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Combining drugs and radiotherapy: from the bench to the bedside

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Abstract

Purpose of review—The current standard care of treatment for glioblastomas (GBM) is never curative and exclusively involves the use of cytotoxics upfront (e.g. radiation and chemotherapy). Current clinical protocols involve use of single agent targeted therapies which inhibit specific pathways. Given the functional redundancies present in human tumors and escape mechanisms, it is highly unlikely that such a monotherapy approach will be successful in the treatment of GBM. Future directions of therapy for GBMs will likely involve the use of therapeutic cocktails including more than one target specific inhibitors based on tumor escape mechanism, genetic, epigenetic and molecular signatures. This review addresses some of the relevant issues.

Recent findings—Correlative clinical studies from various clinical trials and pre-clinical studies have provided the meticulous use of chemotherapeutics and radiation based on molecular profiling of tumors. Alkylating agents such as temozolomide lose their efficacy if DNA repair enzyme expression is up regulated. The alternative strategies include targeting the enzyme or one can use Poly (ADP) ribose inhibitor to inhibit base excision repair (BER) pathway rather than mismatch repair (MMR) pathway. Currently, several inhibitors in this category are in clinical trials. Next, we have addressed new avenues including radiosensitizers, hypoxia, metabolism, angiogenesis, invasive and infiltrative nature of tumors and potential molecular targets which can be exploited for clinical trials. Finally we have included some aspect of genome wide association studies and correlative analysis and the lessons learned to design better clinical trials.

Summary—Advances in profiling the non-coding RNAs, genetic, epigenetic profiles, metabolomics, genomics and proteomics may uncover important resistance mechanisms in GBM. Personalized therapy using various therapeutic cocktails targeting these resistance mechanisms may prove even more effective in the future management of GBMs.

Keywords

Glioblastoma; Clinical trials; Correlative studies; ChemoRadio therapy; Therapeutic targets

Introduction

Malignant gliomas are treatment-resistant tumors for which curative therapies remain elusive. The addition of temozolomide has been found to significantly improve outcome compared to radiation alone, but this improvement, while significant, remains marginal for most. Patients with epigenetic silencing of the *MGMT* gene promoter appeared to benefit the

most from the addition of temozolomide chemotherapy in the upfront setting. Patients whose tumors harbor an unmethylated gene promoter and patients with recurrent tumors are left with few effective treatment options. The wealth of novel therapeutics being discovered and developed for other systemic cancers have created a pipeline of drugs that are promising for investigation in brain tumor patients. Targeted therapies aimed at angiogenesis and at key signal transduction pathways regulating cellular survival represent promising attempts to approach malignant glioma therapy in a new way with potentially improved patient outcomes. This article provides an overview on various targeted therapeutic approaches for malignant gliomas under current investigation, as well as future directions.

Standard care for GBM patients

Current standard care for newly-diagnosed GBM patients is temozolomide therapy (TMZ) + radiation treatment (RT) (1, 2). Both RT and TMZ therapy can induce DNA damage and activate DNA repair mechanisms. TMZ leads to methylation at the O⁶ position of guanine. DNA methylation by TMZ will trigger the activation of mismatch repair (MMR) pathways. In MMR-proficient cells, this results in G2 checkpoint activation leading to G2/M cell cycle arrest and eventually to induction of apoptosis. This process is accompanied by activation of ataxia telangiectasia mutated (*ATM*) and *ATM* and Rad3-related (*ATR*) kinases that phosphorylate checkpoint kinase 1 (Chk1) and checkpoint kinase 2 (Chk2) checkpoint proteins, and p53. Mitotic cells in the G2-M phase due to arrest are more susceptible to DNA damage induced by radiation and hence one would theoretically expect a synergism when TMZ is combined with radiation.

