# Molecularly Targeted Clinical Trials

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**KEYWORDS**

• Glioblastoma • Heterogenicity • Targeted therapy • Blood-brain barrier • Synthetic lethality

**KEY POINTS**

- Glioblastomas are incurable malignant central nervous system cancers with an unmet need for new therapies.
- Intratumoral heterogenicity and redundancy of growth pathways make targeting individual pathways ineffective for most patients.
- The blood-brain barrier presents a challenge for drug delivery.
- Molecularly targeted clinical trial design requires robust biomarkers.
- Molecularly targeted therapies and synthetic lethality may benefit a subset of glioblastoma patients.

## INTRODUCTION

Glioblastoma (GBM) is the most common primary brain tumor in adults, with approximately 12,000 new cases diagnosed each year in the United States.[1] The prognosis for patients with GBM remains dismal, with a median survival with surgery, chemotherapy, and radiation in patients eligible for clinical trials of only 15 months to 22 months.[2,3] Data from population-based registries report a median survival of fewer than 12 months if all patients are included.[4] Despite extensive research, there have not been significant advances in the past 30 years except for temozolomide with radiation therapy.[5,6]

The 2016 World Health Organization guideline update of central nervous system tumors led to recognizing molecular profiling of brain tumors as best practice.[2] Aside from improved clarity of diagnosis, molecular profiling of tumors can identify gene or gene product alterations potentially amenable to targeted therapy. In contrast to traditional chemotherapies, which broadly affect cells in the body, targeted therapies interfere with specific molecular changes unique to the cancer cells. Targeted therapies have shown efficacy in various cancers, including lymphoma, breast, colon, and lung, but have demonstrated success in only a small subset of primary brain tumor patients.[7–10] Because targeting a single mutation does not work for most malignant gliomas, exploiting a larger genomic context may be more effective. Synthetic lethality, or cell death resulting from simultaneous disabling of 2 genes, may be exploited to expand the therapeutic options of glioma patients. First observed by Bridges[11] in the early twentieth century when crossing fruit flies with certain nonallelic genes,[12] this approach as anticancer therapy is exemplified by the use of poly (adenosine diphosphate–ribose) polymerase (PARP) inhibitors in breast cancer patients with germline mutations in BRCA1 and BRCA2.[13,14] Successful utilization of synthetic lethality in GBM will depend on the ability to predict robust synthetic lethal relationships. This article discusses the successes and challenges of targeted

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therapy in brain tumors and reviews synthetic lethality as an attractive new approach to treating brain tumors.

## DISCUSSION

The Food and Drug Administration (FDA) approval of tamoxifen in the 1970s for estrogen receptor–positive breast cancer signaled the start of personalized, targeted cancer medicine. Subsequent decades of research led to the discoveries of a diverse arsenal of new therapies with clinical benefits in various cancers, notably imatinib for Philadelphia chromosome–positive chronic myeloid leukemia.[15,16] Despite the success of molecularly targeted therapies in other cancers, however, these approaches have not demonstrated much success in GBM. This failure has been attributed to a variety of factors, including intratumoral genetic and transcriptional heterogeneity, redundant activating pathways or escape mechanisms, and delivery of a drug to therapeutic levels within tumor tissue through the blood-brain barrier (BBB).[9,10] Recent advances in molecular testing and tumor profiling, however, have led to a resurgence of interest in targeted therapy with the increasing recognition of more robust and potentially actionable alterations.

### *Molecular Classification and Potentially Actionable Alterations in Glioblastoma*

Genomic, transcriptomic, epigenomic, and proteomic analysis of GBM has revealed distinct molecular subtypes with different clinical behaviors and therapeutic implications.[17] Work by The Cancer Genome Atlas (TCGA) classified GBM into 3 subtypes: classical, associated with *EGFR* amplification and *CDKN2A* deletion; mesenchymal, distinguished by *NF1* deletions, elevated endothelial markers (cluster of differentiation 31 (CD31), vascular endothelial growth factor receptor-2 (VEGFR-2)), increased mitogen-activated protein kinase (MAPK) pathway activations, and decreased levels of mechanistic target of rapamycin (mTOR); and proneural, associated with *PDGFRA* amplification, *IDH1* mutation, and proneural development gene expression. A fourth, neural subtype, later was attributed to neural tissue at the margin of the tumor.[18,19]

Other significant genomic alterations identified by TCGA include mutations in *PIK3CA*, *PTEN*, *RB1*, and *TP53*; genomic gains and losses involving *MET*, *CDK6*, *CDK4*, *MDM2*, and *CDKN2A/CDKN2B* codeletion; and oncogenic gene fusions, including fibroblast growth factor receptor 1 (FGFR1)-transforming acidic coiled coil 1 (TACC1), FGFR3-TACC3, Epidermal growth factor receptor (EGFR)-Septin 14 (SEPT14), and neurotrophic tropomyosin receptor kinase (*NTRK*).[19–21] More recently, methylation profiling of an extensive series of GBMs has identified the Receptor tyrosine kinase (RTK1)-type corresponding to the proneural subgroup, RTK2-type, comprising classical and mesenchymal GBM and GBM with Histone Family 3A (H3F3A) alterations as a unique subset.[22] Proteomic investigations of GBM found 2 subclasses with exclusive mutations. Proteomic cluster 1 (GPC1) exclusively had mutations in *EGFR*vIII and *PIK3CA*, whereas the second group, GPC2, was characterized by mutations in *TP53*, *NF1*, *PTEN*, *RB1*, and *EGFR* without the vIII gene fusion variant.[17] More recently, grade 4 gliomas are segregated by their Isocitrate dehydrogenase (IDH) (IDH1/IDH2) mutation status: tumors with wild-type IDH retain the designation of GBM, whereas tumors with IDH mutation now are labeled as grade 4 IDH-mutated astrocytoma.

Drawing from the molecular insights and successes of targeted therapies of other cancers, attempts have been made to extrapolate these successes to GBM, albeit with limited success for most patients.

