Clinical Trials of Small Molecule Inhibitors in High-Grade Glioma

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KEYWORDS

• Glioblastoma • Small molecule inhibitor • Clinical trial

KEY POINTS

- Small molecule inhibitors (SMIs) are highly selective compounds that are generally water soluble with high oral bioavailability. These agents are designed to produce targeted inhibition at the active site of proteins involved with critical pathways in tumor biology.
- Several SMIs, including sunitinib (renal cell carcinoma) and imatinib (chronic myelogenous leukemia), have shown significant therapeutic benefit in clinical trials and are now considered standard of care.
- Several clinical trials of SMIs have been performed in patients with glioblastoma, including drugs targeting epidermal-derived growth factor receptor, platelet-derived growth factor receptor, and vascular endothelial growth factor receptor. To date, there has been no reported clinical benefit associated with the use of currently available agents.
- Increasing insight into the heterogeneous nature of glioblastoma may allow future tailoring of targeted agents for individual patients.

CHEMOTHERAPY: OPPORTUNITIES FOR OPTIMIZATION IN HIGH-GRADE GLIOMA

Modern chemotherapy can be traced to the discovery of the antitumoral properties of nitrogen mustard, a DNA alkylating agent used in chemical warfare.[1] The first trial of nitrogen mustard derivatives, used to treat Hodgkin lymphoma in the 1940s, followed observations of lymphosuppressive and myelosuppressive effects in soldiers exposed to mustard gas. Most historical approaches to treating cancers have incorporated agents that derive a degree of disease specificity by inducing DNA damage in rapidly dividing cells. Chemotherapeutics have traditionally been derived from broadly toxic substances that trigger cascades of programmed cell death in actively dividing tumor cells. However, the sequelae of this strategy are the many nonspecific effects in normal cells with high rates of turnover, such as those in the bone marrow, digestive tract, and hair follicles. Examples of 2 cytotoxic drugs that remain standard of care in primary and recurrent glioblastoma multiforme (GBM) are carmustine (BCNU, Gliadel) and temozolomide (TMZ; Temodar, Temodal).[2–4] These 2 drugs alkylate many cellular functional groups, including sites on guanine and cytosine nucleotides, thereby triggering the DNA damage–detecting checkpoint mechanisms of mitosis that subsequently promote cellular apoptotic cascades.

DNA-damaging approaches are limited in many aggressive tumors, because of mutations resulting in prosurvival traits including defective apoptotic signaling cascades, upregulation of rates of DNA repair, and increased rates of mutation leading to drug resistance. GBM is known to be inherently

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resistant to nearly every standard DNA-damaging chemotherapeutic agent. The lack of significant progress with traditional cytotoxic agents has provided the impetus for a new strategy of targeting specific alterations in signaling pathways responsible for the development and maintenance of GBM. Recent genome-wide analyses of human GBM tumor samples have amassed data regarding the commonly altered, mutated, or amplified genes implicated in GBM development,[5] many of which involve receptor tyrosine kinase (RTK) signaling pathways. Several these overactive signaling pathways, which include upstream receptors as well as downstream targets of activation, are the specific focus of many small molecule inhibitor (SMI) drugs.

WHAT IS AN SMI?

A small molecule drug is a nonpolymeric organic compound, generally fewer than 800 to 1000 Da. SMIs are designed to specifically inhibit the activity of a cellular constituent for therapeutic benefit. In practice, SMIs should be soluble in aqueous solution, lipophilic enough to cross the cellular membrane, and bind specifically to a target of interest to effect some change in cellular function. A particular advantage of these compounds is their potential for high selectivity for an active region of a given target, thus minimizing potential side effects. Major additional benefits inherent to small molecule compounds include the potential for oral bioavailability and, in the specific case of brain tumors, potentially superior passage across the blood-brain barrier relative to larger compounds (such as antibodies). These properties, combined with an ability to screen both new and modified compounds in high-throughput fashion, have led to the role of SMIs as a large proportion of drugs under current clinical study for cancer.