Although the vast majority of alkylation events occur at N3 and N7, it is hypothesized that the cytotoxicity of TMZ results primarily from DNA methylation at the O⁶ position of guanine which represents the minority adduct of TMZ. The resulting O⁶-methylguanine, if left unrepaired, is mutagenic and acts as a trigger for cytotoxicity and apoptosis. These methyl adducts are removed by the ubiquitous DNA repair enzyme MGMT. Epigenetic inactivation of the DNA repair enzyme *MGMT* seems to be the strongest marker for outcome in patients treated with alkylating agent chemotherapy (3). The Median survival of patients with unmethylated *MGMT* is 12.5 months in RT arm which is higher than unmethylated *MGMT* overall and TMZ+RT arm which are 11 and 10 months respectively(4). It is intriguing that methylation of the *MGMT* promoter also appears to be associated with improved outcome in GBM patients treated by radiation alone. Therefore, it is unclear whether *MGMT* methylation is truly a predictive marker in the setting of TMZ treatment, or a more general prognostic marker. Patients whose tumors do not have *MGMT* promoter methylation appear less likely to benefit from the addition of temozolomide chemotherapy and require alternative treatment strategies. Preclinical findings from our lab (5) suggest that TMZ can potentiate RT response in *MGMT*-negative cells through increasing the degree of double-strand DNA damage when a competitive inhibitor of *MGMT* such as O⁶-Benzyl guanine is combined with TMZ. These results provide mechanistic evidence for the important role of *MGMT*-mediated repair of the alkylating agent-induced O⁶-methylguanine-adduct for tumor resistance,

ChemoRadioTherapy

The integration of temozolomide into current treatment protocols of postoperative combination therapy with radiation and drugs in high-grade glioma increases survival benefits. Attempts to optimize the schedule of temozolomide administration and to combine this regimen with additional agents are currently ongoing. Additional trials are examining whether temozolomide and radiotherapy combination regimens should also be the standard of care in patients with anaplastic glioma(6). It has been shown that through a randomized

trial stereotactic radiosurgery (SRS) prior to radiation therapy (RT) had no benefit in GBM patients. A recent report shows the benefits of SRS after RT and at the time of progression. On multivariate analysis, Radiation Therapy Oncology Group (RTOG) class III patients, those with more extensive resections, and those who were not on steroids at the time of SRS had significantly improved OS. The authors suggest that SRS provides a survival advantage when delivered after RT. This benefit may be best appreciated in RTOG class III patients (7). In the phase III EORTC-NCIC study reported, combined initial treatment for glioblastoma with temozolomide and radiotherapy improves survival compared with radiotherapy alone. Addition of chemotherapy early in the disease course and concomitantly with radiotherapy is the best strategy to incorporate new drugs. Some patients had pseudoprogression, which was most likely in those given temozolomide who have a methylated *MGMT* promoter (8). The open-label, dose-escalation, Phase I clinical study evaluated the safety of three dose levels of intravenously administered bortezomib in addition to concurrent radiotherapy and temozolomide. The most frequent toxicities were Grade 1 and 2 stomatitis, erythema, and alopecia. All 27 patients were evaluable for response. At a median follow-up of 15.0 months, 9 patients were still alive, with a median survival of 17.4 months for all patients and 15.0 months for patients with high-grade glioma. Bortezomib is well tolerated and safe combined with temozolomide and radiotherapy when used in the treatment of central nervous system malignancies (9). The open-label, prospective, single-arm, phase II study combined erlotinib with radiation therapy (XRT) and temozolomide to treat glioblastoma multiforme (GBM) and gliosarcoma. All patients received temozolomide during and after XRT. Molecular markers of epidermal growth factor receptor (*EGFR*), *EGFRvIII*, phosphatase and tensin homolog (*PTEN*), and methylation status of the promoter region of the *MGMT* gene were analyzed from tumor tissue. Survival was compared with outcomes from two historical phase II trials. Median survival was 19.3 months in the current study and 14.1 months in the combined historical control studies. Treatment was well tolerated. There was a strong positive correlation between *MGMT* promoter methylation and survival, as well as an association between *MGMT* promoter-methylated tumors and *PTEN* positivity shown by immunohistochemistry with improved survival (10).

