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Targeted delivery of antitumoral therapy to glioma and other malignancies with synthetic chlorotoxin (TM-601)

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Targeted therapies for cancer is a rapidly advancing field, but the identification of tumor-specific ligands has proven difficult. Chlorotoxin (CTX) is a small, 36 amino acid neurotoxin isolated from the venom of the Giant Yellow Israeli scorpion *Leiurus Quinquestriatus.* Interestingly, the peptide has been found to preferentially bind to a variety of human malignancies, but shows little or no binding to normal human tissues. A synthetic version of this peptide (TM-601) has been manufactured and covalently linked to iodine 131 (^{131}I-TM-601) as a means of targeting radiation to tumor cells. Preclinical studies and Phase I clinical trials have been completed in patients with recurrent glioma, a type of malignant brain tumor. These studies demonstrated that intracavitary dosing of ^{131}I-TM-601 appears safe, minimally toxic, and binds malignant glioma with high affinity and for long durations. A Phase II trial of this agent using higher doses of radioactivity and repeated local administrations is underway. In addition, enrolment has begun in a Phase I trial evaluating whether systemically delivered ^{131}I-TM-601 can be used to image metastatic solid tumors and primary gliomas. Due to its small size, selective tumor binding properties, minimal toxicity and relative ease of manipulation, CTX represents a potentially important targeting agent for many cancers.

Keywords: chlorotoxin, glioma, intracavitary, targeted therapy

Expert Opin. Drug Deliv. (2007) 4(2):175-186

1. Introduction: overview of disease

In the US, an estimated 43,800 new cases of primary brain tumors were diagnosed in 2005 [1]. Approximately 18,500 (42%) of these were malignant tumors. Overall, primary brain tumors represented 1.3% of the approximate 1.5 million malignant cancers diagnosed in the US in 2005 (American Cancer Society). The incidence of primary brain tumors appears to be on the rise, although it is unclear if this is attributable to better reporting, or the influence of environmental or genetic factors [2]. Gliomas, including glioblastoma multiforme (GBM), anaplastic astrocytoma, astrocytoma and oligodendroglioma, are the most lethal forms of primary brain tumors [2]. Approximately 13,000 brain cancer deaths attributable to glioma occur annually in the US, where the median survival for GBM is ~ 12 months. For anaplastic astrocytoma and low-grade (WHO grade II) astrocytomas, survival is ~ 3 and 5 – 7 years, respectively [2]. These grim statistics make gliomas among the most deadly forms of cancer. Despite over three decades of intensive research and a variety of chemotherapy, radiotherapy and surgical approaches, the prognosis for patients with these tumors has not changed significantly.

10.1517/17425247.4.2.175 © 2007 Informa UK Ltd ISSN 1742-5247

The lack of meaningful therapies with long-term impact on disease control and survival has led to continued research for better treatment. This paper focuses primarily on the role of chlorotoxin (TM-601) in the treatment of malignant glioma, as this tumor is the primary disease platform in which the technology has been evaluated. Nonetheless, limited data suggest that this technology may be applicable to many other cancers.

2. The standard of care

The standard of care treatment for gliomas is similar to that of many malignancies and includes: i) cytoreductive surgery when feasible; ii) external beam radiation therapy and associated variants; and iii) adjuvant chemotherapy during and after radiation.

2.1 Surgery for primary brain tumors

Open surgical removal of brain tumors has been a mainstay of glioma management for decades [3-6]. The goals of surgery are fourfold: i) to alleviate mass effect and compression of brain structures; ii) to restore normal cerebrospinal fluid pathways; iii) to reduce the tumor burden for other therapies; and iv) to obtain tissue for pathological diagnosis and characterization. Unfortunately, total excision of glioma is rarely possible. Glioma cells are highly invasive, and are commonly observed four or more centimeters away from the primary tumor mass [7]. Most of these cells are interdigitated with normal functioning brain parenchyma, and resection of these regions can result in unacceptable, neurological deficits. Furthermore, although gliomas rarely metastasize outside the CNS, they frequently disseminate widely in both hemispheres of the brain. Tools to aid surgeons in differentiating normal tissue from glial cells at the periphery of tumors can improve the extent of microscopic removal. These methods include the use of tumor fluorescence [8,9], infrared imaging [10], diffusion-weighted tensor imaging of white matter pathways [11], physiological mapping of cortical structures and other methods that improve resection safety. Gross total excision of low-grade gliomas, especially pilocytic tumors is curative in > 80% of cases [4]. In contrast, gross total removal of all other gliomas and most primitive neuroectodermal tumors are not clearly correlated with a higher cure rate. Cytoreductive surgery, which removes at least 85% of the enhancing volume of high-grade glioma, has been correlated with improved length of survival and improved quality of life [3,12], with this effect especially evident for > 98% resection [12,13]. These results indicate that methods to improve the extent to which all macroscopic and microscopic tumor can be removed should result in better outcomes. Further development of these methods should be encouraged. Despite its limitations, surgical resection remains the most effective single therapy for gliomas, especially when tumors recur or progress [12].

2.2 Radiation therapy

Gliomas are not particularly radiosensitive tumors. Radiation doses in excess of 50 Gy are effective in retarding glioma progression, but do not generally produce a cure or long-term control of disease [14]. Increasing doses of radiation have improved efficacy on higher-grade tumors, although most dose escalation studies have failed to demonstrate improved survival. Unfortunately, normal brain parenchyma is also sensitive to radiation effects. The tolerance for brain injury escalates quite rapidly above 70 Gy, effectively limiting total radiation doses to 60 – 70 Gy. The use of radiosensitizers has not been proven to significantly improve the effects of radiation or long-term outcome [15]. Furthermore, radiation injury and radiation-induced cell death (radiation necrosis) is often as damaging as the primary tumor. Despite these limitations, radiation therapy is used almost uniformly in the treatment of gliomas, and is likely to remain a mainstay of glioma therapy in the foreseeable future.

2.3 Chemotherapy

Systemic chemotherapy has proven to be disappointing in the treatment of gliomas. This is partially attributable to the poor distribution of drug in the brain due to the blood–brain barrier (BBB). Many agents, including carmustine, lomustine, procarbazine and temozolamide, have caused a response against high-grade gliomas [16,17]. However, these agents tend to cause fairly limited or partial responses in both an upfront and a recurrent setting. Most recently, the combination of temozolamide with radiation therapy in patients with newly diagnosed GBM, followed by a course of temozolamide, has been found to increase the median survival by 2 months when compared to radiation alone, with ~ 35% of patients surviving beyond 18 months [18]. A sizeable number of chemotherapy trials are underway for glioma, although a thorough review is well beyond the scope of this paper.

