Chapter 20

Topoisomerase Therapy in the Treatment of Brain Tumors

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1. Introduction

The treatment of brain tumors remains a challenge for modern medicine and care. Therapy for these patients is often complicated by site accessibility and the risk of damage to surrounding tissue. The ability of chemotherapies to cross the blood brain barrier has also limited their use as compared with surgical resections and radiation therapy. (Groothuis, 2000) For these reasons, malignant gliomas of the central nervous system (CNS) have a poor prognosis. In fact, the majority of patients with high grade gliomas (glioblastomas) will die within the first couple of years after the diagnosis.(McLendon, 2003) The 5 year survival rate for these patients with glioblastoma is less than 4% with the majority of deaths in the first two years post-diagnosis. (Grossman, 2004; CBTRUS, 2012) This devastating impact has been the impetus behind much of the research that is ongoing into effective therapies to combat these tumors. Over the years many therapies have been studied, but in recent times the increased investigation into specific molecular pathways has led to targeting specific tumor expression patterns and cellular attributes. The enhanced understanding of cell division, including aspects of DNA replication are now being used to target tumor replication and treat many cancers from various tissues. In this fashion, tumors of the CNS should be more specifically targeted so that damage to the surrounding normal tissue is minimized.

2. Epidemiology

Even after treatment, the median survival after the primary diagnosis remains poor. Many multimodal treatment approaches are considered, but few patients have been reported to have

long term survival greater than three years. In a recent study, analysis of 34,664 patients, diagnosed with GBM over the age of 20 from 1972-2008 in the Surveillance, Epidemiology, and End Results (SEER) NCI database, evaluated specific prognostic factors known to influence survival in these patients. (Thumma, 2012) This analysis included racial/ethnic characteristics in the description of specific subpopulations and found that Asian/Pacific Islanders had a better survival compared to the white population ($P<0.001$). Patients diagnosed with GBM during the years of 2000 to 2008 had a superior survival rate when compared with earlier decades ($P<0.001$). Statistically significant improvements in overall survival were also found for patients who received surgical resections, and adjuvant radiation treatment versus no radiation (P values <0.001). Young age was also found to be highly predictive of improved overall survival rates when separated into age groups as well as when studied as a continuous variable. (Thumma, 2012) Thus, there are subpopulations with varied genomic and environmental attributes that may affect survival of CNS tumors. Although these studies did not specifically identify the critical factors that dictate these differences, future studies may capitalize on differences that enhance efficacy.

Despite aggressive therapy for malignant gliomas, recurrence rate is quite high and the prognosis for most patients is extremely poor. The standard approach for high grade gliomas is radiation therapy combined with temozolomide. (Stupp, 2005) Temozolomide added to cranial radiation therapy improved Two year survival to 26.5% vs 10.4% to radiation therapy alone. Toxicity of temozolomide and radiation therapy was minimal. Despite the improved two-year survival with the addition of temozolomide to standard radiation therapy, the vast majority of patients progress and die of their disease. Thus, more effective drugs and approaches are definitely needed. Newer drugs being investigated include topoisomerase I inhibitors. This chapter will review topoisomerase I inhibitors in the treatment of malignant gliomas. Novel approaches using this class of compounds will also be discussed.

3. Current therapies

The DNA topoisomerases are a family of important enzymes involved in different stages of the cell cycle. They are essential nuclear enzymes important in DNA topology, repair, and replication by breaking and rejoining of the DNA double helix. The breakage that they induce in essence unwinds the DNA structure and releases the molecule from its wound configuration. In this configuration, DNA replication as well as transcription can occur in the cell nucleus. Two significant topoisomerase molecules are named topoisomerase I and topoisomerase II. Although these molecules are in the same family, they work in different steps to bind and cause the eventual unwinding of the helical DNA structure. Topoisomerase I is a monomeric protein that induces single stranded breaks in DNA, one strand at a time.(Redinbo, 1998) Topoisomerase II brings about double stranded breaks in DNA since it is a dimer in which each homologous monomer can cleave a strand of DNA.(Wang, 1996) These differences have lead to the development of specific inhibitors to these topoisomerase enzymes. Since these inhibitors actually damage DNA, they are sometimes referred to as poisons in the literature.

