Shi P, Zhang X, Zhou X, Zhou X, Niu X, *et al*: Discovery of a novel small molecule as CD47/SIRPα and PD-1/PD-L1 dual inhibitor for cancer immunotherapy. Cell Commun Signal 22: 173, 2024.
42. Hung CY, Kang EY, Jacek K, Yu C, Zhang X, Zhu Y, Aftabizadeh M, Wong RA, Badie B, Świderski P, *et al*: Multimodal glioma immunotherapy combining TLR9-targeted STAT3 antisense oligodeoxynucleotides with PD1 immune checkpoint blockade. Neuro Oncol 27: 2296-2312, 2025.
43. Xing YL, Panovska D, Park JW, Grossauer S, Koeck K, Bui B, Nasajpour E, Nirschl JJ, Feng ZP, Cheung P, *et al*: BRAF/MEK inhibition induces cell state transitions boosting immune checkpoint sensitivity in $BRAF^{V600E}$-mutant glioma. Cell Rep Med 6: 102183, 2025.
44. Escors D, Gato-Cañas M, Zuazo M, Arasanz H, García-Granda MJ, Vera R and Kochan G: The intracellular signalosome of PD-L1 in cancer cells. Signal Transduct Target Ther 3: 26, 2018.
45. Pfeffer LK, Fischbach F, Heesen C and Friese MA: Current state and perspectives of CAR T cell therapy in central nervous system diseases. Brain 148: 723-736, 2025.

46. Look T, Sankowski R, Bouzereau M, Fazio S, Sun M, Buck A, Binder N, Mastall M, Prisco F, Seehusen F, *et al*: CAR T cells, CAR NK cells, and CAR macrophages exhibit distinct traits in glioma models but are similarly enhanced when combined with cytokines. Cell Rep Med 6: 101931, 2025.
47. Thomas BC, Staudt DE, Douglas AM, Monje M, Vitanza NA and Dun MD: CAR T cell therapies for diffuse midline glioma. Trends Cancer 9: 791-804, 2023.
48. Park DH, Bhojnagarwala PS, Liaw K, Bordoloi D, Tursi NJ, Zhao S, Binder ZA, O'Rourke D and Weiner DB: Novel tri-specific T-cell engager targeting IL-13Rα2 and EGFRvIII provides long-term survival in heterogeneous GBM challenge and promotes antitumor cytotoxicity with patient immune cells. J Immunother Cancer 12: e009604, 2024.
49. Leland P, Degheidy H, Lea A, Bauer SR, Puri RK and Joshi BH: Identification and characterisation of novel CAR-T cells to target IL13Rα2 positive human glioma in vitro and in vivo. Clin Transl Med 14: e1664, 2024.
50. Brown CE, Hibbard JC, Alizadeh D, Blanchard MS, Natri HM, Wang D, Ostberg JR, Aguilar B, Wagner JR, Paul JA, *et al*: Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: A phase 1 trial. Nat Med 30: 1001-1012, 2024.
51. Hou AJ, Shih RM, Uy BR, Shafer A, Chang ZL, Comin-Anduix B, Guemes M, Galic Z, Phyu S, Okada H, *et al*: IL-13Rα2/TGF-β bispecific CAR-T cells counter TGF-β-mediated immune suppression and potentiate anti-tumor responses in glioblastoma. Neuro Oncol 26: 1850-1866, 2024.
52. Dong X, Ren J, Amoozgar Z, Lee S, Datta M, Roberge S, Duquette M, Fukumura D and Jain RK: Anti-VEGF therapy improves EGFR-vIII-CAR-T cell delivery and efficacy in syngeneic glioblastoma models in mice. J Immunother Cancer 11: e005583, 2023.
53. Agliardi G, Liuzzi AR, Hotblack A, De Feo D, Núñez N, Stowe CL, Friebel E, Nannini F, Rindlisbacher L, Roberts TA, *et al*: Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. Nat Commun 12: 444, 2021.
54. Wu A, Ericson K, Chao W and Low WC: NFAT and AP1 are essential for the expression of a glioblastoma multiforme related IL-13Ra2 transcript. Cell Oncol 32: 313-329, 2010.
55. Zhang D, Li AM, Hu G, Huang M, Yang F, Zhang L, Wellen KE, Xu X, Conn CS, Zou W, *et al*: PHGDH-mediated endothelial metabolism drives glioblastoma resistance to chimeric antigen receptor T cell immunotherapy. Cell Metab 35: 517-534.e8, 2023.
56. Monje M, Mahdi J, Majzner R, Yeom KW, Schultz LM, Richards RM, Barsan V, Song KW, Kamens J, Baggott C, *et al*: Intravenous and intracranial GD2-CAR T cells for H3K27M^{+} diffuse midline gliomas. Nature 637: 708-715, 2025.
