Small Molecule Inhibitors in Adult High-Grade Glioma: From the Past to the Future

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Glioblastoma is the most common primary malignant tumor in the brain and has a dismal prognosis despite patients accepting standard therapies. Alternation of genes and deregulation of proteins, such as receptor tyrosine kinase, PI3K/Akt, PKC, Ras/Raf/MEK, histone deacetylases, poly (ADP-ribose) polymerase (PARP), CDK4/6, branched-chain amino acid transaminase 1 (BCAT1), and Isocitrate dehydrogenase (IDH), play pivotal roles in the pathogenesis and progression of glioma. Simultaneously, the abnormalities change the cellular biological behavior and microenvironment of tumor cells. The differences between tumor cells and normal tissue become the vulnerability of tumor, which can be taken advantage of using targeted therapies. Small molecule inhibitors, as an important part of modern treatment for cancers, have shown significant efficacy in hematologic cancers and some solid tumors. To date, in glioblastoma, there have been more than 200 clinical trials completed or ongoing in which trial designers used small molecules as monotherapy or combination regimens to correct the abnormalities. In this review, we summarize the dysfunctional molecular mechanisms and highlight the outcomes of relevant clinical trials associated with small-molecule targeted therapies. Based on the outcomes, the main findings were that small-molecule inhibitors did not bring more benefit to newly diagnosed glioblastoma, but the clinical studies involving progressive glioblastoma usually claimed “noninferiority” compared with historical results. However, as to the clinical inferiority trial, similar dosing regimens should be avoided in future clinical trials.

Keywords: glioblastoma, small molecule inhibitor, molecular mechanism, TKI—tyrosine kinase inhibitor, clinical trial

Abbreviations: GBM, glioblastoma multiforme; TCGA, The Cancer Genome Atlas; GSCs, glioma stem cells; NCCN, National Comprehensive Cancer Network; TMZ, Temozolomide; RT, Radiotherapy; Bev., Bevacizumab; EGFR, Epidermal growth factor receptor; OS, Overall survival; mOS, Median overall survival; PFS, Progressive free survival; mPFS, Median progressive free survival; OR, Response rate; PR, partial response; CR, complete response; SD, stable disease; RTK, Receptor tyrosine kinase; TKI, Tyrosine kinase inhibitor; nRTK, Nonreceptor tyrosine kinase; BBB, Blood-brain barrier; HDAC, Histone deacetylases; mTOR, mammalian target of rapamycin; PTEN, Phosphatase and tensin homolog; PARP, Poly (ADP-Ribose) polymerase; Grb,2Growth factor receptor-bound protein 2; GPCRs, G Protein-Coupled Receptors; NHEJ, Nonhomologous DNA end joining; HR, Homologous recombination repair system; BER, Base excision repair.
INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor. Based on the data from 2011 to 2015 in the United States, the average annual age-adjusted incidence of GBM is 3.21 per 100,000 population, with an overall prevalence of 9.23 per 100,000 population (1). Maximal safe surgical resection followed by radiotherapy with concomitant adjuvant temozolomide has gradually become the standard regimen since 2005 (2). However, after standard therapy, almost every patient recurs within 6 months, and there is no effective therapy for recurrent GBM (3, 4). Although plenty of effort has been made toward studying the mechanisms of pathogenesis and progression of glioma, there was no significant change in treatment regimen and survival for GBM patients. In the past 20 years, targeted cancer therapies were promising methods, and simultaneously, various targeted drugs gradually entered clinical trials and then were approved by the FDA for cancer treatment.

Gliomas were deemed to derive from neural stem cells and, based on different classification criteria, can be generally divided into circumscribed gliomas and diffuse gliomas, or into low-grade (WHO I, II) and high-grade (WHO III, IV), or adult-type and pediatric-type (5–7). Furthermore, adult high-grade diffuse glioma affects the most people and is also the hardest to cure, predominantly including GBM (WHO IV) and anaplastic glioma (AG, WHO III, including AOD: anaplastic oligodendroglioma and AA: anaplastic astrocytoma) and accounts for approximately 80%. In 2008, Parsons et al. computed by the TCGA database and proposed three core alterations of signaling pathways in GBM: P53, retinoblastoma pathway (pRB), and receptor tyrosine kinases (RTKs) (8). Besides, mutation of phosphatase and tensin homolog deleted on chromosome ten (PTEN), neurofibromatosis (NF1), isocitrate dehydrogenase (IDH), B-Raf Proto-Oncogene (BRAF), and chromosome 1p19q co-deleted are common in glioma, even being used for molecular classification and prognosis prediction. In 2016, it was the first time the WHO Classification of CNS Tumors used molecule profiles to define the sub-types of gliomas. It is an important way to translate the abundant knowledge of the molecular mechanisms to clinical applications (9). But regardless of IDH mutation, 1p/19q co-deletion, and H3 K27 mutation, the therapy regimen is similar and the classification method plays a limited role in guiding treatment. On the other hand, all the typical hallmarks of cancer can be detected in GBM, especially angiogenesis, tumor-promoting immune microenvironment, and reprogramming metabolism, which is gradually getting attention (10). The relative mechanisms of the hallmarks become therapeutic targets (Figure 1). Over the last two decades, since the specimen which was capable of generating tumor cells containing multiple lineage markers was reported, the concept of glioma stem cells (GSCs) has been more and more accepted by scientists (11, 12). GSCs are ascribed to a population accounting for heterogeneity in glioma mass and therapeutic resistance through self-renewal and differentiation to variant subpopulations. The elucidation of features of GSCs may be a key step in developing next-generation therapies (13). However, GSCs were not a bulk of homogeneous cells but rather a mosaic of discrete populations with distinct features (14). The complexity of GBM requires us to more deeply understand the mechanisms, in particular, the genetic and phenotypic characteristics of subclones distributed in the mass of tumor (15, 16).

NCCN guidelines on central nervous system cancers indicate that the preferable treatment option for patients with recurrent
GBM is enrollment in clinical trials due to the dismal outcomes of other therapies, including carmustine/lomustine, TMZ, radiotherapy, and bevacizumab (Bev.). There are more than 1,500 clinical trials associated with glioma completed or ongoing, among which contain hundreds of targeted therapies (https://clinicaltrials.gov/, Figure 2). Targeted therapies predominantly include two methods: small molecule and monoclonal antibodies. Simultaneously, various gene therapies such as microRNA, lncRNA, and exosome are a hotspot of research. Compared with monoclonal antibodies, small-molecule inhibitors possess several exclusive characteristics: reduced financial burden, taken orally, and more extensive druggable targets, including intracellular proteins (17). According to the targets of the small molecule inhibitors, we divided them into three main categories: tyrosine-kinase inhibitor (TKI), non-receptor tyrosine kinase (nRTK), intracellular signal transduction pathway inhibitor, and cellular biological process inhibitor. TKIs are still the most studied small-molecule inhibitors. But the outcomes of TKIs for the treatment of GBM and AG are often disappointing. An important reason for this is the complex network of intracellular signaling pathways leading to drug resistance. Besides, interference with biological processes and impact on the hallmarks of tumor growth can inhibit the growth of tumors through different mechanisms. In this review, we revisited the outcomes of recent clinical trials of adult high-grade glioma (mainly including GBM and AG) published in PubMed and the functional mechanisms of small-molecule inhibitors (Additional File 1). We hope to provide advice on the combination of drugs in future clinical trials by understanding the characteristics of different drugs.

**RTK**

Receptor tyrosine kinases (RTKs) and the downstream signal transduction are the most characterized networks associated

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**FIGURE 2** | The time line of publicly published clinical trials of the small-molecule inhibitors.
with glioma pathogenesis and progression. To date, scientists have found more than 60 RTKs such as EGFR, VEGFR, MET, PDGFR, and FGFR (18, 19). Mutations in RTKs are also the most frequent alterations in GBM, namely, copy number variation, structure variation, nucleotide variation, and over-activation of the autocrine growth factor/receptor loop (18, 20). And then RTKs cause deregulation of several important pathways, namely, Ras/MAPK, PI3K/Akt/PTEN, PLC gamma/PKC, and Jak/STAT3 (21, 22). For instance, more than 50% of GBM harbor EGFR amplification and 25% cause EGFR mutation (23). EGFRvIII, a common mutant of EGFR which is continually activated even without ligand binding to the receptor, occurs after the pathogenesis of GBM to change the pattern of tumor growth and increase the intra-tumoral heterogeneity and resistance to targeted therapies (23). PDGFR, VEGFR, and FGFR are the most important RTKs associated with tumor angiogenesis (24). c-Met, also known as mesenchymal–epithelial transition factor, plays an important role in migration, therapy resistance, and vasculogenesis. In addition, Ret, c-Kit (CD177), and Flt3 (CD135) are also the targets of TKIs. The dynamic change of variant RTKs over time and space in tumor entities accounts for the complexity of GBM and therapy resistance after recurrence (3). RTKs also regulate the expression of GSC relevant transcription factors such as OLIG2, SOX2, and ZEB, which are also GSC marker molecules (25, 26).

According to the chronological order, TKIs include three categories, and up to now, the most studied TKIs in glioma are predominantly first and second generation. The first-generation EGFR inhibitors, also known as type I, including erlotinib and gefitinib, can reversely bind to the ATP-binding site of EGFR to inhibit the activity of the receptor (27, 28). The second-generation inhibitors, such as afatinib, neratinib, vandetanib, and dacomitinib, irreversibly inhibit EGFR. Besides EGFR, the second-generation inhibitors can often inhibit multiple other RTKs, such as Her2, PDGFR, MET, and VEGFR (29–32). The third-generation inhibitor, Osimertinib, is used to overcome the resistance due to the T790M mutation, which is usually acquired after treatment with first-generation EGFR inhibitors. But researchers found that the acquired exon 20 C797S mutation would lead to resistance to Osimertinib within 10 months (33). So, the next-generation inhibitor, such as EAI045, is proposed to solve the problem of T790M mutation and C797S mutation inducing resistance (33). sorafenib, anlotinib, cabozantinib, cediranib, imatinib, lenvatinib, sunitinib, pazopanib, and motesanib enter clinical trials in glioma after approval by the FDA for renal cell carcinoma, lung cancer, thyroid cancer, and other solid tumors. They can target PDGFR, FGFR, C-kit, MET, and some nRTKs, which play great roles in the growth of GBM (Figure 3).

Until June 2021, there have been approximately 85 clinical trials of diverse TKIs to treat GBM and AG published in PubMed, including 17 studies for newly diagnosed GBM and 68 studies for progressive GBM. When comparing the results extracted from the publication with the baseline (historic results of 9 important clinical trials), there are 27 of 40 studies that show improvement in radiological response rates (ORs). These studies mainly involved patients with recurrent or progressive GBM. PFS was evaluated as mPFS in 71 studies and as PFS6 in 61 studies. There were 6 of 17 studies involving newly diagnosed GBM and 13 of 63 studies involving recurrent GBM that had an improvement in mPFS compared with baseline. OS was evaluated as mOS in 72 studies and as OS12 in 22 studies. But the most urgent problem is the only improvement of PFS while infrequently increasing the OS (Additional File 1).

Erlotinib is the most studied TKI in glioma. A phase II trial in 2014 that added Bev. and erlotinib to TMZ after completion of radiation involving 59 patients with newly diagnosed GBM showed a amazing result (mPFS: 13.5 m; mOS: 19.8 m), although the study did not reach the primary endpoint of improved OS (34). A study in 2010 using erlotinib as monotherapy for recurrent GBM had 40% PFS6 and 53% OS12 with 14 months of mOS (35). In summary, regardless of

| Agent   | VEGFR | PDGFR | FGFR | RET | C-kit | Flt3 | Other         |
|---------|-------|-------|------|-----|-------|------|---------------|
| Apatinib| ✔     | ✔     | ✔    | ✔   | ✔     |      | Src           |
| Vandetanib| ✔   | ✔     |      |     |       |      |               |
| Sorafenib| ✔   | ✔     | ✔    | ✔   | ✔     |      | Raf           |
| Imatinib | ✔     | ✔     |      |     |       |      | Bcl-Abl       |
| Cediranib| ✔   | ✔     |      |     | ✔     |      |               |
| Sunitinib| ✔     | ✔     |      |     |       | ✔    |               |
| Pazopanib| ✔     | ✔     | ✔    |     | ✔     |      |               |
| Vatalanib| ✔     | ✔     | ✔    |     | ✔     |      |               |
| Regorubicib| ✔   | ✔     | ✔    | ✔   | ✔     |      | CSF-1R        |
| Dasatinib| ✔     | ✔     | ✔    |     | ✔     |      | Bcr-Abl/Src   |
| Ponatinib| ✔     | ✔     | ✔    | ✔   | ✔     |      | Src           |

**FIGURE 3** | A table which summary the major targets of Pan-TKIs.
monotherapy or combined regimen, the result did not reach the expected outcome. And the improvement of ORs was partly deemed to be unauthentic because of improper imaging criteria (McDonald criteria). Combining erlotinib with other small-molecule inhibitors, such as sorafenib and sirolimus, did not play a greater role. Maybe due to the toxicity of sirolimus and its derive temsirolimus, erlotinib combined with sirolimus decreased the PFS and OS in a phase II trial for recurrent malignant glioma (36, 37). Sorafenib is a pan-TKI, and it can also effectively inhibit the Ras pathway. Based on hypothesizing that inhibition of both EGFR and Ras would improve the survival time of patients with recurrent GBM, a phase II trial in 2013 was launched but did not reach the effective hypothesis H0 (30% increase in overall survival time compared with historical controls) (38). More clinical trial results will be helpful to clarify the efficacy of the combination of sorafenib and erlotinib. To date, the studies of erlotinib in glioma have stopped in phase II trials, and there is not enough evidence to show the potential efficacy of erlotinib.

