

# New Treatment Strategies for Malignant Gliomas

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## ABSTRACT

Although survival in patients with malignant gliomas remains limited, there is renewed optimism with the emergence of novel treatment strategies. Cytotoxic agents such as temozolomide and CPT-11 have shown promising clinical activity. Biological treatments for brain tumors, including antisense oligonucleotides, gene therapy, and

angiogenesis inhibitors, are also being evaluated in clinical trials. Delivery strategies have been developed to overcome challenges presented by the blood-brain barrier. These noteworthy treatments, alone or in combination, may ultimately prolong survival and enhance quality of life in this group of patients. *The Oncologist* 1999;4:209-224

## INTRODUCTION

While primary malignant brain tumors account for only 2% of all adult cancers, these neoplasms cause a disproportionate burden of cancer-related disability and death. The five-year survival rates for brain tumors are the third lowest among all types of cancer (pancreas and lung are first and second, respectively). Malignant gliomas (glioblastoma multiforme [GBM] and anaplastic astrocytoma [AA]) comprise the most common types of primary central nervous system (CNS) tumors and have a combined incidence of 5-8/100,000 population. The median survival of patients with malignant gliomas treated conservatively is 14 weeks; by surgical resection alone, 20 weeks; by surgery and radiation, 36 weeks; and by the addition of chemotherapy, 40-50 weeks [1-4]. Although survival for GBM has not changed significantly over the past three decades, the emergence of novel treatment strategies for these tumors has led to heightened interest and optimism among oncologists.

## CLINICAL TRIAL DESIGN

The history of clinical trials for brain tumors is replete with examples of poor study design and ambiguous results (Table 1) [5, 6]. One of the challenges for testing new agents in this disease is the fact that brain tumors are uncommon, one-tenth as frequent as breast or lung cancer. Therefore, an unlimited number of large, prospective, randomized, controlled studies is not possible. As a result, there is reliance on nonrandomized studies as the principal design for the identification of potentially active therapies that should be studied in more definitive, randomized trials.

The decision to proceed with larger, more expensive and time-consuming randomized studies should be based on carefully designed and conducted phase I trials to define the maximum tolerated dose and toxicity, and on phase II trials to define efficacy. Because enzyme-inducing (CYP450) antiepileptic drugs (AED) enhance the metabolism and inactivation of certain chemotherapies, phase I trials of new agents that undergo hepatic metabolism should be conducted in patients with brain tumors stratified into those on CYP450 inducers and those not on such agents with independent dose escalations in each arm [7, 8]. This will avoid the possibility of underdosing patients. Despite careful phase II study design and execution, the possibility of selection bias remains, especially if the outcome is progression-free or overall survival. Objective responses on neuroimaging are more likely to indicate activity of a drug, whereas progression-free survival and overall survival may simply reflect patient selection.

To address these challenges, one major brain tumor collaborative group has adopted a paradigm that includes a

**Table 1.** Historical limitations of brain tumor clinical trials

- ▲ Divergent study entry criteria
- ▲ Inadequate statistical power
- ▲ Use of different outcome measures (tumor response, tumor control, clinical parameters)
- ▲ Inadequate control for known prognostic factors (age, Karnofsky Performance Score, histology)
- ▲ Inadequate control for co-interventions (steroids, treatment at recurrence)

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**Table 2.** Response criteria for phase II studies of supratentorial malignant glioma

Response	Enhancing tumor area	Neurological status	Steroids
Complete response	≥ 95% decrease	Improved or stable	Off
Partial response	50%-94% decrease	Improved or stable	Stable or decreased dose
Progressive disease	≥ 25% increase	Worsened	Stable or increased dose
Stable disease	All other situations	All other situations	All other situations

combined phase I/II design with dose escalation in two independent arms (CYP450 inducers and non-CYP450 inducers); chemotherapy prior to radiation and primary outcome defined as objective radiographic responses. All patients must have residual, enhancing tumor on postoperative neuroimaging. The radiographic responses are defined along the lines of other oncology trials as outlined in Table 2 [9]. This study design optimizes conditions for defining which agents are active or inactive for newly diagnosed malignant gliomas.

New agents that are cytostatic represent another challenge in the design of clinical trials. Since the predominant effect of these agents is stabilization of tumor size, radiographic response rates are a suboptimal outcome measure. Specifically, direct visualization by computerized tomography (CT) or magnetic resonance imaging (MRI) may be insensitive to capillary number, density, blood flow, and tumor metabolic activity. Despite the limitations of progression-free and overall survival in the context of phase II studies, these endpoints currently serve as the primary outcome measures in the assessment of most cytostatic agents. There is a need to identify biological correlates of activity in these types of studies. Data collected from surrogate studies such as positron emission tomography (PET) scanning, MR spectroscopy, and biopsy with attention to blood vessel morphology and number may be relevant in these regards.

Important issues for phase III trials include stratified randomization for the known prognostic factors (Karnofsky Performance Score [KPS], age, histology) to minimize the chance of unbalanced distribution of these factors in the different arms of the study [10]. Centralized neuropathology review and use of pathologic criteria known to have a high inter-rater correlation (WHO criteria = 94% correlation) are also important [11, 12]. High inter-rater reliability ensures accurate case identification and minimizes misclassification bias.

Analysis of phase III outcomes should begin with examination of predefined primary and secondary outcome measures between the different treatment groups. Further analysis may include multivariate modeling to identify subgroups responsive to therapy. Such subgroup analyses should not serve as the basis for definitive treatment recommendations, but instead should be seen as hypothesis-generating for future studies.

Finally, oncology study outcomes (including brain tumor trials) have traditionally consisted of survival and time to progression of disease. However, patient-derived data based on quality of life (QOL) surveys are becoming more common and desirable. Combination of QOL data with survival data (Q-TWiST analyses [time without symptoms or toxicity]) will be an important means of comparing treatments, especially if survival times are similar [13]. Several QOL measures have been validated in malignant glioma patients (FACT-BR [Functional Assessment of Cancer Therapy-Brain subscale] and the EORTC QLQ-C30 [European Organisation for Research and Treatment in Cancer Quality of Life Questionnaire]) and could be incorporated into a phase III study as an outcome [14, 15]. KPS is not a sufficient measure and does not correlate with QOL measures [14, 16].

Attention to the basic principles of clinical trial design will optimize conditions for identifying active agents in phase II studies and allow such drugs to undergo more definitive testing in randomized controlled trials.

## CYTOTOXIC AGENTS

### Methylating Agents

#### Temozolomide

Temozolomide is an imidazotrazinone with activity attributed to the formation of a reactive methyldiazonium cation and methylation of O6-guanine in DNA (Fig. 1). Clinical responses to temozolomide are closely linked to the activity of O6-alkylguanine-DNA alkyltransferase (AGT), a DNA repair protein that removes O6-alkylguanine adducts in DNA [17]. Features of temozolomide that are attractive for use in tumors of the CNS include excellent oral bioavailability and good penetration of the blood-brain barrier (BBB) [18].

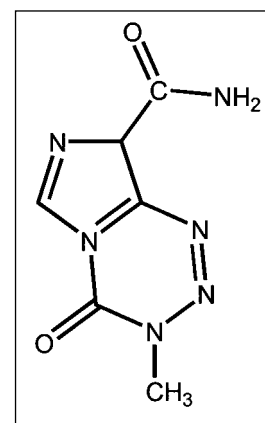


Figure 1. Temozolomide.