Delivery of chemotherapy via time-released polymer wafers is currently the only FDA-approved form of loco-regional chemotherapy [19]. Polyfeprosan 20 with carmustine (Gliadel®; Guilford Pharmaceuticals) is a synthetic, biodegradable polymer wafer containing a 2.4% concentration of carmustine [20]. Typically 6 – 8 wafers are implanted directly along the walls of a resected tumor at the time of surgery. A Phase III trial comparing Gliadel to drug-free wafers demonstrated a slight improvement in median survival of ~ 8 weeks for patients with recurrent GBM [19]. One major advantage of Gliadel is that it avoids many of the untoward side effects of systemic carmustine, such as thrombocytopenia. However, Gliadel is associated with an increased risk of brain edema, steroid dependence, seizures and wound infection. The cost of the wafers roughly approximates to the cost of a 6-week course of carmustine. Efforts to increase the concentration of carmustine in the wafers have lead to unacceptable adverse events, effectively limiting the drug concentration to ≤ 5%. So far, no agent, with the exception of carmustine, is available in this

formulation. Various shapes of the polymer are also being tested to ease method of insertion [20,21]. In addition, several Phase I trials have looked at the effects of combining intracavitary chemotherapy with systemic chemotherapy [22].

In light of the limitations of the treatments described above, tremendous attention has focused on the development of tumor-specific targeted therapies. The broad goal of this technology platform is to deliver therapy directly to the tumor cells, while avoiding or preventing any therapy delivery to normal brain cells. Although this concept is broadly applicable to many malignancies, it is particularly important and challenging for gliomas, due to the fact that they heavily infiltrate into, and interdigitate with normal brain tissue, and that the destruction of parenchyma results in the specific loss of neurological function. Furthermore, the BBB functionally excludes most of the large molecules from entering the brain, making the ability to actually deliver the therapy the key to that therapy's success.

3. Overview of loco-regional targeted therapy so far

3.1 Methods of therapy delivery

Because the BBB effectively limits the size of molecules entering the brain, direct delivery of these targeted therapies via a loco-regional delivery system is imperative for many agents. These delivery systems parallel drug infusion pumps, such as those used for liver infusion or direct injection into a large body cavity, for example intraperitoneal infusion for diffuse ovarian cancer peritoneal metastases.

The most obvious method for loco-regional delivery of drugs to a brain tumor is direct injection of drug into the tumor cavity via an implanted reservoir (e.g., Ommaya or Rickham reservoir), or syringe at the time of surgical resection or biopsy. Once the reservoir has been surgically inserted, delivery of the drug is simple and can be repeatedly performed by almost any physician with minimal training. This approach has been tested with several chemotherapeutic drugs, including carmustine, methotrexate, cisplatin and cyclophosphamide [23], as well as viral vector gene therapies [16,24-27]. Although toxicities have been tolerable, tumor responses have been very disappointing. For chemotherapies, failure is principally attributable to a lack of diffusion into the surrounding parenchyma. Thus, methods to increase the tissue penetration of drugs are being tested. A trial, which involved carmustine dissolved in ethanol (DTI-105) to increase tissue penetration, is the most prominent example of this approach [28].

Intra-carotid injection of chemotherapy supplemented by methods to transiently open the BBB has been studied for many years. BBB disruption is primarily accomplished with mannitol [29] or the bradykinin analogue, RMP-7 [30]. Again, the main goal of this approach is to increase concentrations of drug in the brain by utilizing a first-pass effect. Cisplatin and etopiside have been most frequently employed [31,32], although many agents have been tested [33,34] Success with this method has been variable, and most studies have failed to demonstrate a clear benefit in survival or time to progression. There have been no randomized, prospective trials utilizing this method.

Most recently, significant interest has developed around the use of convection enhanced delivery (CED) of chemotherapies and targeted toxins to brain tumors. CED refers to the process of applying uniform, positive pressure to overcome the natural resistance of the surrounding tissues, and essentially push the drug into the parenchyma. Using very slow infusion rates (0.5 – 4.0 µl/min) over long time periods (3 – 5 days) with a positive pressure, relatively uniform concentrations of drug can be delivered up to 4 cm away from the tip of the infusion catheter, with well-tolerated side effects and a reasonable risk profile [35-40]. CED is a rapidly advancing field, and improvements in catheter placement, drug delivery techniques and drug preparation are all having an impact on the potential efficacy of this method. However, not all molecules will disperse via CED, and there are significant technical challenges, such as charge, size and quaternary structure, which limit the widespread use of CED for glioma therapy.

For the delivery of most targeted therapies, intracavitary administration or CED is likely to continue to play a dominant role as the preferred methods, as they permit direct administration of the therapy to the region of the tumor while avoiding problems of BBB exclusion. However, ideally, a targeted therapy which is effective in treating brain cancer will be able to efficiently cross the BBB and achieve adequate tumor penetration to achieve tumoricidal activity.

3.2 Principles of targeted therapy

Targeted cancer therapies principally depend upon receptor-mediated selective binding to tumor cells. The success of this approach requires high specificity or selectivity of binding to tumor cells or other tumor related cells, such as blood vessels. This can be accomplished by either overexpression of receptors by tumor cells, or preferential expression of receptors not found on normal brain tissues. The targeting agents for these receptors are either i) antibody or antibody-like ligands; ii) proteins that bind the receptor; iii) proteins that bind the cell and are internalized; or iv) large molecules with receptor-specific binding properties.

3.3 Targeted cytotoxin therapy

Targeted therapy of glioma with tumor-specific cytotoxins has recently generated great interest. The basic strategy employed is to identify a cellular toxin, most commonly one derived from bacteria, modify that toxin to maximize antitumoral activity, and deliver the toxin directly to the tumor with a tumor-specific ligand acting as the carrier molecule (see [41] for a review). The most notable of these is a modified variant of *Pseudomonas* exotoxin joined to IL-13 (IL-13 receptors are overexpressed in many gliomas [38]). When this fusion protein binds to glioma cells, it is internalized and results in cellular death. Phase I and Phase II studies with this agent were encouraging [38]. A Phase III

clinical trial of this agent delivered by CED was recently completed. Unfortunately, the results indicated minimal survival benefit over intracavitary chemotherapy, with many toxicities. An alternate strategy employs diptheria toxin, which has been fused to a fragment of the transferrin-receptor antibody and delivered via CED [42]. This agent is undergoing Phase III clinical trials, but the ubiquitous nature of transferrin in the brain is a potential limiting factor. Fusion of diphtheria toxin to human plasminogen activator and IL-13 have also been accomplished [43], but these agents have not yet undergone clinical testing.

3.4 Targeted radioimmunotherapy and radiopeptide therapy

Antibodies to cell-surface antigens have been most commonly employed in clinical settings, and several of these compounds are FDA-approved for clinical use. Examples include trastuzamab, an antibody to the Her-2/Neu receptor found in many breast cancer cells, and ibritumomab tiuxetan, a radiolabelled antibody to the CD20 antigen on lymphoma cells.

This approach has been tested for brain tumors. Examples of this approach in glioma therapy include targeting the EGFR [44], fibronectin [45], and the extracellular matrix molecule, tenascin, with ^{131}iodine radiolabelled ligands. A Phase II trial of ^{131}I-anti-tenascin antibody (tenascin-81C6) injected into a postresection surgical cavity via an intracavitary reservoir (doses of up to 100 mCi), followed by radiotherapy and chemotherapy, was completed in 33 patients with GBM. A median survival of 79.4 weeks was reported [46]. However, imaging studies demonstrated that the antibodies were too large to penetrate beyond a few millimeters into the surrounding brain parenchyma, essentially neutralizing any potential targeting advantage [47]. Furthermore, tenascin is widely expressed throughout the nervous system, and EGFR, although upregulated in some gliomas, is also expressed by normal brain cells, limiting the specificity of these approaches. Both of these compounds are now in Phase II clinical trials in glioma, although they are unlikely to have widespread impact on the disease or treatment strategies in the foreseeable future.