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4. Topotecan

Topotecan (TPT) is an analogue of camptothecin, an FDA approved chemotherapy for many types of cancer.(Bookman, 1998; tenBukkel, 2004) TPT is water soluble, and inhibits an essential role of topoisomerase I, depleting it in tumor cells and resulting in DNA strand breaks that are not utilized or repaired.(Yamashita, 2007) Cells are then stopped in the G2 phase of the cell cycle, and the progression of replication does not occur eventually leading to programmed cell death through apoptotic processes. In subcutaneous xenograft models and in vitro, TPT therapy has shown significant activity against glioblastoma (GBM) which is the most common and malignant type of primary brain tumor.(Ciusani, 2005; Rapisarda, 2004) Topotecan treatment showed some efficacy in preclinical studies, and TPT was found to be distributed in the cerebrospinal fluid after systemic administration, leading to the initiation of clinical trials evaluating the efficacy of TPT monotherapy on patients with GBM. (Ciusani, 2005; Baker, 1996) Data from phase II clinical trials with TPT treatment of both newly diagnosed and recurrent GBM revealed modest tumor responses. It is proposed that the lack of efficacy in treatment may be due to rapid clearance of TPT from the CSF, and rapid inactivation in plasma. (Mi, 1995) Both of these processes provide a survival advantage to the tumors since drug concentrations may not be significant around the tumor site. In order to address this and other mechanisms, the approach to TPT therapy has changed from monotherapy to use in combination with different agents targeting alternate pathways.(MacDonald,1996; Blaney,1996; Reveiz,2012) These combinations may vary in the sequence of drug delivery or may be simultaneous depending on the protocol used.

Topotecan is being studied as a component of combination therapies in primary brain tumors as well as in the efficacy it may have in the treatment of brain metastases. In one recent large scale literature review, approximately 10% to 18% of patients presented with brain metastases (BM) at the time of initial diagnosis of small cell lung cancer (SCLC), and an additional 40% to 50% will develop brain metastases during the course of their disease. To evaluate the effectiveness and toxicity of systemic chemotherapy for the treatment of these types of brain metastases from SCLC, the large scale systematic literature review was conducted for publications up to July 2011.(Reveiz,2012) The literature searched included randomized controlled trials comparing systemic chemotherapy (single agent or combination chemotherapy) vs another chemotherapy regimen, palliative care, whole brain radiotherapy or any combination of these interventions for the treatment of brain metastases as the sole site of progression. (Reveiz,2012) After this extensive search, no significant differences for overall survival (OS) were reported from randomized controlled trials with whole brain radiation therapy, and no significant difference was found between those treated with topotecan and those not treated with topotecan. Hence the treatment efficacy was not established in that review. A second trial found that patients receiving teponoside plus whole brain radiotherapy had a higher complete response rate than those receiving only the topoisomerase inhibitor. Hence, available evidence is insufficient to judge the effectiveness and safety of chemotherapy for the treatment of brain metastasis from small cell lung cancer. This may depend on the characteristics of the primary tumor and the activated mechanisms of metastasis. Future research may better address the

different combination therapies as well as monotherapies in head to head comparisons and trials in populations with primary tumors and metastatic tumors.

Liposomes are microscopic phospholipid particles with a bilayer membrane structure, and are used to encapsulate various anticancer drugs.(Allen, 2004; Drummond,1999,2005) Liposomes have been used to encapsulate TPT, while free TPT was found to be less active against subcutaneous xenografts of cancers than injection of nano-liposomal TPT.(Drummond, 2005;Tardi,2001) Liposomal encapsulation may improve the efficacy of TPT by increasing increasing drug circulatory half-life and by providing the appropriate pH to maintain drug activity.(Tardi, 2001;Burke, 1994) In either case, the effect may be an increased amount of active drug present at the tumor site. In orthotopic, intracranial xenograft models of GBM, nanoliposomal TPT demonstrated superior efficacy when administered directly into the tumor by convection-enhanced delivery (CED).(Tardi, 2001;Saito,2006) CED of nano-liposomal TPT increased TPT half-life in the brain vs free TPT, and conferred a highly significant survival advantage. In a recent study, systemically administered nano-liposomal TPT had enhanced efficacy in 3 orthotopic xenograft models of GBM.(Serwer,2011) Bioluminescence monitoring of tumor growth and therapeutic response, survival benefit to animal subjects, and immunehistochemical analysis of tumor apoptotic response to therapy were used to assess efficacy. Although these results were promising, data from clinical trials would be more significant and applicable to the demonstration of efficacy. Consistent with the inhibitor function, increased DNA strand breaks in TPT-treated tumors, and an increase in activated caspase-3 (marker of programmed cell death) were observed in this study.(Serwer,2011) Delivery of liposomal packaged TPT to tumors increased both of these molecular events, leading to cell death.