The activity of temozolomide is highly dependent on dosing schedule, with multiple administrations being more

**Table 3.** Summary of clinical trials of temozolomide for malignant gliomas

Author	Path	Study	# Patients	Dose	CR(%)	PR(%)	Remarks
Newlands [19]	GBM AA	Ph-I Rec	3	200 i.v.; 5/28 d †	0 (0)	2 (67)	“Dramatic clinical improvement” reported.
Brock [20]	GBM AA	Ph-I Rec	17	75 p.o.; 7 wks *	0 (0)	7 (41)	An additional 6/17 maintained stable disease. Regimen permitted >2 fold drug exposure/cycle over standard dose.
Newlands [21]	GBM AA	Ph-I/II: N Ph-I/II: Rec	27 48	200 p.o.; 5/28 d †	0 (0) 0 (0)	8 (30) 12 (25)	Survival advantage not demonstrated. Seven patients not assessable by neuroimaging.
Bower [22]	GBM AA	Ph-II: N(5) Rec(98)	103	200 p.o.; 5/28 d †	0 (0)	5 (9)	Only 57 of these patients evaluated with MR/CT of brain. 87% maintained stable disease.
Levin [23]	AA	Ph-II: Rec	161	200 p.o.; 5/28 d †	- (-)	42 (42)	Analysis of first 100 patients only. No breakdown of CR versus PR. 24% of patients maintained stable disease.
Friedman [24]	GBM AA	Ph-II: N	33	200 p.o.; 5/28 d †	3 (9)	14 (43)	No survival data. AGT protein expression may identify patients in whom tumors are resistant to temozolomide.

CR = complete response; PR = partial response; N = neoadjuvant (prior to radiation); Rec = recurrent; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; i.v. = intravenously; p.o. = orally; d = days; wks = weeks; †150-200 mg/m<sup>2</sup> daily on days 1-5 each 28-day cycle; \*75 mg/m<sup>2</sup> daily for seven weeks; AGT = O6-alkylguanine-DNA alkyltransferase.

effective than a single dose. It is administered orally at 200 mg/m<sup>2</sup> daily for five days on a four-week cycle. Peak plasma concentration is achieved within 30-60 min of oral administration and the compound has an elimination half-life of one to two hours. Elimination is largely via renal excretion as intact drug and a carboxylic acid metabolite that has equivalent cytotoxicity. Myelosuppression, which is dose limiting at 1,200 mg/m<sup>2</sup>, and nausea and vomiting are the most frequent adverse events [18].

Clinical trials of temozolomide in malignant gliomas are summarized in Table 3 [19-24]. Phase II trials have reported partial responses (PR) in 9%-43% of cases. This activity has been especially promising for AA. New directions for the use of this drug will likely focus on optimizing dosage and delivery to the CNS. For example, temozolomide is compatible with chronic administration as patients have tolerated this drug for up to three years [18]. Additionally, combination with the “pseudosubstrate” O6-BG (O6-benzylguanine) is a promising approach since O6-BG irreversibly inactivates AGT and potentially increases the efficacy of temozolomide treatment [17].

### Topoisomerase I Inhibitors

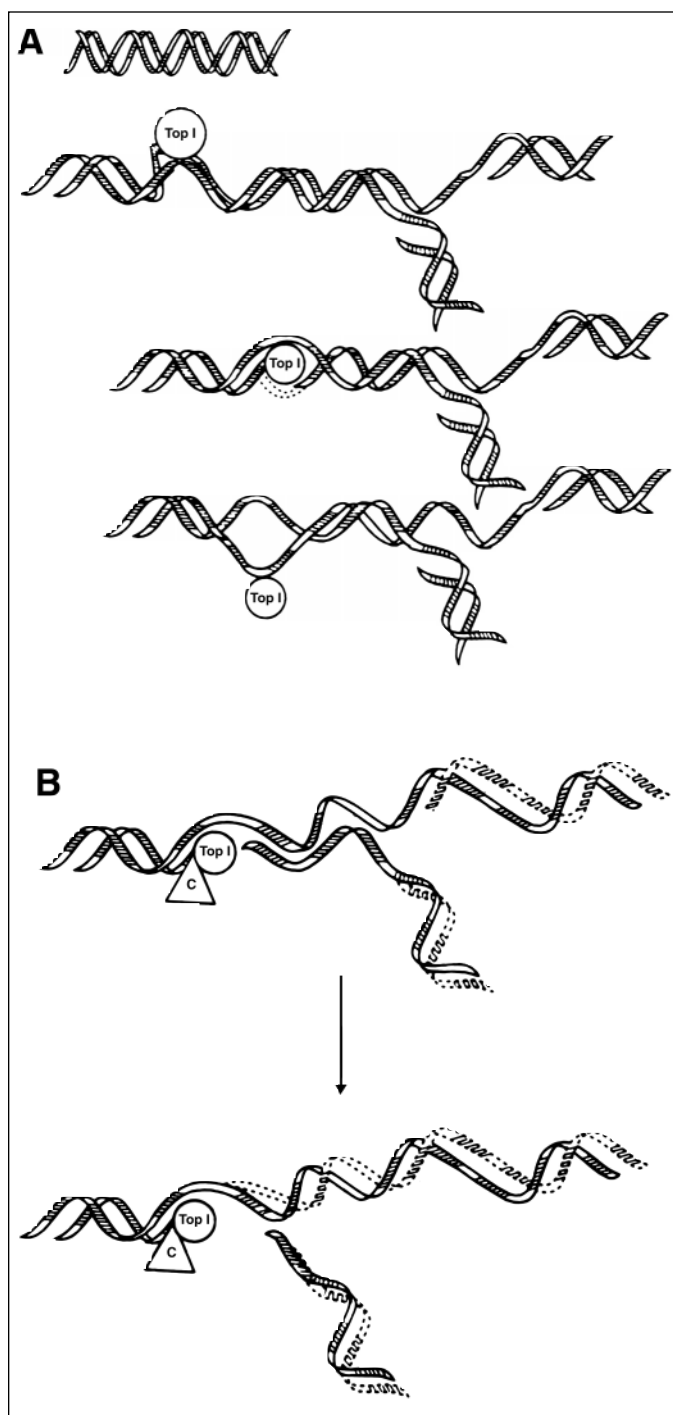
The camptothecin analogs CPT-11, topotecan, and 9-aminocamptothecin (9-AC) exert their cytotoxic effects by inhibiting topoisomerase I. Normally, topoisomerase I tyrosine undergoes a reversible trans-esterification reaction with the 3' end of the DNA strand. This cleaves the strand long enough to allow passage of a newly synthesized strand through the cut, after which time topoisomerase I normally

releases the cleavage. The cytotoxic effect of camptothecins is exerted during replication by their ability to bind and stabilize this DNA-topoisomerase I complex. An irreversible double-stranded DNA break occurs at the time of collision of the replication fork and cleaved DNA strand (Fig. 2). However, the correlation of cellular topoisomerase I levels to drug sensitivity has been difficult to establish [25].