### *Signals of Efficacy in Biomarker-Driven Therapy in Glioblastoma*

Biomarkers are biological molecules indicative of a physiologic state and may include DNA, RNA, protein, or extracellular vesicles.[23] Biomarkers in oncology fall in a spectrum of prognostic (or indicative of a patient's overall outcome) versus predictive (or informative of the expected response to therapeutic intervention).[24] Some biomarkers have both attributes; for example, in breast cancer, HER2 amplification is both prognostic of a poor prognosis due to a more aggressive course without targeted therapy and predictive of therapeutic efficacity with HER2 targeting treatments, such as trastuzumab.[25] Similarly, IDH mutations are a prognostic marker of better survival for glioma patients and may be predictive of response to IDH and PARP inhibitors.[2,10,26,27] Some biomarkers are predictive of a lack of targeted therapy, as exemplified by a lack of efficacy of EGFR inhibitors targeting non–small cell lung cancers with concurrent mutation of EGFR and *K-ras* mutations, and lack of efficacy of *BRAF* inhibitors in mutant colon cancers and GBM with concurrent EGFR and *BRAF* mutations.[24,28] An established and regularly utilized molecular biomarker in GBM is methylguanine methyltransferase (*MGMT*) promotor methylation status. When this DNA repair gene is inactive through methylation of the gene promoter (which occurs in approximately 30% of GBMs), it is

predictive of therapeutic efficacy of alkylating chemotherapy in IDH wild-type gliomas and possibly is prognostic of better survival or at least predictive of the benefit of radiation therapy without chemotherapy.[3,29,30] As discussed previously, robust complementary biomarkers are a necessity for successful targeted treatment and design of clinic trials.[23]

Relative to other cancers, biomarker-driven therapies in GBM are less established and have been mainly unsuccessful. Despite recent setbacks, targeted treatment of driver mutations and gene fusions in GBM has produced clinical benefit in rare subsets of patients exemplified in case reports and basket trials. Many clinical trials with active targeted therapy are under way for GBM patients (**Table 1**).

Most reports of the benefit of targeted therapy in GBM patients have been in driver mutations. *BRAF* mutations have been demonstrated to be a viable therapeutic target in a variety of cancers, including primary brain tumors through inhibition of *BRAF* and *MEK,* which is downstream in this kinase pathway.[31] A basket trial using trametinib, a *MEK* inhibitor, included 5 patients with anaplastic astrocytoma and 6 with GBM. One patient had a partial response, and 5 patients had stable disease, with 2 of the patients having disease stabilization that lasted more than 1 year.[4] Currently, a majority of reported cases of adult brain tumor patients with *BRAF* alterations are heavily pretreated, may have other current tumor-directed treatments, and had mixed use of different combinations of *MEK* and *BRAF* inhibitors making the results difficult to interpret.[28] A trial is under way evaluating the use of the *MEK* inhibitor binimetinib and *BRAF* inhibitor encorafenib in adults with recurrent *BRA*FV600E mutant GBM (NCT03973918). The *IDH* inhibitor ivosidenib has shown prolonged disease control in grade 2 and grade 3 *IDH*-mutant astrocytomas, but it is unknown if there is a benefit with grade 4 *IDH*-mutant astrocytomas.[27,32] Neurofibromatosis type 1–associated GBMs are uncommon and typically arise from lower-grade gliomas. A clinical benefit with MEK inhibitors was observed based on case report experiences.[33,34] Targeting of TSC2 mutation with the *MTOR* inhibitor everolimus in a GBM patient with Li-Fraumeni syndrome also showed a therapeutic response.[35] Gliosarcoma, a subtype of mesenchymal GBM with platelet-derived growth factor receptor (*PDGFR*) and *KIT*/*SCF* autocrine activation loops, has an ongoing phase II trial using sunitinib that targets these pathways (NCT03641326).

Although gene fusions occur in 30% to 50% of GBMs, only a select few have been associated with oncogenic biologic function.[36] Neurotrophic-tropomyosin receptor kinase (*NTRK*) fusions in adults with GBM are rare, but, similarly to other cancers with this alteration, have demonstrated a treatment response in case reports, including 45% volume reduction using entrectinib in a pontine astrocytoma patient harboring *BCAN-NTRK1* fusion, and a partial response of subclonal periventricular lesion from 67 mm × 52 mm to 8 mm × 4 mm using larotrectinib in an adult with recurrent multifocal GBM with an EML4-NTRK3 fusion for 1 month.[37,38] Several basket trials are exploring *NTRK* inhibitors.[39] A pediatric patient with GBM harboring a Receptor-type tyrosine-protein phosphatase zeta-MET proto-oncogene (PTPRZ1-MET) fusion and treated with crizotinib had a partial response. An ongoing trial (NCT02978261) is evaluating the c-Met Inhibitor PLB1001 in patients with PTPRZ1-MET fusion recurrent high-grade gliomas. The targeting of FGFR-TACC fusions also has been explored. A phase 1 trial using the pan-fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor JNJ-42756493 reported a partial response in 2 GBM patients with FGFR3-TACC3 fusion.[40] There are currently are ongoing trials in recurrent glioma with FGFR3-TACC3 fusions (NCT01975701, NCT02824133).

A majority of targeted therapy studies in GBM have been derived from successes in systemic cancer. Even among systemic cancers, however, there is heterogenicity of responses of the same drug to the same mutation, which is not surprising given the heterogeneity in the genetic and epigenetic background in which these mutations occur.[10,41] Concomitant mutations can prevent therapeutic efficacy through the activation of alternative proliferation pathways. For example, EGFR mutations with concurrent *EML4-ALK* fusions or NRAS alterations lead to EGFR tyrosine kinase inhibitor resistance in non–small lung cancer. Similarly, targeted inhibition of *BRAFV600E* yields a response rate in 80% of melanoma versus 5% of colon cancers. It is hypothesized that this results from much higher expression in of EGFR in colon cancers, which results in adaptive feedback reactivation of MAPK signaling, leading to activation of other *RAF* kinases and subsequent resistance.[28]

### *Challenges to Success of Molecularly Targeted Therapy in Glioblastoma*

Throughout the spectrum of cancer, the number of patients eligible for targeted therapy is relatively low, with the number of patients who benefit from targeted therapy even lower. A cross-