57. Liu Z, Zhou J, Yang X, Liu Y, Zou C, Lv W, Chen C, Cheng KK, Chen T, Chang LJ, *et al*: Safety and antitumor activity of GD2-specific 4SCAR-T cells in patients with glioblastoma. Mol Cancer 22: 3, 2023.
58. Sutton MN, Glazer SE, Muzzioli R, Yang P, Gammon ST and Piwnica-Worms D: Dimerization of the 4Ig isoform of B7-H3 in tumor cells mediates enhanced proliferation and tumorigenic signaling. Commun Biol 7: 21, 2024.
59. Vitanza NA, Wilson AL, Huang W, Seidel K, Brown C, Gustafson JA, Yokoyama JK, Johnson AJ, Baxter BA, Koning RW, *et al*: Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: Preliminary first-in-human bioactivity and safety. Cancer Discov 13: 114-131, 2023.
60. Tachi T, Kijima N, Kuroda H, Ikeda S, Murakami K, Nakagawa T, Yaga M, Nakagawa K, Utsugi R, Hirayama R, *et al*: Antitumor effects of intracranial injection of B7-H3-targeted Car-T and Car-Nk cells in a patient-derived glioblastoma xenograft model. Cancer Immunol Immunother 73: 256, 2024.
61. Inthanachai T, Boonkrai C, Phakham T, Pisitkun T, Thaiwong R, Chuthaphakdikun V, Sakunrangsit N, Limprasutr V, Chinsuwan T, Hirankarn N, *et al*: Novel B7-H3 CAR T cells show potent antitumor effects in glioblastoma: A preclinical study. J Immunother Cancer 13: e010083, 2025.
62. Moghimi B, Muthugounder S, Jambon S, Tibbetts R, Hung L, Bassiri H, Hogarty MD, Barrett DM, Shimada H and Asgharzadeh S: Preclinical assessment of the efficacy and specificity of GD2-B7H3 SynNotch CAR-T in metastatic neuroblastoma. Nat Commun 12: 511, 2021.
63. Rosenkrans ZT, Erbe AK, Clemons NB, Feils AS, Medina-Guevara Y, Jeffery JJ, Barnhart TE, Engle JW, Sondel PM and Hernandez R: Targeting both GD2 and B7-H3 using bispecific antibody improves tumor selectivity for GD2-positive tumors. bioRxiv [Preprint]: 2024.05.23.595624, 2024.
64. Eichholz T, Heubach F, Arendt AM, Seitz C, Brecht IB, Ebinger M, Flaadt T, Süsskind D, Richter L, Hülsenbeck I, *et al*: Targeted therapies in retinoblastoma: GD2-directed immunotherapy following autologous stem cell transplantation and evaluation of alternative target B7-H3. Cancer Immunol Immunother 73: 19, 2024.
65. Graef J, Bluemel S, Brenner W, Amthauer H, Truckenmueller P, Kaul D, Vajkoczy P, Onken JS and Furth C: [^{177}Lu]Lu-PSMA therapy as an individual treatment approach for patients with high-grade glioma: Dosimetry results and critical statement. J Nucl Med 64: 892-895, 2023.
66. Gu J, Li J, Xu Y, Zhang G, Xie J, Jia R, Chen W, Lu Z, Chang C, Wen H, *et al*: Preliminary exploration of PSMA CAR-T combined with GD2 CAR-T for the treatment of refractory/relapsed gliomas. J Transl Med 23: 591, 2025.
67. Thakur A, Xu C, Li WK, Qiu G, He B, Ng SP, Wu CL and Lee Y: In vivo liquid biopsy for glioblastoma malignancy by the AFM and LSPR based sensing of exosomal CD44 and CD133 in a mouse model. Biosens Bioelectron 191: 113476, 2021.
68. Zhai Y, Li G, Pan C, Yu M, Hu H, Wang D, Shi Z, Jiang T and Zhang W: The development and potent antitumor efficacy of CD44/CD133 dual-targeting IL7Rα-armored CAR-T cells against glioblastoma. Cancer Lett 614: 217541, 2025.
69. Alwithenani A, Hengswat P and Chiocca EA: Oncolytic viruses as cancer therapeutics: From mechanistic insights to clinical translation. Mol Ther 33: 2217-2228, 2025.
70. Wang S, Yan W, Kong L, Zuo S, Wu J, Zhu C, Huang H, He B, Dong J and Wei J: Oncolytic viruses engineered to enforce cholesterol efflux restore tumor-associated macrophage phagocytosis and anti-tumor immunity in glioblastoma. Nat Commun 14: 4367, 2023.