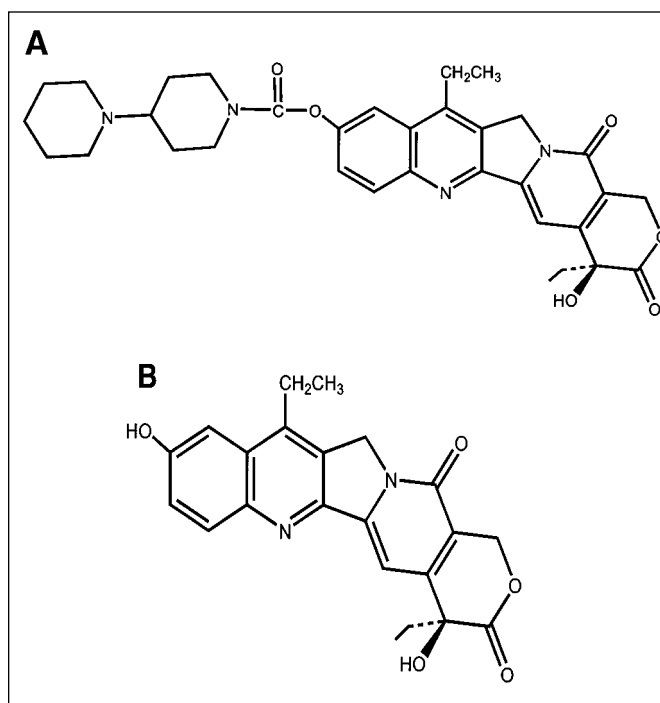
Topoisomerase I inhibitors studied as potential treatments of gliomas include CPT-11, topotecan, and 9-AC. The in vitro activity of these camptothecins in human colon carcinoma HT-29 cells has been compared with the following cytotoxic potency established: SN-38 (metabolite of CPT-11) > camptothecin > 9-AC > topotecan > CPT-11 [26]. In GB-1 and U-87MG glioma cell lines, SN-38 induced apoptosis and demonstrated significantly stronger antitumor effects than did CPT-11 [27].

### CPT-11

CPT-11 undergoes hydrolysis or de-esterification to form the active metabolite SN-38, which is approximately 100-1,000 times as potent as CPT-11 as an inhibitor of topoisomerase I (Fig. 3) [25, 27]. Because SN-38 is 95% bound to plasma proteins compared to only 65% for CPT-11, the precise contribution of SN-38 to the activity of CPT-11 is unclear. SN-38 can be further metabolized to the inactive SN-38 glucuronide (SN-38G) by hepatic UDP-glucuronyltransferase (UDP-GT) [28]. In an animal model, pretreatment with the AED valproic acid resulted in a 99% inhibition of the formation of SN-38G, leading to a 270% increase in the area under the plasma concentration-time



**Figure 2. Topoisomerase I.** **A)** The mechanism of topoisomerase I action. (1) Increasing tension and supercoiling of DNA. (2) Topoisomerase I binds to one DNA strand and cuts it (cleavage reaction). (3) The intact strand of DNA passes through the neck, resulting in the relaxation of the torsional strain. (4) Topoisomerase I reseals the broken strand (religation step) and dissociates from the DNA molecule. **B)** Collision of the replication fork with the camptothecin-stabilized cleavable complex results in an irreversible double-strand break in the DNA. Top I = topoisomerase I; C = camptothecin; solid lines = parent DNA; dotted lines = daughter DNA. Used with permission from [25].



**Figure 3. A) CPT-11. B) SN-38.**

curve of SN-38 compared with controls [29]. This observation has led to the exclusion of patients requiring valproic acid from most clinical trials of CPT-11.

The initial and terminal half-lives of CPT-11 are approximately 6 h and 10-14 h, respectively. Elimination occurs primarily in the bile and secondarily in the urine. The main toxicities observed with CPT-11 are an acute cholinergic syndrome, delayed onset diarrhea, neutropenia, nausea, vomiting, fatigue, and alopecia. The usual dosing schedule is 125 mg/m<sup>2</sup>/week for four weeks followed by a two-week rest [25].

*Hare* investigated more than 40 drugs in a CNS xenograft model using multiple adult and pediatric glioma cell lines and found CPT-11 to be the most active agent tested [30]. An initial clinical trial of CPT-11 in 60 patients with recurrent malignant glioma resulted in 10/49 (20%) PR in GBM patients and 1/8 (12.5%) PR in AA patients [31]. Ten patients with GBM and three with AA demonstrated stable disease beyond two cycles. Single agent CPT-11 for recurrent malignant gliomas is now the subject of three phase II NCI-sponsored trials.

#### Topotecan

Topotecan is a topoisomerase I inhibitor that undergoes a reversible pH-dependent hydrolysis of its lactone ring to produce the pharmacologically active form of the drug (Fig. 4). It is then rapidly converted to its inactive carboxylate form at physiologic pH [32, 33]. Topotecan has a

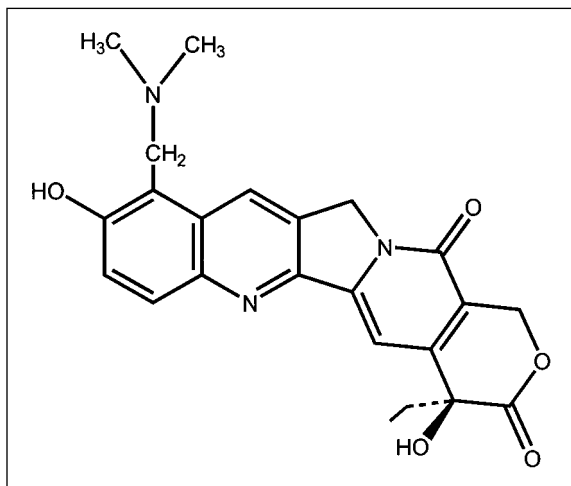


Figure 4. Topotecan.

half-life of two to four h following doses of 0.5 to 1.5 mg/m<sup>2</sup> administered as a 30-min infusion, and approximately 35% of the drug is protein bound [34]. The antitumor effect of topotecan appears to be greater when the drug is administered over a prolonged period of time compared with an intermittent schedule of drug administration [35]. The drug penetrates the BBB with a cerebrospinal fluid (CSF) to serum ratio of 0.3 [36]. The main toxicities of topotecan are leukopenia and thrombocytopenia; diarrhea and vomiting are less than with CPT-11. Elimination is primarily renal and patients with creatinine clearances less than 50 ml per min should not be treated with this drug [25].

The results of clinical trials using topotecan in patients with malignant brain tumors are in Table 4 [37-40]. In these studies, myelosuppression was a significant complication and topotecan was found to have minimal activity in this setting. Because of excellent BBB penetration and the novel mechanism of action, other studies of topotecan in brain tumors are under way, including trials for such potentially chemosensitive

tumors as oligodendrogliomas, brain metastases, and primary CNS lymphoma.

#### 9-Aminocamptothecin (9-AC)

Hochberg conducted a phase I/II dose escalation study of 9-AC in 59 patients, 31 with newly diagnosed GBM and 28 with recurrent high-grade astrocytomas [8]. Although ineffective, the authors noted no grade III or IV myelosuppression in patients receiving concurrent "cytochrome P450 system inducing" AEDs. Although the trial was terminated, plasma levels of the drug may have been insufficient to achieve cytotoxic activity.