Gefitinib is another well-characterized first-generation EGFR inhibitor. Just like erlotinib, it is more effective in cancers with mutated and overactive EGFR. Gefitinib did not show the potential to increase the OS of glioma. In 2012, a phase I/II trial involving 178 newly diagnosed GBM combining gefitinib with radiotherapy showed a disappointing result (mPFS: 4.9 m, mOS: 11.5 m) compared to baseline (38). Two important trials using gefitinib monotherapy for recurrent CNS tumor (NCT00025675, 2003) or combination with radiation for newly diagnosed GBM (NCT00052208, 2003) involving 105 and 158 patients respectively did thus far not show their results. There are no more results to evaluate the efficacy of gefitinib. As such, the outcomes of two classic first-generation EGFR inhibitors are a little disappointing. The insensitivity of GBM to erlotinib and gefitinib is partly due to the different models of EGFR overactivity in GBM cells. The two first-generation TKIs have a higher inhibiting ability of mutant EGFR such as L858R and exon 19 deletion type EGFR, which increases the kinase activity. In contrast to non-small cell lung cancer and adenocarcinoma harboring activating nucleotide mutations within the EGFR kinase domain, GBM overly activates EGFR by the increasing copy number and the substituted mutations occur in the extracellular domain (39) (Figure 4).

Lapatinib can selectively inhibit EGFR and HER2. Lapatinib combined with TMZ is a potential salvage regimen for recurrent ependymoma due to a phase II study (NCT00826241) demonstrating clinical activity with objective responses and prolonged disease control associated with disease-related symptom improvements (40). To date, three clinical trials including 74 patients did not present good results (41–43).

Afatinib is a second-generation irreversible ErbB family inhibitor, mainly used to treat cases of NSCLC that harbor mutations in the EGFR gene. Simultaneously, afatinib can effectively block EGFRvIII, a common mutant occurring in glioma (23, 44). A phase I study (NCT00977431) of afatinib combined with chemoradiotherapy demonstrated a favorable objective rate but simultaneously resulted in a high rate of serious adverse events. In 2015, a parallel-arm phase II trial (NCT00727506) showed that the addition of afatinib significantly decreased the PFS6 compared with TMZ alone (44). In the future, a pulsatile-increased dosing schedule of afatinib will be tested in glioma. It may be feasible to make a difference and reduce the adverse effects (44).

Vandetanib acts as a kinase inhibitor of several receptors, including VEGFR, EGFR, and the RET-tyrosine kinase. Because of the dual inhibition of EGFR and VEGFR2, vandetanib was supposed to play a greater role than combining erlotinib and Bev. in inhibiting angiogenesis. The prevailing rates and PFS usually showed favorable increases. A phase I/II study (NCT00441142) of parallel arm design demonstrated that adding vandetanib to TMZ and radiation can modestly increase the OS (45). These results give researchers the faith to continue their clinical studies. There are more than 7 phase I studies ongoing.

Sorafenib is a classical pan-TKI, targeting VEGF, PDGFR, FGFR, c-Kit, Flt3, and RAF kinases, which are important in the angiogenesis of solid tumors. Pan-TKI is a trend of development of small-molecule inhibitors because of the complex crosstalk between various growth factors and compensatory mechanisms of RTKs. GBM cells can rapidly switch their signaling addiction from reliance on a single RTK to multiple RTK pathways. This may be driven by glioma stem cells (46). In general, sorafenib did not demonstrate sufficient efficacies to improve the outcome of patients with glioma. Many phase II trials (NCT00597493, NCT00544817, and NCT00621686) that have added sorafenib into the Stupp protocol or Bev. showed disappointing outcomes, even those inferior to Bev. monotherapy (47–49). In addition, sorafenib combined with erlotinib can play a synergic role by inhibiting EGFR and other RTKs in theory. But a phase II study (NCT00445588, 2013) using the combination regimen only showed an OS of 5.7 months and a PFS6 rate of 14% (38). Sorafenib combined with mTOR inhibitors such as temsirolimus did not improve the outcome in two phase I/II studies (NCT00335764, NCT00329719). Many clinical trials evaluating targeted combinatorial blockade frequently have disappointing outcomes due to overlapping toxic events, including serious diarrhea, seizure, and rash. Alternative administration schedules will probably reduce toxicity (17).

Imatinib is a specific inhibitor of several tyrosine kinase enzymes, including ABL, c-Kit, and PDGFR by occupying the tyrosine kinase active site to decrease the activity of kinases. PDGFR is deemed as a driven gene in low-grade glioma and is overexpressed in GBM. Imatinib has limited efficacy as monotherapy in GBM, and the chemotherapeutic agent hydroxyurea (HU) was found to play a synergic role when combined with imatinib due to imatinib sensitizing cancer to chemoradiotherapy and the penetrability of the blood–brain barrier (BBB) of HU (50). A multi-center phase II study (NCT00290771) involving 231 patients with progressive GBM showed a good safety profile among patients, which receiving up to 1,000 mg of imatinib and 1,000 mg of hydroxyurea. But the efficacy is not enough to induce anti-tumor (51). A phase III study (NCT00154375) involving 240 patients with TMZ-resistant GBM presented similar results of not meeting the
primary study end point despite a good safety profile (52). Thus far, imatinib has not brought good news for glioma patients, just like in CML.

Cediranib is mainly a VEGFR inhibitor, and later the inhibiting c-Kit was found. Cediranib had a narrower inhibiting spectrum of RTKs than other pan-TKIs. But just because of this, cediranib has the chance to function as anti-VEGF therapy in combination with chemoradiotherapy for newly diagnosed GBM (NCT00662506 in 2008; NCT01062425 in 2010). The adverse effects of multiple TKIs are predominantly driven by the inhibition of VEGFR (17). The anti-angiogenesis and normalization of micro-vessels driven by anti-VEGF such as Bev. are deemed to sensitize patients to radiotherapy by increasing the supply of oxygen (53). The impact of angiogenesis inhibition on tumor distribution of TMZ is also a key factor considering the combination of standard therapy and anti-angiogenesis drugs (54).

Sunitinib inhibits all subtypes of PDGF-Rs and VEGFRs and c-Kit, RET, so that the safety is disquieting (55). Recently, sunitinib has mainly functioned as a monotherapy in clinical trials of CNS tumors, but the outcomes of patients were dismal. A phase II study (NCT00923117) of sunitinib to treat recurrent brain cancer has been terminated because of an unexpected result. The OS of Bev. resistant patients is 0.92 months and that of Bev. naive patients is 1.08 months. The regimen of sunitinib monotherapy caused high adverse event rates of 100% with more than 30% serious adverse events, such as skin, mucosal, and gastrointestinal adverse events (56). Bev. is a unique targeted therapy drug recently approved by the FDA for recurrent GBM. The various TKIs are considered in combination with Bev. or to be used in Bev.-resistant patients, even the VEGFR inhibitors. Continuous schedules enhance the efficacy of sunitinib. How to balance the dose plan and safety is the key factor to acquiring a satisfactory outcome.

![Image](image_url)

**FIGURE 4** | In contrast to lung cancer, glioma majorly harboring amplification of EGFR, instead of nucleotide mutation (cBioPortal). (A) The EGFR alteration frequency in different tumor types based on the PanCancer study “Pan-cancer analysis of whole genomes (ICGC/TCGA, Nature 2020)” in cBioportal database. (B) The collection of genetic alteration of EGFR based on four different studies in cBioportal database.
Pazopanib limits tumor growth by targeting angiogenesis via the inhibition of VEGFR, PDGFR, c-KIT, and FGFR (57, 58). A phase II study (NCT00459381) involving 35 patients with recurrent GBM was disappointing (59). Adding chemotherapeutic drug topotecan with pazopanib did not significantly increase the OS of patients with recurrent GBM (NCT01931098) regardless of prior Bev. exposure or not. Simultaneously inhibition of EGFR and other RTKs increased the efficacy and theoretically led to a series of phase I trials to evaluate the safety and maximum toxic dose of the combination of EGFR inhibitors and pazopanib (60–62). But the accurate efficacy of GBM warrants more studies.

Vatalanib was designed for advanced cancers, especially those that have not responded to chemotherapy. It inhibits all known VEGF receptors, as well as PDGFR-beta and c-kit, but is most selective for VEGFR-2 (63). The outcomes of two phase I trials for newly diagnosed GBM were not poor compared with baseline, but the efficacy of vatalanib for treating GBM needs to be tested in more clinical trials.

Regorafenib, as a sorafenib derivative and pan-TKI of several kinases involved in tumor angiogenesis (VEGFR1–3 and Tie2), oncogenesis (KIT, RET, RAF1, and BRAF genes), the tumor microenvironment (PDGFR and FGFR), and tumor immunity (colony-stimulating factor 1 receptor), is recommended in the newest NCCN guideline for testing in future clinical trials due to (colony-stimulating factor 1 receptor), is recommended in the oldest NCCN guideline for testing in future clinical trials due to oncogenesis (KIT, RET, RAF1, and BRAF genes), the tumor microenvironment (PDGFR and FGFR), and tumor immunity (colony-stimulating factor 1 receptor). In summary, (i) first EGFRi such as gefitinib and erlotinib, and PDGFRi imatinib may play great roles in specific patients with over-acting EGFR (especially EGFR with kinase domain mutation) and PDGFR. However, Pan-TKIs, such as sorafenib, sunitinib, and regorafenib did not show enough efficacy thus far (Table 1). (ii) Afatinib, which block EGFRVIII, a common structure mutation occurring after over-activating wild-type EGFR. The judgment of the time window of afatinib is a challenge of deeply understanding of heterogeneity in GBM temporally and regionally. (iii) VEGFR is a more important target than EGFR for GBM therapy. Anti-angiogenesis is a promising method including vandetanib, cediranib, vatalanib, and cobanzotinib, just like Bev. Normalizing addition, regorafenib can induce autophagy arrest by the PSAT1/PRKAA autophagy-initiating pathway (65). By 2015, it had two US approvals for advanced cancers. However, the conclusion from the phase II study was defective. The outcome of regorafenib was just better than that of the control arm in the trial instead of baseline (66).

Many other TKIs are being tested in clinical trials, namely, anlotinib, cobanzotinib, dovitinib, vatalanib, tandutinib, lenvatinib, and nintedanib, but so far, there are few trials with outcomes of these small-molecule inhibitors. Some TKIs have been approved by the FDA for other types of cancer and have not been widely tested in glioma. For instance, third-generation osimertinib with improved CNS penetration has broader coverage for mutated versions of EGFR.

In summary, (i) first EGFRi such as gefitinib and erlotinib, and PDGFRi imatinib may play great roles in specific patients with over-acting EGFR (especially EGFR with kinase domain mutation) and PDGFR. However, Pan-TKIs, such as sorafenib, sunitinib, and regorafenib did not show enough efficacy thus far (Table 1). (ii) Afatinib, which block EGFRVIII, a common structure mutation occurring after over-activating wild-type EGFR. The judgment of the time window of afatinib is a challenge of deeply understanding of heterogeneity in GBM temporally and regionally. (iii) VEGFR is a more important target than EGFR for GBM therapy. Anti-angiogenesis is a promising method including vandetanib, cediranib, vatalanib, and cobanzotinib, just like Bev. Normalizing

| Small molecule | First author | Phase | Year | Patient number | Therapy | CR | PR | OR | SD | mPFS | PFS6 | mOS | OS12 |
|----------------|--------------|-------|------|----------------|---------|----|----|----|----|------|------|-----|------|
| gefitinib      | Amanda L. Schwer (67) | I     | 2008 | 15             | gefitinib 250 mg pod + radiotherapy | 13.3% | 13.3% | 13.3% | 7 m | 63.0% | 10 m | 40.0% |
| erlotinib      | Jeffrey J. Raizer (35) | I     | 2010 | 32             | erlotinib 150–775 mg pod | 3.3%  | 3.3%  | 6.7%  | –40% | 14 m  | 53.0% |
|                | Jennifer L. Clarke (34) | II    | 2014 | 59             | erlotinib + TMZ + RT + bev. | 13.5 m | 19.8 m |
| afatinib       | David A. Reardon (44) | II (pilot) | 2015 | 119            | afatinib 40 mg pod | 0.0% | 2.4% | 2.4% | 34.1% | 3.0% | 9.8 m | 9.8 m |
|                |               |       |      |                | afatinib 40 mg + TMZ | 2.6% | 5.1% | 7.7% | 35.9% | 10.0% | 8.0 m |
| Vandetanib     | Jan Drappatz (54) | I     | 2010 | 13             | Vandetanib + TMZ+ RT | 90.0% | 8 m  | –75% | 11 m | –80% |
| Cobozaonib     | Patrick Y. Wen (68) | II    | 2018 | 222            | cobozaonib at up to 140 mg/day | 17.6% | 17.6% | 3.7 m | 22.3% | 7.7 m | –35% |
| Imatinib       | David A. Reardon (50) | II    | 2005 | 33             | imatinib mesylate 400–500 mg + hydroxyurea (500 mg twice a day) | 14.4% | 14.4% | 3.7 m | 27.8% | 10.4 m | –40% |
|                | David A. Reardon (69) | I     | 2008 | 65             | imatinib + TMZ | 3 m  | 27.0% | 11.4 m |
|                | David A. Reardon (70) | I     | 2009 | 37             | vatalanib + imatinib + hydroxyurea | 24.3% | 24.3% | 48.6% | 3 m  | 25.0% | 12 m |
| Sorafenib      | David A. Reardon (47) | II    | 2011 | 32             | sorafenib (400 mg twice daily) + temozolomide | 3.1%  | 3.1%  | 46.9% | 1.5 m | 9.4%  | 14.3 m |
| Vorinostat     | Katherine B. Peters (71) | II/III | 2018 | 39            | vorinostat 200–400 mg/d + TMZ + bev. | 5.10% | 38.50% | 43.60% | 6.7 m | 53.80% | 12.5 m | 51.30% |
| Veliparib      | H. Ian Robins (72) | II/III | 2016 | 225            | veliparib +TMZ | 2 m  | 17.00% | 10.3 m | –38% |
the immature vessels in the mass of glioma may play a supporting role in other therapies. (iv) Modest efficacy of inhibiting wild-type EGFR, effectively suppressing VEGFR, FGFR to anti-angiogenesis, along with inhibiting EGFRvIII may play an unexpected role. (v) Blindly using Pan-TKIs such as sorafenib, sunitinib, and regorafenib always did not show better efficacy and rather brought the risk of off-target effects. (vi) The side effects of TMZ and radiation are brain necrosis and myelosuppression, respectively. The side effects of TKIs are diarrhea and rash. Using TKIs and decreasing the dose of chemoradiotherapy may reduce the side effects, especially for patients over the age of 70 or patients with poor performance status to improve the life quality. (vii) The methylation status of the MGMT promoter is an important prognosis factor for TMZ therapy (Stupp regimen, MGMT methylated mOS 23.2 m, mPFS 10.5 m; MGMT unmethylated mOS 16.0 m, mPFS 7.8 m). As for MGMT unmethylated patients, TKIs may play an effective role. In the future, RTKs will still be the prime target for treatment of glioma because of their irreplaceable functions. To seriously appreciate the efficacy of various TKIs, we need more idealized randomized controlled trials just like AVAglio and RTOG 0825 for Bev.