Nano-liposomal topotecan (nLS-TPT) has anti-tumor activity when administered directly to brain tumors by convection-enhanced delivery (CED).(Serwer,2011 poster) As a topoisomerase I inhibitor, topotecan (TPT) must be internalized in order to have a cytotoxic effect, hence increasing cellular internalization may increase the anti-tumor activity of nLS-TPT. Attaching an epidermal growth factor (EGFR)-specific antibody to the nLS-TPT surface increased EGFR-targeting. EGFR activation is considered a proliferative event leading to more cell replication. This receptor is commonly found on cell membranes in order to increase accessibility to ligand binding, hence the antibody used for targeting in this study may increase specific binding to cells that have increased expression of the receptor. Improved targeting rates of TPT-nLS internalization in vitro, and secondly nLS-TPT-EGFR offered superior efficacy compared to nLS-TPT in vivo.(Serwer,2011 poster) When coupled to the antibody, the internalization of the inhibitor was highly and significantly increased as was the rate of internalization of nLS-TPT-EGFR when compared to nLS-TPT in all cells that express EGFR. In vivo studies in both EGFR-expressing mouse models of glioblastoma models showed a substantial dose dependent benefit of nLS-TPT-EGFR treatment compared to nLS-TPT treatment.(Serwer, 2011 and poster) The use of nLS-TPT-EGFR against glioblastomas that overexpress EGFR increases targeting and improves internalization. This may ultimately increase survival by delaying tumor growth.

The prognosis for newly-diagnosed GBM remains poor, and one of the reasons for this is that GBM's have the highest levels of vascular endothelial growth factor (VEGF) and hypoxia

inducing factor-1 alpha (HIF-1 alpha), an important regulator of VEGF. Topotecan therapy may play a role in this signalling pathway by inhibiting HIF-1 alpha in treated tumor cells, limiting tumor vascularization. (Vredenburgh, 2011) A phase II trial in newly diagnosed GBM added bevacizumab and topotecan to standard therapy. 80 newly diagnosed GBM patients received standard radiation therapy and temozolomide with bevacizumab at 10 mg/kg every 14 days was added a minimum of 4 weeks post-op. Two weeks after radiation therapy was completed, 12 monthly cycles of temozolomide, and oral topotecan were given for patients not on an enzyme inducing anti-epileptic drug.(Vredenburgh, 2011) The addition of bevacizumab to temozolomide and radiation followed by temozolomide, bevacizumab and oral topotecan was tolerable and safe. Six patients came off the study with recurrent grade IV thrombocytopenia, one each with grade 2 CNS hemorrhage, wound dehiscence requiring surgery and a GI perforation. Median PFS and OS were not reached at a median follow-up of 8 months but the 6 month EFS was 83%. Hence in this case, multifactorial combination therapies for DNA replication as well as vascularization may aid in treatment efficacy.

5. Irinotecan

Irinotecan is another water soluble topoisomerase inhibitor that is being used clinically for the treatment of tumors. (Hsiang, 1985) A recent prospective, phase II study evaluated the efficacy of irinotecan and bevacizumab in the treatment of recurrent glioblastoma multiforme (GBM). (Møller,2012) In the evaluation of 85 patients with different brain tumors, the investigators used response rate and progression free survival (PFS) in patients who received intravenous bevacizumab (10 mg/kg), and irinotecan (125/340 mg/m^2) every 14 days until progression. The median treatment that these patients received was four cycles. At 8 week intervals, the patients underwent MRI imaging and were evaluated based on the Macdonald response criteria. The following histologies were studied among the 85 patients: GBM (n = 32), glioma WHO gr. III (n = 33), glioma WHO gr. II (n = 12), others (n = 8). For glioblastoma, ORR (overall response rate) was 25%, with 59% achieving stable disease. The median PFS in this study was 5.2 months. Upon evaluating the other types of tumors, for grade III gliomas ORR was 21% and 45% had SD with a median PFS of 3.7 months. Objective responses were not found for any grade II gliomas in this study. (Møller,2012) Since the study included a non-glioma population, the investigators reported that they observed several long PFS times. Bevacizumab and irinotecan combination therapy was well tolerated and moderately efficacious in glioblastoma and glioma of WHO grade III with the majority of patients achieving some disease stabilization. (Møller, 2012) The current studies have evaluated irinotecan alone or in combination (ex:bevacizumab). (Table 1) Future studies should expand on these findings in larger cohorts since progression-free survival was prolonged in non-glioma patients.