71. Zhang H, Du Y, Qi L, Xiao S, Braun FK, Kogiso M, Huang Y, Huang F, Abdallah A, Suarez M, *et al*: Targeting GBM with an oncolytic picornavirus SVV-001 alone and in combination with fractionated radiation in a novel panel of orthotopic PDX models. J Transl Med 21: 444, 2023.
72. Noh MH, Kang JM, Miller AA, Nguyen G, Huang M, Shim JS, Bueso-Perez AJ, Murphy SA, Rivera-Caraballo KA, Otani Y, *et al*: Targeting IGF2 to reprogram the tumor microenvironment for enhanced viro-immunotherapy. Neuro Oncol 26: 1602-1616, 2024.
73. van de Donk NWCJ and Zweegman S: T-cell-engaging bispecific antibodies in cancer. Lancet 402: 142-158, 2023.
74. Zhang C, Röder J, Scherer A, Bodden M, Pfeifer Serrahima J, Bhatti A, Waldmann A, Müller N, Oberoi P and Wels WS: Bispecific antibody-mediated redirection of NKG2D-CAR natural killer cells facilitates dual targeting and enhances antitumor activity. J Immunother Cancer 9: e002980, 2021.
75. Whittle JR, Vieito M, Rohrberg K, Rodriguez-Ruiz ME, Castanon E, Mellinghoff IK, Van Linde M, Cloughesy T, Reardon DA, Chong RA, *et al*: First-in-human study of an EGFRvIII x CD3 T cell bispecific antibody in the treatment of newly diagnosed glioblastoma. Neurooncol Adv 7: vdaf160, 2025.
76. Gedeon PC, Streicker MA, Schaller TH, Archer GE, Jokinen MP and Sampson JH: GLP toxicology study of a fully-human T cell redirecting CD3:EGFRvIII binding immunotherapeutic bispecific antibody. PLoS One 15: e0236374, 2020.
77. Wang HL, Chi N and Zhang HT: Bispecific antibodies in glioblastoma: Mechanistic advances, delivery innovations, and translational challenges in overcoming immune escape. Int J Med Sci 22: 4250-4277, 2025.
78. Herrera M, Pretelli G, Desai J, Garralda E, Siu LL, Steiner TM and Au L: Bispecific antibodies: Advancing precision oncology. Trends Cancer 10: 893-919, 2024.
79. Zarezadeh Mehrabadi A, Tat M, Ghorbani Alvanegh A, Roozbahani F and Esmaeili Gouvarchin Ghaleh H: Revolutionizing cancer treatment: The power of bi- and tri-specific T-cell engagers in oncolytic virotherapy. Front Immunol 15: 1343378, 2024.
80. Zhang Z, Yang N, Lu H, Chen Y, Xu L, Wang Z, Lu Q, Zhong K, Zhu Z, Wang G, *et al*: Improved antitumor effects elicited by an oncolytic HSV-1 expressing a novel B7H3nb/CD3 BsAb. Cancer Lett 588: 216760, 2024.

81. Arnone CM, Polito VA, Mastronuzzi A, Carai A, Diomedi FC, Antonucci L, Petrilli LL, Vinci M, Ferrari F, Salviato E, *et al*: Oncolytic adenovirus and gene therapy with EphA2-BiTE for the treatment of pediatric high-grade gliomas. J Immunother Cancer 9: e001930, 2021.
82. Baugh R, Khalique H, Page E, Lei-Rossmann J, Wan PK, Johanssen T, Ebner D, Ansorge O and Seymour LW: Targeting NKG2D ligands in glioblastoma with a bispecific T-cell engager is augmented with conventional therapy and enhances oncolytic virotherapy of glioma stem-like cells. J Immunother Cancer 12: e008460, 2024.
83. Vazaios K, Hernández López P, Aarts-Riemens T, Daudeij A, Kemp V, Hoeben RC, Straetemans T, Hulleman E, Calkoen FG, van der Lugt J and Kuball J: Unusual partners: γδ-TCR-based T cell therapy in combination with oncolytic virus treatment for diffuse midline gliomas. Int J Mol Sci 26: 2167, 2025.
84. Ma R, Lu T, Li Z, Teng KY, Mansour AG, Yu M, Tian L, Xu B, Ma S, Zhang J, *et al*: An oncolytic virus expressing IL15/IL15Rα combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. Cancer Res 81: 3635-3648, 2021.
85. Tian L, Xu B, Chen Y, Li Z, Wang J, Zhang J, Ma R, Cao S, Hu W, Chiocca EA, *et al*: Specific targeting of glioblastoma with an oncolytic virus expressing a cetuximab-CCL5 fusion protein via innate and adaptive immunity. Nat Cancer 3: 1318-1335, 2022.
86. Zhu Y, Zhang X, Jin J, Wang X, Liu Y, Gao J, Hang D, Fang L, Zhang H and Liu H: Engineered oncolytic virus coated with anti-PD-1 and alendronate for ameliorating intratumoral T cell hypofunction. Exp Hematol Oncol 14: 16, 2025.