**NRTK and Intracellular Signal Transduction Pathways**

Recently, nRTKs have gradually come into the field of view of researchers. Approximately 32 nRTKs have been identified in human cells, such as the SRC family, FAK family, and ABL family. Most of them are located in the cytoplasm, with part of them anchored on the cellular membrane by amino-terminal modification (73, 74). At first, the main function of nRTKs is associated with the immune system, such as activation of T and B cells (75). Despite lacking an RTK-like extracellular ligand-binding domain, nRTKs possess an ATP-binding site and a tyrosine kinase catalytic domain. So nRTKs can function partly as the same as transmembrane tyrosine kinases to regulate cell growth, proliferation, differentiation, adhesion, migration, and apoptosis. Beyond that, as nRTKs are located in the cytoplasm, they can bind proteins, lipids, and DNA through different domains to act broadly. Small-molecule inhibitors play a dominant role in targeted therapy for nRTKs instead of monoclonal antibodies due to their intracellular location and structure. In addition, some proteins functioning as part of the intracellular signal transduction pathway, such as Akt, MEK, and Erk, are also targets of therapy and discussed in this part.

Until June 2021, there are 48 clinical studies (20 for newly diagnosed GBM and 28 for progressive GBM) involving 9 small molecules published in PubMed. The radiological response rates are optimistic but not mTOR inhibitors compared to baseline. Thus far, the PFS and OS have not shown obvious benefits (Additional File 1).

**Src**

Since Francis et al. found the first proto-oncogene v-Src in the Rous sarcoma virus, 11 members of the human Src Kinase family (SFK) have gradually been discovered (76). Among these, c-Src, Yes, Fyn, Lyn, and Lck have functional roles in glioma involved in survival, proliferation, migration, angiogenesis, irradiation therapy resistance, and even stemness maintenance (77–81). Src kinase is the first characterized proto-oncogene (79, 82). The Src Kinase family thus likely functions as a traffic node of a complicated gene regulatory network initiated by membranes such as growth factor receptors, G protein-coupled receptors, and cytokine receptors (83, 84). Then, SRC-family kinases cross-talk with multiple pathways and are involved in the regulation of FAK/STAT3, Wnt/beta-catenin, caspase, cyclin/CDK, and integrin/FAK intracellularly (85–87). In GBM, the activity of Src kinase was significantly increased even though there was little missense mutation or amplification (88). Whereas mutation or loss of PTEN and EGFRvIII mutation would activate Fyn and c-Src, respectively, SRC-family kinase activity increasing is due to upstream deregulation instead of SRC per se (23, 78, 89). CD90 high-expression cells, which are identified as glioma stem cells, are sensitive to Src inhibitors (77). Moreover, Src activation and downstream multiple RTKs partially account for radiotherapy resistance. Si306, an inhibitor of c-Src, can increase the sensitivity to radiotherapy (79). To date, dasatinib and ponatinib have entered clinical trials in the area of glioma. These drugs also belong to pan-TKI.

Dasatinib is a second-generation TKI that can inhibit many nRTKs, including all types of kinases of the SRC family, such as c-Src, Lyn, Fyn, and Yes, which play important roles in the brain. Simultaneously, in a high concentration, it can impair other kinase activities, including BCR-ABL, Eph A2, c-kit, and VEGFR2, particularly PDGFR, which is altered in most diffuse intrinsic pontine gliomas (90, 91). To date, dasatinib has been evaluated along with conventional treatments or other TKIs in phase I and phase II clinical trials for GBM and diffuse pontine glioma in children and adults. The safety and tolerability of dasatinib demonstrated in the studies is disappointing, particularly when combined with lomustine in recurrent GBM and in combination with crizotinib in children with glioma (92, 93). Although theoretically anti-Src therapy can suppress radiation resistance, in a phase II trial involving 217 patients with newly diagnosed GBM, and the combination of dasatinib with concomitant radiation and TMZ did not improve PFS and OS compared with placebo (NCT00869401). Bev. and dasatinib in combination can numerically increase the PFS but not OS compared with Bev. alone (NCT01892177) (94). The above results suggest that it is effective to combine dasatinib with TKIs or various targeted drugs at the level of cellular mechanisms, such as c-MET, PDGFR, and VEGFR inhibitors (95, 96), but useless in clinical trials.

Ponatinib, the third-generation TKI for CML, targets Src and VEGFR, PDGFR, and FGFR, which are three crucial receptors associated with angiogenesis. Therefore, ponatinib is a potent anti-angiogenesis drug. It did not show a promising effect in a phase II study including 15 patients with Bev.-refractory GBM (97).

Bosutinib is a small molecule inhibitor targeting BCR-ABL and Src and is used for treating CML. In 2014, a phase II study involving only 11 patients with recurrent GBM showed a better mOS of 11.7 m compared with baseline. More clinical trials are warranted to evaluate the efficacy of bosutinib (98).
PKC
Protein kinase C is a serine/threonine kinase that includes three subgroups: the classical isoforms, novel isoforms, and atypical isoforms. Among the subgroups, there are tens of isoforms functioning differently in the survival, proliferation, adhesion, migration, and therapy resistance of glioma cells because of the diversity of phosphorylation sites, types of stimuli, and cell environment (99). The traditional signal pathway is the PLC/PIP2/IP3 pathway. But studies in various cancers, including GBM, showed that overexpression of PKC contributes to tumor pathogenesis and seems to be involved in EGFR, PI3K/Akt, Ras/MEK/MAPK, hedgehog, and TNFα signaling, which are deregulated in GBM and are deemed to be potent targets. In addition, PRKD2, a member of the PKC-activated protein kinase family, was identified as a mediator of GBM growth involving decreasing p53 and regulating the phosphorylation of retinoblastoma protein (100). RTKs, p53, and Rb, the three pathways that are commonly deregulated in GBM, crosstalk with PKC (101). Therefore, PKC inhibition is an important approach for the treatment of GBM and many trials test its efficacy. Targeting atypical PKC decreases tumor growth in EGFR inhibitor-resistant mouse models of GBM (102). PKCδ, an isoform of novel PKC, acts as a critical mediator of the maintenance of tumor stem cells through an autocrine loop with positive feedback that is driven by the PKCδ/STAT3/IL-23/JAK signaling axis (103).

Tamoxifen is a selective estrogen receptor modulator used to treat and prevent breast cancer in women (104). Strictly speaking, tamoxifen is not a classical small-molecule inhibitor. In GBM, it functions as a targeted inhibitor of PKC. Because overexpression of PKC in GBM is associated with TMZ and irradiation resistance and tamoxifen has no significant overlapping toxicities with most other drugs, clinical trials combine tamoxifen with traditional therapies for newly diagnosed GBM. Up until now, there have been no definite results for the regimen in phase II trials. A phase II trial of RTOG protocol BR-0021 combining high-dose tamoxifen with radiation did not exhibit improved effects (105). The later trials combining tamoxifen with TMZ and RT also did not have a different outcome.

Enzastaurin is a synthetic bisindolylmaleimide with potent antineoplastic activity. Enzastaurin binds to the ATP-binding site and selectively inhibits PKCβ, a classical isoform involved in the induction of VEGF-stimulated neo-angiogenesis. In glioma, enzastaurin functions as an antiangiogenesis drug, entering clinical trials (106). In 2010, enzastaurin entered a phase III study as monotherapy but showed modest efficacy compared with the control arm of lomustine or baseline (107).

PI3K/Akt/mTOR
PI3K/Akt/mTOR, including the endogenous inhibitors PTEN, TSC1/2, and PHLPP, is one of the most common and most characterized malfunction pathways in glioma (108). High activity in the pathway, in general, portends a poor outcome (109). Since Powis et al. proposed that inhibition of PI3K by wortmannin could repress growth of human tumor xenografts in mice (110), there have been more than 20 types of small-molecule inhibitors entering clinical trials and more than 50 types tested in laboratories. In addition, many inhibitors can regulate the activity of PI3K/Akt to inhibit tumors, though they do not immediately target the pathway, such as oxymatrine, baicalin, and gartanin (111–113). Primarily, targeted therapy for PI3K/Akt/mTOR is mainly proposed to solve the resistance of chemoradiotherapy (114). On the one hand, the pathway is the busiest road of signal transduction that is initiated by growth factors or known as various RTKs and G protein-coupled receptors (GPCRs) on the cytoplasm membrane. On the other hand, it can crosstalk with diverse pathways intracellulary, such as Ras/Raf, Notch, STAT3, and Wnt/catenin. All of the proteins in the pathway, and upstream RTKs, and the endogenous inhibitors of the pathway are usually detected malfunctioning in glioma (114). Whereas in this pathway, every protein is an independent target, there is a combination regimen targeting more than one protein to increase efficacy. Simultaneously, PI3K has four classes and various isoforms with different catalytic-subs which play different roles. Among them, class I is dominant in cancers (114, 115). Therefore, there are pan-PI3K, isoform-selective, and dual PI3K/mTOR inhibitors. The common mutation of PIK3CA (encodes p110α, a 110 kDa catalytic subunit of Class IA) in cancer causes constitutive activation of PI3K and downstream Akt, and it is perhaps a promising target for therapy, but the mutation is not common in glioma despite 90% of GBMs harboring deregulated PI3K (116, 117). Other isoforms also impact the pathogenesis of solid tumors and hematologic malignancies. For instance, PTEN-deficient GBM largely depended on p110α for proliferation and p110β for migration (118). Recently researchers found many inhibitors can increase reactive oxygen species (ROS) to regulate the ROS-JUN-p53 loop and then decrease the level of phosphorylation and activity of PI3K/Akt/mTOR (119, 120). Inhibition of PI3K would promote the expression of the genes associated with glioma stem cells, such as SOX2, OCT4, and MSII (121). This is probably one of the mechanisms of resistance.

Akt, also known as PKB, includes three isoforms, and among these, Akt1 plays a major role in the PI3K/Akt pathway (122). It is not only an indispensable part of the PI3K/Akt pathway but also a serine/threonine-specific protein kinase that activates NF-kB, Bcl-2 family protein, and MDM2 to regulate multiple cellular processes (123). In addition, Akt also accepts the immediate regulatory association driven by other kinases (124). The drugs, which target Akt, combined with TMZ and fractional radiation, gradually enter the field of vision because chemoradiotherapy would increase the level of phosphorous Akt (125, 126).

Lastly, targeting mTOR is the most studied approach for the treatment of glioma. There are two different complexes, regulatory-associated protein mTORC1 and rapamycin-insensitive mTORC2, the latter functioning in resistance to rapamycin by activating Akt in a positive feedback manner (127). 2-hydroxyglutarate (2HG) produced by IDH1/2 mutation promotes mTOR activity by depleting KDM4A and decreasing DEPTOR protein stability (128), presenting another mechanism of IDH1/2 mutation promoting the genesis of glioma and the possibility of a combination IDH inhibitor and mTOR.
inhibitor. Simultaneously, the pathway is a hotspot of studies on microRNA, IncRNA, and exosomes because of the extensive functions of noncoding RNA and PI3K/Akt (129–132). In addition, the downstream targets of the pathway, such as p70S6K, 4EBP1, and eIF-4E, are also druggable targets (133). However, in summary, the approach of targeting PI3K/Akt is failing in glioma clinical trials (Additional File 1). Single-target drugs are usually fed back to activate upstream and other pathways such as Erk through complex networks (124, 133). The content of phosphorous Akt, the status of PTEN, and other proteins may be prognostic factors that indicate the sensitivity to PI3K/Akt inhibitors (134, 135), but to date, the tissue analysis usually does not show that the molecular markers correlate with survival. The occurrence of heterogeneity and stem cells within the tumor adds to these difficulties (121).

**PI3K**

As mentioned above, there are isoform-selecting and pan-PI3K inhibitors. The former includes eganelisib,idelalisib, and alpelisib, but does not enter clinical trials in glioma. Eganelisib is a highly selective inhibitor of the enzyme PIK3C gamma (p110gamma) (136). But p110 gamma is predominantly expressed in the pancreas, skeletal muscle, liver, and heart instead of the brain (137). Idelalisib, approved by the FDA in 2014, blocks selectively P110beta, which is expressed in normal and malignant B-cells. Alpelisib is a PI3K alpha specific inhibitor for the treatment of breast cancer with a PI3KCA mutation after disease progression (138).

The drugs targeting PI3K that have entered clinical trials are mainly Pan-PI3K inhibitors, namely, taselisib, pilaralisib, buparlisib, and copanlisib. Thus far, the trials are mainly MATCH (Molecular Analysis for Therapy Choice) models because PI3K has diverse effects on the regulation of biological processes in a wide variety of human cancers (139). Taselisib is a pan-PI3K inhibitor but has the strongest activity to repress p110alpha (140, 141). Therefore, it is used in cancers with PIK3CA mutations, in particular uterine serous carcinomas and breast cancers, which are accompanied by hormone receptor changes because of the cross-talk between estrogen receptor (ER) and PI3K (141). The relationship between the expression of estrogen receptors and glioma expression has gained attention recently because of sex-specific differences in GBM (142).Copanlisib is predominantly against PI3K-alpha and PI3K-d isoforms (143). A phase II MATCH trial involving the above two pan-PI3K inhibitors is recruiting (NCT02465060).

Pilaralisib, another pan-PI3K inhibitor, has been tested in early clinical trials. A phase I trial indicated that pilaralisib had a favorable safety profile (144), although some researchers thought that the toxicity of pan-PI3K was high (141).