#### Alkylating Agents

##### Oxaliplatin

Cisplatin and carboplatin have been used both i.v. and intra-arterially as first-line chemotherapy for malignant glioma with survival rates similar to BCNU [41, 42]. However, significant myelosuppression (carboplatin) and nephrotoxicity (cisplatin) have limited the usefulness of these drugs. Oxaliplatin ([trans-(L)-1,2-diaminocyclohexane] oxalatoplatinum (II)) is a cytotoxic platinum complex that has shown activity against colorectal cancer in combination and as a single agent in phase II and phase III clinical trials (Fig. 5) [43, 44]. Oxaliplatin's *dach* ring complex results in the formation of platinum-DNA adducts, which are more effective at blocking DNA replication than those formed by cisplatin. Because ototoxicity, nephrotoxicity, cardiac toxicity, and alopecia have not been observed in adults treated with oxaliplatin alone, this agent is an

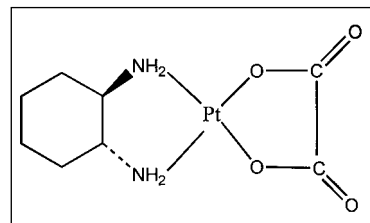


Figure 5. Oxaliplatin.

Table 4. Summary of clinical trials using topotecan in malignant gliomas

Author	Path	Study	# Patients	Dose	CR(%)	PR(%)	Remarks
Macdonald [37]	GBM (15) AA (16)	Ph-II Rec	31	1.5 i.v.; 5/21 d †	0 (0)	2 (6)	68% maintained stable disease.
Blaney [38]	GBM	Ph-II Rec	9	7.5 i.v.; 1/21 d *	0 (0)	0 (0)	Two patients maintained stable disease for 12 weeks and one for 16 weeks.
Eisenhauer [39]	GBM AA	Ph-II Rec	12	1.5 i.v.; 5/21 d †	1 (8)	1 (8)	—
Kyritsis [40]	GBM AA	Ph-II Rec	29	0.4 i.v.; 28 d cy *	0 (0)	3 (12)	15% of patients with stable disease.

CR = complete response; PR = partial response; Rec = recurrent; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; i.v. = intravenously; d = days; † 1.5 mg/m<sup>2</sup> daily on days 1-5 each 21-day cycle; \* 5.5-7.5 mg/m<sup>2</sup> as a single 24-h continuous infusion on day 1 of each 21-day cycle; ★ Continuous i.v. infusion every 28 days with a starting dose of 0.4 mg/m<sup>2</sup>/day.

attractive candidate for use in brain tumors [45, 46]. The cumulative dose limiting toxicity in adults is sensory neuropathy (12%). In those patients who developed sensory neuropathy of grade 2 or higher, symptoms regressed in 82% at four months and completely resolved in 41% at eight months after the drug was discontinued [45].

Experience with oxaliplatin for treatment of malignant gliomas is limited. *Misset* reported a PR in two of six patients (33%) with GBM [47]. *Soulie* reported a complete response (CR) in one of nine patients (11%) with recurrent GBM [48]. Both of these series incompletely defined pre-treatment status and patient outcomes and used varying dosages of oxaliplatin. A phase II trial of neoadjuvant oxaliplatin at 130 mg/m<sup>2</sup> every three weeks is planned for newly diagnosed GBM.

## BIOLOGICAL AGENTS

### Protein Kinase C Inhibitors

Protein kinase C (PKC) is a phospholipid-dependent, cytoplasmic, serine threonine kinase responsible for signal transduction in response to various growth factors, hormones, and neurotransmitters. Once activated, PKC phosphorylates proteins and triggers many cellular responses including membrane transport, gene expression, and cellular differentiation/proliferation [49]. PKC inhibition has been investigated as a therapeutic strategy for malignant gliomas because of its critical intermediary role in the malignant transformation, proliferation, and invasiveness of glial cells [50, 51]. Two methods of PKC inhibition that have been studied in clinical trials for malignant gliomas are i.v. treatment with antisense oligonucleotides and oral tamoxifen.

### Antisense Oligonucleotides

Oligonucleotides are short sequences of nucleotides (usually at least 15 bases in length) designed to hybridize with complementary messenger RNA (mRNA) and prevent translation of the RNA message at the ribosome (Fig. 6). Unmodified oligonucleotides are unstable in the circulation primarily due to degradation by ubiquitous cellular nucleases, and have a half-life of about five min [52]. Substituting one of the oxygens in the phosphate groups with a sulfur atom (phosphorothioate modification) makes these fragments (S-oligos) resistant to cleavage, increases the half-life to approximately one hour, and allows for continuous i.v. infusion. Upon administration, S-oligos are presumed to enter cells by endocytosis [53].

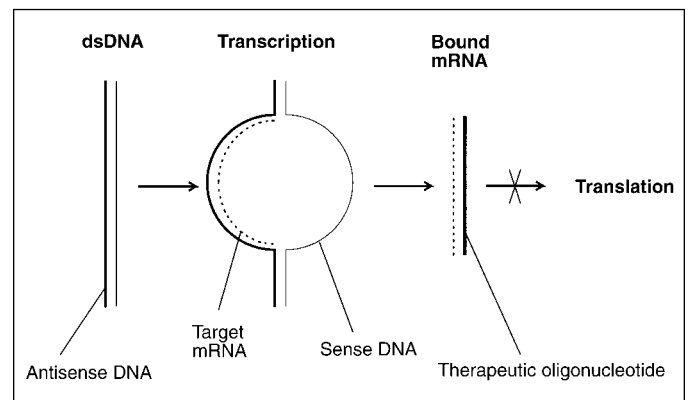
ISIS 3521 is a phosphorothioate oligonucleotide that binds to the 3' untranslated region of PKC mRNA with high affinity and inhibits the production of protein kinase C- $\alpha$ , a PKC isoenzyme, by promoting cleavage of the hybridized

molecule [54]. ISIS 3521 is the subject of an ongoing phase II trial for treatment of recurrent malignant glioma.

There are several potential limitations associated with the systemic administration of antisense oligonucleotides. First, the delivery of antisense compounds to tumor cells beyond an intact BBB may be impeded due to the highly negative charge, acid lability, and large molecular weight of S-oligos [55]. Second, this therapeutic compound accumulates in the liver, kidneys, and throughout the reticuloendothelial system and relatively small amounts may be left to traverse the BBB and enter the tumor. Continuous i.v. infusion may be necessary to achieve sufficient and sustained delivery of antisense oligonucleotides to the tumor [52]. Effective treatment of tumor cells exhibiting cell-cycle dependent susceptibility to an antisense compound (depending on the molecular target) may require this delivery method. Third, multiple genes are important in cell proliferation, invasiveness, and survival. Targeting PKC alone may not result in cytotoxicity or sustained tumor response. Finally gene expression changes over time and blocking one critical cell pathway may activate yet another. Some of these limitations may be bypassed by direct tumoral infusion.

### Tamoxifen

Tamoxifen is an agent widely used for adjuvant treatment of breast carcinoma. When administered in sufficient doses, tamoxifen yields an estrogen receptor-independent antineoplastic effect by inhibiting PKC [56]. Tamoxifen also induces transforming growth factor beta 1 and inhibits ouabain-sensitive Na-K ATPase, Mg-ATPase, calmodulin dependent protein kinase, and certain calcium channels [57, 58]. The clinical relevance of these mechanisms has not been fully defined.



**Figure 6. Antisense RNA.** Antisense oligonucleotides bind the target to the messenger RNA (mRNA) sense strand, thus blocking successful translation of the corresponding protein. High-affinity binding (formation of the RNA duplex) results in gene inactivation either through steric blocking of the ribosome complex or by triggering mRNA cleavage by RNase H. This diagram presumes that the target gene is regulated by transcription from the sense RNA strand.