87. Puigdelloses M, Garcia-Moure M, Labiano S, Laspidea V, Gonzalez-Huarriz M, Zalacain M, Marrodan L, Martinez-Velez N, De la Nava D, Ausejo I, *et al*: CD137 and PD-L1 targeting with immunovirotherapy induces a potent and durable antitumor immune response in glioblastoma models. J Immunother Cancer 9: e002644, 2021.
88. Ayele K, Feng X and Saha D: A novel oncolytic HSV co-expressing IL-12 and anti-PD-1 for glioblastoma. Mol Ther Oncol 32: 200810, 2024.
89. Bommareddy PK, Wakimoto H, Martuza RL, Kaufman HL, Rabkin SD and Saha D: Oncolytic herpes simplex virus expressing IL-2 controls glioblastoma growth and improves survival. J Immunother Cancer 12: e008880, 2024.
90. Abdessalem MA and Adham SA: Research advancements in nanoparticles and cell-based drug delivery systems for the targeted killing of cancer cells. Oncol Res 33: 27-44, 2024.
91. Lim SH, Yee GT and Khang D: Nanoparticle-based combinational strategies for overcoming the blood-brain barrier and blood-tumor barrier. Int J Nanomedicine 19: 2529-2552, 2024.
92. Wang K: Innovative nanoparticle-based therapeutic strategies against glioblastoma multiform: A focus on enhanced delivery systems and efficacy. Front Bioeng Biotechnol 13: 1601673, 2025.
93. Zhu YF, He MQ, Lin CS, Ma WH, Ai Y, Wang J and Liang Q: Multifunctional nanocarrier drug delivery systems: From diverse design to precise biomedical applications. Adv Healthc Mater 15: e02178, 2026.
94. Zhu Y, Jiang Y, Meng F, Deng C, Cheng R, Zhang J, Feijen J and Zhong Z: Highly efficacious and specific anti-glioma chemotherapy by tandem nanomicelles co-functionalized with brain tumor-targeting and cell-penetrating peptides. J Control Release 278: 1-8, 2018.
95. Ruan W, Jiao M, Xu S, Ismail M, Xie X, An Y, Guo H, Qian R, Shi B and Zheng M: Brain-targeted CRISPR/Cas9 nanomedicine for effective glioblastoma therapy. J Control Release 351: 739-751, 2022.
96. Zhu Z, Zhai Y, Hao Y, Wang Q, Han F, Zheng W, Hong J, Cui L, Jin W, Ma S, *et al*: Specific anti-glioma targeted-delivery strategy of engineered small extracellular vesicles dual-functionalised by Angiopep-2 and TAT peptides. J Extracell Vesicles 11: e12255, 2022.
97. Zou C, Liu X, Wang W, He L, Yin A, Cao Z, Zhu M, Wu Y, Liu X, Ma J, *et al*: Targeting GDF15 to enhance immunotherapy efficacy in glioblastoma through tumor microenvironment-responsive CRISPR-Cas9 nanoparticles. J Nanobiotechnology 23: 126, 2025.
98. Jiménez-Boland D, Robles-Fernández A, Martín-Rodríguez A, Cuadros MÁ, Traverso JÁ, Sánchez-Moreno P and Bramini M: Breaking barriers in glioblastoma targeting through advanced nanoparticle cell membrane coating. ACS Appl Mater Interfaces 17: 35288-35303, 2025.
99. Saladino GM, Mangarova DB, Nernekli K, Wang J, Annio G, Varniab ZS, Khatoon Z, Ribeiro Morais G, Shi Y, Chang E, *et al*: Multimodal imaging approach to track theranostic nanoparticle accumulation in glioblastoma with magnetic resonance imaging and intravital microscopy. Nanoscale 17: 9986-9995, 2025.
100. Tushe A, Marinelli E, Musca B, Ventura A, Zumerle S, Slukinova O, Zampardi G, Volpin F, Bonaudo C, Della Puppa A, *et al*: Drug-loaded nanoparticles induce immunogenic cell death and efficiently target cells from glioblastoma patients. Nanomedicine (Lond) 20: 1223-1234, 2025.
101. Domingo-Diez J, Foti A, Casanova-Carvajal Ó, Marrodán L, Granado N, Satriano C, Martínez-Murillo R, Serrano-Olmedo JJ and Ramos-Gómez M: Effect of photothermal therapy using gold nanoparticles conjugated with hyaluronic acid in an intracranial murine glioblastoma model. Int J Nanomedicine 20: 9327-9346, 2025.