Buparlisib is the most studied pan-PI3K inhibitor in glioma. It presented a promising prospect in preclinical studies. At low concentrations, buparlisib can inhibit the migration and invasion of GBM cells, and at higher concentrations and with a longer duration of drug exposure, it can promote apoptosis of cells (145, 146). It is controversial how the status of PTEN influences the efficacy of buparlisib. Koul showed that PTEN and EGFR status did not correlate with sensitivity, but mutation or wild type of p53 could change the method of death of tumor cells after treatment with buparlisib (147). But Xie et al. found that the buparlisib activity of anti-p110beta was poor and that may limit the efficacy of treating PTEN-deficient GBM (118). Buparlisib was also combined with other targeted therapies such as HSP90 (an Hsp90 inhibitor) and ABT-737 (a Bcl-2 inhibitor) because inhibiting the proteins in other pathways would cause compensatory overactivation of PI3K/Akt (148, 149). Yet, buparlisib is combined with other therapies in phase I or phase II clinical trials (150–152). To date, the safety profile of buparlisib is deemed poor, and the regimen did not show enough efficacy to improve the outcome.

**Akt**

Akt inhibitors, including ipatasertib, capivasertib, MK2206, aferesertib, and perifosine, thus far are less studied in glioma. Ipatasertib is currently in phase II trials for the treatment of solid tumors. Capivasertib and aferesertib have mainly been tested in preclinical studies. MK2206 acts as an allosteric Akt inhibitor and is a highly selective inhibitor of pan-Akt, namely, of all three Akt isoforms Akt1, Akt2, and Akt3 (153). MK2206 can induce apoptosis and autophagy, increasing the efficacy of gefitinib (154). Narayan et al. showed that MK2206 at a low concentration (1 μM) reduced the phosphorylation of Thr308 and Ser473 residues of AKT in both adherent GBM cells and spheroids to sensitize them to irradiation and TMZ. At a high concentration (>5 μM), it inhibited invasion and migration of cells (155). But in PTEN-deficient cells, MK2206 could not decrease the level of phosphorylation of mTOR and S6K, the effectors of the PI3K/Akt pathway, nor induce apoptosis or autophagy.

**mTOR**

mTOR inhibitors are the most studied small molecules in the pathway. Now there are three generations of inhibitors and more than 70 clinical trials. Everolimus, a generation I mTOR inhibitor, is a medication used as an immunosuppressant to prevent rejection of organ transplants and in the treatment of renal cell cancer and other tumors, approved by the FDA for various conditions. Everolimus is more selective for the mTORC1 protein complex (156). It binds to its protein receptor FKBP12, which directly interacts with mTORC1 with little impact on the mTORC2 complex (156). Besides, everolimus could inhibit the production of glutathione from glutamine and glutamate and then inhibit the repair of DNA damage caused by carboplatin (157). But everolimus can lead to a hyper-activation of the kinase Akt via inhibiting the mTORC1 negative feedback loop while not inhibiting the mTORC2 positive feedback to Akt. And it has been evaluated in GBM for more than a decade with 26 trials registered on ClinicalTrials.gov (158). But a randomized phase II clinical trial RTOG 0913 (NCT01062399) presented a disappointing result: that adding everolimus into standard therapy reduced mOS by 4.7 months relative to the control arm (159). This leads us to consider that the penetration through the BBB, the toxicity of everolimus, and the activity of mTORC2 are influential but uncontrollable (158). But the next two early phase studies in pediatric patients demonstrated the manageable
toxicity profile of everolimus (160, 161). On this basis, we think the prospect of mTOR inhibitors is promising. The PI3K/Akt/mTOR is a busy traffic node in cells, hence it is an important target just like RTKs and Ras/Raf/MAPK. The combination of mTOR inhibitors and targeted inhibition of Ras and PDGFR α is still a possible way to treat glioma (160, 162). The dual inhibitor of mTORC1 and mTORC2 may be a vulnerability in cancer (163). In addition, everolimus plays an irreplaceable role in the treatment of tuberous sclerosis complex and is mainly used for neurofibromatosis type 1-associated pediatric low-grade glioma and subependymal giant cell astrocytoma.

Sirolimus, also known as rapamycin and initially developed as an antifungal agent, functions as an immunosuppressive small-molecule inhibitor by inhibiting activation and sensitivity to interleukin-2 (IL-2) of T and B cells through inhibition of mTOR (164). Although it is a generation I inhibitor of mTOR like everolimus, it can also inhibit mTORC2. However, this has the adverse effect of increasing the risk of type 2 diabetes (165). The effect of sirolimus is complex, even though many studies have indicated that sirolimus can enhance mouse lifespan in a sex-specific manner (166). Temsirolimus, a derivative and prodrug of sirolimus, is converted to sirolimus (rapamycin) in vivo. But temsirolimus also shows exclusive activity on its own, and the trials in glioma mainly use temsirolimus instead of sirolimus. Outcomes of temsirolimus in clinical trials are frequently worse than those in the control arm. The combination of temsirolimus and TKIs, such as erlotinib and sorafenib, limits the dose of temsirolimus to one-tenth of MTD so as to achieve acceptable safety because of the side effects of temsirolimus. Thus, the regimen should be tested in more studies combining mTOR inhibitors and TKIs (37, 167, 168). Combining temsirolimus and perifosine, an Akt inhibitor, theoretically inhibits mTORC1 and supresses the activity of Akt, which is activated by mTORC2 in a back-feed manner. The regimen was tolerable in a phase I study (169).

**Ras/Raf/MEK/Erk**

Ras/Raf/MEK/Erk is another busy traffic road in cells in addition to PI3K/Akt. Firstly, Ras is a protein superfamily of small GTPases and, in general, is responsible for cell proliferation (170). Ras is one of the most frequently altered proteins in cancer, but rarely in glioma (171). However, it is not a common target of therapy for cancer despite the many efforts contributed to the relative studies (172). Raf kinases are a family of three serine/threonine-specific protein kinases, including A-Raf, B-Raf, and C-Raf. BRAF became the focus of research recently since a large portion of human tumors carry oncogenic ‘driver’ mutations in the BRAF gene, with 11% of glioma cell lines harboring BRAF mutations (171, 173). The activating mutation in BRAF is a common change in pediatric low-grade gliomas (174). The BRAF V600E mutation inducing the constitutive activity of Raf is frequently detected in low-grade pleomorphic xanthoastrocytoma, ganglioglioma, extra-cerebellar pilocytic astrocytoma, and epithelioid GBMs instead of other types of high-grade glioma (175). But the overactivation of Ras/Raf is highly frequent in GBM. The BRAF V600E mutation is a successful target in melanoma, non-small cell lung cancer, and thyroid cancer. In high-grade gliomas, it may become a biomarker of benefit from dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) dual-targeted therapy (176). MEK is also known as MAPK, and Erk is a classical MAPK, a classical cascade amplification pathway. The pathway connects extracellularly initiated signals from receptors on the surface of cells to the DNA in nuclei to regulate multiple biological processes. In addition, MEK and MAPK can function as kinases to immediately phosphorylate other proteins. Deregulation of the MAPK/Erk pathway is a necessary step in the malignant transformation of many cancers.

In summary, the intracellular signal transduction pathway is a significant embodiment of the complexity of biological regulation. On the one hand, every kinase can accept regulation from upstream kinase and activate downstream kinase to form a complicated network. This network is critical for maintaining the rule cell behavior. However, the overactivation of the pathway is usually due to upstream RTKs and GPCRs but not the abnormality of proteins comprising the pathway. Therefore, we suppose that it is not an ideal target for tumor treatment. Perhaps, targeting the intracellular signal transduction pathway would play an assistant role if the tumor showed significant overactivation or TKIs caused the second pathway overactivation by tissue analysis. The utilization of these inhibitors still requires biomarkers for precision medicine.

**PROTEINS REGULATING CELLULAR BIOLOGICAL PROCESSES**

In addition to the kinome, there are several proteins regulating cellular biological processes. The small molecules inhibiting the proteins are usually highly selective but difficult to target. There were 11 small molecules that were involved in 20 clinical trials that were published in PubMed (Additional File 1).

**HDAC**

Epigenetic changes refer to alternations affecting the expression of genes and the phenotypes of cells but not changing the sequence of DNA. It plays an important role in multiple aspects of pathogenesis and therapy resistance (177). In general, post-translational acetylation of histones modulates the structure of chromatin by relaxing the latter to promote the binding of transcription factors with motifs in genes and then increase the expression level of tumor suppressor genes (178). In addition, GSCs have a distinct pattern of epigenetic alterations, including DNA methylation and histone modifications (13). The process is controlled by the balance between the histone acetyltransferases (HATs) and histone deacetylases (HDACs).

There are at least four classes of HDACs, including various isoforms (179). Different isoforms function in various ways. This indicates that HDAC inhibitors targeting multiple isoforms may be more effective (180–184). Class I and class II were studied more thoroughly, and the disruption of HDACs was observed in multiple cancers, particularly diffuse intrinsic pontine gliomas with H3K27M mutations (182, 185–187). HDAC inhibitors...
decondensing chromatin can prevent DNA double-strand break repair and regulate the stemness of GBM cells to induce radio sensitization (180, 188, 189). HDAC inhibitors also regulate the post-translational acetylation of proteins in the cytoplasm to influence the function of the latter, which is involved in angiogenesis, stemness, immune regulation, and ultimately inducing apoptosis. For example, many studies suggested that HDACs could downregulate the activity of p53, a classical tumor suppressor protein (185, 190, 191). HDACs block the NF-κB pathway by acetylation to inhibit resistance to TMZ and radiation (192, 193). But there was a study that indicated HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) favored the acquisition of TMZ resistance by increasing recruitment of SP1, C-JUN, NF-κB, and p300 within the relaxing MGMT promoter region (194). HDAC inhibitors repress EGFR/EGFRVIII expression inducing the expression of FOXO1—a tumor suppressor—in MYC-driven medulloblastoma cells (195, 196). HDACs also influence the polarization of microglia mediated by glioma cells (197). In addition, Nguyen et al. found that HDAC inhibitors blunted glycolysis in a c-Myc dependent manner and lowered ATP levels to elicit metabolic reprogramming to dependence on fatty acid oxidation (198). In summary, HDACs impact multiple processes through epigenetic changes. In the past few years, there have been a number of studies in which HDAC inhibitors were combined with radiation, chemotherapy, and other targeted drugs, presenting exciting efficacy in vitro and in vivo (192, 199–201). Because it is pharmacologically much simpler to inhibit an enzyme than to induce one, HDAC inhibition has gained enormous clinical interest as an anticancer strategy (177). At present, HDAC inhibitors as anti-tumor drugs are mainly used for the treatment of hematological neoplasms. Some HDAC inhibitors, such as valproic acid, have been used as anti-seizure drugs in GBM (202). To date, besides vorinostat, entinostat, Panobinostat, and VPA, many new HDAC inhibitors are still found and being studied preclinically. Interestingly, HDAC inhibitors are also being studied for their potential to induce viral HIV-1 expression in latently infected cells and disrupt latency (203).

Vorinostat, also known as SAHA, binds to the active sites of HDACs and acts as a chelator for zinc ions, which are also found in the active sites of HDACs. It acts on classes I, II, and IV of HDAC (204). In addition, SAHA could trigger autophagy in GBM stem cells through the Akt/mTOR pathway. Many clinical trials are ongoing or completed, but most are phase II or earlier. Based on preclinical results, vorinostat-combined drug with chemoradiotherapy, Bev., or bortezomib (a proteasome inhibitor) (205, 206). In a phase II north central cancer treatment group study involving 66 patients with recurrent GBM, vorinostat monotherapy showed nice tolerability but modest drug activity (207), and a phase I/II trial of vorinostat combined with traditional therapy for newly diagnosed GBM presented the same result. Because of the compromised efficacy presented in recent trials, there are additional trials registered in NIH except for a phase I trial of pembrolizumab and vorinostat combined with temozolomide for newly diagnosed GBM. Now preclinical studies focus on molecule markers such as IDH1 mutation, pChek2, and Bcl-XL, imaging parameters, which indicate sensitivity to vorinostat and other HDAC inhibitors (208–210), or to identifying some targeted inhibitors, such as melatonin, chloroquine, and gamitrinib, which can function as synergic effects with HDAC inhibitors (201, 211).

Romidepsin, also known as FK228 or Istodax, is a natural product obtained from bacteria. It acts as a prodrug, with the disulfide bond undergoing reduction within the cell to release a zinc-binding thiol. Thiol binds to a zinc atom in the binding pocket of Zn-dependent HDAC to block its activity. So, in theory, it acts on HDACs of the zinc-dependent classes I, IIa, IIb, and IV (179). Romidepsin decreases the expression of p21 to induce apoptosis (212). Wu et al. found that romidepsin increased the sensitivity to TMZ by blocking the PI3K/Akt/mTOR pathway (213). A phase I/II trial of romidepsin for adults with recurrent malignant glioma failed (214).

Panobinostat, trade name Farydak, is a hydroxamic acid and acts as a non-selective HDAC inhibitor. There are plenty of studies to research the activity in the treatment of diffuse intrinsic pontine glioma, a lethal pediatric brain cancer usually harboring mutations altering the epigenetic regulatory histone tail (H3 K27M) (215). Panobinostat can increase H3 acetylation and H3K27 trimethylation to partial rescue of the H3K27M-induced global hypomethylation phenotype (216). At present, Panobinostat is being tested in phase I clinical trials for diffuse intrinsic pontine glioma and GBM. Its potential of anti-angiogenesis led to combination with Bev. but failed in phase II (217).

Valproic acid is presently extensively used for epilepsy, bipolar disorder, and migraine headaches. In addition to inhibiting HADCs, it can affect GABA levels and voltage-gated sodium channels (218). Retrospective trials indicated that patients with GBM receiving valproic acid during the traditional regimen of TMZ and radiation had an improved prognosis by sensitizing to chemoradiotherapy (219–222). But Berendsen et al. proposed that epileptogenic GBM is a favorable prognostic factor regardless of using antiepileptic drugs or not (223). In 2016, a retrospective trial including 1,869 patients showed that valproic acid or levetiracetam, another anti-epileptic drug, did not improve the outcome of newly diagnosed GBM (224). To date, the activity of valproic acid in GBM is controversial. A phase II trial involving 43 patients with high-grade gliomas who received valproic acid and TMZ plus radiation indicated no increase in mOS (NCT00302159).