6. Safety, adverse effects, and methods to minimize toxicity

Malignant gliomas are highly proliferative, resistant to therapy and often recurrent post radiation and chemotherapy. Lack of adequately tumoricidal concentrations of chemothera-

peutic drugs in tumor cells may be be one of the primary causes of treatment failure in solid tumors. The blood-brain barrier (BBB) restricts the concentrations of chemotherapeutic drugs that may reach the tumor site. Methods that deliver drugs in a systemic fashion may increase toxicity and create off target site complications. Convection-enhanced delivery (CED) is a method that may provide drugs to the tumor in a more targeted fashion through the interstitial space.(Barker,1998; Bobo,1994) This approach results into more drug being delivered into tumors and surrounding brain through stereo-tactically placed catheters connected to pumps. (Barker,1998; Bobo,1994) By providing a continuous, low grade positive-pressure microinfusion that distributes drugs by bulk flow, CED may result in high local concentrations and reduce systemic toxicity. (Bruce,2011)

Topotecan, a topoisomerase I inhibitor, is cytotoxic to glioma cells and nontoxic to normal brain, and its levels are higher in glioma cells and tumor tissue than in normal brain. Topotecan is a natural-product drug with high molecular weight which allows it to minimally traverse the BBB from the brain to the systemic circulation. (Kaiser,2000;Bruce,2000;Borris,1998, Matsumoto, 1999) Now there is evidence that in colon tumors topotecan treatment results in the downregulation of hypoxia-inducible factor-1α (HIF1A) target genes along with an inhibition of HIF1A protein accumulation. (Guerin, 2012) Hence, topoisomerase I inhibitors may also influence the tumor environment by decreasing tumor angiogenesis leading to tumor size stabilization, although clinical trials of topotecan delivered intravenously had minimal effects on tumors. (Fridman,1999) In other studies, CED in rat in vivo models prolonged survival and had significant antitumor efficacy. (Kaiser,2000; Bruce,2000)

Recently, a prospective, dose-escalation phase Ib study of CED of topoisomerase-I was conducted in sixteen patients with recurrent malignant gliomas with a median age of 50 years. Ten patients had glioblastoma multiforme, and the other 6 had World Health Organization grade III glial tumors with an average enhancing volume of 16.1 cm^3. (Bruce,2011) Standard MRI/CT-guided stereotactic biopsy was used to histologically confirm the presence of recurrent malignant glioma. In this study, the investigators evaluated toxicity and quality of life (QOL) effects and confirmed antitumor activity radiographically. The change in contrast-enhancing volume of tumor on MRI was used to assess tumor response to treatment. Three response categories were used to characterize the tumors: early response; as a decrease in contrast-enhancing volume of >50% through the first 3 to 6 months after therapy, progressive disease; as increasing contrast-enhancing volume (>25%) at ≥1 month after therapy until surgical resection or death, and pseudoprogression; an increase in the contrast-enhancing volume of >50% followed by regression of enhancement and edema (changes had to be sustained for at least 4 weeks with patients on a stable or decreasing dose of steroids).(Bruce, 2011) The investigators noted significant antitumor activity in these tumors through radiographic changes, and treatment with CED topotecan prolonged overall survival. The maximum tolerated dose that can be used for phase II studies was determined in this trial. Drug-associated toxicity was minimal and topotecan convection-enhanced delivery had activity at concentrations that were nontoxic to normal brain. (Bruce,2011)

CED locally administered topotecan treatment was tested on a rat model of glioblastoma that is induced by intracerebral injection of PDGF (platelet-derived growth factor)-IRES (internal

ribosome entry site) expressing retrovirus. (Lopez, 2011) Glial progenitor cells recruited to the tumor and the transformed tumor cells were analyzed by histopathology. Glial progenitor cells are proliferate within gliomas and contribute to the growth of the tumor. (Appolloni, 2009 OGDEN) This pro-growth process is influenced via PDGF signaling, another common proliferative pathway. (Assanah, 2006 Assanah, 2009) The transformed cell population was reduced about 10-fold and recruited progenitors by about 80-fold. A significant survival advantage was found in treated animals and this improved with greater treatment duration. (Lopez, 2011) In addition, the distribution of topotecan was traced with MRI of a tracer molecule and corresponded with regions of glial progenitor ablation. The decrease in progenitor recruitment was most likely due to the ablation of recruitable progenitor cells. These results showed that in a model of growth factor influenced gliomas, tumor cells and the induced progenitor cells are eradicated by topoisomerase inhibition based treatments. Hence, future characterization of tumors through these methods may enhance the efficacy of treatments in specific subpopulations. Topotecan administration by convection-enhanced delivery has significant antitumor activity at concentrations that are nontoxic to normal brain. The potential for use of this therapy as a generally effective treatment option for malignant gliomas will be tested in subsequent phase II and III trials. (Bruce, 2011)

Topoisomerase II (epipodophyllotoxin) has been implicated in the pathogenesis of treatment-related myelodysplastic syndrome/ treatment-related acute myelogenous leukemia (t-MDS/t-AML). (Baehring, 2012) Once patients develop these t-MDS/t-AML disorders they are treated with supportive care, including transfusion of blood products and administration of antibiotics; 5-azacytidine, decitabine, and lenalidomide are approved for the treatment of selected patients with MDS in the United States. Hence, some of the side effects of topoisomerase inhibition therapy for CNS tumors must be further investigated in additional head to head clinical studies with other monotherapies or combinations that may alleviate adverse effects. Additional ways to reduce or minimize toxicity include close monitoring of blood counts and limiting long term usage of the drug.