Most SMIs find efficacy via inhibition of function; as a result, most of these drugs are targeted toward reducing flux through overactive oncogenic pathways. Many of the SMIs in current clinical use have been identified either serendipitously or through in vitro screens focusing on a desired biologic activity. Several SMIs, designed to inhibit specific kinases that are upregulated in many cancers, have recently changed standard clinical practice for several solid tumors. Examples include lapatinib (Tykerb) for metastatic breast cancer,[6] sunitinib (Sutent) for metastatic renal cell carcinoma (RCC),[7] and sorafenib (Nexavar), which has proved efficacious in both advanced hepatocellular carcinoma[8] and advanced RCC.[9] Perhaps the best-known example of a targeted agent is the tyrosine kinase inhibitor imatinib mesylate (Gleevec). This small molecule was designed to inhibit a mutated RTK fusion protein, Bcr-Abl, the constitutive activation of which has long been known to cause chronic myelogenous leukemia (CML). In what is considered to be the proof-of-principle success story for SMIs in cancer, patients with chronic-phase CML treated with imatinib generally experience dramatic remission with few side effects.[10]

The remainder of this article focuses on specific SMIs designed to target signaling pathways previously associated with malignant glioma (reviewed in **Fig. 1**) and reviews preliminary results of clinical trials using these drugs.

EPIDERMAL-DERIVED GROWTH FACTOR RECEPTOR

Perhaps the best example of increased RTK signaling in GBM is that of the epidermal-derived growth factor receptor (EGFR), which increases activation of the downstream RAS and PI3K intracellular signaling cascades. Early studies suggested that around 40% of GBM show EGFR amplification and protein overexpression leading to increased pathway flux.[11] Furthermore, approximately 40% of GBM with EGFR amplification also harbor activating EGFR mutations.[12] These findings have been recently supported through integrated genome analysis from The Cancer Genome Atlas (TCGA) Research Network study, which found that 41 of 91 (45%) sequenced tumors harbored EGFR alterations.[13]

Several SMIs designed to inhibit EGFR and its mutant variants have been or are currently under investigation in GBM, including erlotinib (Tarceva), gefitinib (Iressa), lapatinib, and AEE788, as well as a host of monoclonal antibodies outside the scope of this article. Although the drugs seem to be well tolerated, most early single-agent trials of EGFR inhibitors have failed to show significant therapeutic benefit in GBM. In one phase II study, 13% of patients remained progression free for 6 months in response to gefitinib monotherapy.[14] However, results from trials in lung cancer, in which improved clinical and radiographic responses to gefitinib have correlated with documented mutations in the EGFR kinase regions,[15] have not been similarly recapitulated in patients with GBM.[16] Preliminary data from trials focusing on erlotinib[17,18] were slightly more encouraging than the results from gefitinib trials,[14,19] suggesting potentially greater activity of this compound against the constitutively active EGFRvIII mutant receptor[20] frequently found in GBM,[21,22] but limited overall efficacy was seen. Despite disappointing early trials comparing single-agent administration of erlotinib versus temozolomide or BCNU to treat GBMbeing,[23,24]