Belinostat, trade name Beleodaq, previously known as PXD101, is a hydroxamate-based pan-HDAC inhibitor that induces apoptosis by upregulation of p21 and through multiple pathways just like other HDAC inhibitors (225). There is currently no glioma clinical trial registered with the NIH.

**CDK4/6**

Deregulation of cyclin-dependent kinase 4/6 (CDK4/6) is detected in various types of cancers, including glioma (226). Targeted inhibition of CDK4/6 has emerged as an efficient approach for treating breast cancer, with three small-molecule inhibitors approved by the FDA: ribociclib, palbociclib, and...
abemaciclib (227). CDK4/6 inhibitors primarily arrest the cell cycle in the G1 phase by regulating the CDK4/6–retinoblastoma (Rb)–E2F pathway to inhibit proliferation. However, as more and more studies are conducted in vitro and in vivo, the new targets of CDK4/6 inhibitors and other pathways involved in CDK4/6 have been proposed, involving cellular metabolism, autophagy, and immune evasion, despite the high selectivity of third-generation CDK4/6 inhibitors (228). The on-target and off-target mechanisms of CDK4/6 inhibitors have been reviewed by Denisa et al. (227). According to the TCGA database, the CDK4/6-Rb-E2F axis is deregulated in about 80% of GBMs, including endogenous inhibitors of CDKs, such as p16, an INK4 family protein (229). A large-scale data analysis on comprehensive genomic profiling indicated that 47.1% of brain gliomas harbored at least one cyclin alteration (230). In glioma, the activity of CDK4/6 inhibitors depends on the wild type of Rb. Mutation or deletion of Rb causes resistance to the drug. The deficiency of endogenous inhibitors, such as p16 and p18, or known as CDKN2A and CDKN2C, is a strong predictor of sensitivity to CDK4/6 inhibitors (231, 232). Besides, SHH and MYC amplification are the most characteristic pathways involved in the pathogenesis of glioma (234). Previous studies found that the combination of CDK4/6 and RTK inhibitors, such as mTOR inhibitors or c-Met/Trk inhibitors, could play a synergy (229, 235), possibly because the monotherapy of TKIs or CDK inhibitors would activate each other. Simultaneously, inhibition of CDK4/6 can delay radiation resistance and TMZ resistance by repressing double-strand break repair (236, 237). In general, the trials of CDK4/6 inhibitors are mainly in the early phase.

Palbociclib is the first and most studied CDK4/6 inhibitor in glioma therapy. But acquired resistance is still a problem that urgently needs to be addressed (238). On the one hand, the deregulation of the CDKs/Rb/E2F axis is a source of resistance to palbociclib. For example, gliomas with deletion or mutation of Rb are naturally insensitive to CDK4/6 inhibitors (232), but the wild-type Rb cancers would overexpress the Rb protein to gradually promote resistance to palbociclib (239). In addition, the isoforms of cyclins and CDKs, such as CDK2 and cyclin E, can play compensatory roles (227). However, a study found that palbociclib only induced reversible quiescence but not irreversible senescence in glioma stem cells (240). Thus far, palbociclib entered phase II trials for refractory or recurrent GBM, but for the moment it did not show enough efficacy to improve the outcome.

Ribociclib is used along with an aromatase inhibitor (such as letrozole). Some studies indicated the good CNS penetration of ribociclib (241). Ribociclib demonstrates a synergistic effect when combined with ALK or MEK inhibitors in the treatment of neuroblastoma (242). A phase I/II study of ribociclib following radiation therapy in children with newly diagnosed diffuse intrinsic pontine glioma indicated that the regimen was feasible (243).

Based on CDK4/6 inhibitor suppressing immune evading, the regimen of abemaciclib plus cancer immunotherapy, such as humanized antibody targeting the programmed cell death protein 1 (PD-1) receptor of lymphocytes pembrolizumab, has entered clinical trials. Thus far, there has been no result.

**PARP**

Poly (ADP-Ribose) polymerase (PARP) is a family of enzymes that catalyze the synthesis of linear or branched polymers of ADP-ribose (PAR) using NAD+ as substrate, and functions as radiosensitizer (244). Once PARP detects single-strand DNA breaks, it binds to DNA through the DNA-binding domain to induce structural change. Then it initiates the synthesis of a polymeric adenosine diphosphate ribose chain. The autopolyADP-ribosylation of PARP on the auto-modification domain is a signal that recruits DNA ligase III (LigIII), DNA polymerase beta (polβ), and scaffolding proteins such as X-ray cross-complementing gene 1 (XRCC1) to repair the damaged DNA, a process known as base excision repair (BER) (245). Poly (ADP-ribose) glycohydrolase (PARG) degrades the PAR chain to recycle the PARP. Thus far, there are 18 members in the family, but PARP1 and PARP2 play major roles in repairing DNA, especially the first one. Beyond that, the other members perform other functions, and the PARylation of proteins as an important and ubiquitous post-translational modification involving histones and various transcription factors can also regulate multiple processes of cells, such as caspase-independent apoptosis by translating apoptosis-inducing factors into the nucleus (246–248). The high expression of PARP-1 was detected in multiple types of cancers (248). In the beginning, studies demonstrated that PARP inhibitors repressed the activity of PARP to hinder the BER system by competing with the substrate NAD+ (249). This would cause the accumulation of DNA damage of N-methylpurines (N7-methylguanine and N3-methyladenine) generated by TMZ and then eventually increase the sensitivity to TMZ and other alkylating agents. Notably, in the preclinical studies, the function of sensitization is still remarkable in MGMT-proficient and MMR-deficient glioma cells, which in general are regarded as drug-resistant cells (250–252). Higuchi et al. found that the TMZ sensitizer role of the PARP inhibitor was independent of base excision repair. There are perhaps other pathways by targeting which PARP inhibitors enhance the efficacy of chemoradiotherapy (253). For example, sustained inhibition of PARP-1 activity delays GBM recurrence by enhancing radiation-induced senescence (253). Similarly, PARP inhibitors can sensitize cells to ionizing radiation even in glioma stem cells, which can promote resistance to radiation by overexpressing PARP (254, 255), because the radiation generates single-strand DNA to exert clinical effect (256). Another important anti-tumor mechanism of PARP inhibitors is “synthetic lethality.” In some breast and ovarian cancers with BRCA mutations, meaning the deficiency of the homologous recombination repair system (HR), BER driven by PARP functions as compensation. So, the inhibition of PARP can maximize the effect of anti-tumor (257, 258). In glioma, deletion of PTEN, commonly occurring in primary GBM, can impact genomic stability by regulating the expression of RAD51, an important homologous recombination repair component (259). In in vitro, the cell lines with PTEN deficiency are more sensitive to PARP inhibitors than the wild-
type PTEN cell lines (244). Inducing DNA damage is an important method to treat tumors. Except for chemotherapy and radiotherapy, topoisomerase inhibitors, topotecan and irinotecan, and inhibitors of DNA-dependent protein kinase (DNA-PK), a key enzyme involved in nonhomologous DNA end joining (NHEJ), can combine with PARP inhibitors to enhance the efficacy of antitumors (244). Recently, studies have demonstrated that IDH1/2 mutations could not only impair the HR system but also compromise the BER associated with PARP by decreasing NAD+ availability. This may be a reason for patients with IDH1/2 mutations to have a better prognosis relative to IDH wild type when they receive chemoradiotherapy, and it also renders tumor cells sensitive to PARP inhibitors (260–262).

Simultaneously, wild type p53, associated with cell cycle checkpoints and DNA repair, is supposed to indicate sensitivity to PARP inhibitors (263, 264). HDAC can repress DNA damage repair by epigenetic downregulation, which can play a synergistic role with PARP inhibitors (265). The inhibition of PARP can probably be used as an adjuvant therapy and there have been some clinical trials ongoing based on this theory. The loss of p53-binding protein 1 (53BP1), an antagonism factor of BRCA1, and the promoter of NHEJ can cause resistance to PARP inhibitors (266).

Olaparib, particularly in glioma harboring IDH mutation, the clinical trials were mainly carried out within recent 3 years, and there are no results thus far. OPARATIC trial, a phase I trial, demonstrated that olaparib could reliably penetrate recurrent GBM at radio sensitizing concentrations (267). Niraparib was tested in a phase II trial as a radiosensitizer (NCT04715620). Junko et al. showed that niraparib has the strongest potency in trapping PARP compared to olaparib and veliparib (268). In addition, pamiparib, talazoparib, and rucaparib which have been tested in other cancers.

In conclusion, the proteins regulating some specific cell processes, such as epigenetic changes, cell cycle, and DNA repair, may make a difference in glioma treatment. But in general, the relevant clinical trials are in their early phase. These inhibitors play functions by different mechanisms to have chance to combine with TKIs in future clinical trials.

CONCLUSION

Small-molecule inhibitors did not change the survival of GBM patients in clinical trials. But at present, it is not reasonable to define small-molecule inhibitors as a failure. On the one hand, most trials involving recurrent GBM would stop therapy after the progression of the tumor. But the mPFS of recurrent GBM is only 2 months. It causes the duration of the small molecule therapies to be less than 2 months. The short exposure duration limits the efficacy of small molecules. In addition, the efficacy of small molecules includes relieving edema, improving of neurological function, and improvement of life quality. On the other hand, anatomical characteristics of the brain restrain the delivery and efficacy of drugs. Heterogeneity within the GBM mass, and specifically mosaicism of glioma stem cells, is a critical factor in maximizing efficacy. In clinical practice, the tailored therapy associated with molecular subtyping in different regions of a tumor mass and the change of recurrence is more useful than personal therapy. The time and space obstacles could be tackled by several methods, such as new approaches to drug delivery of drugs and the scheduling of drug administration. In the future, as the finding of new targets and new technologies for drug discovery replaces the “Me-too” model, small molecules will play a more functional role in the treatment of cancer and other diseases.

AUTHOR CONTRIBUTIONS

WH performed the selection of literature, drafted the manuscript, and prepared the figures and tables. DG designed this review, critically instructed the writing, and revised the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.911876/full#supplementary-material

Additional File 1 | Table S1.xlsx: A summary of outcomes in clinical studies in adult patients with glioblastoma.

Additional File 2 | Table S2.xlsx: Some clinical trials being conducted.

REFERENCES

1. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS.: CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. Neuro-Oncology (2018) 20(suppl_4):v1–v86. doi: 10.1093/neuonc/noy131

2. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of Radiotherapy With Concomitant and Adjuvant Temozolomide Versus Radiotherapy Alone on Survival in Glioblastoma in a Randomised Phase III Study: 5-Year Analysis of the EORTC-NCIC Trial. Lancet Oncol (2009) 10(5):459-66. doi: 10.1016/S1470-2045(09)70025-7
41. Thiesen B, Stewart C, Tsoa M, Kamel-Reid S, Schiaquevich P, Mason W, et al. A Phase II/III Trial of GW572016 (Lapatinib) in Recurrent Glioblastoma Multiforme: Clinical Outcomes, Pharmacokinetics and Molecular Correlation. Cancer Chemother Pharmacol (2010) 65:353–61. doi: 10.1007/s00280-009-1041-6

42. Karavasilis V, Kotoula V, Pentheroudakis G, Televantou D, Lambaki S, Chrisafi S, et al. A Phase I Study of Temozolomide and Lapatinib Combination in Patients With Recurrent High-Grade Gliomas. J Neurol (2013) 260:1469–80. doi: 10.1007/s10072-014-6812-z

43. Reardon DA, Groves MD, Wen PY, Nabhors L, Mikkelsen T, Rosenfeld S, et al. A Phase II/III Trial of Pazopanib in Combination With Lapatinib in Adult Patients With Relapsed Malignant Glioma. Clin Cancer Res (2013) 19:980–8. doi: 10.1158/1078-0432.CCR-12-1707

44. Reardon DA, Nabors LB, Mason WP, Perry JR, Shapiro J, Waven P, et al. Phase I: Randomized Phase II Study of Afinatin, an Irreversible ErbB Family Blocker, With or Without Protracted Temozolomide in Adults With Recurrent Glioblastoma. Neuro Oncol (2015) 17:430–9. doi: 10.1093/neuonc/nou160.48

45. Lee EQ, Kaley TJ, Duda DG, Schiff D, Lassman AB, Wong ET, et al. A Phase II Study of Sorafenib/Regorafenib and Lapatinib Interact to Kill CNS Tumor Cells. J Cell Physiol (2015) 230 (1):131–9. doi: 10.1002/jcp.24689

46. Reardon DA, Vredenburgh JJ, Desjardins A, Peters K, Gururangan S, Sampson JH, et al. Effect of CYP3A-Inducing Anti-Epiletics on Sorafenib Exposure: Results of a Phase II Study of Sorafenib Plus Daily Temozolomide in Adults With Recurrent Glioblastoma. J Neuropsychiatr (2011) 101:57–66. doi: 10.1037/101060-010-0217-6

47. Hansworth JD, Ervin T, Friedman E, Priego V, Murphy PB, Clark BL, Lamar RE: Concurrent Radiotherapy and Temozolomide Followed by Temozolomide and Sorafenib in the First-Line Treatment of Patients With Glioblastoma Multiforme. Cancer (2010) 116:3663–9. doi: 10.1002/cncr.25275

48. Galanis E, Anderson SK, Lafky JM, Uhm JH, Giannini C, Kumar SK, et al. Phase II Study of Bevacizumab in Combination With Sorafenib in Recurrent Glioblastoma (N0776): A North Central Cancer Treatment Group Trial. Clin Cancer Res (2013) 19:4816–23. doi: 10.1158/1078-0432.CCR-13-0708

49. Reardon DA, Egorin MJ, Quinn JA, Rich JN, Gururangan S, Vredenburgh JJ, et al. Phase II Study of Imatinib Mesylate Plus Hydroxyurea in Adults With Recurrent Glioblastoma Multiforme. J Clin Oncol (2005) 23(36):9395–68. doi:10.1200/JCO.2005.03.2185