7. Emerging topoisomerase therapies and combinations

Genz-644282 is a new a non-camptothecin topoisomerase I inhibitor (Kurtzberg, 2011) in clinical development. Efficacy for this novel agent was tested and compared with the standard anticancer drugs; irinotecan, docetaxel, and dacarbazine in human tumor xenografts of colon cancer, renal cell carcinoma, non-small cell lung cancer, and melanoma. Genz-644282 had superior or equal antitumor activity than the standard drug comparators, although brain tumor models were not utilized. (Kurtzberg) Genz-644282 and its metabolites induce Top1 cleavage at similar, as well as unique genomic positions, compared with camptothecin which traps topoisomerase I (Top1)-DNA cleavage complexes. Protein-linked DNA breaks are induced by Genz-644282, and cleavage complexes persist longer after compound removal than camptothecin treatment. (Sooryakumar, 2011) The agent was tested against the pediatric preclinical testing program (PPTP) panel as well as *in vivo* using at its maximum tolerated dose (MTD) of 4 mg/kg (3 times per week × 2 schedule re-

peated at day 21).(Houghton, 2012) Testing was also conducted in model systems in order to determine a dose regulated response. Treatment exhibited potent cytotoxic activity in vitro models, and in vivo it induced and maintained complete responses in all 6 solid tumor models at MTD. At lower doses of 2 mg/kg it induced complete responses and maintained complete responses in tumor models relatively insensitive to Topotecan although responses were not observed at lower doses. A significant correlation was found between predictive response scores in baseline mRNA tumor gene expression profiles and the observed in vivo responses to Genz-644282. Future research on clinical activity in children impacted by CNS tumors will depend on tolerated drug exposures and safety profiles. (Houghton, 2012)

Topotecan has been studied in combination with other therapies that may increase treatment efficacy in brain tumors. In a recent study, the combination of nanoliposomal topotecan (nLs-TPT) and pegylated liposomal doxorubicin (PLD) was delivered with CED as treatment for malignant brain tumors. (Yamashita, 2007) Both drugs decreased proteins and enzymes with roles in cell replication in vitro, with some synergistic effects. Doxorubicin is also used to inhibit topoisomerase II, and although these studies used implantation of tumor cells in animals, the investigators conducted a survival study in which animals in the control group and the single agent groups had a median survival that was less than the median survival of the combination group. In this study combination therapy use two agents that were both encapsulated in liposomes. Furthermore, the use of CED was promoted as an enhanced drug delivery method, increasing drug availability at the brain tumor site and leading to tumor death.

New phase III randomized control trials incorporating the addition of bevacizumab for newly diagnosed GBM patients may be informative and increase treatment efficacy. (Vredenburg, 2011) In combination with the use of specific molecular biomarkers, data from these trials may clarify the role of anti-angiogenesis agents such as bevacizumab in combination therapy with topoisomerase inhibitors. The incorporation of molecular signatures elicited by therapies such as irinotecan will create a more descriptive situation of the tumor microenvironment, and lead to the elucidation of additional therapeutic targets. (Guerin, 2012)

Additional research into the use of low-dose etoposide (topoII inhibitor) with an oncolytic herpes simplex virus increased survival of mice-bearing intracranial human GSC–derived tumors. (Cheema) These results were found without adverse side effects, possibly leading to this as an effective combination strategy to treat resistant and recurrent GBM in the future. (Cheema)

In a recent study of both a neuroblastoma and astrocytoma cell line that were resistant to chemotherapy (eg. temozolomide) and radiation treatment, investigators found that a novel cytotoxic compound was toxic to these these cells but not to human primary astrocytes. This compound is an analog of thiobarbituric acid and is effective in subcutaneous and intracranial mouse tumor models with a good safety profile. (Lee, 2011) The mechanism of action of the lead compound has topoisomerase IIa inhibition activity but does not inhibit topoisomerase I activity. These types of studies may lead to the development of new agents that can overcome some of the tumor resistance mechanisms in temozolomide and radiation resistant astrocytomas.