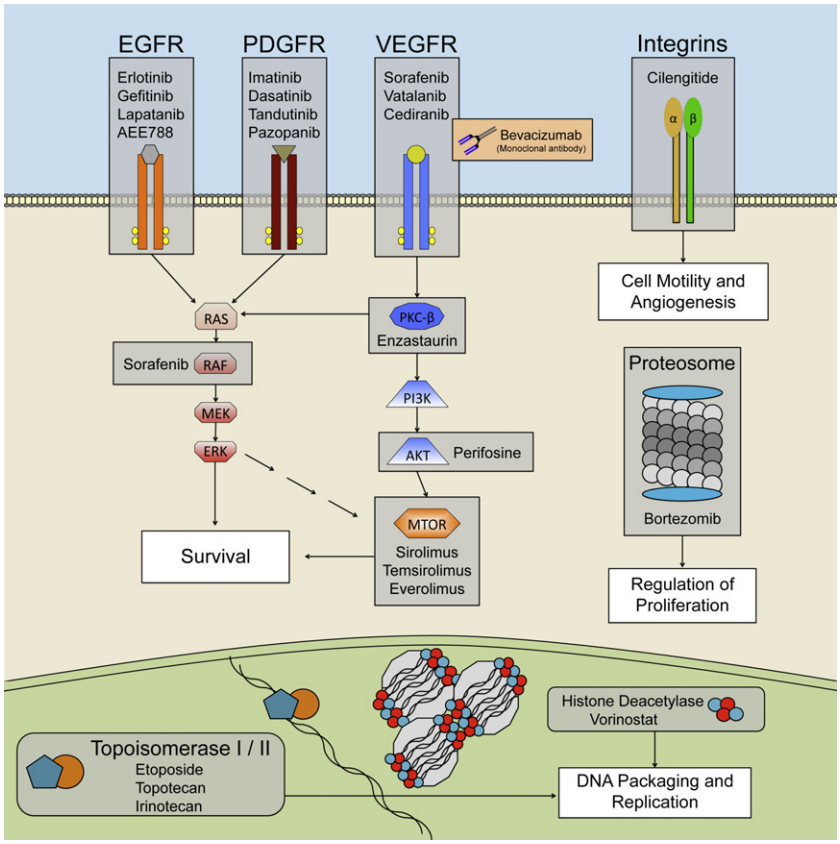

Fig. 1. Molecular targets of interest for SMIs in glioblastoma. EGFR, epidermal-derived growth factor receptor; PDGFR, platelet-derived growth factor receptor, VEGFR, vascular endothelial growth factor receptor.

further study to understand the potential cytostatic effects of EGFR inhibitors on GBM is warranted. To this end, EGFR inhibitors have been incorporated into multidrug trials including standard therapies (radiation therapy [RT] and TMZ) to determine any synergistic effect. One recent phase I/II trial showed no benefit of adding erlotinib to standard RT/TMZ protocols,[25] whereas another phase II trial combining erlotinib with TMZ before and after RT showed increased median survival (19.1 months) relative to historical controls (14.1 months).[26] It is likely that the variety of alterations observed in the RTK signaling axis of GBM means that some patients will find benefit from EGFR inhibition, whereas many will not, and also that combination therapies might be designed to address the most common alterations. Further studies using erlotinib as a component of first-line therapy may continue to elucidate this concept.

PLATELET-DERIVED GROWTH FACTOR RECEPTOR

Platelet-derived growth factor receptor (PDGFR) is another RTK signaling molecule with documented upregulation of expression in a subset of GBM.[27] Inhibitors of PDGFR include the drugs imatinib, dasatinib (Sprycel), tandutinib, and pazopanib (Votrient). Imatinib, an inhibitor of PDGFR as well as other selected RTKs (including KIT and ABL), is indicated for the treatment of CML and gastrointestinal stromal cell tumors. As mentioned earlier, response to imatinib in these previously untreatable tumors is often dramatic. However, imatinib has generally failed to show similar efficacy as a single-agent therapeutic drug in GBM[28–30] despite studies that detected intact imatinib within the GBM tissue.[31] A recent European Organization for Research and Treatment of Cancer (EORTC) study using imatinib monotherapy in 112 patients with recurrent gliomas showed evidence of radiological response in the form of a reduction of postcontrast T1 gadolinium enhancement but did not show a concomitant improvement in clinical outcomes. It was concluded that, in the range of 600 to 1000 mg/d, imatinib shows a good safety profile but lacks antitumor activity in most patients with recurrent glioma.[32] Two phase II studies of recurrent GBM, using a combination of hydroxyurea (HU) plus imatinib, suggested that this combination strategy was well

tolerated by patients and showed evidence of response in excess of expectations.[33,34] The positive results in these phase II trials led to completion of a randomized phase III trial comparing combination imatinib plus HU therapy with HU alone in progressive patients with TMZ-resistant tumors. However, no difference in progression-free survival (PFS) was seen between the 2 arms, with median PFS in both groups being only 6 weeks. Six-month PFS (PFS-6) was also similar at 5% and 7% respectively.[35]

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR

Angiogenesis, a phenomenon encompassing the creation of new blood vessels from existing vasculature, is a pathologic characteristic of GBM. This process is, in part, driven by the expression of the regulatory protein vascular endothelial growth factor (VEGF) and its receptor (VEGFR). The apparent need for angiogenesis in tumors, compared with the stable vascular networks present in other tissues, has implicated VEGFR signaling as an attractive target for inhibiting tumor growth, which is particularly relevant for GBM, a tumor in which increased vascular density and VEGF levels are associated with poor prognosis.[36] Given recent clinical success with bevacizumab (Avastin), a humanized monoclonal antibody against VEGF, there has been increasing focus on exploring SMIs targeting VEGF/VEGFR in GBM.