**Table 5.** Summary of clinical trials using tamoxifen in recurrent malignant gliomas

Author	Path	Study	# Patients	Dose	Med Survival*	Remarks
Vertosick [59]	GBM (29) AA (3)	Ph-I	32	20 mg p.o. BID	17 wks KPS $\leq$ 60 8 wks KPS $\geq$ 70 21 wks	7/32 pts remained stable on tamoxifen for >6 mos.
Couldwell [60]	GBM (6) AA (5)	Ph-I	11	160-200 mg p.o. daily	24 wks	PR in three pts—these responders survived longer than 12 mos with clinical improvement.
Vertosick [61]	GBM (53)	Ph-I	26 18 9	40 mg p.o. daily 80 mg p.o. daily 160 mg p.o. daily	12 wks 18 wks Not reached at time of publication	Significant reduction in peritumoral edema.
Freeman [62]	Brain stem gliomas (5)	Anecdotal	5	Not reported	Not reported	PR in 4/5 pts. SD in one pt. Remissions of up to 26 mos.
Couldwell [63]	GBM (20) AA (12)	Ph-II	32	80 (F)-100 (M) mg p.o. BID	GBM 29 wks AA 64 wks	PR in four AA and four GBM pts with SD in six as measured by MRI and PET.
Pollack [64]	GBM/AA	Ph-I	7 7	60 mg p.o. BID 100 mg p.o. BID	11 wks	SD in four pts for at least three months. Longest survivor was 17 months.

\*After initiation of therapy.

Pts = patients; Ph = phase; PR = partial response; SD = stable disease; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; p.o. = orally; mos. = months; wks = weeks; M = male; F = female; KPS = Karnofsky Performance Status; PET = positron emission tomography.

Because of the sensitivity of glioma cell lines to tamoxifen-induced PKC inhibition, this drug has been the subject of several clinical trials (Table 5) [59-64]. These trials have enrolled patients with largely inoperable, recurrent malignant gliomas. Median survivals have ranged from 11-64 weeks from the initiation of tamoxifen therapy with partial radiographic responses varying widely between series (0%-80%) [65].

There appears to be a consistent relationship between higher tamoxifen doses, higher radiographic response rates, and longer survival [61]. This is exemplified by *Couldwell's* description of a 49-year-old male with recurrent glioblastoma treated with tamoxifen 20 mg orally twice a day [60]. Six weeks later, progressive disease was documented on brain MRI and the patient was then treated with tamoxifen 100 mg orally twice a day. This resulted in clinical improvement, a radiographic PR, and a greater than nine-month survival.

Micromolar concentrations of serum tamoxifen can be achieved within several days by "loading" with 1,000 mg per day prior to the administration of the maintenance dose [64]. Tamoxifen has been shown to attain high concentrations in brain metastases and surrounding brain tissues in patients with breast cancer [66]. Levels of tamoxifen within the middle of the in vitro therapeutic range have also been demonstrated in a tumor biopsy specimen from a patient with malignant glioma treated with this drug [60].

PKC inhibition for the adjuvant treatment of malignant gliomas is a strategy still under investigation. Another PKC inhibitor under development is staurosporine. This drug is more effective at halting the proliferation of glioma cell

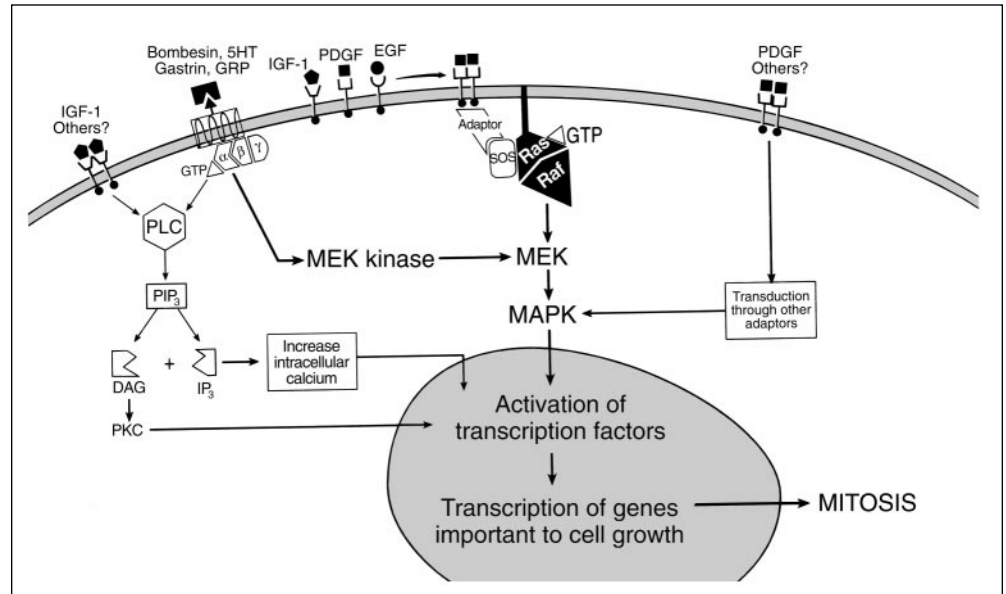
lines at lower doses than tamoxifen [67]. Clinical trials with this agent have not yet been reported.

#### Cell Signal Transduction Inhibitors

Peptidomimetic drugs developed to target critical intermediaries in cell signal transduction pathways represent another novel class of antineoplastic drugs. The main focus has been on inhibiting ras, a family of GTP-binding cytoplasmic proteins with a pivotal role in the development and progression of many human cancers (Fig. 7). Permanent activation of the ras-signaling pathway requires insertion of proteins into the plasma membrane. This in turn requires attachment of a farnesyl group (15 carbon lipid tail) to the protein, a reaction catalyzed by the enzyme, farnesyl transferase. Inhibition of this step by a farnesyl transferase inhibitor (FTI) is a potentially useful antineoplastic strategy [68, 69]. In animal models, tetrapeptide FTIs specific for ras have successfully interrupted the transmission of signals from activated cell surface growth factor receptors to downstream intracellular partners. FTIs are cytostatic and have demonstrated in vitro activity against a number of human tumor cell lines.

There is a sound rationale for the study of FTIs in malignant gliomas, as up to 70% of these tumor specimens overexpress ras oncoproteins [70]. Moreover, ras-dependent receptors (epidermal growth factor receptor, platelet-derived growth factor/receptor, and insulin growth factor/receptor-1) have been implicated in brain tumorigenesis [71]. Finally, because FTIs are cytostatic and known to have synergistic effects on tumor cell lines when used in conjunction with standard chemotherapeutic agents, the most effective clinical application of these drugs may be as part of a multiple drug combination [72].

**Figure 7. Cell signal transduction.** Ras is illustrated attached to the plasma membrane by a farnesyl anchor. This protein occupies a pivotal position in cellular replication. However, some ras-associated receptors such as PDGF are known to act via multiple pathways. The existence of multiple parallel pathways of signal transduction is a theoretical limitation of farnesyl transferase inhibitors as antineoplastic therapy. Used with permission from [68].



## Immunotherapy

### Cytokines

Interferons possess direct tumor cytotoxicity and a capacity for immune modulation. They may act indirectly to recruit and activate leukocytes, augment expression of cell surface molecules, and induce the production of other intermediate cytokines. Studies have demonstrated that human interferon alpha and beta inhibit tumor growth in rodent glioma models, and a large number of phase I and II clinical trials investigating these interferons have been reported over the past decade [73-76]. Limitations of these studies have included selection bias, inadequate sample size, and incompletely documented progression-free survival and radiographic response rates. Despite these shortcomings, other groups have reported encouraging response rates and survival times [77-79].