DNA repair inhibitors in combination therapy

A class of agents currently being tested in clinical trials in combination with temozolomide therapy consists of pseudosubstrates for *MGMT* to act as a suicide inhibitor of DNA methyltransferase activity. The lead compounds in this class have been *O*6-benzylguanine and lomeguatrib (AstraZeneca, London, UK); the latter is also known as *O*6-(4-bromophenyl)guanine or PaTrin-2. Resistance to *O*6-alkylating agents can be overcome in preclinical models by depletion of *MGMT* and a relationship exists between *MGMT* activity and resistance to chloroethylating nitrosoureas and methylating agents in tumour cells grown *in vitro* and in xenograft models. Dose-dense TMZ also holds the promise of depleting *MGMT* activity. The RTOG and EORTC are investigating this strategy in a prospective clinical trial (RTOG 0525/EORTC 26052-22053). The primary endpoint of this protocol is to determine if increasing the both the dose intensity and exposure duration (21/28 days) of temozolomide given after concomitant TMZ/RT improves overall survival. This is also the first trial on prospectively stratifying for *MGMT* methylation status. The correlative biology work behind the RTOG 0525/Intergroup study is one of the most comprehensive in the setting of GBM and will serve to determine the predictive/prognostic value of *MGMT* promoter methylation and molecular profiles associated with outcomes in newly-diagnosed GBM patients treated by RT+TMZ. The overall goal of the study is to utilize tissue available from the clinical trial to determine the relationships between chromosome 10 and *MGMT* promoter methylation status and prognosis in glioblastoma. In addition to this, expression of other key markers will also be analyzed. All these markers will be combined to develop a

clinically useful predictive model associated with distinct prognoses or therapeutic response. Furthermore, it has been proposed to identify novel molecular correlates that further define the sub-groups of glioblastoma and to highlight potential therapeutic targets in the groups that are unlikely to respond to standard treatments.

O6-Benzylguanine (O6-BG) and lomeguatrib have been tested in phase I–II clinical trials and biologically effective doses have been established for both agents. In a clinical trial using O6-BG by South West Oncology Group (SWOG) (11), patients with methylated *MGMT* had a median survival of 12.6 months and patients with unmethylated *MGMT* had a median survival of 10.6 months. Median progression-free survivals were 4.5 and 3.1 months respectively, for the methylated and unmethylated groups. The subgroup of patients without promoter methylation may be more likely to benefit from treatment with O⁶BG.

H-14273,S0001, a phase III study of RT and BCNU (carmustine) with and without O6-Benzylguanine (O6-BG) for newly diagnosed GBM. Other clinical trial includes combining radiation therapy with carmustine and O6-BG. It is not yet known whether radiation therapy and carmustine are more effective with or without O6-benzylguanine (NCT00017147). The combination of temozolomide with inhibitors of poly(ADP-ribose) polymerase 1 (*PARP1*) shows efficacy in pre-clinical studies (12). The role of *PARP* in DNA repair has not been fully elucidated and additional roles for *PARP* in DNA damage signaling or repair might explain the increased toxicity of combination treatments. There are several phase I/II trials with *PARP* inhibitors such as AZD-2281 (Astra Zeneca), AGO14699 (Pfizer), INO-1001 (Inotek), ABT-888 (Abbott) and *BER* inhibitor TRC-102 (Tracon Pharma). The success of the treatment rationale depends on the overall biological role of and necessity for *PARP* in cancer cells that are trying to repair the DNA damage induced by temozolomide.