SMIs developed to inhibit VEGFR include the drugs vatalanib and cediranib (tentative trade name Recentin), both of which have shown promise in early clinical trials. Vatalanib inhibits both the VEGFR and platelet-derived growth factor receptor (PDGFR), and has shown moderate effect when used alone or in combination with TMZ or lomustine to treat recurrent GBM in phase I/II multicenter trials.[37,38]

A recent study designed to target both EGFR and VEGFR using a combination of erlotinib and bevacizumab (a monoclonal antibody) was well tolerated in patients, but showed no benefit in increasing PFS compared with that of historical regimens containing bevacizumab.[39,40]

A phase II trial of cedirinib, which inhibits many forms of VEGFR, recently showed evidence of activity with a PFS-6 of 27.6%, normalization of vasculature, and reduction of edema in patients with GBM.[41] Because serial sampling of GBM tissue is generally not possible, this study used multiple MRI-based methods to measure functional tissue response to cedirinib over time. These methods included measurements of vessel size, permeability, gadolinium enhancement, and diffusion-weighted imaging (DWI) characteristics. The results showed rapid reduction in vessel size, blood volume, and permeability to gadolinium contrast agents, with a corresponding reduction in vasogenic edema. The noninvasive nature of MRI allowed repeated measurement and temporal characterization of the vascular changes, showing them to begin as early as 24 hours after treatment, and to begin to reverse at day 28,although effects such as the reduction in vascular permeability persisted for up to 4 months. Measurement of circulating biomarkers also provided insight into the efficacy of cedirinib in this trial: following VEGFR inhibition, the concentration of circulating VEGF ligand increased. In addition, the use of MRI and biomarker measurement provided valuable insight into the duration of effects of the small molecule inhibition of VEGFR with cedirinib, suggesting that careful timing of combinatorial therapies (including cytotoxic drugs) might be critical to their success.

INHIBITION OF INTRACELLULAR SIGNALING CASCADES

Overall, the TCGA study found that 88% of all GBM harbored 1 or more mutations increasing the activity of the RTK signaling axis and flux through downstream RAS and PI3K pathways.[13] In addition to EGFR activity, increased signaling of the ERBB2, c-MET, and PDGFR RTKs can all result in activation of RAS and PI3K; whereas signaling through VEGF activates both pathways via PKC-β. However, increased activity of RTKs are not the only drivers of RAS and PI3K signaling. RAS and PI3K are themselves upregulated in many GBM, whereas their endogenous inhibitors NF1 and PTEN (phosphatidylinositol phosphate 3'-phosphatase) are often mutated or lost.[13,42] Loss of PTEN inhibition has been shown to remove sensitivity to EGFR inhibition by erlotinib and gefitinib[43] and is a powerful negative prognostic factor.[44] Subsequent signaling molecules in the 2 pathways have been identified and are also deregulated and mutated, resulting in increased flux through the pathways. The characterization of these downstream alterations gives rise to approaches other than simply targeting more or different cell surface RTKs.

RAS/RAF

RAS signaling ultimately activates the transcription factor extracellular signal-regulated kinase (ERK) by way of the intermediate proteins RAF and MEK. Tipifarnib (Zarnestra) is a farnesyl transferase–inhibiting drug shown to reduce signaling through the RAS pathway. Although early phase I trials determined that tipifarnib is well tolerated by

patients with GBM, early phase II trials failed to show a benefit of tipifarnib when added to TMZ and RT. The RAF protein is among those inhibited by the drug sorafenib, currently being studied in GBM. In addition to its effects on RAF, sorafenib also shows inhibitory effects on VEGFR and PDGFR.[45] However, despite potentially complimentary antitumor effects, trials combining sorafenib with TMZ and RT have thus far failed to show benefit, although the drug combination was well tolerated in patients.[46] Similarly, studies of recurrent GBM treated concurrently with sorafenib and TMZ have been unsuccessful in improving outcomes.[47] There are several additional active trials of sorafenib that may provide important information about the effect of combining sorafenib with other agents including erlotinib, the RAS inhibitor tipifarnib, and the mTOR inhibitor temsirolimus (Torisel). A study proposing treatment of recurrent GBM with sorafenib plus the mTOR inhibitor evirolimus (Afinitor, Zortress) has recently been approved, but is not yet recruiting patients.