50. Reardon DA, Dresemann G, Taillibert S, Campone M, van den Bent M, Clement P, et al. Multicentre Phase II Studies Evaluating Imatinib Plus Hydroxyurea in Patients With Progressive Glioblastoma. Br J Cancer (2009) 101(12):1995–2004. doi: 10.1038/sj.bjc.6605411

51. Dresemann G, Weiler M, Rosenthal MA, Wedding U, Wagner W, Engel E, et al: Imatinib in Combination With Hydroxyurea Versus Hydroxyurea Alone as Oral Therapy in Patients With Progressive Pretreated Glioblastoma Resistant to Standard Dose Temozolomide. J Neuropsychiatr (2010) 96(3):393–402. doi: 10.1037/s11060-009-9976-3

52. Jain RK: Normalization of Tumor Vasculature: An Emerging Concept in Antiangiogenic Therapy. Sci (New York NY) (2005) 307(5706):58–62. doi: 10.1126/science.1104819

53. Drappatz J, Norden AD, Wong ET, Doherty LM, Lafrankie DC, Ciampa A, et al. Phase I Study of Vandetanib With Radiotherapy and Temozolomide for Newly Diagnosed Glioblastoma. Int J Radiat Oncol Biol Phys (2010) 78:85–90. doi: 10.1016/j.ijrobp.2009.07.1741

54. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and Safety of Sunitinib in Patients With Advanced Gastrointestinal Stromal Tumour After Failure of Imatinib: A Randomised Controlled Trial. Lancet (Lond Engl) (2006) 368(9544):1329–38. doi: 10.1016/j.ijrobp.2006.09.0446

55. Kreitl TN, Smith P, Sul J, Salgado C, Iwamoto FM, Shih JH, et al. Continuous Daily Sunitinib for Recurrent Glioblastoma. J Neuroonc (2013) 111:41–8. doi: 10.1007/s11060-012-0988-z

56. Zivi A, Cerbone L, Recine F, Sternberg CN, Safety and Tolerability of Pazopanib in the Treatment of Renal Cell Carcinoma. Expert Opin Drug Saf (2012) 11:851–9. doi: 10.1517/14740338.2012.721208

57. Verweij J, Sleijfer S, Pazopanib, a New Therapy for Metastatic Soft Tissue Sarcoma. Expert Opin Pharmaco (2013) 14(7):929–35. doi: 10.1517/14656566.2013.780030

58. Lombardi G, De Salvo GL, Brandes AA, Eoli M, Ruda R, Faedi M, et al. Regorafenib Compared With Lorumistine in Patients With Relapsed Glioblastoma (REGOMA): A Multicentre, Open-Label, Randomised, Phase II Trial. Lancet Oncol (2015) 16(1):106–22. doi: 10.1016/S1470-2045(14)62078-7

59. Sivin KS, Prabhu KS, Achkar IW, Kuttikrishnan S, Khan AQ, et al. Role of Non Receptor Tyrosine Kinases in Hematological Malignances.
Huang et al. Small-Molecule Inhibitors in Glioma

85. Wei J, Ma L, Li C, Pierson CR, Finlay JL, Lin J. Targeting Upstream Kinases

87. Villarroel A, Del Valle-Peórez B, Fuertes G, Curto J, Ontiveros N, García de

92. Broniscer A, Jia S, Mandrell B, Hamideh D, Huang J, Onar-Thomas A, et al.

94. Galansis, Anderson SK, Twothy EL, Carrero XV, Dixon JG, Tran DD, et al. A

95. Greish K, Jasim A, Paraythay N, Abdelghany S, Alkhateeb A, Taurin S, et al. Micellar Formulations of Crizotinib and Dasatinib in the Management of Glioblastoma Multiforme. *J Drug Target* (2018) 26(8):692–708. doi: 10.1080/1061186X.2017.1419357

96. Truffaux N, Philippe C, Paulsson J, Andreu-Folch, Guido Rinella, et al. Preclinical Evaluation of Dasatinib Alone and in Combination With Cabaotinib for the Treatment of Diffuse Intrinsic Pontine Glioma. *Neuro-Oncology* (2015) 17(7):953–64. doi: 10.1093/neuonc/nou330

97. Lee EQ, Muzikansky A, Duda DG, Gaffey S, Dietrich J, Nayak L, et al. Phase II Trial of Ponatinib in Patients With Bevacizumab-Refractory Glioblastoma. *Glioma Med-US* (2018) 8(13):5988–94. doi: 10.1002/tmg2.3156

98. Nwanze FO, Njoku CI, Onwubiko VA, Ezumah CN, Asiegbuyi OA, et al. Phase 2 Trial of Radiation Plus High-Dose Tamoxifen for Glioblastoma. *Neuro Oncol* (2015) 17(7):953–64. doi: 10.1093/neuonc/not303

99. Funato K, Hayashi T, Echizen K, Negishi L, Shimizu N, Koyama-Nasu R, et al. SIRT2-Mediated Inactivation of PT3 is Required for Glioblastoma Tumorigenicity. *EMBO Rep* (2018) 19(11):e45587. doi: 10.15252/embr.201745587

100. Kasche E, Carrera-Silva EA, Perry AS, Carrero XW, Dixon JG, Tran DD, et al. Kinomic Exploration of Temozolomide and Radiation Resistance in Glioblastoma Multiforme Xenolines. *Radiother Oncol* (2014) 111(3):468–74. doi: 10.1016/j.radonc.2014.04.010

101. Bernhart E, Damm S, Heffter P, Wintersperger A, Asslaber M, Frank S, et al. Silencing of Protein Kinase D2 Induces Glioma Cell Senescence via P53-Dependent and -Independent Pathways. *Neuro Oncol* (2014) 16(7):933–45. doi: 10.1093/neuonc/not030

102. Kim RK, Suh Y, Hwang E, Yoo KC, Choi KS, An S, et al. Pkcζ Phosphorylates and Inhibits the Function of the CIC Tumor Suppressor Receptors. *Exp Cell Res* (1994) 76(2):263–9. doi: 10.1016/0014-4821(94)90334-4

103. Kim RK, Suh Y, Hwang E, Yoo KC, Choi KS, An S, et al. Pkcζ Phosphorylates and Inhibits the Function of the CIC Tumor Suppressor Receptors. *Exp Cell Res* (1994) 76(2):263–9. doi: 10.1016/0014-4821(94)90334-4

104. Gierach GL, Curtis RE, Pfeiffer RM, Mullooly M, Ntowe EA, Hoover RN, et al. Phase III Study of Enzastaurin Compared With Lomustine in Patients With Recurrent Glioblastoma. *J Clin Oncol* (2010) 28(22):3156–62. doi: 10.1200/JCO.2009.23.2595

105. Robins HI, Won M, Seiferheld WF, Schultz CJ, Choucair AK, Brachman DG, et al. Phase 2 Trial of Radiation Plus High-Dose Tamoxifen for Glioblastoma Multiforme: RTOG Protocol BR-0021. *Cancer Med-US* (2019) 8(13):4734–47. doi: 10.1002/cmm.3156

106. Seystahl K, Weller M. Is There a World Beyond Bevacizumab in Targeting Gliomas? *Neuro-Oncology* (2017) 19(7):571. doi: 10.1093/neuonc/not306

107. Expert Opinion for the Treatment of Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia in Adults. *Expert Opin Investig Drugs* (2012) 21(5):605–17. doi: 10.1517/13543874.2012.670219

108. Zhao H, Wang J, Shao W, Wu C, Chen Z, To ST, et al. Recent Advances in the Use of PI3K Inhibitors for Glioblastoma Multiforme: Current Preclinical and Clinical Development. *Mol Cancer* (2017) 16(1):100. doi: 10.1186/s12943-017-0670-3

109. Broniscer A, Jia S, Mandrell B, Hamideh D, Huang J, Onar-Thomas A, et al. Phase 1 Trial, Pharmacokinetics, and Pharmacodynamics of Dasatinib Combined With Crizotinib in Children With Recurrent or Progressive High-Grade and Diffuse Intrinsic Pontine Glioma. *Pediatr Blood Cancer* (2018) 65(7):e27035. doi: 10.1002/pbc.27035
119. Lin CJ, Chen TL, Tseng YY, Wu GJ, Hsieh MH, Lin YW, et al. Honokiol Mediated Inhibition of Growth Factor Signaling. *Sci (New York NY)* (2015) 367(1):58

120. Zhang L, Wang H, Ding K, Xu J. FTY720 Induces Autophagy-Related Pathway. *Acta Biochim Pol* (1995) 42(4):395–403. doi: 10.18388/abp.1995.4893

121. Jones NM, Rowe MR, Shepherd PR, McConnell MJ. Targeted Inhibition of mTOR and YAP Serve as Prognostic Markers and Therapeutic Targets in Gliomas. *Liu Invest* (2017) 97(11):18–63. doi: 10.1016/j.lanfibust.2017.70

122. Hsu PP, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, et al. The mTOR-Regulated Phosphoproteome Reveals a Mechanism of Mtorc1-Phosphoinositide-3-Kinase Inhibitor With High Unbound Exposure and Robust in Vivo Activity. *Acta Biochim Pol* (2018) 71. doi: 10.1016/j.actbio.2017.06.058

123. Fan QW, Nicolaides TP, Weiss WA. Inhibiting eBPI in Glioblastoma. *Clin Cancer Res* (2018) 24(1):14–21. doi: 10.1158/1078-0432.CCR-17-0042

124. von Aachen B, Weller M, Kaulich K, Gratzmacher D, Zacher A, Fabbro D, et al. Synergistic Growth Inhibition Mediated by Dual PI3K/mTOR Pathway Targeting and Genetic or Direct Pharmacological AKT Inhibition in Human Glioblastoma Models. *J Neurochem* (2020) 153(4):510–24. doi: 10.1111/jnc.14899

125. Yu Z, Xie G, Zhou G, Cheng Y, Zhang G, Yao G, et al. miR-489 Inhibits Proliferation, Cell Cycle Progression and Induces Apoptosis of Glioma Cells via Targeting SPIN1-Mediated PI3K/AKT Pathway. *BioMed Pharmacother* (2019) 117:1354–66. doi: 10.1016/j.biopha.2018.11.133

126. Zhu J, Li K, Yu L, Chen Y, Cai Y, Jin J, et al. Targeting Phosphatidylinositol 3-Kinase. *Cancer Res* (2015) 75(7):1929–38. doi: 10.1158/1078-0432.CCR-14-2442

127. Duzgun Z, Eroglu Z, Biray Avci C. Role of mTOR in Glioblastoma. *Glioma Cell Proliferation and Invasion Through the mTOR/HIF-1 Signaling Pathway by Targeting Cab39. Hum Gene Ther Clin Dev* (2016) 27:12701–12704. doi: 10.1038/ncomms12700

128. Burris HAR. Overcoming Acquired Resistance to Anticancer Therapy: Focus on the PI3K/AKT/mTOR Pathway. *Breast Cancer Res* (2007) 10:274–8. doi: 10.1186/bcr1853

129. Li Y, Ma X, Wang Y, Li G. miR-489 Inhibits Proliferation, Cell Cycle Progression and Autophagy Related Pathway as a Target for Anticancer Drug Development. *Acta Biochim Pol* (1995) 42(4):395–403. doi: 10.18388/abp.1995.4893

130. Nan Y, Guo H, Guo L, Wang Y, Ren B, Yu K, et al. miRNA-451 Inhibits Glioma Cell Proliferation and Invasion Through the mTOR/HIF-1α/VEGF Signaling Pathway by Targeting Cab39. *Hum Gene Ther Clin Dev* (2018) 29(3):356–66. doi: 10.1089/humc.2017.0551

131. Xu DH, Chi GN, Zhao CH, Li DY. Long Noncoding RNA MEG3 Inhibits Proliferation and Migration But Induces Autophagy by Regulation of Sirt7 and PI3K/AKT/mTOR Pathway in Glioma Cells. *J Cell Biochem* (2018). doi: 10.1002/jcb.28026

132. Yao J, Wang Z, Cheng Y, Ma C, Zhong Y, Xiao Y, et al. M2 Macrophage-Derived Exosomal microRNAs Inhibit Cell Migration and Invasion in Gliomas Through PI3K/AKT/mTOR Signaling Pathway. *J Trans Med* (2021) 19(1):99. doi: 10.1186/s12967-021-02766-w

133. Rodon J, Tabernero J. Improving the Armamentarium of PI3K Inhibitors: From Selective AKT Inhibition to Dual PI3K/AKT Pathway Targeting and Genetic or Direct Pharmacological AKT Inhibition in Human Glioblastoma Models. *Am J Transl Res* (2017) 97(11):18–63. doi: 10.1016/j.lanfibust.2017.70

134. Burger MT, Pecchi S, Wagman A, Ni ZJ, Knapp M, Hendrickson T, et al. Synergistic Growth Inhibition Mediated by Dual PI3K/mTOR Pathway Targeting and Genetic or Direct Pharmacological AKT Inhibition in Human Glioblastoma Models. *J Neurochem* (2020) 153(4):510–24. doi: 10.1111/jnc.14899

135. Gao Y, Li L, Zheng H, Zhou C, Chen X, Hao B, et al. KIF3C is Associated With Favorable Prognosis in Glioma Patients and may be Regulated by PI3K/AKT/mTOR Pathway. *J Neurooncol* (2020) 146(3):513–21. doi: 10.1007/s11060-020-03399-7

136. Zhu J, Li K, Yu L, Chen Y, Cai Y, Jin J, et al. Targeting Phosphatidylinositol 3-Kinase Gamma (PI3Kγ): Discovery and Development of its Selective Inhibitors. *Med Res Rev* (2021) 41(3):1599–621. doi: 10.1002/med.21770

137. Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, et al. Cloning and Characterization of a G Protein-Activated Human Phosphoinositide-3 Kinase. *Sci (New York NY)* (1995) 269(5224):690–3. doi: 10.1126/science.7624799

138. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Ruggero H, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. *N Engl J Med* (2019) 380:1929–40. doi: 10.1056/NEJMoa1813904

139. Burger MT, Pecchi S, Waganm A, Ni ZJ, Knapp M, Hendrickson T, et al. Identification of NVP-BKM120 as a Potent, Selective, Orally Bioavailable Class I PI3 Kinase Inhibitor for Treating Cancer. *ACS Med Chem Lett* (2011) 2(10):774–9. doi: 10.1021 mcl200156t