Rajkumar reported results of a phase I study evaluating radiation combined with recombinant interferon alpha-2A and BCNU as initial therapy for patients with high-grade glioma [80]. Five of nine patients evaluable for radiographic response had a PR, with a median survival of the entire cohort approaching four years. In a phase II study evaluating alpha interferon and BCNU for patients with recurrent high-grade glioma, Brandes reported on 21 patients who had not received prior chemotherapy [81]. A PR was obtained in 7/21, and 6/21 maintained stable disease, although overall median survival was seven months. Both of these studies reported "substantial but acceptable" constitutional symptoms.

### Adoptive Immunotherapy

The cellular immune response in malignant glioma patients is significantly depressed as demonstrated by

impaired blastogenic response of peripheral blood lymphocytes and reduced interleukin 2 (IL-2) production and IL-2 receptor expression of mitogen-stimulated T cells. Peripheral blood lymphocytes from glioma patients can be activated in vitro by IL-2, and these lymphokine-activated killer cells (LAK cells) are capable of killing both autologous and allogenic glioma cells [82].

Hayes treated 15 recurrent malignant glioma patients with intracavitary LAK cells and IL-2 in six-week cycles through a modified Ommaya reservoir placed at the time of reoperation [83]. Four radiographic responses (two CR and two PR) and a median survival of 53 weeks after reoperation were reported. Eight of these patients survived more than one year. These data should be interpreted cautiously as some patients received surgery and/or chemotherapy subsequent to LAK/IL-2 administration.

Plautz reported 10 patients with progressive primary or recurrent malignant glioma who were treated with systemic T cell adoptive immunotherapy [84]. These patients were vaccinated with irradiated autologous tumor cells, and T cells from draining inguinal lymph nodes were then harvested, stimulated, and expanded. Following i.v. T cell transfer therapy, radiographic regression that lasted at least six months was demonstrated in two patients with recurrent tumors, and one patient demonstrated stable disease that lasted more than 17 months. Four of eight patients with recurrent tumor were alive more than one year after surgery for recurrence.

The source, specificity, and number of T cells are essential determinants of efficacy. There are significant limitations of this treatment strategy. First, diminished immune responses are generated against antigens introduced into the CNS. Second, the BBB effectively impedes T cells from reaching

their target. Third, many gliomas release substances such as tumor growth factor  $\beta$  and IL-10 that cause immunosuppression. Finally, the brain might not tolerate the inflammation associated with an immune reaction [84].

### Gene Therapy

Gene therapy is an attractive strategy for the treatment of brain tumors because of the lack of systemic toxicity and the ease of application during stereotactic procedures or craniotomy. Direct introduction of genes without any cellular or viral vector can be accomplished via aerosol, systemic delivery, or microcellular injection. Indirect gene delivery by transplantation of genetically engineered cells or inoculation of a recombinant defective virus is more clinically relevant and constitutes a highly efficient means of transferring DNA to a target cell [85].

The most common experimental paradigm for genetic treatment of brain tumors has been delivery of the herpes simplex virus thymidine kinase (*HSV-tk*) gene to the tumor using an adenovirus vector (Fig. 8). Adenoviruses are highly stable, nonenveloped, double-stranded DNA-containing viruses with a low rate of genomic instability and, therefore, low risk of insertional mutagenesis. Adenoviruses transfer their DNA by binding to a specific cell surface receptor, entering the cytoplasm by endocytosis, and then forming a pore in the endosome to translocate genetic material to the nucleus [86].

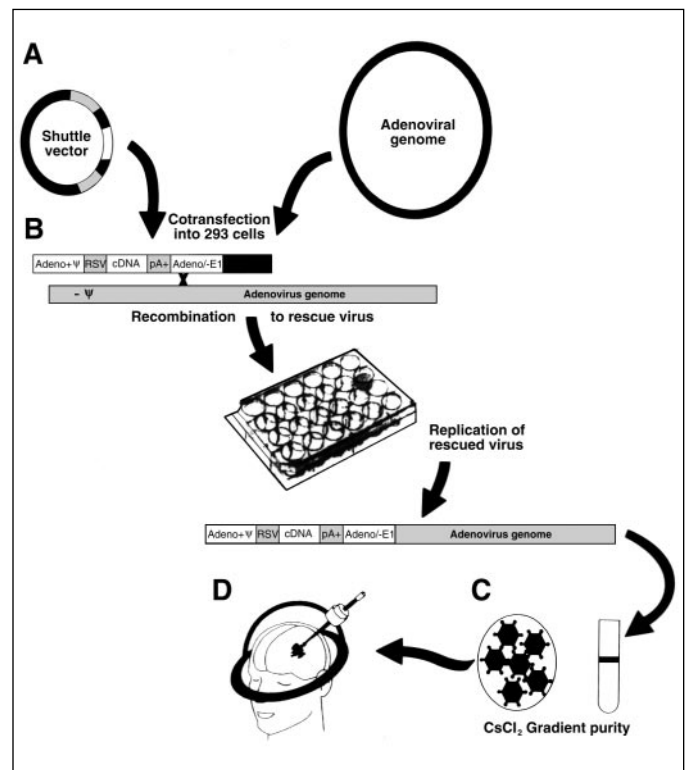
In principle, the *HSV-tk* construct should only be delivered to dividing cells (i.e., tumor cells and not neurons). The thymidine kinase that is being produced by these cells can phosphorylate nucleoside analogs such as ganciclovir to form nucleotide-like precursors that will block replication of DNA. Although the transduction process alone is not cytotoxic, cellular production of thymidine kinase confers susceptibility to those cells subsequently exposed to ganciclovir. The so-called “bystander effect” is the result of diffusion of phosphorylated nucleosides away from dying cells to adjacent nontransduced tumor cells resulting in their death [86].

Ram treated 15 patients with recurrent malignant brain tumors using intratumoral injection of murine cells modified to produce retroviral vectors containing the *HSV-tk* construct [87]. Nine of these 15 patients had GBM and were treated to either a single focus or multiple foci of disease. Three of the nine patients exhibited either a CR or PR, with smaller lesions most likely to respond. Potential limitations of this strategy include lack of transduction of distant tumor cells, transduction of endothelial cells, and immunologic rejection of the murine vector cells.

Izquierdo used retrovirus-mediated gene therapy to treat five patients with anaplastic glioma and two of these patients showed a PR [88]. In a follow-up study the investigators

reported that they had been unable to reduce the tumor size of recurrent glioblastoma patients with tumor volumes larger than 100 cm<sup>3</sup> by applying the standard *HSV-tk/ganciclovir* therapy or to prolong patient survival for more than eight months [89]. These observations underscore the need for more effective delivery and distribution strategies.

Ideally, gene therapy with a replication-competent adenoviral vector should result in intracellular viral replication and exclusive cytolysis of targeted cancer cells. In theory, newly released virions from a lysed cell could then infect both neighboring and distant cells but selectively replicate only in cancer cells. ONYX-015 (dl1520) is an adenovirus construct designed to be differentially lethal to tumor cells with mutated or deleted *p53*, a tumor suppressor gene important in the early transformation of most gliomas [90].