8. Predictive biomarkers and tests

The development of molecular markers which predict response to chemotherapy is an important aspect of current neuro-oncology research. The studies and subsequent tests that may assess the status of these biomarkers are ongoing, but few molecules are being tested at this time. O 6-methylguanine-DNA methyltransferase MGMT promoter methylation is the only proved marker of glioblastoma. (Weller,2010) This DNA repair enzyme antagonizes the genotoxic effects of alkylating agents. The expression of MGMT in tumor cells is a marker for significant resistance to temozolomide therapy and other treatments. (Liu, 2006; Donson, 2007; Hegi, 2005) MGMT promoter methylation is the key mechanism of MGMT gene silencing and predicts a favorable outcome in patients with glioblastoma who are exposed to alkylating agent chemotherapy. (Weller,2010) The predictive or prognostic value of MGMT promoter methylation may differ depending on glioma subtypes, and the extent to which testing should be incorporated into routine clinical practice is still under investigation.

A recent study assessed the effect of topoisomerase expression on glioblastoma survival and the mechanisms involved. (Arivazhagan,2012) In an effort to correlate outcome with gene expression, the transcript levels of all isoforms of the topoisomerase family in all grades of diffuse astrocytoma were assessed in this prospective study of patients with glioblastoma treated by a uniform treatment procedure. Transcript levels of TOP2A, TOP2B, and TOP3A were up regulated significantly in GBM in comparison with lower grades of astrocytoma and normal brain samples. The mRNA levels of TOP2A correlated significantly with survival of the patients, and better prognosis in GBM patients. Temozolomide (Arivazhagan,2012) was also a TOP2A inhibitor, and TOP2A transcript levels determined the chemosensitivity of glioblastoma to temozolomide therapy. Very high levels of TOP2A were considered a good prognostic indicator in GBM patients receiving temozolomide chemotherapy. Methylation of the MGMT promoter was found to be the strongest predictor of outcome and benefit from temozolomide chemotherapy. (Stupp,2009) Analysis of progression free survival revealed an advantage solely for patients whose tumor had a methylated MGMT promoter and who were treated with temozolomide and radiotherapy. Hence, in this patient analysis relied on combination therapy was as opposed to monotherapy. These types of results may serve as the impetus for the identification of new genetic biomarkers for GBM and the development of therapies targeting new molecular targets.

9. Conclusions

The treatment of primary malignancies of the CNS continues to be a challenging problem since their treatment is complicated due to anatomical site and the intricacy of the blood brain barrier. Topoisomerase I (topo I) inhibitors, in addition to temozolomide and nitrosourea compounds, represent one promising one treatment option. While preclinical studies in glioma models were promising, clinical trials with topo I inhibitors with topotecan and CPT-11 showed only modest benefit in phase II clinical trials. Children with anaplastic astrocytoma

or glioblastoma (Turner, 2002) appear to benefit more with higher response rates than adults with the same tumors. (Friedman,1999; Batchelor, 2004)

The combination of topo I inhibitors with drugs that block angiogenesis including VEGF appear promising, and further studies are needed in the establishment of efficacy, and the development of treatment strategy. However, serious toxicity such as CNS hemorrhage and leukoencephalopathy may occur with these agents. (Ozcan,2006) Further clinical trials are needed to better define the patients at risk for these major side effects.

Another future approach is combining topo I inhibitors together with targeted agents and antiangiogenesis drugs. For example, NF-κB activation in glioma cells may be induced by campothecins leading to inhibition of apoptosis in these cells. It has been shown that over-production of IL-1B can sustain NF-κB activation (Morandi,2006) and agents that inhibit NF-κB activation may increase the susceptibility of glioma cells y to apoptosis induced by campothecin. (Weaver,2003)

Other agents that may increase the efficacy of topo I drugs include the new chemotherapy drug, irofulven. (Woo,2005) PKC inhibitors in combination with CPT-11 have also shown promise in laboratory studies. (Chen TC, 2003) These studies demonstrated an increase in apoptosis and decrease in proliferation in glioma cell lines when exposed to both agents. A decrease in the antiapoptotic protein bcl-2 and an increase in the proapoptotic bax protein may be propagate this mechanism of apoptosis.

Other novel approaches include the use of agents to increase penetration of topotecan into glioma cells. (Carcaboso, 2010) It is possible that one mechanism of drug resistance to topotecan is increased pumping of drug out of the cell. It is known that topotecan is a substrate of the ATP-binding cassette (ABC) transporters P-glycoprotein and breast cancer resistance protein (BCRP/ABCG2). In mice it has been shown that the epidermal growth factor receptor tyrosine kinase inhibitor, Gefitinib, can increase intracellular drug penetration into glioma cells. Similar approaches in the treatment of brain tumors may increase drug availability to the tumor environment.

Immunotherapy for treatment of malignant gliomas has usually been unsuccessful. One new approach is to add topotecan to enhance immune clearance of gliomas. (Wei J,2009) Preclinical studies with the human glioma cell line U-87 using topotecan showed that the drug can upregulate functional Fas receptors and the resulting upregulated Fas expression can increase susceptibility to cytotoxic T cell killing. These findings will have to be substantiated through additional clinical studies and testing.