PI3K

It has been suggested that aberrant activation of the PI3K pathway is universal in human cancer. PI3K pathways regulate several malignant phenotypes including resistance to apoptosis, cell growth, proliferation, and invasion, and PI3K activation is associated with poor prognosis in GBM.[48,49] PI3K signaling activates AKT and subsequently mTOR via phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to produce phosphatidylinositol-3,4,5-trisphosphate (PIP3). Perifosine is an AKT inhibitor that has shown promise in preclinical studies and is currently being tested in phase II trials.[50] mTOR can also be activated downstream of RAS and is therefore an example of a confluence of the RAS and PI3K pathways. The mTOR-inhibiting drugs sirolimus (Rapamycin, Rapamune) and temsirolimus have been studied in human GBM, though dramatic growth inhibition has not been seen.[51–53] Two single-agent phase II trials of temsirolimus showed altered radiological response after monotherapy, but failed to show a prolongation of survival.[52,53] These trials suggest that mTOR inhibition alone is likely to be insufficient for effective GBM therapy, although these agents continue to have potential as components of multimodal approaches.

β-PROTEIN KINASE C

The β-protein kinase C (PKC-β) signaling molecule is implicated in promoting activity of both the RAS and PI3K pathways after activation through VEGFR. Expression of this receptor, and its subsequent activation of PKC-β, leads to downstream promotion of the prosurvival and progrowth pathways described earlier. The drug enzastaurin, which inhibits PKC-β activity,[54] has been shown to affect total flux through the PI3K and RAS pathways and was determined in preclinical studies to be a good candidate drug for trials in GBM. A recent phase II trial showed a strong radiological response in 26% of patients treated with enzastaurin for recurrent glioma.[55,56] This success was used as rationale to begin a phase III clinical trial comparing enzastaurin with the alkylating drug lomustine (CCNU, CeeNU) for the treatment of recurrent GBM. Although this study was terminated early because of the lack of an increase in median overall survival (OS) or median PFS compared with lomustine, the enzastaurin was better tolerated, suggesting the potential for inclusion of enzastaurin into combination therapies.[57–60] To this end, a study combining enzastaurin with RT and TMZ for the treatment of primary GBM is ongoing (clinicaltrials.gov), and enzastaurin is also being combined with the antiangiogenic antibody bevacizumab and the alkylating agent carboplatin (Paraplatin) in a study for the treatment of recurrent GBM.

OTHER AGENTS

Several SMIs have been developed to target intracellular proteins that are not implicated in the RTK-RAS-PI3K signaling axis. These SMIs include inhibitors of topoisomerase I and II, histone deacetylase, integrins, and the proteasome.

Topoisomerase inhibitors include the drugs etoposide, topotecan, irinotecan, edotecarin, rubitecan, pyrazoloacridine, karenitecin, and gimatecan, many of which have shown efficacy in various types of cancer. Topoisomerase-inhibiting drugs block function of the cellular enzymes topoisomerase I and II, which bind to DNA and result in breakage and ligation of the phosphodiester backbone during the S phase of the cell cycle, allowing unwinding (and, thus, unpackaging) of supercoiled DNA. It is thought that topoisomerase-inhibiting drugs block the ligation step, and thereby produce single-stranded and double-stranded DNA breaks that compromise integrity and result in checkpoint arrest and cellular apoptosis. Several single-agent studies of irinotecan have shown discouraging results in patients with recurrent malignant gliomas.[61–65] Despite this, the unique cytotoxic action of these drugs has made them an attractive potential component of multimodal therapy, especially in

patients who have recurrent disease or those whose methylguanine methyltransferase (MGMT) status suggests that they will be resistant to TMZ therapy. Trials combining topoisomerase inhibitors with other therapies have shown some benefit to the addition of these drugs, and the combination of irinotecan and bevacizumab has been shown to have some effect in treating recurrent GBM in a phase II study.[66,67] Another study using etoposide in place of irinotecan showed similar results,[68] supporting the case for combinatorial approaches to using topoisomerase inhibitors in GBM.

Histone deacetylase enzymes (HDAC) remove acetyl groups from histones, allowing for the unpackaging of supercoiled DNA, which is normally stored condensed and wound around the histone proteins. This process is important in DNA replication as well as DNA repair; inhibition of HDAC results in cell cycle arrest and apoptosis in cancer cells. Pretreatment of patients with cancer with HDAC inhibitors has been shown to sensitize GBM cells to RT and DNA-damaging chemotherapeutics.[69–71] In phase II trials of recurrent GBM, vorinostat induced inhibition of HDAC and showed modest single-agent activity. As a result of this finding, and considering the method of action of HDAC, various clinical trials of vorinostat combined with RT, TMZ, erlotinib, bevacizumab, or irinotecan are underway.