Along a similar vein, tumor-specific monoclonal antibodies conjugated to ^{131}iodine for leptomeningeal carcinomatosis or ^{90}yttrium for glioma have been clinically tested [48,49]. Although somewhat promising for a diffuse process such as leptomeningeal carcinomatosis, the glioma data indicated a brachytherapy-like dose delivered to ~ 2 cm beyond the cavity, with no meaningful survival data available. From these studies, it is unclear if monoclonal antibodies offer any distinct advantage over non-tumor-specific, radiolabelled proteins such as albumin, which serves as a radiation carrier without targeted binding.

4. Chlorotoxin basic science

Chlorotoxin (CTX) is a small neurotoxin isolated from the venom of the Giant Yellow Israeli scorpion *Leiurus Quinquestriatus*. Chlorotoxin is a 36 amino acid (molecular weight of 3950 Da) peptide containing a single tyrosine residue that is available for radioiodination, eight cysteine residues, and four disulfide bonds, yielding a tightly folded, tertiary structure (Figure 1). CTX was originally described as a chloride-ion channel blocker [50,51]. In nature, CTX functions as one component of the venom that acts as a paralytic agent for small insects and other invertebrates. The scorpion bites an insect, which induces paralysis so the scorpion can either eat the insect or potentially escape a dangerous situation. When crayfish are injected with CTX, they become completely immobilized for a period of ~ 2 min, and then resume normal locomotion. This response is dose-dependent and highly reproducible, thus providing an important biological assay for CTX and any engineered modifications of its structure.

CTX was first identified as having tumor-specific targeting based on physiological studies in which CTX was applied to cell cultures of normal (glial cell) and malignant (glioma) astrocytes [50-53]. This finding was subsequently confirmed in an extensive assortment of tissue explants obtained from human gliomas, and other tumors, but most non-malignant organs and tissues, and non-transformed cells, did not demonstrate significant binding (Table 1 [54], and Figure 2, TransMolecular, Inc., internal data).

Although *in vitro* cell culture and immunostaining data support the view that CTX binds a tumor cell surface receptor, the exact receptor has been difficult to identify. Initial studies demonstrated that a human glioma cell line contained 1300 high-affinity binding sites per cell [51]. Given that electrophysiological experiments showed that CTX inhibits chloride ion fluxes across the cell membrane of glioma cells, it was initially suggested that the receptor was associated with the chloride channel [50-52,55]. Subsequent analysis, using a recombinant polyhistidine-tagged version of CTX, indicated that the binding site may be a matrix metalloproteinase, MMP2 [56]. MMP2 is considered a chloride channel-associated receptor, and is believed to indirectly regulate the chloride channels expressed by gliomas, but not normal human cells [50-52,55]. CTX decreases MMP2 enzyme activity. However, demonstrating that TM-601 (CTX manufactured by solid-phase peptide synthesis) specifically interacts with MMP2 has proved difficult, and studies are ongoing to further elucidate cell-surface and intracellular binding partners for TM-601. More recent experiments have demonstrated that TM-601 is indeed tumor-specific in its binding, and is internalized by tumor cells (D Jacoby, unpublished data). Nonetheless, the exact mechanism of action of CTX on tumor cells or the surrounding cellular environment has yet to be fully elucidated.

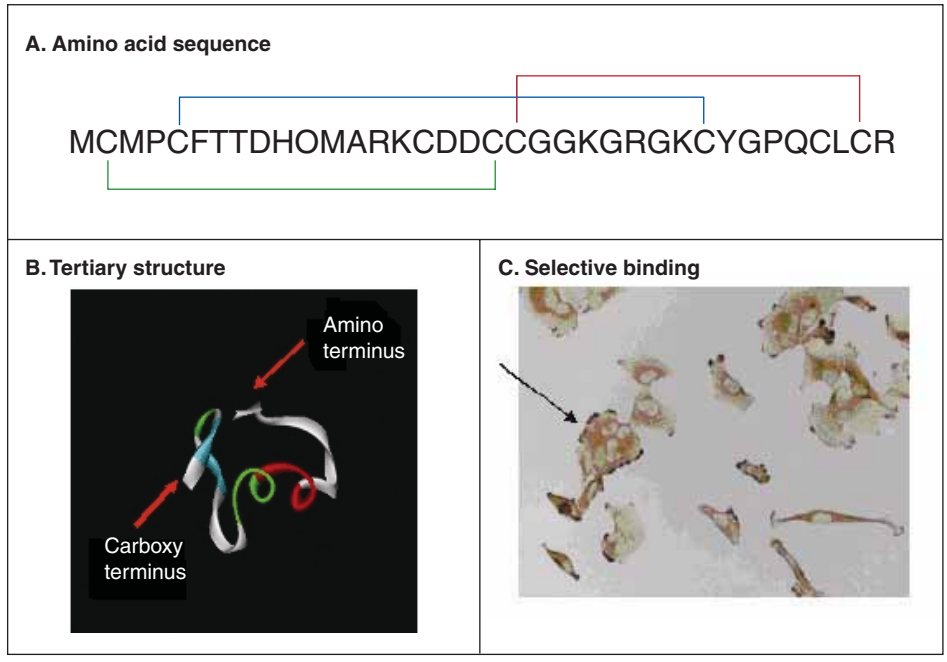

Figure 1. **CTX structure and binding. A**. Amino acid sequence demonstrating four disulfide bridges. **B**. Tertiary structure of CTX. **C**. Cell-surface binding of biotinylated TM-601 to fixed HeLa cervical cancer cells.
CTX: Chlorotoxin.
The arrow in **C.** indicates the specific binding of TM-601 to the cell surface.

Using *in vitro* invasion assays, CTX has been shown to inhibit invasion in a dose-dependent fashion [56]. Taken together, *in vitro* studies suggest that through its inhibitory affect on ion efflux, CTX prevents cell shrinkage, thereby diminishing the ability of glioma cells to migrate through tight extracellular spaces in brain tissue. Furthermore, by decreasing MMP2 activity, CTX prevents proteolytic degradation of extracellular matrix, thus preventing the release of glioma cells from the constraints of cellular interactions with extracellular matrix [57].

5. Preclinical studies

Chlorotoxin has been synthetically produced (TM-601). It can be produced in large quantities for reasonable costs, and the amino acid structure can be modified as needed. Mass spectrometry and HPLC assays indicate that the compound is identical to the naturally occurring compound in shape and structure. The crayfish biological assay shows an identical response with the synthetic compound as with the naturally appearing one. To confirm selective binding of TM-601 to tumors, biotinylated TM-601 has been shown to specifically interact with a large number of tumor biopsies, and TM-601 does not appreciably bind to normal tissues (Table 1, Figure 2). Despite its selective binding properties, TM-601 does not kill glioma cells *in vitro* when given as a single agent. Therefore, TM-601 was initially clinically developed as a targeting agent for delivering a therapeutic payload.