**Table 1**
**Active glioblastoma trials using molecularly targeting agents**

| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| NCT02761070 | Temozolomide, bevacizumab | VEGFA | Bevacizumab Alone vs Dose-dense Temozolomide Followed by Bevacizumab for Recurrent Glioblastoma, phase III | Phase 3 |
| NCT02678975 | Disulfiram, alkylating agents | ALDH2, DBH | Disulfiram in Recurrent Glioblastoma | Phase 2, Phase 3 |
| NCT02573324 | Temozolomide, ABT-414, placebo for ABT-414 | EGFR | A Study of ABT-414 in Subjects with newly diagnosed Glioblastoma GBM With Epidermal Growth Factor Receptor (EGFR) Amplification | Phase 2, Phase 3 |
| NCT02152982 | Temozolomide, veliparib | PARP1, PARP2 | Temozolomide With or Without Veliparib in Treating Patients With Newly Diagnosed Glioblastoma Multiforme | Phase 2, Phase 3 |
| NCT03025893 | Sunitinib, lomustine | PDGFRB, FLT4, KDR, FLT3, KIT, FLT1, CSF1R, PDGFRA, STMN4 | A Phase II/III Study of High-Dose, Intermittent Sunitinib in Patients With Recurrent Glioblastoma Multiforme | Phase 2, Phase 3 |
| NCT03970447 | Temozolomide, lomustine, regorafenib | STMN4, FGFR2, PDGFRB, ABL1, *BRAF*, RAF1, FLT4, KDR, KIT, FGFR1, RET, FLT1, NTRK1, PDGFRA, EPHA2, TEK, DDR2, MAPK11, FRK | A Trial to Evaluate Multiple Regimens in Newly Diagnosed and Recurrent Glioblastoma | Phase 2, Phase 3 |
| NCT02525692 | ONC201 | DRD2, AKT1, MAPK1 | Oral ONC201 in Recurrent GBM, H3 K27M glioma, and Midline Glioma | Phase 2 |
| NCT03363659 | Disulfiram, temozolomide | ALDH2, DBH | Disulfiram and Copper Gluconate With Temozolomide in Unmethylated Glioblastoma Multiforme | Phase 2 |
| NCT03973918 | Encorafenib, binimetinib | BRAF V600, MAP2K1, MAP2K2 | Study of Binimetinib With Encorafenib in Adults With Recurrent BRAF V600-Mutated HGG | Phase 2 |

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| NCT03919071 | Dabrafenib mesylate, trametinib dimethyl sulfoxide | BRAF, MAP2K1, MAP2K2, MAPK1 | Dabrafenib Combined With Trametinib After Radiation Therapy in Treating Patients With Newly-Diagnosed High-Grade Glioma | Phase 2 |
| NCT02981940 | Abemaciclib | CDK4, CDK6 | A Study of Abemaciclib in Recurrent Glioblastoma | Phase 2 |
| NCT03746080 | Plerixafor, temozolomide | CXCR4 | Whole Brain Radiation Therapy With Standard Temozolomide Chemo-Radiotherapy and Plerixafor in Treating Patients With Glioblastoma | Phase 2 |
| NCT03600467 | SEVI-D (seviteronel in combination with dexamethasone) | CYP17A1 | Activity of Seviteronel in Patients With Androgen Receptor (AR)-Positive Glioblastoma | Phase 2 |
| NCT03618667 | GC1118 | EGFR | GC1118 in Recurrent Glioblastoma Patients With High EGFR Amplification | Phase 2 |
| NCT02844439 | Tesevatinib | EGFR, ERBB1, HER2, ERBB2, VEGFR, EPHB4 | Study of Tesevatinib Monotherapy in Patients With Recurrent Glioblastoma | Phase 2 |
| NCT03216499 | HIF-2$\alpha$ inhibitor PT2385 | EPAS1 | HIF-2 Alpha Inhibitor PT2385 in Treating Patients With Recurrent Glioblastoma | Phase 2 |
| NCT04051606 | Regorafenib | FGFR2, PDGFRB, ABL1, BRAF, RAF1, FLT4, KDR, KIT, FGFR1, RET, FLT1, NTRK1, PDGFRA, EPHA2, TEK, DDR2, MAPK11, FRK | Regorafenib in Bevacizumab Refractory Recurrent Glioblastoma | Phase 2 |
| NCT02926222 | Regorafenib, lomustine | FGFR2, PDGFRB, ABL1, BRAF, RAF1, FLT4, KDR, KIT, FGFR1, RET, FLT1, NTRK1, PDGFRA, EPHA2, TEK, DDR2, MAPK11, FRK, STMN4 | Regorafenib in Relapsed Glioblastoma | Phase 2 |
| NCT02137759 | Standard temozolomide, belinostat | HDAC1, HDAC2, HDAC3, HDAC6 | MRSI to Predict Response to RT/TMZ + Belinostat in GBM | Phase 2 |
| NCT02977780 | Temozolomide, neratinib, CC-115, anemaciclib | HER2, ERBB2, EGFR, MTOR, CKD4, CDK6 | INdividualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT) | Phase 2 |