Another novel approach is to increase drug delivery across the blood brain barrier. Liposomes incorporating Tamoxifen and wheat germ agglutinin have improved the transport of topotecan across the blood brain barrier in brain tumor-bearing rats. (Du 2009) In these studies, improved survival may be related to the enhanced effect of Tamoxifen by inhibiting efflux of multidrug resistant proteins in the blood brain barrier and/or an enhanced effect by the wheat germ agglutinin via endocytosis in the blood brain barrier and in the brain tumor.

Another method to deliver more drug into brain tumors is by a convection-delivery system. This approach may be advantageous by potentially increasing drug delivery into the brain

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tumor while reducing systemic side effects. Regression of malignant gliomas by this convection-enhanced delivery system has been reported recently. (Bruce, 2011) Future phase II trials are being planned with this technique since the maximum tolerated dose has been established.

Finally, there are newer generations of topo I inhibitors being evaluated and several have entered into clinical trials in human patients. These newer topo I inhibitors include Diflomotecan, Karenitecin, Silatecan, PEG-camptothecin, Rubitecan, 9-aminocamptothecin, Exatecan mesylate, Lurtotecan, and Gimatecan. (Pommier, 2006) A phase II trial of Rubitecan in patients with glioblastoma showed disappointing results. (Raymond,2002) A phase I and pharmacokinetic study of Karenitecin in patients with recurrent malignant gliomas was recently reported. (Grossman, 2008)

Since the vast majority of patients with malignant gliomas die of their disease, it is clear that newer and more effective drugs are needed. There has been renewed interest in topoisomerase I inhibitors in brain tumors using innovative drug carriers or drug delivery systems. In addition, novel topoisomerase I inhibitors are promising and are currently being explored and investigated.

Investigator	n	Treatment	Response Rate	Ref.
Møller et al.	85	IV bevacizumab (10 mg/kg), irinotecan (125/340 mg/m^2) every 14d until progression	ORR (overall response rate) was 25%, with 59% achieving stable disease; median PFS of 5.2 months	Møller, 2012
Bruce et al.	16	Dose escalation of Ib study of CED of topotecan	Significant antitumor activity by radiographic changes, and treatment with prolonged OS	Bruce, 2011
Vredenburgh et al.	80	Add. bevacizumab to temozolomide and radiation followed by temozolomide, bevacizumab, topotecan	Median PFS and OS not reached at a median follow-up of 8 months but the 6 month EFS was 83%	Vredenburgh, 2011
Colavolpe et al.	25	After combined treatment with bevacizumab and itinotecandetermine the independent prognostic value of (FDG)-PET on PFS and OS of recurrent histologically proven high grade glioma, compared with other documented prognostic variables. Imaging was performed within 6 weeks of starting chemotherapy	Median PFS and OS were 4 months (range, 0.9-10.4 months) and 7.2 months (range, 1.2-41.7 months), respectively. At 6 months, PFS and OS rate were 16.0% and 72.0%. FDG uptake was the most powerful predictor of both PFS and OS	Colavolpe, 2012
Paldino et al.	15	Determine the prognostic significance of changes in parameters derived from diffusion tensor imaging (DTI) that occur in response to treatment with bevacizumab and	DTI detected a change in Apparent diffusion coefficient (ADC) within FLAIR signal abnormality (FSA). Patients with a change in ADC within FSA had significantly shorter	Paldino, 2012