140. Ndbukak CO, Heffron TP, Staben ST, Baumgardner M, Blaquiere N, Bradley E, et al. Discovery of 2-[3-[1-Isopropyl-3-Methyl-1H-1,2-Diazol-5-Yl]-5,6-Dihydrobenzof][Imidazol]-1,2-D] [-1,4]Oxazepin-9-Yl]-1H-Pyrazol-1-Yl]-2-Methylpropanamide (GDC-0032): A β-Sparing Phosphoinositide 3-Kinase Inhibitor With High Unbound Exposure and Robust in Vivo Antitumor Activity. *J Med Chem* (2013) 56(11):4597–610. doi: 10.1021/ jm3006362

141. Carrano A, Juarez JJ, Incontri D, Ibarra A, Guerrero Cazares H. Sex-Specific Differences in Glioblastoma. *Cells-Basel* (2021) 10(7):1783. doi: 10.3390/cells10071783

142. Carrano A, Juarez JJ, Incontri D, Ibarra A, Guerrero Cazares H. Sex-Specific Differences in Glioblastoma. *Cells-Basel* (2021) 10(7):1783. doi: 10.3390/cells10071783

143. Munoz J, Follows GA, Nastoupol J, Copanlis for the Treatment of Malignant Lymphoma: Clinical Experience and Future Perspectives. *Target Oncol* (2021) 16(3):295–308. doi: 10.1007/s11523-021-00862-9

144. Edelman G, Rodon J, Lager J, Castell C, Jiang Y, Van Allen EM, et al. Phase I Trial of a Tablet Formulation of Pilaralisib, a Pan-Class I PI3K Inhibitor, in Patients With Advanced Solid Tumors. *Oncologist* (2018) 23:401–38. doi: 10.1634/theoncologist.2017-0691
145. Foster KA, Jane EP, Premkumar DR, Morales A, Pollack IF. NVP-BKM120 Potentiates Apoptosis in Tumor Necrosis Factor-Related Apoptosis-Inducing-Ligand-Resistant Glioma Cell Lines via Upregulation of Noxa and Death Receptor 5. Int J Oncol (2015) 47(2):566–16. doi:10.3892/ijo.2015.3035

146. Speranza MC, Nowicki MO, Behera P, Cho CF, Chiocca EA, Lawler SE. Speranza MC, Nowicki MO, Behera P, Cho CF, Chiocca EA, Lawler SE. NVP-BKM120: A Phosphatidylinositol-3-Kinase Inhibitor With Anti-Invasive Properties in Glioblastoma. Sci Rep (2016) 6:20189. doi:10.1038/srep20189

147. Koul D, Fu J, Shen R, Lafortune TA, Wang S, Tiao N, et al. Antitumor Activity of NVP-BKM120—a Selective Pan Class I PI3 Kinase Inhibitor Showed Differential Forms of Cell Death Based on P53 Status of Glioma Cells. Clin Cancer Res (2012) 18(1):184–95. doi:10.1158/1078-0432.CCR-11-1559

148. Wachsmberger PR, Lawrence YR, Liu Y, Rice B, Feo N, Leiby B, et al. Hsp90 Inhibition Enhances PI3-Kinase Inhibition and Radiosensitivity in Glioblastoma. J Cancer Res Clin Oncol (2014) 140(4):573–82. doi:10.1007/s00432-014-1594-6

149. Jane EP, Premkumar DR, Morales A, Foster KA, Pollack IF. Inhibition of Phosphatidylinositol 3-Kinase/AKT Signaling by NVP-BKM120 Promotes ABT-737-Induced Toxicity in a Caspase-Dependent Manner Through Mitochondrial Dysfunction and DNA Damage Response in Established and Primary Cultured Glioblastoma Cells. J Pharmacol Exp Ther (2014) 350(1):22–35. doi:10.1124/jpet.114.212910

150. Rosenthal M, Clement PM, Campone M, Gil-Gil MJ, DeGroot J, Chinot O, Huang et al. Small-Molecule Inhibitors in Glioma
151. Foster KA, Jane EP, Premkumar DR, Morales A, Pollack IF. NVP-BKM120 Potentiates Apoptosis in Tumor Necrosis Factor-Related Apoptosis-Inducing-Ligand-Resistant Glioma Cell Lines via Upregulation of Noxa and Death Receptor 5. Int J Oncol (2015) 47(2):566–16. doi:10.3892/ijo.2015.3035

152. Speranza MC, Nowicki MO, Behera P, Cho CF, Chiocca EA, Lawler SE. Speranza MC, Nowicki MO, Behera P, Cho CF, Chiocca EA, Lawler SE. NVP-BKM120: A Phosphatidylinositol-3-Kinase Inhibitor With Anti-Invasive Properties in Glioblastoma. Sci Rep (2016) 6:20189. doi:10.1038/srep20189

153. Koul D, Fu J, Shen R, Lafortune TA, Wang S, Tiao N, et al. Antitumor Activity of NVP-BKM120—a Selective Pan Class I PI3 Kinase Inhibitor Showed Differential Forms of Cell Death Based on P53 Status of Glioma Cells. Clin Cancer Res (2012) 18(1):184–95. doi:10.1158/1078-0432.CCR-11-1559

154. Wachsmberger PR, Lawrence YR, Liu Y, Rice B, Feo N, Leiby B, et al. Hsp90 Inhibition Enhances PI3-Kinase Inhibition and Radiosensitivity in Glioblastoma. J Cancer Res Clin Oncol (2014) 140(4):573–82. doi:10.1007/s00432-014-1594-6

155. van den Bent M, Azaro A, De Vos F, Sepulveda J, Yung W, Wen PY, et al. A Phase IIb/II, Open-Label, Multicentre, Randomised Study. ESMO Open (2020) 5(4):e000672. doi:10.1136/esmoopen-2020-000672

156. Hainsworth JD, Becker KP, Mekhail T, Crowdharya SA, Eakle JF, Wright D, et al. Phase II/II Study of Bevacizumab With NVP-BKM120, an Oral PI3K Inhibitor, in Patients With Recurrent Solid Tumors (Phase I) and Relapsed/Refractory Glioblastoma (Phase II). J Neurol Oncol (2019) 144 (2):303–11. doi:10.1007/s11601-019-03227-7

157. Cheng Y, Ren X, Zhang Y, Patel R, Sharma A, Wu H, et al. eIF2-Kinase Dictates Cross-Talk Between Autophagy and Apoptosis Induced by Immune Inhibition, Thereby Modulating Cytotoxicity of Novel Akt Inhibitor MK-2206. Cancer Res (2011) 71(7):2654–63. doi:10.1158/0008-5472.CAN-10-2889

158. Cheng Y, Zhang Y, Zhang L, Ren X, Huber-Keener KJ, Liu X, et al. MK-2206, a Novel Allosteric Inhibitor of Akt, Synergizes With Gefitinib Against Multinucleate Glioma via Modulating Both Autophagy and Apoptosis. Mol Cancer Ther (2012) 11(1):154–64. doi:10.1158/1535-7163.MCT-11-0060

159. Narayan RS, Fedrigo CA, Brands E, Dik R, Stalpers LJ, Baumert BG, et al. The Allosteric AKT Inhibitor MK2206 Shows a Synergistic Interaction With Chemotherapy and Radiotherapy in Glioblastoma Spheroid Cultures. BMC Cancer (2017) 17(1):204. doi:10.1186/s12885-017-3193-9

160. Arriola Apelo SI, Neuman JC, Baar EL, Syed FA, Cummings NE, Brak HK, et al. Alternative Rapamycin Treatment Regimens Mitigate the Impact of Rapamycin on Glucose Homeostasis and the Immune System. Aging Cell (2016) 15(1):28–38. doi:10.1111/acin.12405

161. Poore B, Yuan M, Arnold A, Price A, Alt J, Rubens JA, et al. Inhibition of Mtorc1 in Pediatric Low-Grade Glioma Depletes Glutathione and Therapeutically Synergizes With Carboplatin. Neuro Oncol (2019) 21 (2):252–63. doi:10.1093/neuonc/noy150

162. Babak S, Mason WP, mTOR Inhibition in Glioblastoma: Requiem for a Dream? Neuro Oncol (2018) 20(5):584–5. doi:10.1093/neuonc/noy034

163. Chinnaiyan P, Won M, Wen PY, Rojiani AM, Werner-Wasik M, Shih HA, et al. A Randomized Phase II Study of Everolimus in Combination With Chemoradiation in Newly Diagnosed Glioblastoma: Results of NRG Oncology RT0G 0913. Neuro Oncol (2018) 20(5):666–73. doi:10.1093/neuonc/noy029

164. Mikjla Z, Yadav VN, Cartaxo RT, Siada R, Thomas CC, Cummings JR, et al. Everolimus Improves the Efficacy of Daetinib in Pdgfr-Driveren Glioma. J Clin Invest (2020) 130(10):3313–25. doi:10.1172/JCI133310
180. Marampon F, Megiorni F, Camero S, Crescioli C, McDowell HP, Sierra R, et al. HDAC4 and HDAC6 Sustain DNA Double Strand Break Repair and Stem-Like Phenotype by Promoting Radioresistance in Glioblastoma Cells. Cancer Lett (2017) 397:1–11. doi: 10.1016/j.canlet.2017.03.028

181. Zhang Z, Wang Y, Chen J, Tan Q, Xie C, Li C, et al. Silencing of Histone Deacetylase 2 Suppresses Malignancy for Proliferation, Migration, and Invasion of Glioblastoma Cells and Enhances Temozolomide Sensitivity. Cancer Chemother Pharmacol (2016) 78(6):1289–96. doi: 10.1007/s00280-016-1188-2

182. Ecker J, Oehme I, Mazitschek R, Korshunov A, Kool M, Hielscher T, et al. Glioma Stem Cell Activity and Mediates HDAC Inhibitor Response. DCI, Garros-Regulez L, et al. High Expression of MKP1/DUSP1 Counteracts Stemness in In Vitro and In Vivo Models of Oncogenesis (2017) 6(12):401. doi: 10.1038/s41389-017-0003-9

183. Pak E, MacKenzie EL, Zhao X, Pazyra-Murphy MF, Park P, Wu L, et al. A Large-Scale Drug Screen Identifies Selective Inhibitors of Class I HDACs as a Potential Therapeutic Option for SHH Medulloblastoma. Neuro Oncol (2019) 21(9):1150–63. doi: 10.1093/neuonc/noz089

184. Huang et al. Small-Molecule Inhibitors in Glioma

185. Buyandelger B, Bar EE, Hung KS, Chen RM, Chiang YH, Liou JP, et al. Combined Therapy of AXL and HDAC Inhibition Reverses Resistance to Temozolomide in Preclinical Models of Glioblastoma. J Hematol Oncol (2018) 11(1):32. doi: 10.1186/s40405-018-0576-6

186. Anastas JN, Zee BM, Kalin JH, Kim M, Guo R, Alexandrescu S, et al. Sequential Changes in Histone Modifications Shape Transcriptional Responses Underlying Microglia Polarization by Glioma. GLIA (2021) 69 (1):109–23. doi: 10.1002/glia.23887

187. Maleszewska M, Steranka A, Smiech M, Kaza B, Planic P, Dabrowski M, et al. PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma. Cancer Cell (2016) 29(3):311–23. doi: 10.1016/j.ccell.2016.02.011

188. Pal S, Kozono D, Yang X, Fendler W, Fitts W, Ni J, et al. Dual HDAC and PI3K Inhibition Abrogates Nf-κB and FOXM1-Mediated DNA Damage Response to Radio sensitize Pediatric High-Grade Gliomas. Cancer Res (2018) 78(14):4007–21. doi: 10.1158/0008-5472.CAN-17-3691

189. Singh MM, Johnson B, Venkatarayan A, Flores ER, Zhang J, Su X, et al. Preclinical Activity of Combined HDAC and KDMIA Inhibition in Glioblastoma. Neuro Oncol (2015) 17(11):1463–73. doi: 10.1093/neuonc/nov179

190. Buyandelger B, Bar EE, Hung KS, Chen RM, Chiang YH, Liou JP, et al. Histone Deactylase Inhibitor MPT0B291 Suppresses Glioma Growth In Vitro and In Vivo Models of Human Glioblastoma. J Cancer Res Clin Oncol (2019) 145(2):393–409. doi: 10.1007/s00432-018-2800-8

191. Li ZY, Li QZ, Chen L, Chen BD, Wang B, Zhang XJ, et al. Histone Deactylase Inhibitor RGP109 Overcomes Temozolomide Resistance by Blocking NF-κB-Dependent Transcription in Glioblastoma Cell Lines. Neurochem Res (2016) 41(12):3192–205. doi: 10.1007/s11064-016-2043-5

192. Kitange GJ, Mladek AC, Carlson BL, Schroeder MA, Pokorny JL, Cen L, et al. Combined HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma. Cancer Cell (2016) 29(3):311–23. doi: 10.1016/j.ccell.2016.02.011

193. Pont LM, Naipal K, Kloezeman JJ, Venkatesan S, van den Bent M, van Gent alves RM, Agnes JP, Delgado M, de Souza PO, Thomé MP, Heimfahr L, et al. Late Autophagy Inhibitor Chloroquine Improves Efficacy of the Histone Deacetylase Inhibitor SAHA and Temozolomide in Gliomas. Biochem Pharmacol (2019) 163:440–50. doi: 10.1016/j.bcp.2019.03.015

194. Plattner S, Kwobora Y, Suseiro S, Yamashita D, Tanaka M, Tanaka A, et al. Valproic Acid Reduces Hair Loss and Improves Survival in Patients Receiving Temozolomide-Based Radiation Therapy for High-Grade Glioma. Eur J Clin Pharmacol (2017) 73(3):357–63. doi: 10.1007/s00228-016-2167-1

195. Rasmussen TA, Schmitz Sgard A, Brinkmann C, Wightman F, Lewin SR, Melchjorsen J, et al. Comparison of HDAC Inhibitors in Clinical Development: Effect on HIV Production in Latently Infected Cells and T-Cell Activation. Hum Vacc Immunother (2013) 9(5):993–1001. doi: 10.4161/hv.23800