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**Table 1**
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| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| NCT02885324 | Cabozantinib | KDR, RET, MET | Pilot Study of Cabozantinib for Recurrent or Progressive High-Grade Glioma in Children | Phase 2 |
| NCT03581292 | Temozolomide, veliparib | PARP1, PARP2 | Veliparib, Radiation Therapy, and Temozolomide in Treating Patients With Newly Diagnosed Malignant Glioma Without H3 K27 M or BRAFV600 Mutations | Phase 2 |
| NCT03212274 | Olaparib | PARP1, PARP2, PARP3 | Olaparib in Treating Patients With Advanced Glioma, Cholangiocarcinoma, or Solid Tumors With IDH1 or IDH2 Mutations | Phase 2 |
| NCT03661723 | Pembrolizumab, bevacizumab | PDCD1, VEGFA | Pembrolizumab and Reirradiation in Bevacizumab Naive and Bevacizumab Resistant Recurrent Glioblastoma | Phase 2 |
| NCT02626364 | Crenolanib | PDGFRA, PDGFRB, FLT3 | Study of Crenolanib in Recurrent/ Refractory Glioblastoma With PDGFRA Gene Amplification | Phase 2 |
| NCT01817751 | Sorafenib tosylate, valproic acid, sildenafil citrate | PDGFRB, BRAF, RAF1, FLT4, KDR, FLT3, KIT, FGFR1, RET, FLT1 | Sorafenib Tosylate, Valproic Acid, and Sildenafil Citrate in Treating Patients With Recurrent High-Grade Glioma | Phase 2 |
| NCT03522298 | Paxalisib (GDC-0084) | PIK3CA | Safety, Pharmacokinetics and Efficacy of Paxalisib (GDC-0084) in Newly-diagnosed Glioblastoma | Phase 2 |
| NCT03027388 | LB-100 | PP2A | Protein Phosphatase 2A Inhibitor, in Recurrent Glioblastoma | Phase 2 |
| NCT01582269 | LY2157299 monohydrate, lomustine, placebo | STMN4 | A Study in Recurrent Glioblastoma (GBM) | Phase 2 |

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| NCT03149003 | DSP-7888 dosing emulsion, bevacizumab | VEGFA | A Study of DSP-7888 Dosing Emulsion in Combination With Bevacizumab in Patients With Recurrent or Progressive Glioblastoma Following Initial Therapy | Phase 2 |
| NCT01903330 | ERC1671, granulocyte macrophageecolony stimulating factor (GM-CSF), cyclophosphamide, oral control (sucrose pill)<br>Injectable control (sodium chloride injection United States Pharmacopeia [0.9%]), bevacizumab | VEGFA | ERC1671/GM-CSF/ Cyclophosphamide for the Treatment of Glioblastoma Multiforme | Phase 2 |
| NCT03532295 | Epacadostat, bevacizumab | VEGFA | INCMGA00012 and Epacadostat in Combination With Radiation and Bevacizumab in Patients With Recurrent Gliomas | Phase 2 |
| NCT03743662 | Bevacizumab, nivolumab | VEGFA, PDCD1 | Nivolumab With Radiation Therapy and Bevacizumab for Recurrent MGMT Methylated Glioblastoma | Phase 2 |
| NCT03463265 | ABI-009, bevacizumab, temozolomide, lomustine, marizomib | VEGFA, STMN4 | ABI-009 (Nab-Rapamycin) in Recurrent High Grade Glioma and Newly Diagnosed Glioblastoma | Phase 2 |
| NCT01004874 | Bevacizumab, temozolomide, topotecan | VEGFA, TOP1, TOP1MT | Avastin/Radiation (XRT)/ Temozolomide (Temodar) Followed by Avastin/Temodar/ Topotecan for Glioblastoma | Phase 2 |
| NCT01062425 | Cediranib maleate, temozolomide | VEGFR1, VEGFR2, VEGFR3 | Temozolomide and Radiation Therapy With or Without Cediranib Maleate in Treating Patients With Newly Diagnosed Glioblastoma | Phase 2 |
| NCT02974621 | Cediranib, cediranib maleate, olaparib | VEGFR1, VEGFR2, VEGFR3, PARP1, PARP2, PARP3 | Cediranib Maleate and Olaparib Compared to Bevacizumab in | Phase 2 |

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**Table 1**
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| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| | | | Treating Patients With Recurrent Glioblastoma | |
| NCT03856099 | TTAC-0001 | VEGFR2 | TTAC-0001 Phase II Trial With Recurrent Glioblastoma Progressed on Bevacizumab | Phase 2 |
| NCT03673787 | Ipatasertib, atezolizumab | AKT1, PDCD1 | A Trial of Ipatasertib in Combination With Atezolizumab | Phase 1, Phase 2 |
| NCT02715609 | Disulfiram, copper gluconate, temozolomide | ALDH2, DBH | Disulfiram/Copper With Concurrent Radiation Therapy and Temozolomide in Patients With Newly Diagnosed Glioblastoma | Phase 1, Phase 2 |
| NCT03158389 | APG101, alectinib, idasanutlin, atezolizumab, vismodegib, temsirolimus, palbociclib | ALK, SMO, MTOR, CDK4, CDK6 | NCT Neuro Master Match (NOA-20) | Phase 1, Phase 2 |
| NCT02586857 | ACP-196 | BTK | A Phase 1 b/2, Multicenter, Open-label Study of ACP-196 in Subjects With Recurrent Glioblastoma Multiforme (GBM) | Phase 1, Phase 2 |
| NCT02942264 | Zotiraciclib (TG02), temozolomide | CDK1, CDK2, CDK7, JAK2, CDK9, FLT3, FLK2, STK1 | Zotiraciclib (TG02) Plus Dose-Dense or Metronomic Temozolomide Followed by Randomized Phase II Trial of Zotiraciclib (TG02) Plus Temozolomide vs Temozolomide Alone in Adults With Recurrent Anaplastic Astrocytoma and Glioblastoma | Phase 1, Phase 2 |
| NCT01790503 | PLX3397, temozolomide | CSF1R | A Phase 1 b/2 Study of PLX3397 + Radiation Therapy + Temozolomide in Patients With Newly Diagnosed Glioblastoma | Phase 1, Phase 2 |
| NCT04121455 | Olaptesed pegol | CXCL12 | | Phase 1, Phase 2 |