102. Thirumurugan S, Dash P, Liu X, Tseng YY, Huang WJ, Li Y, Zhao G, Lin C, Murugan K, Dhawan U and Chung RJ: Angiopep-2-decorated titanium-alloy core-shell magnetic nanoparticles for nanotheranostics and medical imaging. Nanoscale 14: 14789-14800, 2022.
103. Şahin Ş, Kaya-Tilki E, Baysal M and Öztürk AA: Preparation and evaluation of temozolomide loaded PLGA nanoparticles for the treatment of glioblastoma multiforme. Sci Rep 15: 36194, 2025.
104. You H, Zhang S, Zhang Y, Chen Q, Wu Y, Zhou Z, Zhao Z, Su B, Li X, Guo Y, *et al*: Engineered bacterial outer membrane vesicles-based doxorubicin and CD47-siRNA co-delivery nanoplatform overcomes immune resistance to potentiate the immunotherapy of glioblastoma. Adv Mater 37: e2418053, 2025.
105. Zhang Y, Qin S, Song T, Huang Z, Lv Z, Zhao Y, Jiao X, Sun M, Zhang Y, Xie G, *et al*: A novel dual-targeting strategy of nanobody-driven protein corona modulation for glioma therapy. Acta Pharm Sin B 15: 4917-4931, 2025.
106. Meng X, Zhao Y, Han B, Zha C, Zhang Y, Li Z, Wu P, Qi T, Jiang C, Liu Y and Cai J: Dual functionalized brain-targeting nanoinhibitors restrain temozolomide-resistant glioma via attenuating EGFR and MET signaling pathways. Nat Commun 11: 594, 2020.
107. He W, Li X, Morsch M, Ismail M, Liu Y, Rehman FU, Zhang D, Wang Y, Zheng M, Chung R, *et al*: Brain-targeted codelivery of Bcl-2/Bcl-xl and Mcl-1 inhibitors by biomimetic nanoparticles for orthotopic glioblastoma therapy. ACS Nano 16: 6293-6308, 2022.
108. Branco F, Cunha J, Mendes M, Vitorino C and Sousa JJ: Peptide-hitchhiking for the development of nanosystems in glioblastoma. ACS Nano 18: 16359-16394, 2024.
109. Jin Y, Zhang B, Li J, Guo Z, Zhang C, Chen X, Ma L, Wang Z, Yang H, Li Y, *et al*: Bioengineered protein nanocarrier facilitating siRNA escape from lysosomes for targeted RNAi therapy in glioblastoma. Sci Adv 11: eadr9266, 2025.
110. Cen B, Zhang J, Pan X, Xu Z, Li R, Chen C, Wang B, Li Z, Zhang G, Ji A and Yuan Y: Stimuli-responsive peptide/siRNA nanoparticles as a radiation sensitizer for glioblastoma treatment by co-inhibiting RELA/P65 and EGFR. Int J Nanomedicine 19: 11517-11537, 2024.
111. Zheng Z, Chen J, Kang Y, Ding Z, Jin C, Gan Z and Yu Q: Dual-responsive nanoparticle system for enhanced blood-brain barrier crossing and glioblastoma penetration. Biomaterials 327: 123730, 2026.
112. 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.
113. Chen WJ, Gupta D, Yang M, Yang F, Feng N, Song J, Wood MJA, Qiu L and Chen J: A purposefully designed pH/GSH-responsive MnFe-based metal-organic frameworks as cascade nanoreactor for enhanced chemo-chemodynamic-starvation synergistic therapy. Small 19: e2303403, 2023.
114. Obrador E, Moreno-Murciano P, Oriol-Caballo M, López-Blanch R, Pineda B, Gutiérrez-Arroyo JL, Loras A, Gonzalez-Bonet LG, Martinez-Cadenas C, Estrela JM and Marqués-Torrejón MÁ: Glioblastoma therapy: Past, present and future. Int J Mol Sci 25: 2529, 2024.
115. Sabu A, Liu TI, Ng SS, Doong RA, Huang YF and Chiu HC: Nanomedicines targeting glioma stem cells. ACS Appl Mater Interfaces 15: 158-181, 2023.
116. Wang X, Prager BC, Wu Q, Kim LJY, Gimple RC, Shi Y, Yang K, Morton AR, Zhou W, Zhu Z, *et al*: Reciprocal signaling between glioblastoma stem cells and differentiated tumor cells promotes malignant progression. Cell Stem Cell 22: 514-528.e5, 2018.

117. Xu X, Zheng Y, Luo L, You Z, Chen H, Wang J, Zhang F, Liu Y and Ke Y: Glioblastoma stem cells deliver ABCB4 transcribed by ATF3 via exosomes conferring glioblastoma resistance to temozolomide. Cell Death Dis 15: 318, 2024.