Radiosensitizers

DNA-dependent protein kinase (*DNAPK*), a member of the PIKK family, is important for DSB repair by non-homologous end joining following ionizing radiation. Cells defective in *DNAPK* are highly sensitive to ionizing radiation indicating that inhibition of *DNAPK* might sensitize tumors to radiation treatment. Other small molecules that reversibly inhibit *DNAPK* kinase activity at low micromolar concentrations have been synthesized. They are currently in transition from late preclinical development to early clinical trials. In particular, NU7441 (Northern Institute for Cancer Research, Chemistry Section, University of Newcastle upon Tyne) has been shown to sensitize cells to topoisomerase II poisons and can also function as a radiosensitizer in a manner consistent with *DNAPK* inhibition. Both gemcitabine and its metabolite difluorodeoxyuridine (dFdU) are potent radiosensitizers. The aim of a phase 0 study was to investigate whether gemcitabine passes the blood-tumor barrier, and is phosphorylated in the tumor by deoxycytidine kinase (dCK) to gemcitabine nucleotides in order to enable radiosensitization, and whether it is deaminated by deoxycytidine deaminase (dCDA) to dFdU. Gemcitabine was administered at 500 or 1000 mg/m² 1–4 hours before surgery to 10 GBM patients. By measuring the gemcitabine in plasma and the tumor the authors concluded that gemcitabine passes the blood-tumor barrier in GBM patients. In tumor samples, both gemcitabine and dFdU concentrations are high enough to enable radiosensitization, which warrants clinical studies using gemcitabine in combination with radiation (13).

In theory, DNA repair inhibitors could be used to impair the repair of replication lesions that are present in tumour cells and convert them into fatal replication lesions that specifically kill cancer cells. For example, there is evidence that the increased expression or activity of oncogenes can induce replication stress (14, 15). In such tumors it might be possible to use DNA repair inhibitors to make existing cancer-specific replication lesions more toxic, resulting in fatal replication lesions selectively killing oncogene expressing cancer cells.

More advanced cancers are exposed to another source of replication stress owing to the tumour microenvironment. Tumors are often hypoxic, which has been shown to disrupt DNA synthesis. These conditions cause replication lesions that activate the *ATM*- and *ATR*-mediated checkpoint response. Furthermore, DNA repair is downregulated in hypoxic cells, which cumulatively contributes to the genetic instability observed in these cells. In hypoxic cancer cells, therefore, inhibitors of the checkpoint response could prove to be more efficient than inhibitors of DNA repair. A more challenging treatment strategy is the inhibition of the repair of tumour-specific replication lesions and conversion of these into fatal lesions.

Hypoxia and glucose metabolism in the solid tumor

One of the most recognized reasons for altered tumour metabolism is the physiological stresses that exist within the tumour due to hypoxia, acidosis and increased interstitial fluid pressure. Tumour cells respond to these conditions (microenvironment), and adapt their metabolism to adjust the oxygen (O_2) demand to meet the limited supply. Perhaps, they respond to this by activating the hypoxia-inducible factor 1 (*HIF1*). The *HIF1* activation shifts the energy production by increasing glycolysis and decreasing mitochondrial function. *HIF1* can be regulated by other factors such as oncogene activation or loss of tumour suppressors. For example, *HIF1* accumulates in tumour cells after activation of oncogenes such as *Ras*, *SRC*, *PI3K*, etc., or loss of tumour suppressors such as *VHL* or *PTEN*, even under normoxic conditions. *HIF1* stimulates glycolytic energy production by transactivating genes involved in extracellular glucose import such as *GLUT1* and enzymes responsible for the glycolytic breakdown of intracellular glucose such as phosphofructokinase 1 (*PFK1*) and aldolase. Glycolysis is a process that breaks glucose down to pyruvate and generates two molecules of *ATP*. This low-yield energy production is sufficient to supply *ATP* for cellular energetics if the supply of glucose is adequate. Pyruvate can be further broken down at the mitochondria through the process of oxidative phosphorylation, which uses O_2 and generates CO_2 , H_2O and about 18 additional molecules of *ATP*. *HIF1* down regulates oxidative phosphorylation within the mitochondria by transactivating genes such as pyruvate dehydrogenase kinase 1 (*PDK1*) and MAX interactor 1 (*MXI1*). In order to produce more *ATP* glucose uptake by tumor tissues will be more than the normal tissue. In the hypoxic cell pyruvate is not used by mitochondria but instead is converted to lactate by lactate dehydrogenase (*LDH*) to be released into the extracellular space. *HIF1* can channel glucose into glycolysis by increasing the amounts of the enzymes involved in this process. All 12 enzymes necessary for glycolysis are directly regulated by *HIF1* such that the entire process is coordinately stimulated by *HIF1*.