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| | | | Glioblastoma Treatment With Irradiation and Olaptesed Pegol (NOX-A12) in MGMT Unmethylated Patients | |
| NCT00669669 | Carmustine, O6-benzylguanine, plerixafor, temozolomide | GSR, CXCR4 | O6-Benzylguanine-Mediated Tumor Sensitization With Chemoprotected Autologous Stem Cell in Treating Patients With Malignant Gliomas | Phase 1, Phase 2 |
| NCT00555399 | Vorinostat, isotretinoin, temozolomide | HDAC1, HDAC2, HDAC3, HDAC6 | Vorinostat, Isotretinoin and Temozolomide in Adults With Recurrent Glioblastoma Multiforme (GBM) | Phase 1, Phase 2 |
| NCT00731731 | Temozolomide, vorinostat | HDAC1, HDAC2, HDAC3, HDAC6 | Vorinostat, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme | Phase 1, Phase 2 |
| NCT03684811 | FT-2102, azacitidine, gemcitabine and cisplatin | IDH1, DNMT1 | A Study of FT 2102 in Participants With Advanced Solid Tumors and Gliomas With an IDH1 Mutation | Phase 1, Phase 2 |
| NCT01434602 | Everolimus, sorafenib | MTOR, PDGFRB, BRAF, RAF1, FLT4, KDR, FLT3, KIT, FGFR1, RET, FLT1 | Phase I-II Everolimus and Sorafenib in Recurrent High-Grade Gliomas | Phase 1, Phase 2 |
| NCT03150862 | BGB-290, temozolomide | PARP1, PARP2 | A Study Assessing Pamiparib With Radiation and/or Temozolomide (TMZ) in Subjects With Newly Diagnosed or Recurrent Glioblastoma | Phase 1, Phase 2 |
| NCT03914742 | PARP inhibitor BGB-290, temozolomide | PARP1, PARP2 | BGB-290 and Temozolomide in Treating Patients With Recurrent Gliomas With IDH1/2 Mutations | Phase 1, Phase 2 |
| NCT03782415 | MN-166, temozolomide | PDE3, PDE4, PD10, PDE11 | Study to Evaluate Ibudilast and TMZ Combo Treatment in Newly Diagnosed and Recurrent Glioblastoma | Phase 1, Phase 2 |

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| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| NCT02331498 | Pazopanib | PDGFRB, FLT4, KDR, KIT, FLT1, PDGFRA, FGF1, FGFR3, ITK, SH2B3 | Phase I/II Study of Pazopanib + Temozolomide in Patients With Newly Diagnosed Glioblastoma Multiforme | Phase 1, Phase 2 |
| NCT03466450 | PF-04449913, temozolomide oral capsule | SMO | Glasdegib (PF-04449913) With Temozolomide Newly Diagnosed Glioblastoma | Phase 1, Phase 2 |
| NCT02765165 | USL311, lomustine | STMN4 | Phase 1/2 Study of USL311 Alone and in Combination With Lomustine in Subjects With Advanced Solid Tumors and Relapsed/Recurrent Glioblastoma Multiforme (GBM) | Phase 1, Phase 2 |
| NCT02770378 | Temozolomide, aprepitant, minocycline, disulfiram, celecoxib, sertraline, captopril, itraconazole, ritonavir, auranofin | TACR1, VEGFA, ALOX5, rpsl, rpsD, MMP9, CASP1, CASP3, CYCS, ALDH2, DBH, PTGS2, PDPK1, SLC6A4, SLC6A3, ACE, MMP2, MMP9, ERG11, CYP51A1, pol, IKBKB, PRDX5 | A Proof-of-concept Clinical Trial Assessing the Safety of the Coordinated Undermining of Survival Paths by 9 Repurposed Drugs Combined With Metronomic Temozolomide (CUSP9v3 Treatment Protocol) for Recurrent Glioblastoma | Phase 1, Phase 2 |
| NCT03119064 | Nanoliposomal irinotecan, temozolomide | TOP1, TOP1MT | BrUOG 329 GBM Onyvide With TMZ | Phase 1, Phase 2 |
| NCT02611024 | Lurbinectedin (PM01183), irinotecan | TOP1, TOP1MT | Pharmacokinetic Study of PM01183 in Combination With Irinotecan in Patients With Selected Solid Tumors | Phase 1, Phase 2 |
| NCT03678883 | 9-ING-41, gemcitabine—21-d cycle, doxorubicin, lomustine, carboplatin, nab-paclitaxel, paclitaxel, gemcitabine—28 d cycle, irinotecan | TOP2A, STMN4, TUBB1, MAP2, BCL2, MAP4, MAPT, TOP1, TOP1MT | 9-ING-41 in Patients With Advanced Cancers | Phase 1, Phase 2 |
| NCT03213002 | Capecitabine, temozolomide | TYMS | Oral Capecitabine and Temozolomide (CAPTEM) for Newly Diagnosed GBM | Phase 1, Phase 2 |

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| NCT01349660 | Bevacizumab, BKM120 | VEGFA | Combination of BKM120 and Bevacizumab in Refractory Solid Tumors and Relapsed/Refractory Glioblastoma Multiforme | Phase 1, Phase 2 |
| NCT02330562 | MRZ, bevacizumab | VEGFA | Stage 1: Marizomib + Bevacizumab in WHO Gr IV GBM; Stage 2: Marizomib Alone; Stage 3: Combination of Marizomib and Bevacizumab | Phase 1, Phase 2 |
| NCT04004975 | Anlotinib | VEGFR2, VEGFR3 | Clinical Study on the Treatment of Recurrent Glioblastoma With Anlotinib | Phase 1, Phase 2 |
| NCT04421378 | Selinexor, temozolomide (temozolomide), lomustine (CCNU) | XPO1, STMN4 | A Study of Selinexor in Combination With Standard of Care Therapy for Newly Diagnosed or Recurrent Glioblastoma | Phase 1, Phase 2 |
| NCT01430351 | Mefloquine, memantine hydrochloride, metformin hydrochloride, temozolomide | ADORA2A, HBA1 | Temozolomide, Memantine Hydrochloride, Mefloquine, and Metformin Hydrochloride in Treating Patients With Glioblastoma Multiforme After Radiation Therapy | Phase 1 |
| NCT02270034 | Crizotinib | ALK, MET | Study to Evaluate Safety and Activity of Crizotinib With Temozolomide and Radiotherapy in Newly Diagnosed Glioblastoma | Phase 1 |
| NCT03535350 | Ibrutinib, temozolomide (temozolomide) | BTK | Ibrutinib With Radiation and Temozolomide in Patients With Newly Diagnosed Glioblastoma | Phase 1 |
| NCT03224104 | TG02, temozolomide | CDK1, CDK2, CDK7, JAK2, CDK9, FLT3, FLK2, STK1 | Study of TG02 in Elderly Newly Diagnosed or Adult Relapsed Patients With Anaplastic Astrocytoma or Glioblastoma | Phase 1 |
| NCT03231501 | Epitinib succinate | EGFR | HMPL-813 in Treating Patients With Glioblastoma | Phase 1 |
| NCT02101905 | Lapatinib, lapatinib ditosylate | EGFR, ERBB2 | Lapatinib Ditosylate Before Surgery in Treating Patients With Recurrent High-Grade Glioma | Phase 1 |