Integrins are a class of cell adhesion proteins that are important regulators of motility and angiogenesis in glioma cells.[72] The recent development of cilgenitide, an inhibitor of the a_vb_3 and a_vb_5 integrins has shown some promise in recently completed trials in recurrent[73] as well as newly diagnosed GBM.[74] However, it has been suggested that the improvements seen in these 2 trials were possible artifacts of the progressively improved care common to all add-on TMZ therapies, rather than a result of cilengitide itself.[75] Accrual of patients for an international phase III trial to test the effects of addition of cilengitide to the standard therapy for TMZ and RT in newly diagnosed GBM has just been completed. This CENTRIC study should answer many of the questions regarding the benefit of adding cilengitide to standard therapy when treating GBM.[74]

The ubiquitin-proteasome complex is a critical regulatory element in the scheduled degradation of cell cycle proteins involved in balancing proliferation and apoptosis.[76] Inhibition of the proteasome disrupts the cyclic degradation of these regulatory proteins, and can induce cell growth arrest leading to the induction of apoptosis. The proteasome-inhibiting drug bortezomib (Velcade) showed growth arrest in glioma cells in vitro, and is currently being tested in several phase II trials in combination with TMZ or other targeted agents. However, one such phase II trial showed no significant benefit from the combination of the HDAC inhibitor vorinostat (Zolinza) with bortezomib.[77]

FUTURE DIRECTIONS

An ever-increasing armamentarium of SMI drugs has resulted from increasing understanding of the altered signaling inherent to many cancers, and many of these agents are dramatically changing care in previously untreatable diseases such as CML and gastrointestinal stromal tumors. Given the dismal prognosis of high-grade glioma, there has been considerable hope that newer drugs would improve outcomes in this aggressive cancer. However, as outlined earlier, few of these new targeted agents have shown significant or prolonged survival benefits in initial studies of patients with GBM.

There are several potential explanations for current failures with the use of SMIs for GBM. Because of the diverse genetic bases of these tumors, a variety of mutations involving oncogene and tumor suppressor pathways may drive tumor progression in individual patients and likely require multiple agents (targeting both antigrowth and proapoptotic functionality) for clinically relevant antitumor effects. Given coactivation and downstream overlap of multiple oncogenic RTK within GBM, it is likely that inhibition of any RTK can be compensated by increased activation of another. For example, PDGFR and c-MET receptors are engaged after EGFR inhibition and maintain downstream pathway activation.[78] Taken together, these findings suggest that multiple targeted agents used in combination might be required to effectively attenuate RTK signal transmission in GBM.[79]

In addition, although increasing OS is the most pertinent goal of any treatment strategy for cancer, this is only 1 of several relevant clinical endpoints to evaluate drug efficacy. Given that many SMIs are cytostatic in nature, it is likely that their effects on tumor cells could be overlooked when using traditional metrics of prolonged survival and radiological response. To this end, the development of biomarkers and molecular imaging tools to report on drug concentration and activity at the site of interest are important goals that would provide valuable information aiding the design of future studies.

In addition, large-scale studies to better characterize the genetic changes associated with GBM will be critically important for integrating the understanding of common molecular alterations and

subsequently tailoring specific therapy. In an example of such an approach, recent data from TCGA Research Network has reclassified GBM into 4 distinct subtypes based on abnormalities in commonly altered signaling pathways.[80] The differentiators include upregulation of EGFR and PDGFR-, loss of NF1, and activation of IDH1. This identification of GBM subtypes seems to predict those patients who will respond best to aggressive conventional therapies, versus those who respond only poorly or not at all. This example of a clinically relevant stratification approach, based on commonly altered pathways in GBM, provides researchers with a strong foundation on which to design pathway-targeted combination regimens. This information also serves to identify the patient subtypes most likely to derive benefit from aggressive traditional therapies, versus those who will not and should therefore be encouraged to enroll in trials of experimental therapeutics as first-line agents.

Regarding overall approach, integration of the known effects of targeted agents to provide redundancy in signaling inhibition should be a major focus of future trial design. It may be shown that slowing overactive oncogenic cascades through small molecule inhibition may not provide enough antitumor benefit to improve overall outcomes, emphasizing that this approach may be best applied in conjunction with current cytotoxic treatments such as the current standard therapies TMZ and RT. In other cases, certain SMI drugs such as the topoisomerase inhibitors can induce cytotoxicity through novel mechanisms and could feasibly provide alternative treatment options for recurrent patients or those with inherent resistance to TMZ caused by MGMT status.

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