Investigator	n	Treatment	Response Rate	Ref.
		irinotecan in patients with recurrent GM through serial 1.5 T MRI. Axial single-shot echo planar DTI was obtained on scans performed 3 days and 1 day prior to and 6 wks. after initiation of therapy.	overall survival (p=0.032) and progression free survival (p=0.046) than those with no change.	
Desjardins et al.	32	PII trial of combined protracted daily temozolomide 50 mg/m(2) and biweekly bevacizumab (10mg/kg) IV for patients with recurrent glioblastoma who had previously received radiation therapy and temozolomide. Underwent physical examination and brain MRI every 8 weeks.	6month PFS rate 18.8% (95% [CI], 7.6%-33.7%), median PFS 15.8 wks. Median OS of 37 wks.,6-m OS rate of 62.5% (95% CI, 43.5%-76.7%), 12-m OS rate of 31.3% (95% CI, 16.4%-47.3%). Patients progressed; locally (52%), diffuse pattern (38%), distant (10%). Regimen had some activity and was well tolerated but results obtained were inferior to those observed in studies of bevacizumab monotherapy and of combination with irinotecan. Patient population was more heterogeneous and pretreated more heavily than in previous studies.	Desjardins, 2012
Reardon et al.	40	Phase II, open, label, single arm trial on efficacy of carboplatin, irinotecan, and bevacizumab among bevacizumab-naïve, recurrent GBM patients. Patients received carboplatin (area under the plasma curve [AUC] 4 mg/ml-min) on day one, while bevacizumab (10 mg/kg) and irinotecan (340 mg/m(2) for patients on CYP3A-enzyme-inducing anti-epileptics [EIAEDs] and 125 mg/m(2) for patients not on EIAEDs) administered on days 1 and 14 of every 28-day cycle. Evaluated after each of the first 2 cycles and then after every other cycle. Treatment continued until progressive disease, unacceptable toxicity, non-compliance, or voluntary withdrawal.	All patients had progression after standard therapy, patients (40%) had a KPS of 90-100, while 68% were at first progression. PFS-6 rate was 46.5% (95% CI: 30.4, 61.0%) and median OS of 8.3 months [95% CI: 5.9, and 10.7 months]. Addition of carboplatin and irinotecan to bevacizumab significantly increases toxicity but does not improve anti-tumor activity to that achieved historically with single-agent bevacizumab among bevacizumab-naïve, recurrent GBM patients.	Reardon, 2012

Investigator	n	Treatment	Response Rate	Ref.
Reardon, et al.		Phase II, open, label, single arm trial on efficacy of carboplatin, irinotecan, and bevacizumab among recurrent glioblastoma (GBM) patients after prior progression on bevacizumab therapy. Received carboplatin (area under the plasma curve [AUC] 4 mg/ml-min) on day 1, bevacizumab (10 mg/kg) and irinotecan (340 mg/m(2) for patients on CYP3A enzyme-inducing anti-epileptics [EIAEDs] and 125 mg/m(2) for patients not on EIAEDs) were administered on days 1 and 14 of every 28-day cycle. Patients were evaluated after each of the first 2 cycles and then after every other cycle. Treatment continued until progressive disease, unacceptable toxicity, noncompliance, or voluntary withdrawal.	All patients had progression on at least 1 prior bevacizumab regimen and 56% enrolled after either 2nd or 3rd overall progression. Median OS was 5.8 months (95% [CI], 4.0-7.0 months) and PFS-6 rate was 16% (95% CI, 5.0%-32.5%). Carboplatin, irinotecan, and bevacizumab was associated with modest activity and adequate safety among these patients.	Reardon, 2011
Parekh et al.	8	Retrospectively reviewed the records of patients <21 yrs. of age with recurrent or progressive WHO grade 3-4 gliomas who were treated with bevacizumab containing regimens at institution between January 1/2006-9/2008.6 patients received irinotecan, temozolomide and bevacizumab, one patient received irinotecan and bevacizumab, and one patient received CCNU and bevacizumab.	3 patients had stable disease for 30-93 weeks, 5 patients progressed within 17 wks., median PFS was 15 weeks, 6-m PFS was 38%. Contrast enhancing disease responded or remained stable in 5/7 patients, and non-enhancing disease progressed in 3/4 patients. Bevacizumab was well tolerated when used in combination with conventional chemotherapy (irinotecan in most cases). PFS in cohort was much shorter and the response rate was inferior in this small cohort of patients when compared with published adult data, but bevacizumab regimens may have efficacy in a subset of pediatric patients with predominantly contrast-enhancing disease.	Parekh, 2011
Pope et al.	85	Evaluated patterns of tumor progression in patients with recurrent	79% treated with single-agent BEV and 70% of patients treated with	Pope, 2011

Investigator	n	Treatment	Response Rate	Ref.
		glioblastoma who were treated with bevacizumab (BEV) alone or in combination with irinotecan (CPT-11) while participating in the BRAIN study. Iindependent neuroradiologist reviewed MRI scans in patients who received BEV or BEV+CPT-11 while on BRAIN. 28% of patients who participated had nonlocal disease at baseline.	BEV+CPT-11 experienced disease progression while on BRAIN. Most patients did not have a change in radiographic pattern (i.e., "no shift") at the time of progression. 82% of BEV patients had no shift and BEV +CPT-11 patients (53%, $\chi(2)$ p = 0.0004), and a greater proportion of BEV+CPT-11 (39%) compared with BEV (16%) experienced local-to-diffuse tumor pattern at progression ($\chi(2)$ p = 0.002). Patients treated with BEV or BEV +CPT-11 who had local-to-local or local-to-diffuse progression patterns had similar efficacy outcomes, including objective response, PFS, and OS.	

Table 1. Recent studies of topoisomerase inhibitors as monotherapies or in combination with other chemotherapeutics for the treatment of brain tumors

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