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**Table 1**
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| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| NCT02423525 | Afatinib | EGFR, ERBB2, ERBB4 | Safety Study of Afatinib for Brain Cancer | Phase 1 |
| NCT02974738 | PT2977 | EPAS1 | A Trial of PT2977 Tablets In Patients With Advanced Solid Tumors | Phase 1 |
| NCT03374943 | KB004 | EPHA3 | A Trial of KB004 in Patients With Glioblastoma | Phase 1 |
| NCT00102648 | Lonafarnib, temozolomide | Ftase | Lonafarnib and Temozolomide in Treating Patients With Glioblastoma Multiforme That Is Recurrent or Did Not Respond to Previous Treatment With Temozolomide | Phase 1 |
| NCT03452930 | Tinostamustine | HDAC1, HDAC2, HDAC3, HDAC6 | Tinostamustine With or Without Radiation Therapy in Treating Patients With Newly Diagnosed MGMT-Unmethylated Glioblastoma | Phase 1 |
| NCT00268385 | Temozolomide, vorinostat | HDAC1, HDAC2, HDAC3, HDAC6 | Vorinostat and Temozolomide in Treating Patients With Malignant Gliomas | Phase 1 |
| NCT02381886 | IDH305 | IDH1 | A Study of IDH305 in Patients With Advanced Malignancies That Harbor IDH1R132 Mutations | Phase 1 |
| NCT03514069 | Ruxolitinib, temozolomide | JAK1, JAK2 | Ruxolitinib With Radiation and Temozolomide for Grade III Gliomas and Glioblastoma | Phase 1 |
| NCT02133183 | Sapanisertib | MTOR | Sapanisertib Before and After Surgery in Treating Patients With Recurrent Glioblastoma | Phase 1 |
| NCT02142803 | Sapanisertib | MTOR | TORC1/2 Inhibitor MLN0128 and Bevacizumab in Treating Patients | Phase 1 |

| | | | | |
|---|---|---|---|---|
| | | | With Recurrent Glioblastoma or Advanced Solid Tumors | |
| NCT02238496 | Perifosine, temsirolimus | MTOR | Perifosine and Torisel (Temsirolimus) for Recurrent/Progressive Malignant Gliomas | Phase 1 |
| NCT03749187 | PARP inhibitor BGB-290, temozolomide | PARP1, PARP2 | BGB-290 and Temozolomide in Treating Isocitrate Dehydrogenase (IDH)1/2-Mutant Grade I-IV Gliomas | Phase 1 |
| NCT03426891 | Pembrolizumab, vorinostat, temozolomide | PDCD1, HDAC1, HDAC2, HDAC3, HDAC6 | Pembrolizumab and Vorinostat Combined With Temozolomide for Newly Diagnosed Glioblastoma | Phase 1 |
| NCT04205357 | Sulfasalazine | PTGS1, PTGS2, ALOX5, CHUK, IKBKB, SLC7A11, ACAT1, TBXAS1, PLA2G1B | Sulfasalazine and Stereotactic Radiosurgery for Recurrent Glioblastoma | Phase 1 |
| NCT03463733 | Hydroxyurea, temozolomide | RRM1 | Hydroxy-urea and Temozolomide in Patients With a Recurrent Malignant Brain Tumor (Glioblastoma) | Phase 1 |
| NCT03587038 | OKN 007, temozolomide | SULF2 | OKN-007 in Combination With Adjuvant Temozolomide Chemoradiotherapy for Newly Diagnosed Glioblastoma | Phase 1 |
| NCT02192359 | Irinotecan, irinotecan hydrochloride | TOP1, TOP1MT | Carboxylesterase-Expressing Allogeneic Neural Stem Cells and Irinotecan Hydrochloride in Treating Patients With Recurrent High-Grade Gliomas | Phase 1 |
| NCT02644291 | Mebendazole | TUBA1A, TUBB4B | Phase I Study of Mebendazole Therapy for Recurrent/Progressive Pediatric Brain Tumors | Phase 1 |
| NCT01729260 | Mebendazole | TUBA1A, TUBB4B | Mebendazole in Newly Diagnosed High-Grade Glioma Patients Receiving Temozolomide | Phase 1 |
| NCT02669173 | Capecitabine, bevacizumab | TYMS, VEGFA | Capecitabine + Bevacizumab in Patients With Recurrent Glioblastoma | Phase 1 |

(*continued on next page*)

**Table 1**
**(*continued*)**

| NCT Number | Drugs | Targets | Title | Phases |
|---|---|---|---|---|
| NCT03722342 | TTAC-0001 and pembrolizumab combination | VEGFR2 | TTAC-0001 and Pembrolizumab Combination phase1b Trial in Recurrent Glioblastoma | Phase 1 |
| NCT01849146 | Adavosertib, temozolomide | WEE1 | Adavosertib, Radiation Therapy, and Temozolomide in Treating Patients With Newly Diagnosed or Recurrent Glioblastoma | Phase 1 |
| NCT04216329 | Selinexor, temozolomide | XPO1 | Selinexor (KPT-330) in Combination With Temozolomide and Radiation Therapy in Patients With Newly Diagnosed Glioblastoma | Phase 1 |

sectional study reported that 8.33% of 609,640 patients in 2018 were eligible for targeted treatment, but only 4.9% of all patients had a clinical benefit.[42] These numbers likely are even lower in GBM due to a multitude of issues, discussed previously, including impaired drug delivery because of the BBB, intratumoral genetic and transcriptional heterogeneity, redundant activating pathways or escape mechanisms, and inherent therapeutic resistance.[10,43]