118. Uneda A, Kurozumi K, Fujimura A, Fujii K, Ishida J, Shimazu Y, Otani Y, Tomita Y, Hattori Y, Matsumoto Y, *et al*: Differentiated glioblastoma cells accelerate tumor progression by shaping the tumor microenvironment via CCN1-mediated macrophage infiltration. Acta Neuropathol Commun 9: 29, 2021.
119. Lee H, Kim B, Park J, Park S, Yoo G, Yum S, Kang W, Lee JM, Youn H and Youn B: Cancer stem cells: Landscape, challenges and emerging therapeutic innovations. Signal Transduct Target Ther 10: 248, 2025.
120. Tu Y, Xu L, Fu G, Wang J, Xia P, Tao Z, Ye Y, Hu J, Cai W, Zhu H, *et al*: Lactylation-driven USP4-mediated ANXA2 stabilization and activation promotes maintenance and radioresistance of glioblastoma stem cells. Cell Death Differ 32: 1648-1663, 2025.
121. Liu L, Liu Z, Liu Q, Wu W, Lin P, Liu X, Zhang Y, Wang D, Prager BC, Gimple RC, *et al*: LncRNA INHEG promotes glioma stem cell maintenance and tumorigenicity through regulating rRNA 2'-O-methylation. Nat Commun 14: 7526, 2023.
122. Hirano S, Uneda A, Otani Y, Suruga Y, Imoto R, Hokama M, Umeda T, Ikemachi R, Nishigaki S, Tsuboi N, *et al*: Dual molecular therapy targeting tumor cell heterogeneity improves therapeutic efficacy in glioblastoma. iScience 28: 113456, 2025.
123. Jensen KV, Hao X, Aman A, Luchman HA and Weiss S: EGFR blockade in GBM brain tumor stem cells synergizes with JAK2/STAT3 pathway inhibition to abrogate compensatory mechanisms in vitro and in vivo. Neurooncol Adv 2: vdaa020, 2020.
124. Xie C, Wu J, Yang H, Gu A, Cao W, Yao X, Li Z, Niu Y, Li J, Xu Z and Cen B: GSCs differentiation model-informed nanotherapy: Dual-functional brain-targeting liposomes with iRGD modification for co-delivery of osimertinib and bortezomib to combat radioresistant glioblastoma. Cell Death Dis 16: 738, 2025.
125. Cahill KE, Morshed RA and Yamini B: Nuclear factor-κB in glioblastoma: Insights into regulators and targeted therapy. Neuro Oncol 18: 329-339, 2016.
126. Ramar V, Guo S, Wang G and Liu M: The pivotal role of NF-κB in glioblastoma: Mechanisms of activation and therapeutic implications. Int J Mol Sci 26: 7883, 2025.
127. Vagapova E, Kozlov M, Lebedev T, Ivanenko K, Leonova O, Popenko V, Spirin P, Kochetkov S and Prassolov V: Selective inhibition of HDAC class I sensitizes leukemia and neuroblastoma cells to anticancer drugs. Biomedicines 9: 1846, 2021.
128. McNeill RS, Canoutas DA, Stuhlmiller TJ, Dhruv HD, Irvin DM, Bash RE, Angus SP, Herring LE, Simon JM, Skinner KR, *et al*: Combination therapy with potent PI3K and MAPK inhibitors overcomes adaptive kinome resistance to single agents in preclinical models of glioblastoma. Neuro Oncol 19: 1469-1480, 2017.
129. Eckerdt FD, Bell JB, Gonzalez C, Oh MS, Perez RE, Mazewski C, Fischietti M, Goldman S, Nakano I and Platanias LC: Combined PI3Kα-mTOR targeting of glioma stem cells. Sci Rep 10: 21873, 2020.
130. Bozzato E, Tsakiris N, Paquot A, Muccioli GG, Bastiancich C and Préat V: Dual-drug loaded nanomedicine hydrogel as a therapeutic platform to target both residual glioblastoma and glioma stem cells. Int J Pharm 628: 122341, 2022.
131. Magrath JW, Raney WR and Kim Y: In vitro demonstration of salinomycin as a novel chemotherapeutic agent for the treatment of SOX2-positive glioblastoma cancer stem cells. Oncol Rep 44: 777-785, 2020.
132. Liu M, Ji Z, Jain V, Smith VL, Hocke E, Patel AP, McLendon RE, Ashley DM, Gregory SG and López GY: Spatial transcriptomics reveals segregation of tumor cell states in glioblastoma and marked immunosuppression within the perinecrotic niche. Acta Neuropathol Commun 12: 64, 2024.
133. Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, Anderson KJ, Abiola O, Aldape K, Alfaro KD, *et al*: Longitudinal molecular trajectories of diffuse glioma in adults. Nature 576: 112-120, 2019.