Normal tissue uses glycolysis to generate approximately 10% of the cell's *ATP*, while most energy is produced in mitochondria accounting. In tumor sections, over 50% of the cellular energy is produced by glycolysis and the remainder being generated at the mitochondria. Interestingly, this shift occurs even when there is enough O_2 present to support mitochondrial function (aerobic glycolysis). The reliance of tumour cells on glycolysis for energy production causes them to consume more glucose because of the low efficiency of glycolysis in generating *ATP*. All 12 enzymes necessary for glycolysis are directly regulated by *HIF1*. To date, most anticancer strategies targeting metabolism have tried to exploit the increased glycolytic activity of the tumor, rather than the reduced mitochondrial function. Two agents that have advanced to clinical trials are the non-metabolizable competitive inhibitor of glucose 2-deoxyglucose (2-DG) (16, 17) and the putative hexokinase inhibitor lonidamine (18, 19). Phase I/II clinical trials indicated that the combination of 2-DG with RT was well tolerated. The patient compliance to the combined treatment was very good up to a 2-DG dose of 250 mg/kg. No significant damage to the normal brain tissue was observed during follow-up in seven out of ten patients who received complete treatment and survived between 11 and 46 months after treatment. Oral administration of 2-DG combined

with RT (5 Gy/fraction/week) is safe and could be tolerated in glioblastoma patients without any acute toxicity and late radiation damage to the normal brain. Further clinical studies to evaluate the efficacy of the combined treatment are warranted.

Anti-Angiogenesis

Angiogenesis contributes to the progression of cancer from a dormant *in situ* lesion to a more invasive phenotype. Although impressive radiographic responses have been observed with various anti-angiogenic agents, there is some question of whether these represent actual anti-tumor effect in addition to a reduction in peri-tumoral edema. Various mechanisms contribute to the resistance to anti-angiogenic therapies(20), including the increased expression of other pro-angiogenic factors; a process that is termed angiogenic rescue. *VEGFA* is required for endothelial-cell homeostasis. Some of the adverse effects of anti-VEGF-VEGFR agents have therefore been attributed to the deprivation of quiescent endothelial cells from *VEGFA*-maintenance signals. Tumour vasculature is structurally and functionally abnormal, presumably from the imbalance of signaling from pro- and anti-angiogenic growth factors emanating from tumors. The tumor microenvironment leads to interstitial hypertension, hypoxia and acidosis, contributing to increased treatment resistance in many tumor types. Judicious application of anti-angiogenic therapy could transiently 'normalize' the tumour vasculature and thus improve the delivery and effectiveness of cytotoxic agents and radiation given during the normalization window. Bevacizumab is an FDA approved drug and has been widely used in combination with radio- and chemotherapy. Table-I provides the current clinical trials using Antiangiogenic agent in combination with chemotherapy and or radiation in glioblastomas

One hundred and twenty-five patients were treated with bevacizumab in addition to standard TMZ/RT in a phase II trial at Duke University(21, 22). The primary goal of the study was improvement in median progression-free survival. After a median follow-up of 15 months of follow-up, 61 percent of patients in the trial were progression free. According to available data from the first 75 patients, 14 dropped out of the study due to toxicity and there were seven deaths, two of which were associated with the treatment. Thus, an antiangiogenic agent might have more activity for the older age group than in patients of younger age or neuronal molecular profile (23). The Phase III study RTOG 0825 will be a landmark study in the setting of newly diagnosed GBM patients. This study is unique because of its initial correlative analysis using tumor tissue from patients for *MGMT* expression. Based on the molecular markers, patients will be randomized. Bevacizumab treatment utilizes an intravascular approach which would eliminate the concerns regarding drug delivery through the blood-brain or blood-tumor barrier. Currently there are other clinical trials in which bevacizumab is used in combination with traditional cytotoxic agents such as temozolomide, irinotecan, etc.. It is hoped that bevacizumab improves perfusion in tumors and enhance the delivery of cytotoxic drugs. An important issue in the use of antiangiogenesis agents, like bevacizumab, is the evaluation of response and determination of clinical utility. Gadolinium-contrast MRIs may give false responses, because antiangiogenic agents decrease extravasation of the gadolinium. A better clinical tool may be fluorothymidine (FLT) positron emission tomography (PET). FLT-PET metabolic response was a better predictor of overall survival than gadolinium-contrast MRI response.