The BBB presents a unique challenge in that it restricts the entry of more than 95% of FDA-approved drugs into the central nervous system, thereby preventing the delivery of therapeutic drug concentrations to brain cancer. Accordingly, targeted molecular therapies considered for clinical trials should demonstrate therapeutic levels within the brain and the entire tumor volume (both enhancing and nonenhancing).[9]

The genetic and transcriptional heterogenicity of GBM presents a challenge to targeting therapy in that subpopulations can respond to selective evolutionary pressures of targeted therapy, thereby resulting in treatment resistance.[10] Single-cell analysis studies found that frequently there are multiple subtypes (mesenchymal, classical, and so forth) within 1 GBM, including a population harboring stem cell properties.[43] Perceived potentially actionable alterations could be passenger mutations, instead of driver mutations amenable to therapy.[44] A notable example is that EGFR is overexpressed in 50% to 60% of GBM patients making it historically an attractive target. EGFR tyrosine kinase inhibitors and monoclonal antibody targeting EGFR, however, have failed to show clinical activity.[10] A later attempt to address intertumoral heterogenicity with a combination of EGFR tyrosine kinase inhibitor and mTOR inhibitors lead to dose-limiting toxicity and no therapeutic response.[20] Additionally, initial responses to the targeting of driver mutations often lack a durable treatment effect that has been reported in many cases.[28,38,45]

Further complicating the picture and targeted therapy for cancer, in general, are recent investigations showing that off-target toxicity rather than the on-target effects are responsible for the antitumor efficacy. A study using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 mutagenesis evaluated a set of cancer drugs and drug targets and found that the effectiveness of drugs was unaffected by the loss of its putative target, indicating that these compounds kill cells via off-target effects. Therefore, providing experimental validation of the mechanism of action of cancer drugs in the preclinical setting would be critical before embarking on a clinical trial. Such verification may help decrease the number of therapies tested on humans that fail to provide any clinical benefit.[46] These challenges underscore the need to develop complementary approaches to direct targeting.

### *Synthetic Lethality and Future Approaches to Biomarker-Driven Strategies in Glioblastoma*

A large number of currently active clinical trials (see **Table 1**) include a molecular targeting component and can be broadly divided into 2 classes: (1) trials whose eligibility criteria are based on the specific genetic alterations being targeted (for example, *BRAF*V600E, *EGFR*vIII, and *IDH1* R132H), and (2) trials that target pathways frequently amplified over the disease course or as a response to treatment (for example, angiogenesis pathways or DNA repair pathways). The number of patients who can benefit from targeting specific genetic alterations in GBM is small. Many alterations in GBM are loss of function mutations or deletions, which makes their direct targeting difficult. The situation can be partially alleviated by expanding molecular testing to include gene expression profiling. The WINTHER trial (NCT01856296), which enrolled primarily patients with colon, head, and neck, and lung cancer, demonstrated that transcriptomic profiling can expand personalized cancer treatment.[47,48] The success of targeting amplified pathways requires elucidation of the biological mechanisms that are being affected by targeting, identification of predictive biomarkers of response, and inclusion of such biomarkers' status in the eligibility criteria to identify the patients most likely to benefit from the therapy. As illustrated by the failure of many antiangiogenesis therapies to elicit a sustained response in GBM, targeting biological pathways essential for survival is likely to activate compensatory mechanisms that ensure cell survival.[49] In this situation, treatment can be effective only when such compensation is disabled either by the disease (inactivating mutation or gene deletion) or by targeted therapy.

Molecular targeting often works best where the requirement for the target is increased in cancer cells compared with normal cells, due to either intrinsic genetic or epigenetic changes in the cancer cells or extrinsic microenvironmental changes.[50,51] One such dependency that can be exploited for therapeutic benefit is the dependency between 2 synthetic lethal partner genes: the loss of each gene individually can be tolerated by the cell, but their simultaneous loss leads to cell death (**Fig. 1**). For cancer cells in which 1 of the synthetic lethal partners is lost (via mutation or

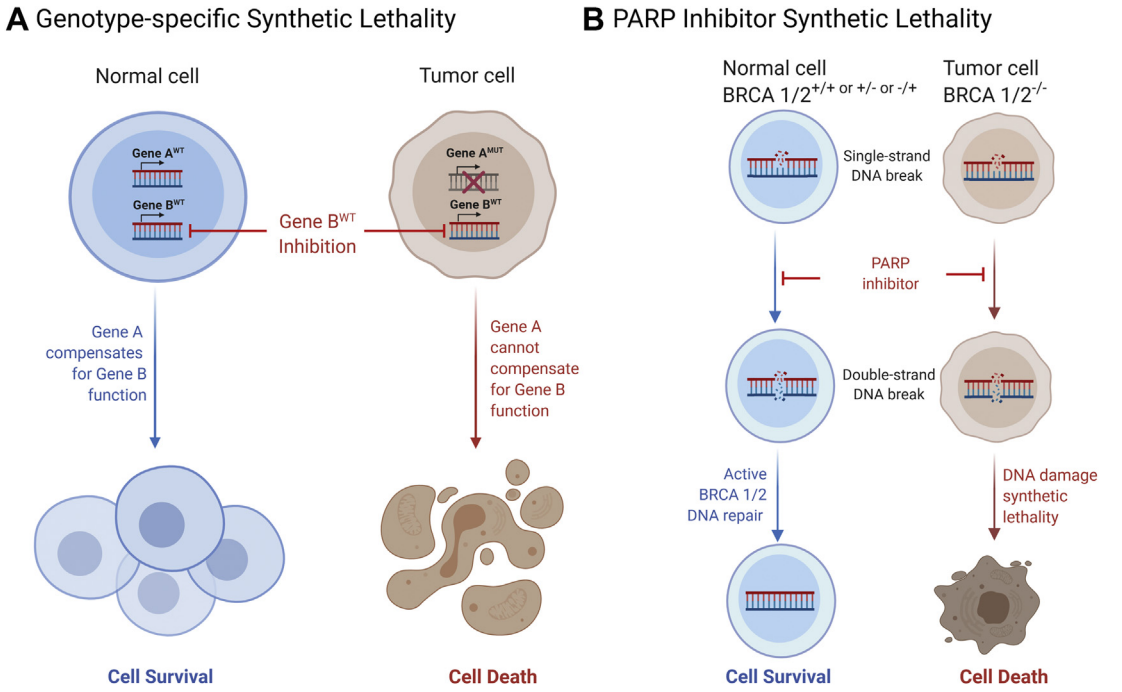


**Fig. 1.** Synthetic lethality. (*A*) Synthetic lethality arises when simultaneous loss of 2 genes results in cell death. (*B*) PARP inhibition is a selective anticancer therapy for BRCA1/2 mutant cancer cells. Created with BioRender.com. MUT, mutant; WT, wild-type.