TM-601 was explored preclinically as a candidate for targeting gliomas with ^{131}iodine. As with many other studies that show tumor-specific binding, TM-601 binds selectively to glioblastoma tumors, and only minimal binding to normal brain tissues has been observed (Figure 3). Radiolabelled TM-601 is able to cross blood–brain and tissue barriers (TransMolecular, Inc., internal data), and preclinical studies have also demonstrated the stability and safety of radio-iodinated TM-601. In addition, repeated attempts to raise a humoral immune response against TM-601 in rabbits have not been successful, and no animal toxicities were observed for non-labelled TM-601 doses in excess of those planned for human use.

6. Clinical studies

A Phase I study to evaluate the safety, tolerability, biodistribution and dosimetry of intracavitary ^{131}I-TM-601 in adult patients with recurrent high-grade glioma has been completed [58]. In this trial, the dose of ^{131}iodine was kept fixed at 10 mCi, and the dose of chlorotoxin was escalated from 0.25 mg, to 0.50 mg and 1.0 mg, in three dosing panels containing 6 patients each. ^{131}Iodine was chosen as a therapeutic agent because it had been demonstrated to have efficacy against gliomas and had been utilized in previous, radiolabelled antibody trials. Furthermore, the ^{131}iodine radiopeptide facilitated dosimetry and biodistribution studies.

Table 1. Summary of various human tissues stained with TM-601.

Tissue types	Cases	Results	Tissue type	Cases	Results
Primary brain tumors (glioma)			Other tumors		
Glioblastoma multiforme WHO Grade IV	31	31 positive	Breast cancer	14	13 positive, 1 negative
Anaplastic astrocytoma WHO Grade III	7	7 positive	Breast cancer metastases	11	11 positive
Low-grade astrocytoma WHO Grade II	4	4 positive	Kidney cancer	3	3 positive
Pilocytic astrocytoma WHO Grade I	14	13 positive, 1 negative	Liver cancer	3	3 positive
Other ungraded gliomas	5	4 positive, 1 negative	Lung cancer	3	3 positive
Oligodendroglioma	8	8 positive	Lymphoma	2	2 positive
Gliosarcoma	2	2 positive	Ovarian cancer	3	3 positive
Ganglioglioma	5	5 positive	Pancreatic cancer	3	3 positive
Meningioma	25	20 positive, 5 negative	Prostate cancer	9	8 positive, 1 negative
Ependymona	3	3 positive			
Other normal or diseased brain tissue			***Normal human tissues***		
Alzheimer's brain	8	8 negative	Breast	2	1 negative, 1 positive
Parkinson's/schizophrenic brain	4	4 negative (2 each)	Colon	2	2 negative
Normal brain or uninvolved tissue of brain cancer patients	29	21 negative 8 positive*	Endometrium/ myometrium	3	3 negative
Epilepsy/gliosis/stroke brain	6	6 negative‡	Eyeball (cross-section)	1	1 negative
Neuroectodermal tumors			Heart	2	2 negative
Medulloblastoma	4	4 positive	Kidney	3	3 negative¶
Neuroblastoma	9	8 positive	Adrenal gland	3	3 negative
		1 negative	Liver	2	2 negative
Ganglioneuroma	4	4 positive	Lung	3	3 negative
Melanoma (metastatic)	11	11 positive	Lymph node	3	1 positive, 2 negative
Melanoma (primary)	5	5 positive	Meninges	3	3 negative
Pheochromocytoma	6	5 positive, 1 negative	Muscle (skeletal)	2	2 negative
			Thyroid	1	1 negative
Ewing's sarcoma	2	2 positive	Pancreas	3	1 positive, 2 negative
Primitive neuroectodermal tumors	2	2 negative	Prostate	3	1 positive, 2 negative
Small cell lung carcinoma	6	5 positive, 1 negative	Spleen	2	2 negative
Schwannoma	4	4 positive	Stomach	2	2 negative
Other brain tumors			Ovary	2	2 negative
Epidermoid cysts	5	1 positive, 4 negative	Skin	6	6 negative
Brain tumors of unknown pathology	9	9 positive	Testes	2	2 negative
Pituitary gland of glioblastoma multiforme pt.	2	2 positive			
Metastatic tumors to brain	17	15 positive, 2 negative§			

*Samples from normal brains or from areas of a glioblastoma multiforme patient's brain diagnosed not to be involved in glioblastoma multiforme.

‡Areas of glial cell proliferation show a few cells binding TM-601.

§Metastatic tumors of unknown tissue origin; some may not be related to neuroectodermal tissue.

¶A few positive cells were observed.

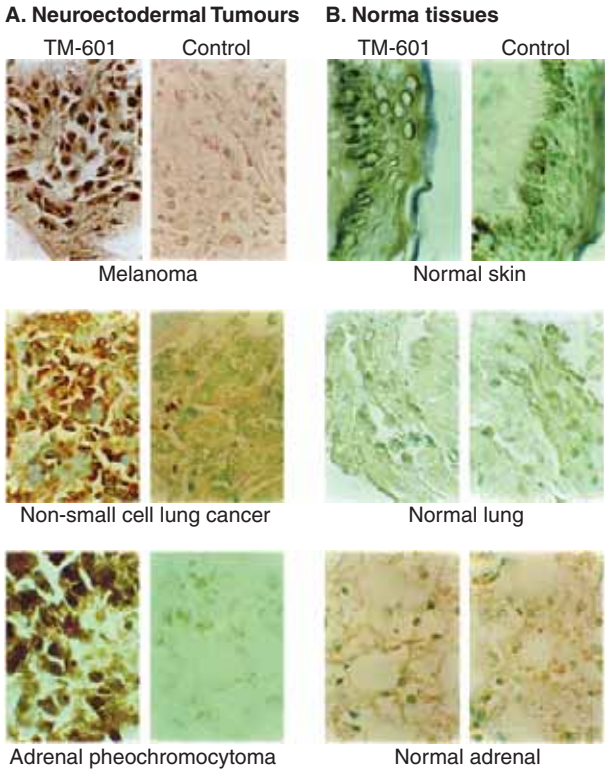

Figure 2. Panel A: representative examples of human tumor tissues histochemically stained with biotinylated TM-601 (Panel A, left column) or buffered saline (Panel A, right column). Panel B: representative examples of human normal tissues matched to human tumor tissues in Panel A histochemically stained with biotinylated TM-601 (Panel B, left column) or buffered saline (Panel B, right column). After primary incubation with biotinylated TM-601 or buffered saline (as a peroxidase reagent staining control), the tissues in Panel A and B were incubated with peroxidase-labelled streptavidin, followed by the peroxidise substrate, to produce a brown color in samples, which bound the biotinylated TM-601. An intense brown color, indicative of positive staining, is only seen in tumor tissues exposed to biotinylated TM-601 (Panel A, left column), and not normal tissues (Panel B, right column).