Invasive, Infiltrative and recurrent Tumors

This process of invasion includes increased synthesis and secretion of several proteases, such as cysteine, serine and metalloproteinases, to degrade extracellular-matrix (ECM) components selectively. Strategies to prevent the expression and / or down regulation of proteases at the molecular level have led to significant reduction / inhibition of tumor

invasion, tumor growth and angiogenesis. Reports (24) indicate that these proteases interact with each other and can directly and indirectly facilitate the expression of other proteases. As such, matrix degrading proteases seem to be extremely promising targets for therapy. Regardless of the upstream mechanism of neoplasia such as *EGFR* amplification, *p53* mutation, *PTEN* loss or cyclin alterations, proteases might constitute a 'final common pathway' for tumour invasion. Anti-proteolytic agents might provide additive or synergistic treatment benefits if used in combination with conventional therapeutics. Cilengitide is a cyclic peptide antagonist of integrins $\alpha v \beta 3$ and $\alpha v \beta 5$ which is currently evaluated as a novel therapeutic agent for recurrent and newly diagnosed glioblastoma. Preliminary clinical results indicate a preferential benefit from cilengitide added to temozolomide-based radiochemotherapy in patients with O(6)-methyltransferase (*MGMT*) gene promoter methylation. Accordingly, the authors have also examined whether the *MGMT* status determines glioma cell responses to cilengitide alone or in combination with temozolomide. Neither ectopic expression of *MGMT* in *MGMT*-negative cells nor silencing the *MGMT* gene in *MGMT*-positive cells altered their response to cilengitide alone or cilengitide in combination with temozolomide. These data suggest that the beneficial clinical effects derived from cilengitide in vivo may arise from altered perfusion which promotes temozolomide delivery to glioma cells (25). Cilengitide is active and synergizes with external beam radiotherapy in preclinical GBM models. Among newly diagnosed GBM patients, single-arm studies incorporating cilengitide into standard external beam radiotherapy/temozolomide have shown encouraging activity with no increased toxicity and have led to a ongoing large randomized Phase III trial (26, 27).

Glioma Genesis and Genome wide association

The more frequently observed chromosomal changes are at 1p, 7, 8q, 9p, 10, 12, 13, 19, 20, and 22. Integrated genomic analysis of GBM has provided a novel view of the genetic landscape of glioblastomas (28). The authors report that patients with *IDH1* mutations on chromosome 2q33 had a significantly improved prognosis, with a median overall survival of 3.8 years as compared to 1.1 years for patients with wild type *IDH1*. Tumors with *IDH1* or *IDH2* mutations had distinctive genetic and clinical characteristics, and patients with such tumors had a better outcome than those with wild-type *IDH* genes (29). Comprehensive genomic characterization of GBM genes and core pathway analysis using "TCGA" pilot project with 206 glioblastomas (30) provides new insights into the roles of *ERBB2*, *NF1*, *TP53*, *PIK3R1*, *MGMT*, etc. demonstrating that it can rapidly expand knowledge of the molecular basis of cancer. It is reasonable to speculate from this type of study, future therapeutic decisions should be made on the patterns of mutations in tumor and eventually one has to use therapeutic cocktails based on specific patterns of mutation. Further a recent report has made use of this cancer genome atlas. The authors have concluded that haploinsufficiency of the tumor suppressor *ANXA7* due to monosomy of chromosome 10 provides a clinically relevant mechanism to augment *EGFR* signaling in glioblastomas beyond that resulting from amplification of the *EGFR* gene (31). The summary of clinical trials currently accruing patients with other sub-type of therapeutic agents is listed in Table-1.