**Figure 8. Gene therapy.** A) The shuttle vector containing the expression cassette with the foreign gene of interest is cotransfected with the plasmid containing the adenoviral genome. B) The vector containing the adenovirus genome is missing the packaging sequence and cannot produce virus. The shuttle vector also cannot replicate and produce virus because it is missing a large piece of the adenoviral genome. For virus production, the shuttle vector containing the packaging sequence and the expression cassette must recombine with the adenovirus genome. C) The recombinant virus can replicate in human embryonic kidney 293 cells. Replicating virus is easily identified by the lysis of cells in tissue culture. After growth and plaque purification, large quantities of recombinant adenovirus can be processed over a cesium chloride density gradient. D) After dialysis, replication-defective adenovirus can be used for in vivo transfections. Used with permission from [86].

A key feature of the ONYX construct is the genetic deletion of an adenoviral protein (E1B 55K) that binds to the N-terminus of *p53* and blocks its activity. Since E1B 55K is deleted in this construct, the cellular *p53* system can respond to “therapeutic” infection by promoting cell-cycle arrest in *p53*-positive cells (a nonlethal infection) or cytolysis in *p53*-negative cells [91]. Initially encouraging cell line and animal study results using ONYX-015 virus have been tempered, however, by recent reports of wild-type *p53* dependent cytolysis [92-95]. *Fueyo* has also demonstrated that overexpression of E2F-1, a promoter of inappropriate cell entry into the S-phase that is upregulated by ONYX-015, triggers apoptosis and suppresses tumor growth in vitro and in vivo independently of cellular *p53* status [96]. These new data suggest that molecular mechanisms concerning replication competent adenoviral vectors need to be defined prior to use in a clinical setting.

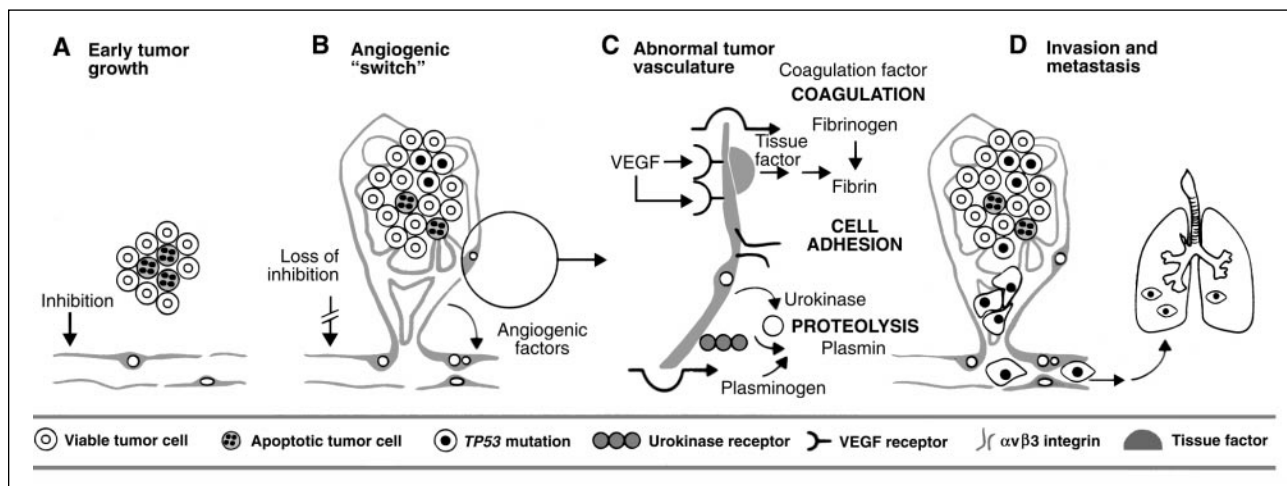
### Angiogenesis Inhibitors

Tumors promote the formation of new blood vessels when they surpass 1-2 ml in greatest diameter (less than  $10^6$  cells) (Fig. 9) [97, 98]. This is accomplished by altering the physiologic balance between positive and negative regulators of angiogenesis [99]. Tumor neovascularization occurs through a number of mechanisms including overexpression and mobilization of angiogenic proteins from the extracellular matrix and recruitment of host cells such as macrophages, which in turn produce their own angiogenic proteins [100]. Key angiogenic proteins include vascular endothelial growth factor (VEGF), basic fibroblast growth factor

(bFGF), platelet-derived growth factor (PDGF), and tenascin. Endogenous inhibitors of angiogenesis include angiostatin, endostatin, and thrombospondin [101].

When the angiogenic process is triggered, a cascade of events including activation of endothelial cells, proteolytic degradation of the extracellular matrix and basement membrane, proliferation and migration of endothelial cells, endothelial tube formation, and fusion/reassembly of the extracellular matrix occur [102]. Vascular basement membrane degradation allows endothelial cells to migrate into the surrounding tissue and form vascular structures [103]. The basement membrane consists of both fibrous and nonfibrous proteins including heparan sulfate proteoglycans, which can bind and enable growth factors such as bFGF and VEGF [104]. This complex degradative process involves many enzymatic systems that result in the release of stored growth factors and, in turn, promote further angiogenesis. Matrix metalloproteinases, serine proteases (plasminogen activators), and cathepsins are among the enzyme classes implicated in this process [105].

Most experience with antiangiogenesis treatments in brain tumors has been with matrix metalloproteinase inhibitors (MMPi). The MMPs are a family of over a dozen secreted and membrane-bound zinc endopeptidases. They require activation by other proteolytic enzymes in order to digest an extracellular matrix. MMPs are upregulated in primary and metastatic brain tumors and correlate with malignant progression [104, 106]. One of the MMPs, MMP-9, is thought to play a critical role in



**Figure 9. Tumor angiogenesis.** A) Small tumor, less than  $1 \text{ mm}^3$  in diameter with high rate of apoptosis, cannot grow further without new blood supply. B) Hypoxic environment and genetic instability allow evolution of tumor clones with loss of *p53* function. These cells have lower apoptotic rate, and produce angiogenic factors, inducing new vasculature (angiogenic “switch”). There is also reduction in antiangiogenic factors. C) Tumor vasculature is abnormal. Leaky vessels allow passage of fibrinogen, and tissue factor is expressed on tumor endothelium producing fibrin deposits. VEGF receptors and urokinase receptor integrins are upregulated. Endothelial cells are dividing. D) These processes allow invasion and metastasis to distant sites. Used with permission from [101].

tumor cell invasion, and, in one study, was detected in the CSF of patients with brain tumors but not in control patients [103].

Marimastat is an orally available MMPI that blocks the ability of tumor cells to disrupt the extracellular matrix, prevents the ingrowth of new blood vessels, and inhibits glial tumor growth and spread in animal cancer models [107]. A multicenter phase III trial of Marimastat in recurrent malignant gliomas has recently been completed.

Thalidomide is an antiangiogenic agent that decreases the expression of beta integrin subunits produced by leukocytes. Because these integrins are crucial for cell-matrix interactions, thalidomide is felt to inhibit cell migration accounting for its antiangiogenic (and teratogenic) activity [108]. Its clinical utility as a long-term treatment for malignant gliomas remains under investigation. *Yung* reported findings from a phase II trial of the antiangiogenic agent thalidomide in patients with recurrent high-grade gliomas [109]. Of the 32 evaluable patients, there were two PRs, two minor responses, and a median time to progression of eight weeks. The assessment of cytostatic antiangiogenesis agents as potential treatments for malignant glioma poses significant challenges in clinical trial design, as discussed earlier in **Clinical Trial Design**.