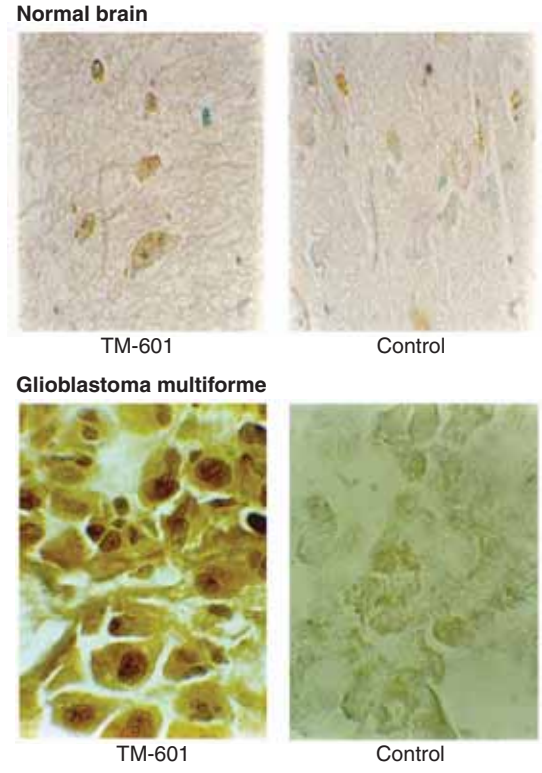

Figure 3. Representative examples of human normal brain and glioblastoma multiforme tumor tissues were histochemically stained with biotinylated TM-601 (left) or buffered saline (right). After primary incubation with biotinylated TM-601 or buffered saline (as a peroxidase reagent staining control), the tissues were incubated with peroxidase-labelled streptavidin followed by the peroxidase substrate to produce brown color in samples, which bound the biotinylated TM-601. Intense brown color, indicative of positive TM-601 staining, is only seen in the tumor tissue.

The compound, ^{131}I-TM-601 was injected into the resection cavity of 18 patients with recurrent, high-grade gliomas via an Ommaya reservoir 2 weeks after surgery. Radiation doses to normal organs were clinically insignificant (Table 2). In contrast, mean radiation dose to within 2 cm of the cavity wall was 81 cGy/mCi (median 49) and ranged 12 – 275 cGy/mCi (Table 2). These values are substantially higher than doses to the whole body (mean 0.4 cGy/mCi) and to any other organ. Furthermore, the biological $t_{½}$ of ^{131}I-TM-601 in the tumor cavity margin was longer than in any other organ, including the normal brain, indicating long-term retention of the drug in and around the injection site (Table 2). The median biological half-life in cavity margin was 70 (range 32 – 193) h, 80 (range 25 – 86) h and 55 (range 41 – 62) h for patients receiving 0.25, 0.50 and 1.0 mg peptide, respectively.

Gamma camera imaging showed the ^{131}I-TM-601 localized to, and remained primarily concentrated in and around, the patient's surgical cavity for all 5 days of imaging (a typical image is shown in Figure 4).

7. Patient follow-up, toxicity and response to therapy

^{131}I-TM-601 was well-tolerated by all patients. There were no grade III or IV toxicities related to the study drug or method of administration in the immediate and/or long-term follow-up period. There were no patient complaints related to the study drug or method of administration.

Table 2. Organ dose and half-life after single injection of 10 mCi ^{131}I-TM-601 in humans.

Organ	cGy/mCi (range)	$T_{biol\ 1/2}$ h (range)
Whole body	0.4 (0.2 – 0.7)	47 (31 – 81)
Stomach	0.7 (0.2 – 1.3)	25 (17 – 40)
Kidney	0.9 (0.3 – 1.8)	26 (18 – 39)
Thyroid	5.3 (0.8 – 22.9)	NA
Normal brain	1.1 (0.3 – 2.4)	2 (21 – 80)
Marrow (blood half-life)	0.3 (0.1 – 0.4)	29 (9 – 41)
Bladder wall	3.2 (1.5 – 6.5)	NA
Tumor cavity wall	81.0 (12 – 275)	69 (25 – 193)

NA: Not available

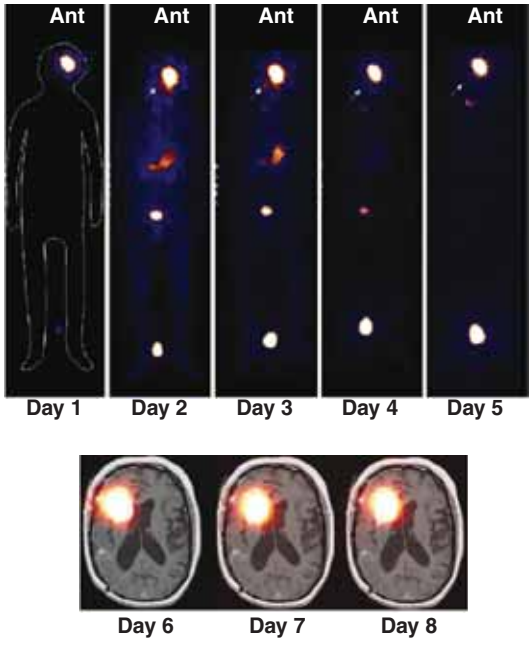

Figure 4. Total body planar and brain SPECT imaging of ^{131}I-TM-601 following a single intracavitary 10 mCi dose. The bright region noted between the legs in the total body planar images is a 1 mCi standard. Retention of the peptide at the tumor cavity site (left side of head) is evident on days 1 – 5, with minimal uptake anywhere else in the body. The loss of a signal in the images roughly approximated the physical decay constant of ^{131}I, indicating long-term retention and minimal biological elimination at the tumor site, with rapid elimination elsewhere. Axial SPECT scans co-registered to MRI indicate long-term retention at the tumor cavity site, with a volume of distribution that approximates well to the tumor volume.

Three patients had serious adverse events possibly or probably related to study medication reported within 22 days of administration. These included the following events: fever, chills, upper extremity paresthesias accompanied by mild cerebral edema on CT, progression in left-sided weakness, infection of the tumor resection cavity and osteomyelitis. Additional serious adverse events reported by investigators to have possible attribution to the study agent beyond the initial 22-day observation period included one patient with generalized seizure and one patient with headache, face droopiness, dysathria and instability.

Over the course of the 180-day observation period, there were a total of seven deaths. Two patients with GBM survived for > 30 months. Median survival was 27 weeks from surgery for all three dosing groups. Histochemistry of the tumor tissue from all patients tested so far stained intensely positive for TM-601.

An analysis of the imaging data obtained for a subset of the patients enrolled in this trial was also performed [59]. The purpose of this analysis was to determine if radiolabelled CTX might be a useful tool for imaging the extent of glioma invasion, as it is tumor-specific and small enough to diffuse away from the primary tumor site. This study demonstrated that ^{131}I-TM-601 SPECT scan estimates of tumor volume were midway between estimates on T2-weighted and T1-weighted contrast-enhanced MRI scan, a result consistent with known glioma invasion patterns. This study provides promise that CTX may be useful for tumor imaging and tumor therapy.