Correlative Analysis- Lessons Learned from Clinical Trials

Among the available large amount of papers in correlative analysis we identified some of the key papers. Immunohistochemical staining in 268 cases of newly diagnosed glioblastoma revealed elevated p-MAPK expression was most strongly associated with poor response to radiotherapy (32). Chinnaiyan et. al., used 153 samples from RTOG clinical trials and found that there were no statistically significant differences between pretreatment patient characteristics and nestin expression. There was no statistically significant difference

in either overall survival or progression-free survival (PFS) demonstrated, although a trend in decreased PFS was observed with high nestin expression ($p = 0.06$) (33). Publications from our lab include *EGFR* expression levels do not appear to have prognostic value (26), PI3K and survivin pathways (34-38). A phase II study of erlotinib plus temozolomide during and after radiation therapy (10), molecular markers of epidermal growth factor receptor (*EGFR*), *EGFRvIII*, phosphatase and tensin homolog (*PTEN*), and methylation status of the promotor region of the *MGMT* gene were analyzed from tumor tissue. There was a strong positive correlation between *MGMT* promotor methylation and survival, as well as an association between *MGMT* promotor-methylated tumors and *PTEN* positivity shown by immunohistochemistry with improved survival. The authors analyzed the *ANGPT1/ANGPT2* balance in the context of therapeutic outcome in 62 patients with primary glioblastomas (39). The results of the study suggest that the *ANGPT1/ANGPT2* balance has prognostic value in patients with primary GBMs. A phase II trial consisting of 60 patients with recurrent malignant astrocytomas was treated with bevacizumab and irinotecan. In this patient cohort, tumor expression levels of *VEGF*, the molecular target of bevacizumab, were associated with radiographic response, and the upstream promoter of angiogenesis, hypoxia, determined survival outcome, as measured from treatment initiation (37). RTOG 0211 revealed that the addition of gefitinib to radiation was well-tolerated, but survival was not significantly improved compared to historical controls. Based on data from a retrospective analysis of recurrent GBM treated with *EGFR TKIs*, we analyzed whether subgroups with specific molecular signatures are more likely to benefit from anti-EGFR therapies (40, 41). The predictive values of 12 molecules integral to EGFR signaling either have been examined (*EGFR*, p*EGFR*, *EGFRvIII*, *PTEN*, p*AKT*, p*MAPK*, p*mTor*, *IGFR1*, *NFKB*, *Survivin*, *MGMT*, and p*Src*). Neither total *EGFR*, *EGFRvIII*, nor *PTEN* expression as single markers were significantly associated with either overall (OS) or PFS in GBM patients treated on RTOG 0211. Patients expressing high versus low levels of p*AKT* had significantly shorter survival times ($p = 0.047$). In the upfront setting, activation of *AKT* signaling appears to be associated with adverse outcome in Gefitinib-treated GBM patients.

Conclusion

Therapeutic targeting of tumors on the basis of molecular analysis is yet to fulfill expectations. This may be partly due to diagnosis, broad distinctions, subjective assessment, lack of predictive power and finding consistencies that can be therapeutically targeted. Further, the nature of critical signaling pathways, compensatory redundancies, and the inherent interrelationship of molecular pathways appear to limit the therapeutic efficacies of monotherapies in GBMs. Co-targeting multiple dependent pathways in an intelligent manner may offer superior results. The intrinsic molecular and genetic heterogeneity of GBMs would argue that a “one-glove fits all” approach may prove suboptimal in this disease. Accordingly, an improved understanding of the underlying molecular biology of these tumors will ultimately lead to personalized care of GBM patients. The use of combined agents in a meticulous manner based on genomic and proteomic profiles will improve the survival outcome and better quality of life. Therapies have to be designed to gain in duration of survival in terms of years rather than current gain of survival in months.