134. Wei X, He Y, Yu Y, Tang S, Liu R, Guo J, Jiang Q, Zhi X, Wang X and Meng D: The multifaceted roles of BACH1 in disease: Implications for biological functions and therapeutic applications. Adv Sci (Weinh) 12: e2412850, 2025.
135. Wang X, Sun Q, Liu T, Lu H, Lin X, Wang W, Liu Y, Huang Y, Huang G, Sun H, *et al*: Single-cell multi-omics sequencing uncovers region-specific plasticity of glioblastoma for complementary therapeutic targeting. Sci Adv 10: eadn4306, 2024.
136. Fan X, Liu J, Xie S, Tan H, Mi Z, Zhang P, Ma X, Liang Q, Yang M, Ming Y and Rong P: HSA-templated synergistic platform boosts radiotherapy via enhanced radiosensitization and ferroptosis induction. Drug Resist Updat 83: 101293, 2025.
137. Zeng Q, Liu M, Wang Z, Zhou R and Ai K: Enhancing radiotherapy-induced anti-tumor immunity via nanoparticle-mediated STING agonist synergy. Mol Cancer 24: 176, 2025.
138. Patel UA, Shi MY, Kazan JM, Nixon KCJ, Ran X, Nair SN, Huang O, Song L, Aparnathi MK, He MY, *et al*: CRISPR screen identifies HDAC3 as a novel radiosensitizing target in small cell lung cancer. Mol Cancer Ther 25: 183-195, 2026.
139. Yong Y, Zhang C, Gu Z, Du J, Guo Z, Dong X, Xie J, Zhang G, Liu X and Zhao Y: polyoxometalate-based radiosensitization platform for treating hypoxic tumors by attenuating radioresistance and enhancing radiation response. ACS Nano 11: 7164-7176, 2017.
140. Zhou X, Liu H, Zheng Y, Han Y, Wang T, Zhang H, Sun Q and Li Z: Overcoming radioresistance in tumor therapy by alleviating hypoxia and using the hif-1 inhibitor. ACS Appl Mater Interfaces 12: 4231-4240, 2020.
141. Hashimoto T, Tsubota K, Hatabi K and Hosoi Y: FDX1 regulates the phosphorylation of ATM, DNA-PKcs Akt, and EGFR and affects radioresistance under severe hypoxia in the glioblastoma cell line T98G. Int J Mol Sci 26: 3378, 2025.
142. Mao M, Li Z, Zheng W, Xia H, Fei Z, Yao Q and Zheng R: Collagen type I alpha 1 induces radio-resistance in hypoxic glioblastoma cells by promoting autophagy. J Neurooncol 175: 287-301, 2025.
143. Dong H, Guo Y, Shi J and Hu P: Tumor disaggregation sensitizes radio-therapy for low rectal tumor. Biomaterials 330: 124029, 2026.
144. Pu S, Li J, Wu C, Song J, Sun P, Kuai R and Li L: Mineralized lipid nanoparticles containing gadolinium enable integrated X-ray fluorescence computed tomography imaging and radiotherapy. Nano Lett 25: 15824-15831, 2025.
145. Wu J, Li N, Ma T, Lin D, Zhou J, Song Y, Cheng W, Wu A and Jiao N: Magnetic continuum robot-mediated intracranial delivery of peptide nanophotosensitizers for glioblastoma photodynamic therapy. Biomater Adv 178: 214458, 2026.
146. Klebowski B, Płaziński W, Płazińska A, Gałuszka-Bulaga A, Krzempek D, Baran J and Depciuch J: The effect of surface modification of ultrasmall palladium nanoparticles (Pd NPs) with polyethylene glycol and glucose on cellular uptake and radiosensitizing potential-comprehensive in vitro and molecular dynamics studies. Colloids Surf B Biointerfaces 268: 116055, 2026.
147. Kim B, Kang H, Park J, Park S, Youn H and Youn B: Adenylate kinase 4 promotes radioresistance by suppressing radiation-induced ferroptosis through metabolic regulation in glioblastoma. Apoptosis 31: 87, 2026.
148. Feng W, Liu Y, Zhang Q, Hu S, Xie D, Tan P, Lei Y, Chen C, Ren C and Du S: GDF15 drives glioblastoma radioresistance by inhibiting ferroptosis and remodeling the immune microenvironment. Int J Biol Sci 21: 6794-6807, 2025.
149. Zhu A, Shao S, Hu J, Tu W, Song Z, Liu Y, Liu J, Zhang Q and Li J: Hydrogen sulfide-generating semiconducting polymer nanoparticles for amplified radiodynamic-ferroptosis therapy of orthotopic glioblastoma. Mater Horiz 12: 973-986, 2025.