A Phase II trial of this agent is underway for patients with recurrent glioma. The goals of this trial are to determine the safety of multiple dose administrations, at 40 mCi/0.8 mg per dose, and to test the potential efficacy of this agent. The first portion of the study (now completed) consisted of a dose escalation schema in which individual patients each received three doses of ^{131}I-TM-601, each dose 1 week apart. The specific activity of the radiolabelled peptide was fixed, with the dose varying between 20 mCi/0.4 mg and 40 mCi/0.8 mg. There were no major toxicities observed, and the FDA permitted the trial to advance to the next stage of the trial, in which patients are randomized to receive either 3 or 6 weekly intracavitary injections of ^{131}I-TM-601 40 mCi/0.8 mg. This trial is being conducted at 17 centers in the US and is expected to complete accrual in the first half of 2007. As with the initial phase of the trial, no deaths

or major toxicities directly attributable to the therapy have been reported, and the trial is ongoing.

An intravenous imaging trial is also underway. Patients with documented progressive cancer of any origin (CNS or systemic) are included, with the primary end point being the ability to visualize specific uptake of the agent on total body imaging. If so, this would suggest that ^{131}I-TM-601 or variants could be used as both a cancer imaging agent and as a systemic targeted therapy. The trial is being conducted at four sites. So far, uptake in regions of metastatic disease has been documented, but as formal dosimetry analyses have not been completed, the data are still preliminary. As patients with brain metastases enter the trial in the future, it will be of great interest to determine if systemically delivered ^{131}I-TM-601 crosses the BBB sufficiently for potentially diagnostic or therapeutic responses.

8. Expert opinion

Targeted cancer therapeutics is a large and ever-expanding field. New molecular targets and tumor-specific ligands are being identified at a very fast pace. Despite these advances, the chances of any single target or ligand making its way into clinical testing is small, with an even smaller chance of impacting therapy. Issues ranging from the inability to commercially produce a targeting agent that meets good manufacturing practices, to intolerable toxicities, and lack of efficacy, all contribute to this issue. In our opinion, CTX represents a novel and exciting platform for cancer therapy and imaging. CTX meets many of the criteria of an ideal targeting agent (Table 3). It is small and highly compact in structure. This means it can cross the BBB and is far more likely to diffuse deep into solid tumors than antibodies, mini-bodies, or other such larger molecule targeting agents. CTX is synthetically manufactured under good manufacturing practices and can be easily modified by amino acid substitution and pre- or postfolding modification. It contains a single tyrosine residue for iodination and can also be modified by covalent linkage for the conjugation of other imaging or therapy payloads. Because it is a naturally occurring, biological peptide that was evolutionarily derived from an invertebrate with no human biology design, it fortuitously demonstrates binding to human malignancies, but is otherwise alien to human tissues. It therefore appears to have no innate toxicity in humans, despite repeated administrations. The underlying interaction of CTX with tumor cells remains to be fully elucidated, and is the subject of much research. CTX may be shown to have antitumoral activity, even in the absence of a therapeutic payload. Several *in vitro* studies suggest that CTX may inhibit cellular migration by blocking ion fluxes, altering cell shape and preventing degradation of extracellular matrix [57]. However, the *in vivo* effect of unlabelled CTX on tumor growth and metastases has not been thoroughly explored. Finally, the ability of CTX to selectively bind to tumor cells may be exploited if the peptide is found to have autofluorescent properties, making it a useful agent for intra-operative tumor identification. Overall, the small size, lack of antigenicity, and lack of toxicity, make CTX an extremely attractive peptide for ongoing development in the field of cancer therapy and imaging.

All clinical trials of CTX have utilized radioactive iodine as the therapeutic payload. ^{131}Iodine was chosen as the initial agent for several practical reasons, including its well-known toxicities, previous use in glioma therapy, and the ability to perform dosimetry and biodistribution studies. However, a radiolabelled compound raises obvious concerns based on the disappointing results of other agents, such as the antitenascin antibody 81C6. There are several critical factors that differentiate CTX in this regard. First, recent data suggest that CTX is internalized by tumor cells, with no observable binding to blood vessels, or other cell types. This means that the radioactivity will be delivered more closely to the source. Second, published studies and ongoing imaging trials indicate that CTX is small enough to diffuse into solid tumors and into the brain parenchyma with intracavitary or intravenous injections. This means that CTX penetrates beyond the tumor resection cavity and truly provides targeted therapy, rather than the brachytherapy-like effect seen with non-diffusible molecules such as radiolabelled 81C6. Finally, methods such as CED are likely to be extremely effective for a small molecule such as CTX, allowing even more extensive dissemination into the brain parenchyma for targeting of invading cells. This could result in more selective delivery of radiation than that available with other tested methods. Large antibodies such as 81C6 are very poorly delivered by CED.

Taken together, these results suggest that CTX has many advantages over other radioactive, targeted therapies for glioma. Nonetheless, ^{131}iodine does deliver a radiation dose that extends beyond the boundaries of an individual cell, resulting in radiation exposure to normal tissue, and this may be a limiting factor in the utility of this approach. Alternate strategies such as the use of more focal radiation sources, for example ^{90}yttrium, remain viable options. In addition, the utilization of other target toxins, such as variants of the *Psuedomonas* or diptheria toxin, may provide even better therapeutic benefit. These benefits must be counterbalanced by the fact that the toxins are large molecules, which could greatly limit drug delivery. These strategies are being considered carefully.

An additional point to consider is the potential for intravenous therapy with ^{131}I-CTX. As CTX is small, rapidly cleared and does not bind normal cells, it is unlikely to have an impact on healthy tissues, as there is not sufficiently prolonged exposure of these cells to the radioactivity to result in a major effect, unless extremely high doses are administered. The Phase I trial data, and preliminary data from the Phase II trial have not identified radiation necrosis as an important finding in any patients, with over 90% of the peptide entirely cleared from the body within

Table 3. Advantages and disadvantages of chlorotoxin as a targeted therapy of glioma and other tumors.

Advantages	Disadvantages	Other issues
Small size – diffusible – crosses the BBB – lack of immunogenicity	Does not kill tumor alone – needs payload therapy or co-administration of other therapy – mechanism of action unclear	The only peptide under clinical trial for glioma May be effective against many other malignancies
Synthetic manufacturing – easily modified – can add linkers and payloads more easily	Clears very rapidly from systemic circulation – May impede use as intravenous agent – May require infusion or repeated administration	Basic science needs to better define peptide function Multiple platforms or options for clinical use, difficult to define the best strategy
No binding to normal human tissues – no obvious toxicities – tumor-specific action		
Useful for imaging and therapy applications		

BBB: Blood-brain barrier.

24 – 36 h. These data certainly support the notion that systemic or CED delivered therapy is unlikely to cause significant damage to healthy tissue.