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Table-1

Molecularly Targeted Clinical Trials currently accruing patients

Intervention	Identifier	Phase
Anti-Angiogenic Agents		
Temozolomide + Radiation Therapy ± Bevacizumab	NCT00884741	III
Bevacizumab + Temodar and Tarceva After Radiation Therapy and Temodar	NCT00525525	II
Concurrent Radiation Therapy, Temozolomide, and Bevacizumab Followed by Bevacizumab/Everolimus	NCT00805961	II
Bevacizumab and Irinotecan or Bevacizumab and Temozolomide With Concomitant Radiotherapy	NCT00817284	II
Gliadel Followed by Bevacizumab + Irinotecan	NCT00735436	II
Radiation therapy, temozolomide and Bevacizumab followed by Bevacizumab, temozolomide, and irinotecan	NCT00597402	II
Bevacizumab and Erlotinib After Radiation Therapy and Temozolomide	NCT00720356	II
Bevacizumab, Temozolomide and Hypofractionated Radiotherapy	NCT00782756	II
Cilengitide, Temozolomide & Radiotherapy	NCT00689221	III
Protease Inhibitors		
Vorinostat (SAHA) + Bortezomib (PS-341) + surgery	NCT00641706	II
Avastin + Bortezomib	NCT00611325	II
Tamoxifen and Bortezomib	NCT00112762	II
Bortezomib and Temozolomide	NCT00544284	I
Nelfinavir Mesylate, Radiation Therapy, and Temozolomide	NCT00915694	I
EGFR/HER/VEGF/PDGF/RTK/mTOR Inhibitors		
Sunitinib	NCT00923117	II
Traceva (Erlotinib) + Bevacizumab + Temozolomide	NCT00525525	II
Lapatinib + Surgery	NCT00103129	II
Cediranib (Recentin) + Lomustine	NCT00777153	III
Imatinib Mesitylate + Hydroxy Urea	NCT00154375	III
Sorafenib + Temozolomide + Radiation Therapy	NCT00734526	I/II
Temsirolimus, Temozolomide, and Radiation Therapy	NCT00316849	I
Radio sensitizers		
Neuradiab + Bevacizumab	NCT00906516	II
MGMT / Farnesyl Transferase / Histone Deacetylase /others		
Vorinostat, Isotretinoin and carboplatin	NCT00555399	I/II
Temozolomide + Carboplatin	NCT00021307	I/II
Temozolomide + O6-benzylguanine	NCT00612989	I
Farnesyl transferase inhibitor (Sarasar or Zarnestra) + Temozolomide	NCT00102648	I

Table-2

Clinical Trials currently accruing patients

Intervention	Identifier	Phase
Protease Inhibitors		
Vorinostat (SAHA) + Bortezomib (PS-341) + surgery	NCT00641706	II
Avastin + Bortezomib	NCT00611325	II
Tamoxifen and Bortezomib	NCT00112762	II
Bortezomib and Temozolomide	NCT00544284	I
Nelfinavir Mesylate, Radiation Therapy, and Temozolomide	NCT00915694	I
EGFR/HER/VEGF/PDGF/RTK Inhibitors		
Sunitinib	NCT00923117	II
Traceva (Erlotinib) + Bevacizumab + Temozolomide	NCT00525525	II
Lapatinib + Surgery	NCT00103129	II
Cediranib (Recentin) + Lomustine	NCT00777153	III
Imatinib Mesitylate + Hydroxy Urea	NCT00154375	III
Sorafenib + Temozolomide + Radiation Therapy	NCT00734526	I/II
Radio sensitizers		
Neuradiab + Bevacizumab	NCT00906516	II
MGMT / Farnesyl Transferase / Histone Deactylase /others		
Vorinostat, Isotretinoin and carboplatin	NCT00555399	I/II
Temozolomide + Carboplatin	NCT00021307	I/II
Temozolomide + O6-benzylguanine	NCT00612989	I
Farnesyl transferase inhibitor (Sarasar or Zarnestra) + Temozolomide	NCT00102648	I