## DELIVERY STRATEGIES

### RMP-7

The BBB impedes passage of circulating compounds that are hydrophilic, ionized, greater than 18 Å in diameter, or more than 180 Da in molecular weight [110]. This excludes many conventional chemotherapy drugs such as cyclophosphamide and the anthracyclines. As the integrity of the BBB is partially compromised in brain tumor-associated blood vessels, it is controversial whether hydrophilic drugs have difficulty traversing this physiologic barrier. However, restoration of the BBB by coadministration of corticosteroids may impede delivery of these agents [111]. Mannitol disruption of the BBB was described almost two decades ago and continues to be a technically difficult method used for the delivery of high-dose chemotherapy [112]. More recently, RMP-7, a bradykinin analogue, has been developed to transiently increase BBB permeability while avoiding some of the risks inherent with the mannitol procedure.

RMP-7 stimulates endothelial B2 receptors, which results in intracellular calcium influx, contraction of capillary endothelial cells, and loosening of tight junctions [113]. In addition, RMP-7 increases vesicular transport and transcellular penetration. These effects led to increased permeability of the BBB in animal studies with lanthanum, a

139 molecular weight tracer substance [114]. RMP-7 has a longer plasma half-life than bradykinin although it exerts its effects over a narrow time frame so that the timing of chemotherapy administration in relation to RMP-7 is critical [115]. RMP-7 preferentially “opens” the BBB in the tumor area [116]. Despite this, vasogenic edema as an adverse event rarely occurs unless serum proteins extravasate into the brain parenchyma. Transient decreases in arterial blood pressure have been observed with high-dose RMP-7 administration in a swine model, but the drug appears to be well tolerated otherwise [113].

The infusion of intracarotid RMP-7 followed by carboplatin in a rat glioma model produced longer survival than in those rats treated with carboplatin alone [115]. The amount of carboplatin used in this experiment was substantially less than an equivalent i.v. dose in humans. RMP-7 also increased permeability to carboplatin in dexamethasone-treated tumors although to a lesser extent than rats not exposed to steroids. In an irradiated dog brain model, RMP-7 appeared to have a selective effect on an impaired BBB, but did not appear to affect the extent or volume of radiation-induced cerebral edema [117].

*Gregor* reported preliminary data from two phase II trials investigating RMP-7 administered with i.v. carboplatin in recurrent malignant glioma patients [118]. A statistically significant survival hazard ratio of approximately 2 in favor of the RMP-7/carboplatin-treated patients was seen in the 87 patients enrolled. There was also an implication that these patients had improved QOL.

### Polymers

Since 90% of malignant gliomas recur within 1-2 cm of the original site, local therapy may be an effective strategy [119]. This observation has served as the basis of focal radiation treatments such as brachytherapy, proton beam therapy, and radiosurgery. Another method is the use of polymers to deliver drugs via diffusion from micropores in the polymer matrix or by the release of drug from within the interstices of a degradable matrix.

One BCNU polymer design is a 1.45-cm diameter wafer disk that consists of a biodegradable polymer component (poly bis(p-carboxyphenoxy) propane and sebacic acid or PCPP-SA) uniformly impregnated with 7.7 mg of BCNU (1,3-bis (2-chloroethyl)-1-nitrosourea). The usual dose is eight wafers, which are to be placed in the margins of the surgical resection cavity. BCNU is released from the wafer over two to three weeks and subsequently diffuses into surrounding brain tissue to produce an antineoplastic effect by alkylating DNA and RNA [120].

Although comparable human data are lacking, recent work by *Fung* with intraparenchymal BCNU impregnated

polyanhydride pellets in cynomolgus monkeys revealed high drug levels (0.5-3.5 mM) within 3 mm of the implant over a period of approximately one month [121]. Pharmacokinetic studies demonstrated that BCNU area under the concentration time curve (AUC) was 4-1,200 times higher than the AUC achieved with i.v. administration of a higher dose. The applicability of this animal study is unclear.

In a randomized, double-blind, placebo-controlled clinical trial in adults undergoing surgical resection for recurrent malignant glioma, 222 patients were assigned to receive surgically implanted biodegradable polymer disks with or without 3.85% BCNU (by weight) [122]. Among patients with glioblastoma, treatment with placebo polymer resulted in 64% mortality at six months, compared to a 44% six-month mortality for those treated with the BCNU polymer ( $p = 0.02$ ). Limitations of this study include the fact that no survival advantage was shown over historical controls treated with i.v. BCNU, BCNU polymer produced no survival prolongation in patients with pathologic diagnoses other than GBM, and maximal feasible resection and initial KPS were strong predictors of survival irrespective of treatment with the BCNU implants. Furthermore, the clinical relevance of the six-month comparison is questionable. Follow-up data demonstrated no significant difference in survival between BCNU polymer and placebo groups at approximately 40 weeks. Dose-escalation trials incorporating wafers with higher concentrations of BCNU by weight are ongoing.

A prospective, randomized, double-blind study of BCNU polymer versus placebo at the time of initial surgery for malignant gliomas was recently reported by *Valtonen* [120]. All 32 patients in the study received involved field radiation therapy following surgery. For 27 patients with grade IV tumors, the median time from surgery to death was 40 weeks for the placebo group and 53 weeks for the active treatment group ( $p = 0.008$ ). The two-year survival for patients receiving BCNU polymer was 30% as compared to 6% in the placebo-controlled polymer patients. Adverse events in the BCNU polymer arm were relatively few. These results should be interpreted with caution as the original study planned to enroll 100 patients and was stopped prematurely due to administrative issues; there were more AAs in the BCNU polymer arm; and there was no comparison to i.v. BCNU.

Many other chemo- and immunotherapies are being developed for interstitial delivery [123, 124]. Carboplatin is one

such candidate that has shown activity against gliomas when administered i.v. but has limited use because of myelotoxicity. Carboplatin polymer was implanted in an experimental glioma rat model [125]. In this setting, median survival increased threefold over controls and it was shown that the best intracranial polymer dose was significantly more effective than the best systemic dose tested. Similarly with cisplatin, mean survival was significantly prolonged as compared to control animals and animals treated with placebo polymer. At autopsy no evidence of viable tumor was noted in the animal survivors [126]. The relevance of these animal models to human glioma patients remains uncertain.

## CONCLUSION

Malignant gliomas remain a poorly understood form of cancer associated with high rates of morbidity and mortality. Nevertheless, prospects for the future are better than ever before. Developments in molecular biology have led to a clearer understanding of the mechanisms of tumor development, growth, and resistance to therapy. As a result, new treatment strategies are emerging that target steps in the molecular pathogenesis of these tumors. Antiangiogenesis agents, antisense oligonucleotides, and signal transduction inhibitors are all examples of such therapies now entering clinical trials. Improved cytotoxic agents that penetrate the BBB (topoisomerase I inhibitors, temozolomide, oxaliplatin) are other promising therapeutic agents. Finally, strategies to circumvent the BBB (polymers, bradykinin analogues, gene therapy) are important advances that have also shown efficacy in early clinical trials. Future treatment strategies for malignant gliomas will likely involve synergistic combinations of agents aimed at different pathways in the molecular pathogenesis of this type of cancer.

The pace and breadth of discovery in molecular biology promise a steady supply of novel agents as well as refinements of existing ones. One of the important challenges for the future is the development and implementation of sound clinical research methods that will enable investigators to identify active treatment regimens. Although the traditional outcomes of survival and time to progression remain important, incorporation of neuropsychological outcomes and valid, reliable QOL instruments assume great importance for future studies. Extending and improving QOL should be the complementary goals of any new agent for malignant gliomas.

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