In general, targeted therapies for glioma have had a lackluster and uncertain history. A recent trial of *Pseudomonas* exotoxin fused to IL-13 and delivered with CED to patients with recurrent brain tumors demonstrated tremendous promise [38], but the clinical realities of the trial demonstrated the compound to be very toxic in high doses and difficult to reliably deliver to the tumor volume to achieve meaningful doses. In addition, radiolabelled antibodies have been quite disappointing in CNS tumors and most other solid tumors due to poor penetration of the antibodies. In contrast, targeted antibodies such as rituxan have been effective for hematological malignancies. The field will undoubtedly continue to evolve, with more tumor targets identified. Small peptide- and protein-based targeting seems to be a much more appealing approach than antibody-based therapies, as there are fewer immune reactions, and probably less toxicity.

7.1 Conclusion

CTX represents a novel and versatile advance in the field of targeted therapeutics for glioma and other malignancies, in that it is a peptide-based targeting agent, rather than an engineered antibody or antibody fragment. The small size of the peptide makes crossing the BBB possible, and it is likely that it is highly diffusible. Due to the fact that its evolutionary development has little link to human biology, it does not appear to bind to normal human tissues, thereby dramatically reducing the possibility for toxicities, and so far no immunogenic responses have been noted. On a more cautionary note, CTX does not directly kill tumor cells, which means that if it is to be effective, a meaningful payload therapy will be critical. The identification and testing of CTX further enhances the search for similar toxins in nature that may play equally intriguing roles in the treatment of human diseases. This is likely to provide a promising pathway for new drug discovery over the coming decades.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. CBTRUS: (2000) *CBTRUS. Statistical Report: Primary Brain Tumors in the United States. (*1992-1997).
2. OHGAKI H, KLEIHUES P: Epidemiology and etiology of gliomas. *Acta Neuropathol. (Berl.)* (2005) 109(1):93-108.
3. LAWS ER, PARNEY IF, HUANG W *et al.*: Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the Glioma Outcomes Project. *J. Neurosurg.* (2003) 99(3):467-473.
4. JOHANNESEN TB, LANGMARK F, LOTE K: Progress in long-term survival in adult patients with supratentorial low-grade gliomas: a population-based study of 993 patients in whom tumors were diagnosed between 1970 and 1993. *J. Neurosurg.* (2003) 99(5):854-862.
5. JEREMIC B, MILICIC B, GRUJICIC D *et al.*: Clinical prognostic factors in patients with malignant glioma treated with combined modality approach. *Am. J. Clin. Oncol.* (2004) 27(2):195-204.
6. BUCKNER JC: Factors influencing survival in high-grade gliomas. *Semin. Oncol.* (2003) 30(6 Suppl. 19):10-14.
7. KELLY PJ, DAUMAS-DUPORT C, KISPERT DB *et al.*: Imaging-based stereotaxic serial biopsies in untreated intracranial glial neoplasms. *J. Neurosurg.* (1987) 66(6):865-874.
8. GRIEB P: [5-Aminolevulinic acid (ALA) and its applications in neurosurgery]. *Neurol. Neurochir. Pol.* (2004) 38(3):201-207.
9. STUMMER W, NOVOTNY A, STEPP H *et al.*: Fluorescence-guided resection of glioblastoma multiforme by using

5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. *J. Neurosurg.* (2000) 93(6):1003-1013.

10. GORBACH AM, HEISS JD, KOPYLEV L, OLDFIELD EH: Intraoperative infrared imaging of brain tumors. *J. Neurosurg.* (2004) 101(6):960-969.
11. WITWER BP, MOFTAKHAR R, HASAN KM *et al.*: Diffusion-tensor imaging of white matter tracts in patients with cerebral neoplasm. *J. Neurosurg.* (2002) 97(3):568-575.
12. SAWAYA R: Extent of resection in malignant gliomas: a critical summary. *J. Neurooncol.* (1999) 42(3):303-305.
13. BUCCI MK, MAITY A, JANSS AJ *et al.*: Near complete surgical resection predicts a favorable outcome in pediatric patients with nonbrainstem, malignant gliomas: results from a single center in the magnetic resonance imaging era. *Cancer* (2004) 101(4):817-824.
14. FISCHBACH AJ, MARTZ KL, NELSON JS *et al.*: Long-term survival in treated anaplastic astrocytomas. A report of combined RTOG/ECOG studies. *Am. J. Clin. Oncol.* (1991) 14(5):365-370.
15. SHORT SC: External beam and conformal radiotherapy in the management of gliomas. *Acta Neurochir. Suppl.* (2003) 88:37-43.
16. GROSSMAN SA, BATARA JF: Current management of glioblastoma multiforme. *Semin. Oncol.* (2004) 31(5):635-644.
17. SHAPIRO WR, SHAPIRO JR: Biology and treatment of malignant glioma. *Oncology (Huntingt.)* (1998) 12(2):233-240; discussion 240, 246.
18. STUPP R, MASON WP, VAN DEN BENT MJ *et al.*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* (2005) 352(10):987-996.

• Recent paper that defines current standard of care for high grade gliomas and prognosis. Empahsizes need for better treatments.

19. BREM H, PIANTADOSI S, BURGER PC *et al.*: Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. *Lancet* (1995) 345(8956):1008-1012.
20. BREM H, GABIKIAN P: Biodegradable polymer implants to treat brain tumors. *J. Control. Release* (2001) 74(1-3):63-67.
21. WESTPHAL M, LAMSZUS K, HILT D: Intracavitary chemotherapy for glioblastoma: present status and future directions. *Acta Neurochir. Suppl.* (2003) 88:61-67.
22. GURURANGAN S, COKGOR L, RICH JN *et al.*: Phase I study of Gliadel wafers plus temozolomide in adults with recurrent supratentorial high-grade gliomas. *Neuro-oncol.* (2001) 3(4):246-250.
23. GARFIELD J, DAYAN AD, WELLER RO: Postoperative intracavitary chemotherapy of malignant supratentorial astrocytomas using BCNU. *Clin. Oncol.* (1975) 1(3):213-222.
24. KLATZMANN D, VALERY CA, BENSIMON G *et al.*: A phase I/II study of herpes simplex virus type 1 thymidine kinase "suicide" gene therapy for recurrent glioblastoma. Study Group on Gene Therapy for Glioblastoma. *Hum. Gene Ther.* (1998) 9(17):2595-2604.
25. RAINOV NG: A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum. Gene Ther.* (2000) 11(17):2389-2401.
26. SHAND N, WEBER F, MARIANI L *et al.*: A phase 1-2 clinical trial of gene therapy for recurrent glioblastoma multiforme by tumor transduction with the herpes simplex thymidine kinase gene followed by ganciclovir. GLI328 European-Canadian Study Group. *Hum. Gene Ther.* (1999) 10(14):2325-2335.
27. TRASK TW, TRASK RP, AGUILAR-CORDOVA E *et al.*: Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. *Mol. Ther.* (2000) 1(2):195-203.
28. HASSENBUSCH SJ, NARDONE EM, LEVIN VA, LEEDS N, PIETRONIGRO D: Stereotactic injection of DTI-015 into recurrent malignant gliomas: phase I/II trial. *Neoplasia* (2003) 5(1):9-16.
29. NEUWELT EA, DAHLBORG SA: Chemotherapy administered in conjunction with osmotic blood-brain barrier modification in patients with brain metastases. *J. Neurooncol.* (1987) 4(3):195-207.
30. BLACK KL, CLOUGHESY T, HUANG SC *et al.*: Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 ethylenediamine tetraacetic acid into human gliomas. *J. Neurosurg.* (1997) 86(4):603-609.
31. TFAYLI A, HENTSCHEL P, MADAJEWICZ S *et al.*: Toxicities related to intraarterial infusion of cisplatin and etoposide in patients with brain tumors. *J. Neurooncol.* (1999) 42(1):73-77.
32. MADAJEWICZ S, CHOWHAN N, ILIYA A *et al.*: Intracarotid chemotherapy with etoposide and cisplatin for malignant brain tumors. *Cancer* (1991) 67(11):2844-2849.
33. FEUN LG, LEE YY, YUNG WK, SAVARAJ N, WALLACE S: Intracarotid VP-16 in malignant brain tumors. *J. Neurooncol.* (1987) 4(4):397-401.
34. GREENBERG HS, ENSMINGER WD, CHANDLER WF *et al.*: Intra-arterial BCNU chemotherapy for treatment of malignant gliomas of the central nervous system. *J. Neurosurg.* (1984) 61(3):423-429.
35. LIEBERMAN DM, LASKE DW, MORRISON PF, BANKIEWICZ KS, OLDFIELD EH: Convection-enhanced distribution of large molecules in gray matter during interstitial drug infusion. *J. Neurosurg.* (1995) 82(6):1021-1029.
36. GILLIES GT, SMITH JH, HUMPHREY JA, BROADDUS WC: Positive pressure infusion of therapeutic agents into brain tissues: mathematical and experimental simulations. *Technol. Health Care* (2005) 13(4):235-243.
37. HADACZEK P, KOHUTNICKA M, KRAUZE MT *et al.*: Convection-enhanced delivery of adeno-associated virus type 2(AAV2) into the striatum and transport of AAV2 within monkey brain. *Hum. Gene Ther.* (2006) 17(3):291-302.
38. KUNWAR S: Convection enhanced delivery of IL13-PE38QQR for treatment of recurrent malignant glioma: presentation of interim findings from ongoing phase 1 studies. *Acta Neurochir. Suppl.* (2003) 88:105-111.