deletion), targeting the second partner provides an effective and selective anticancer strategy: the cancer cells cannot tolerate the loss of the second partner, whereas normal cells largely are unaffected.[50] The first-discovered and most effective to-date anticancer therapy that exploits synthetic lethal interactions is inhibition of PARP (PARP1/PARP2) in breast cancer patients with germline mutations in BRCA1 and BRCA2.[13,14] PARP senses single-strand breaks in DNA and induce DNA damage response; its inhibition leads to accumulation of single and double-strand breaks in DNA. Loss-of-function mutations of BRCA1/2, which are required for homologous recombination and DNA break repair, renders cells unable to repair the accumulated DNA damage, and induces apoptosis (see **Fig. 1**). PARP inhibition has been considered a therapeutic approach in the context of non-BRCA1/2 mutations in situations where cells have increased reliance on homologous recombination for survival (either due to cytotoxic stress induced by treatment or reactive oxygen species, or other DNA repair enzyme mutations). This concept is being tested in brain tumors. For example, NCT03212274 is trialing PARP inhibition in advanced IDH1/2 mutated gliomas (because 2-hydroxygluterate produced by neomorphic IDH has been reported to suppress homologous recombination), and NCT02152982 is trialing PARP inhibition in combination with temozolomide in newly diagnosed GBM.[26]

Several preclinical studies have demonstrated the potential of targeting other synthetic lethal interactions as anticancer therapies.[51–53] Barbie and colleagues,[51] for example, discovered that *TBK1* (encoding the tank binding kinase) is essential in *KRAS* mutation–driven cell lines. Chan and colleagues[52] showed that cancers with microsatellite instability depend on *WRN* helicase. The depletion of WRN-induced double-stranded DNA breaks and promoted apoptosis and cell-cycle arrest selectively in these models.[52] To date, however, few clinical studies have managed to exploit such interactions beyond PARP inhibition.[54] Some of the challenges associated with successfully translating these principles include the difficulty in experimentally determining synthetic lethal interactions, which theoretically entails knocking down all possible pairs of genes; the inability of preclinical models to fully recapitulate the patient disease; and the existence of multiple compensatory mechanisms, which lowers the magnitude of response (leading to synthetic sickness rather than death when a pair of genes is downregulated). In particular, the magnitude of the response may itself be dependent on a larger

molecular context rather than be uniform across tumor subtypes.[55] Similarly, subclonal heterogeneity affects the likelihood of response.[56]

Given the limitations of preclinical testing and the extensive tumor heterogeneity, predicting robust synthetic lethal relationships is imperative for successfully translating the promise of synthetic lethal targeting. The advent of high-throughput screening and gene editing technologies facilitates large-scale screening and identification of synthetic pairs in vitro models.[57–60] The limitations of the experimental approaches are being overcome through computational and machine learning approaches that leverage knowledge from yeast screens, protein-protein information networks, metabolic and functional pathways, and biological principles.[61–63] The accumulation of large multiomics patient-tumor derived data sets from projects like TCGA enables novel integrative computational approaches that strengthen predictions through evidence from orthogonal data sources.[64–66] For example, Lee and colleagues'[65] approach of identification of clinically relevant synthetic lethality (ISLE) sequentially filters putative synthetic lethal pairs by taking into consideration evidence from cell line screens, evidence of negative pressure for selection of disabled putative pairs as gauged by lower than expected frequency of encountering such pairs in patient tumors, evidence of lower viability of tumors that exhibit disabled putative pairs and that can be assessed through the association of such disabled pairs with longer overall survival of the patients harboring such tumors, and evolutionary relatedness of the genes in a pair, which can indicate similarity of function.[65] Crucially, approaches like ISLE enable predicting targeted drug response for individual samples based on the genomic or transcriptomic status of the target's predicted synthetic lethal partners in the sample, effectively stipulating and improving the eligibility criteria for patient enrollment in clinical trials.

## SUMMARY

Despite the advances and successes of molecularly targeted therapies in other malignancies, GBM remains among the most difficult to treat cancers, due to its robust heterogenicity and presence of the BBB preventing adequate delivery of most systemically administered agents. Traditional molecular targeted therapies work only in rare subsets of patients harboring a tumor with a true driver genomic alteration that continues to be required for tumor cell survival. Such driver targets, however, are unlikely to be identified for most brain tumors. Therefore, complementary approaches that incorporate a larger genomic context in the decision process may overcome the limitations of direct targeting and deserve further investigation. The maturation of a master protocol incorporating multicenter clinical trial designs as exemplified by National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) (encompassing 40 treatment arms and spanning more than 1100 clinical centers) combined with advances in next-generation sequencing technologies that are enabling extensive molecular profiling of tumors are providing unprecedented opportunities to make the next-generation brain cancer trials transformative.

## CLINICS CARE POINTS

- Molecular evaluation of GBM is the standard of care for diagnostic clarity and identification of potential druggable alterations
- Targeted therapy benefits few GBM patients due to immense molecular heterogeneity
- Delivery of targeted drugs at therapeutic concentrations often is impeded by the BBB, making it essential to demonstrate therapeutic levels of drug within the brain and entire tumor volume in preclinical studies
- The therapeutic benefit seen with a small subset of GBM patients indicate that robust molecular markers and patient selection are critical
- Novel complementary treatment approaches based on synthetic lethal interactions may expand the promise of precision oncology

## DISCLOSURE

The authors have nothing to disclose.

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