196. Richon V. Cancer Biology: Mechanism of Antitumour Action of Vorinostat (Suberoylanilide Hydroxamic Acid), a Novel Histone Deacetylase Inhibitor. Br J Cancer (2021) 125:533–90. doi: 10.1038/s41464-021-24977-1

197. Mirzaei E, Jaekle KA, Maurer MJ, Reid JM, Ames MM, Hardwick JS, et al. Phase II Trial of Vorinostat in Combination With Bortezomib in Recurrent Glioblastoma: A North Central Cancer Treatment Group Study. Neuro Oncol (2012) 14(2):215–21. doi: 10.1093/neuonc/nor198

198. Chinnaiyan P, Chowdhary S, Potthast L, Prabhu A, Tsai YY, Sarcar B, et al. Phase II Trial of Vorinostat Combined With Bevacizumab and CPT-11 in Recurrent Glioblastoma. Neuro Oncol (2012) 14(1):93–100. doi: 10.1093/neuonc/nor178

199. Tosi U, Kommidt H, Adeyuan O, Guo H, Maachani UB, Chen N, et al. PET-Image-Guided HDAC Inhibition of Pediatric Diffuse Midline Glioma Improves Survival in Murine Models. Sci Adv (2020) 6(30):eabc4405. doi: 10.1126/sciadv.abb4405

200. Kim GH, Choi SY, Oh TI, Kan SY, Kang H, Lee S, et al. IDH1(R132H) Causes Resistance to HDAC Inhibitors by Increasing NANOG in Glioblastoma Cells. Int J Mol Sci (2020) 21(11):42679. doi: 10.3390/ijms211142679

201. Pont LM, Naipal K, Kloezeman JJ, Venkatesan S, van den Bent M, van Gent

202. Sun GJ, Kim SH, Kwak S, Park SH, Song JH, Jung JH, et al. Inhibition of TFE3 Oligomerization by Co-Treatment of Melatonin With Vorinostat Promotes the Therapeutic Sensitivity in Glioblastoma and Glioma Stem Cells. J Pineal Res (2019) 66(3):e12556. doi: 10.1111/jpn.12556

203. Savo H, Mirakami H, Kumatagi M, Nakasato M, Yamuchi S, Matsuyama N, et al. Histone Deactylase Inhibitor, FK228, Induces Apoptosis and Suppresses Cell Proliferation of Human Glioblastoma Cells In Vitro and
In Vivo. Acta Neuropathol (2004) 107(6):523–31. doi: 10.1007/s00401-004-0841-3

V. Y. Dong L, Bao S, Wang M, Yun Y, Zhu R, FK228 Augmented Temozolomide Sensitivity in Human Glioma Cells by Blocking PI3K/ AKT/mTOR Signal Pathways. BioMed Pharmacother (2016) 84:462–9. doi: 10.1016/j.biopharm.2016.09.051

Iwamoto FM, Lamborn KR, Kuhn JG, Wen PY, Yung WK, Gilbert MR, et al. A Phase I/II Trial of the Histone Decetylase Inhibitor Romidepsin for Adults With Recurrent Malignant Glioma: North American Brain Tumor Consortium Study 03-03. Neuro Oncol (2011) 13(5):509–16. doi: 10.1093/neo/nor017

Vitanza NA, Biery MC, Ferguson E, Zheng Y, Girard EJ, et al. Optimal Therapeutic Targeting by HDAC Inhibition in Biopsy-Derived Treatment-Naïve Diffuse Midline Glioma Models. Neuro Oncol (2021) 23 (3):376–86. doi: 10.1093/neouca249

Grasso CS, Tang Y, Truffaux N, Berlow NE, Liu D, Deblzy MA, et al. Functionally Defined Therapeutic Targets in Diffuse Intrinsic Pontine Glioma. Nat Med (2015) 21(6):555–9. doi: 10.1038/nm.3855

Lee EQ, Reardon DA, Schif D, Drapatz J, Muzikansky A, Grimm SA, et al. The Phase II Study of Panobinostat in Combination With Bevacizumab for Recurrent Glioblastoma and Anaplastic Glioma. Neuro Oncol (2015) 17 (6):682–7. doi: 10.1093/neuonc/nou350

Ghodke-Puranyak Y, Thorn CF, Lamba JK, Leeder JS, Song W, Birbaum AK, et al. Valproic Acid Pathway: Pharmacokinetics and Pharmacodynamics. Pharmacogenet Genom (2013) 23 (4):236–41. doi: 10.1097/FGC.0b013e2823e5a082

Barker CA, Bishop AJ, Chang M, Beal K, Chan TA. Valproic Acid Use During Radiation Therapy for Glioblastoma Associated With Improved Survival. Int J Radiat Oncol Biol Phys (2013) 86(3):504–9. doi: 10.1016/j.ijrobp.2013.02.012

Redjal N, Reinhagen C, Le A, Walcott BP, McDonnell E, Dietrich J, et al. Valproic Acid, Compared to Other Antiepileptic Drugs, Is Associated With Improved Overall and Progression-Free Survival in Glioblastoma But Worse Outcome in Grade II/III Gliomas Treated With Temozolomide. J Neurooncol (2016) 127(3):505–14. doi: 10.1007/s11060-016-2054-8

Weller M, Gorlia T, Cairncross JG, van den Bent MJ, Mason W, Belanger K, et al. A Randomized Phase III Study of EB1089 in Newly Diagnosed Diffuse Midline Glioma Models. Neuro Oncol (2015) 18(3):R15. doi: 10.1093/neuroonc/not057

Portman N, Alexandrou S, Carson E, Wang S, Lim E, Cadron CE. Overcoming CDK4/6 Inhibitor Resistance in ER-Positive Breast Cancer. Endocr Relat Cancer (2018) 25(1):R35–86. doi: 10.1530/ERC-18-0317

Guaducci C, Bonechi M, Boccalini G, Benelli M, Risi E, Di Leo A, et al. CDK4/6 Inhibition Suppresses Tumour Growth and Enhances the Effect of Temozolomide In Glioma Cells. Cell Mol Med (2020) 24(9):5135–45. doi: 10.1111/cmm.15156

Hashizume R, Zhang A, Mueller S, Prados MD, Lulla RR, Goldman S, et al. CDK4/6 Inhibition in GBM. Mol Neurobiol (2013) 47 (7):561–7. doi: 10.1007/s12035-013-8575-6

Morris-Hanov O, Marazita MC, Romorini L, Iasa L, Fernandez-Espinosa DD, Selever GE, et al. Palbociclib Effectively Halts Proliferation But Fails to Induce Senescence in Patient-Derived Glioma Stem Cells. Mol Neurobiol (2019) 56(11):4360–9. doi: 10.1007/s12035-019-1633-z

Tien AC, Li J, Bao X, Derogatis A, Kim S, Mehta S, et al. A Phase 0 Trial of Ribociclib in Recurrent Glioblastoma Patients Incorporating a ‘Tumor Pharmacodynamic- and Pharmacokinetic-Guided Expansion Cohort. Clin Cancer Res (2019) 25(19):5777–86. doi: 10.1158/1078-0432.CCR-19-0133

Wood AC, Krytska K, Ryles HT, Inafrairi N, Sano R, Hansel TD, et al. Dual AKD and CDK4/6 Inhibition Demonstrates Synergy Against Neuroblastoma. Clin Cancer Res (2017) 23(11):2856–68. doi: 10.1158/1078-0432.CCR-16-1114
Potential Therapeutic Target. *Neuro Oncol* (2011) 13(11):1171–7. doi: 10.1093/neuonc/nor115

Galla A, Caloggera A, Condorelli R, Fraggetta F, La Corte A, Ridolfi F, et al. PARP-1 Protein Expression in Glioblastoma Multiforme. *Eur J Histochim* (2012) 56(1):e9. doi: 10.4081/ehj.2012.e9

Scalia M, Satriano C, Greca R, Stella AM, Rizzarelli E, Spina-Purrello V. PARP-1 Inhibitor DPQ and PJ-34 Negatively Modulate Proinflammatory Commitment of Human Glioblastoma Cells. *Neurochem Res* (2013) 38(1):50–8. doi: 10.1007/s11064-012-0887-x

Tentori L, Leonetti C, Scarsella M, D’Amati G, Vergati M, Portarena I, et al. Systemic Administration of GPI 15427, a Novel Poly(ADP-Ribose) Polymerase-1 Inhibitor, Increases the Antitumor Activity of Temozolomide Against Intracranial Melanoma, Glioma, Lymphoma. *Clin Cancer Res* (2003) 9:5370–9.

Tentori L, Portarena I, Torino F, Serrati M, Navarra P, Graziani G. Poly(ADP-Ribose) Polymerase Inhibitor Increases Growth Inhibition and Reduces G2/M Cell Accumulation Induced by Temozolomide in Malignant Glioma Cells. *GLIA* (2002) 40(1):44–54. doi: 10.1002/glia.10113

Daniel RA, Rozanska AL, Mulligan EA, Drew Y, Thomas HD, Castelbuono DJ, et al. Central Nervous System Penetration and Enhancement of Temozolomide Activity in Childhood Medulloblastoma Models by Poly(ADP-Ribose) Polymerase Inhibitor AG-016699. *Br J Cancer* (2010) 103(10):1588–96. doi: 10.1038/sj.bjc.6605946

Hipichi F, Nagashima H, Ning J, Koerner M, Wakimoto H, Calhul DP. Restoration of Temozolomide Sensitivity by PARP Inhibitors in Mismatch Repair Deficient Glioblastoma is Independent of Base Excision Repair. *Clin Cancer Res* (2020) 26(7):1690–9. doi: 10.1158/1078-0432.CCR-19-2000

Venere M, Hamerlik P, Wu Q, Rasmussen RD, Song LA, Vasani A, et al. Therapeutic Targeting of Constitutive PARP Activation Compromises Stem Cell Phenotype and Survival of Glioblastoma-Initiating Cells. *Cell Death Differ* (2014) 21(2):258–69. doi: 10.1038/cdd.2013.136

Lugli N, Kamilieri I, Halazonetis TD. PARP Inhibitors and IR Join Forces to Strike Glioblastoma-Initiating Cells. *Cell Death Differ* (2014) 21(2):192–3. doi: 10.1038/cdd.2013.172

Dunegy FA, Löser DA, Chalmers AJ. Replication-Dependent Radiosensitization of Human Glioma Cells by Inhibition of Poly(ADP-Ribose) Polymerase: Mechanisms and Therapeutic Potential. *Int J Radiat Oncol Biol Phys* (2008) 72(4):1188–97. doi: 10.1016/j.ijrobp.2007.03.011

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific Killing of BRCA2-Deficient Tumours With Inhibitors of Poly(ADP-Ribose) Polymerase. *Nature* (2005) 434(7035):913–7. doi: 10.1038/nature03443

Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnston DA, Richardson TB, et al. Targeting the DNA Repair Defect in BRCA Mutant Cells as a Therapeutic Strategy. *Nature* (2005) 434(7035):917–21. doi: 10.1038/nature03445

McEllin B, Camacho CV, Mukherjee B, Hahn B, Tomimatsu N, Bachoo RM, et al. PTEN Loss Compromises Homologous Recombination Repair in Astrocytes: Implications for Glioblastoma Therapy With Temozolomide or Poly(ADP-Ribose) Polymerase Inhibitors. *Cancer Res* (2010) 70(13):5457–64. doi: 10.1158/0008-5472.CAN-09-4295

Sulkowski PL, Corso CD, Robinson ND, Scanlon SE, Purshouse KR, Bai H, et al. 2-Hydroxyglutarate Produced by Neomorphic IDH Mutations Suppresses Homologous Recombination and Induces PARP Inhibitor Sensitivity. *Sci Transl Med* (2017) 9(375):eaal2463. doi: 10.1126/scitranslmed.aal2463

Lu Y, Kwinkiewicz J, Liu Y, Tech K, Frady LN, Su YT, et al. Chemosensitivity of IDH1-Mutated Gliomas Due to an Impairment in PARP1-Mediated DNA Repair. *Cancer Res* (2017) 77(7):1709–18. doi: 10.1158/0008-5472.CAN-16-2773

IDH-Mutant Tumors Vulnerable to PARP Inhibition. *Cancer Discov* (2017) 7(4):F4. doi: 10.1158/2159-8290.CD-NB2017-026

Sabbatino F, Fusciello C, Somma D, Pacelli R, Poudel R, Pepin D, et al. Effect of PS3 Activity on the Sensitivity of Human Glioblastoma Cells to PARP-1 Inhibitor in Combination With Topoisomerase I Inhibitor or Radiation. *Cytom A* (2014) 85(11):953–61. doi: 10.1002/cyt.a.22563

Sizemore ST, Mohammad R, Sizemore GM, Noswsoon S, Yu H, Ostrowski MC, et al. Synthetic Lethality of PARP Inhibition and Ionizing Radiation is PS3-Dependent. *Mol Cancer Res* (2018) 16(7):1092–102. doi: 10.1158/1541-7786.MCR-18-0106

Rasmussen RD, Gajjar MK, Jensen KE, Hamerlik P. Enhanced Efficacy of Combined HDAC and PARP Targeting in Glioblastoma. *Mol Oncol* (2016) 10(3):751–63. doi: 10.1016/j.molonc.2015.12.014

Wang YT, Yuan B, Chen HD, Xu L, Tian YN, Zhang A, et al. Acquired Resistance of Phosphatase and Tensin Homolog-Deficient Cells to Poly(ADP-Ribose) Polymerase Inhibitor and Ara-C Mediated by 53BP1 Loss and SAMHD1 Overexpression. *Cancer Sci* (2018) 109(3):821–31. doi: 10.1111/cas.13477

Hanna C, Kurian KM, Williams K, Watts C, Jackson A, Carruthers R, et al. Pharmacokinetics, Safety, and Tolerability of Olaparib and Temozolomide for Recurrent Glioblastoma: Results of the Phase I OPARATIC Trial. *Neuro Oncol* (2020) 22(12):1840–50. doi: 10.1093/neuonc/noaa104

Murai J, Huang SN, Das BB, Renaud A, Zhang Y, Doroshow JH, et al. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. *Cancer Res* (2012) 72(21):5888–99. doi: 10.1158/0008-5472.CAN-12-2753

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