•• Initial report of CED targeted toxin therapy for glioma.

39. LESNIAK MS, BREM H: Targeted therapy for brain tumours. *Nat. Rev. Drug Discov.* (2004) 3(6):499-508.

40. LIDAR Z, MARDOR Y, JONAS T *et al.*: Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study. *J. Neurosurg.* (2004) 100(3):472-479.

41. MURO K, DAS S, RAIZER JJ: Convection-enhanced and local delivery of targeted cytotoxins in the treatment of malignant gliomas. *Technol. Cancer Res. Treat.* (2006) 5(3):201-213.

42. LASKE DW, ROSSI P: Phase II multicenter trial of intratumoral/interstitial therapy with TransMID in patients with refractory and progressive glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA). *Neuro-oncol.* (2002) 4(Suppl. 2):558.

43. RUSTAMZADEH E, HALL WA, TODHUNTER DA *et al.*: Intracranial therapy of glioblastoma with the fusion protein DTAT in immunodeficient mice. *Int. J. Cancer* (2007) 120(2):411-419.

44. KUAN CT, WIKSTRAND CJ, BIGNER DD: EGFRvIII as a promising target for antibody-based brain tumor therapy. *Brain Tumor Pathol.* (2000) 17(2):71-78.

45. RAVIC M: Intracavitary treatment of malignant gliomas: radioimmunotherapy targeting fibronectin. *Acta Neurochir. Suppl.* (2003) 88:77-82.

46. REARDON DA, AKABANI G, COLEMAN RE *et al.*: Phase II trial of murine $^{(131)}$I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J. Clin. Oncol.* (2002) 20(5):1389-1397.

47. AKABANI G, REIST CJ, COKGOR I *et al.*: Dosimetry of ^{131}I-labeled 81C6 monoclonal antibody administered into surgically created resection cavities in patients with malignant brain tumors. *J. Nucl. Med.* (1999) 40(4):631-638.

48. HOPKINS K, CHANDLER C, EATOUGH J, MOSS T, KEMSHEAD JT: Direct injection of 90Y MoAbs into glioma tumor resection cavities leads to limited diffusion of the radioimmunoconjugates into normal brain parenchyma: a model to estimate absorbed radiation dose. *Int. J. Radiat. Oncol. Biol. Phys.* (1998) 40(4):835-844.

49. RIVA P, FRANCESCHI G, FRATTARELLI M *et al.*: Loco-regional radioimmunotherapy of high-grade malignant gliomas using specific monoclonal antibodies labeled with 90Y: a phase I study. *Clin. Cancer Res.* (1999) 5(10 Suppl.):3275s-3280s.

50. ULLRICH N, BORDEY A, GILLESPIE GY, SONTHEIMER H: Expression of voltage-activated chloride currents in acute slices of human gliomas. *Neuroscience* (1998) 83(4):1161-1173.

51. SOROCEANU L, GILLESPIE Y, KHAZAELI MB, SONTHEIMER H: Use of chlorotoxin for targeting of primary brain tumors. *Cancer Res.* (1998) 58(21):4871-4879.

52. ULLRICH N, GILLESPIE GY, SONTHEIMER H: Human astrocytoma cells express a unique chloride current. *Neuroreport* (1996) 7(5):1020-1024.

53. ULLRICH N, SONTHEIMER H: Biophysical and pharmacological characterization of chloride currents in human astrocytoma cells. *Am. J. Physiol.* (1996) 270(5 Pt 1):C1511-C1521.

54. LYONS SA, O'NEAL J, SONTHEIMER H: Chlorotoxin, a scorpion-derived peptide, specifically binds to gliomas and tumors of neuroectodermal origin. *Glia* (2002) 39(2):162-173.

• Important analysis of binding characteristics of CTX for tumor therapy.

55. SOROCEANU L, MANNING TJ, Jr., SONTHEIMER H: Modulation of glioma cell migration and invasion using Cl(-) and K(+) ion channel blockers. *J. Neurosci.* (1999) 19(14):5942-5954.

• This report provides critical basic data on binding characteristics of CTX.

56. DESHANE J, GARNER CC, SONTHEIMER H: Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. *J. Biol. Chem.* (2003) 278(6):4135-4144.

57. MCFERRIN MB, SONTHEIMER H: A role for ion channels in glioma cell invasion. *Neuron Glia Biol.* (2006) 2(1):39-49.

58. MAMELAK AN, MORGAN R, RAUBITSCHEK A *et al.*: Phase I/II trial of intracavitary ^{131}I-TM-601 in adult patients with recurrent high grade glioma. *Neuro-oncol.* (2003) Abstr. RA-19.

•• Phase I clinical trial of CTX in humans, describing most complete experience so far.

59. HOCKADAY DC, SHEN S, FIVEASH J *et al.*: Imaging glioma extent with ^{131}I-TM-601. *J. Nucl. Med.* (2005) 46(4):580-586.

•• Evaluation of clinical imaging characteristics of radiolabelled CTX.

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