150. Zhang Y, Tian J, Wu S, Zhou Y, Bao Z, Zhu Y, Wang P, Liu Z, Li P, Tao Z, *et al*: FOSL1-PRMT1 transcriptional-epigenetic circuit promotes glioblastoma radioresistance via calcyphosine-mediated DNA repair and invasion. Mol Biomed 6: 147, 2025.
151. Fan X, Yang Y, Li X, Yu L, Wang Y, Wang Z, Wang S, Duan W and Chen J: BRD4 inhibition sensitizes glioblastoma to radiotherapy by suppressing super-enhancer-driven COL1A1. Oncogene 44: 4462-4474, 2025.
152. Ding Y, Zhang ZY, Ezhilarasan R, Modrek AS, Graciani M, Karp J, McManus G, Jambhale A and Sulman EP: NANP targeting radiosensitizes glioblastoma through TNFR1 sialylation-driven mesenchymal shift. Nat Commun 17: 4130, 2026.
153. Qiu Y, Zhang J, Liu J, Wang Z, Ma Z, Wang M, Wang Q, Liu X, Yan D and Zhang Z: Prolactin-releasing hormone receptor (PRLHR) enhances radiosensitivity and exacerbates DNA damage in glioblastoma post-irradiation by inhibiting Y-box-binding protein-1 (YBX1) nuclear translocation: A novel perspective on precision radiotherapy. Mol Biomed 7: 102, 2026.
154. Zhong F, Zhang N, Wang Y, He X and Tu B: MST1 enhances radio-sensitivity in glioblastoma by suppressing autophagy and promoting apoptosis. Am J Cancer Res 15: 4136-4149, 2025.

155. Nishiwaki T, Kudo U, Nakamura S, Kuse Y, Harakawa Y, You F, Shoda K, Yamada T, Takei H, Egashira Y, *et al*: Synergistic antitumor and radiosensitizing effects of α-sulfoquinovosyl-ac ylpropanediol (SQAP) via PI3K/Akt inhibition and DNA repair impairment in glioblastoma. J Neurooncol 175: 1131-1146, 2025.
156. Wang J, Li K, Wang Y, Zhang J, Peng X and Ji N: Niche-specific epigenetic interventions in the spatially heterogeneous glioblastoma microenvironmental landscape: Strategies for radiotherapy enhancement. Front Mol Biosci 12: 1704311, 2025.
157. Jiang Z, Jin X, Xue H, Zhang J, Zeng L, Zhou Y, Pan Y, Zhang J and Shao C: TMEM164 enhances radioresistance of GBM cells by inhibiting the FASN-NADPH-ROS axis. J Neurooncol 175: 1011-1026, 2025.
158. Ren X, Cui X, Lin S, Wang J, Jiang Z, Sui D, Li J and Wang Z: Co-deletion of chromosome 1p/19q and IDH1/2 mutation in glioma subsets of brain tumors in Chinese patients. PLoS One 7: e32764, 2012.
159. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, Koelsche C, Sahm F, Chavez L, Reuss DE, *et al*: DNA methylation-based classification of central nervous system tumours. Nature 555: 469-474, 2018.
160. Appay R, Dehais C, Maurage CA, Alentorn A, Carpentier C, Colin C, Ducray F, Escande F, Idbaih A, Kamoun A, *et al*: CDKN2A homozygous deletion is a strong adverse prognosis factor in diffuse malignant IDH-mutant gliomas. Neuro Oncol 21: 1519-1528, 2019.
161. Cui X, Zhao J, Li G, Yang C, Yang S, Zhan Q, Zhou J, Wang Y, Xiao M, Hong B, *et al*: Blockage of EGFR/AKT and mevalonate pathways synergize the antitumor effect of temozolomide by reprogramming energy metabolism in glioblastoma. Cancer Commun (Lond) 43: 1326-1353, 2023.
162. Yuan F, Wang Y and Ma C: Current WHO guidelines and the critical role of genetic parameters in the classification of glioma: Opportunities for immunotherapy. Curr Treat Options Oncol 23: 188-198, 2022.
163. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, *et al*: MGMT gene silencing and benefit from temozolomide in glioblastoma. NEngl J Med 352: 997-1003, 2005.
164. Bagley SJ, Desai AS, Fraietta JA, Silverbush D, Chafamo D, Freeburg NF, Gopikrishna GK, Rech AJ, Nabavizadeh A, Bagley LJ, *et al*: Intracerebroventricular bivalent CAR T cells targeting EGFR and IL-13Rα2 in recurrent glioblastoma: A phase 1 trial. Nat Med 31: 2778-2787, 2025.
165. Nathanson DA, Gini B, Mottahedeh J, Visnyei K, Koga T, Gomez G, Eskin A, Hwang K, Wang J, Masui K, *et al*: Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science 343: 72-76, 2014.

Copyright © 2026 Zhao et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.