TARGETED THERAPY FOR BRAIN TUMOURS

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Although previously considered untreatable, brain tumours no longer carry the same prognosis as they did even a decade ago. Recent advances in drug delivery to the central nervous system have not only bypassed physiological constraints such as the blood–brain barrier, but have, in fact, changed the course of treatment for patients with malignant brain tumours. The creation of targeted therapies, which spare normal tissue and destroy tumour cells, is changing the field of neuro-oncology. In this article, we review recent developments in the delivery of drugs to tumours of the central nervous system, discuss current trends and directions in the development of novel drugs and delivery systems, and present new and cutting-edge strategies for overcoming the challenges ahead.

SURGICAL DEBULKING
A surgical procedure in which part of the tumour is removed, as opposed to the entire tumour.

BLOOD–BRAIN BARRIER
A state of physiological, metabolic and biochemical processes that distinguish the cerebral capillary endothelium from the endothelium of systemic organ systems. It is formed by tight junctions of cerebral capillary endothelial cells.

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doi:10.1038/nrd1414

Brain tumours represent a heterogeneous group of central nervous system (CNS) neoplasms. The World Health Organization (WHO) recognizes approximately 100 different types of brain tumours classified according to pathological diagnosis. In general, however, these tumours can be classified into either primary or secondary tumours, depending on whether they originate in the brain or simply spread to the central nervous system from elsewhere. Approximately half of all primary brain tumours are glial-cell neoplasms, and more than three quarters of all glial tumours are astrocytomas. Astrocytomas differ in their pathological and clinical behaviour: some astrocytomas are classified as low-grade tumours, meaning they are slow growing, whereas others, such as glioblastoma multiforme (GBM), represent the most aggressive type of tumour known to occur within the CNS (FIG. 1).

The natural history of patients with GBMs has intensified research in the area of drug discovery and drug delivery to the CNS. Conventional therapy for glioblastomas consists primarily of SURGICAL DEBULKING followed by radiation therapy. The median survival after surgical intervention alone is six months and the addition of radiation therapy extends the median survival to nine months[1,2]. In this setting, controlled and local delivery of chemotherapeutic agents via polymers has significantly improved the survival of patients with malignant brain tumours. The use of polymers for local drug delivery further expands the spectrum of chemotherapeutic drugs available for the treatment of neoplasms in the CNS and facilitates the use of novel biological approaches, such as gene therapy, for the treatment of gliomas.

Barriers to CNS drug delivery

Therapy of brain tumours has been limited by a lack of effective methods of drug delivery. Most conventional methods for delivering drug molecules, such as oral ingestion or intravenous injection, fail to achieve therapeutic concentrations of drugs within intracranial tumours, despite reaching toxic systemic levels. The presence of the BLOOD–BRAIN BARRIER (BBB), the blood–cerebrospinal fluid barrier and the blood–tumour barrier strategically prevent the effective therapy of brain tumours.

The tight junctions of the cerebral capillary endothelium, which form the anatomic basis of the BBB, effectively restrict the influx of molecules from the bloodstream into the brain. As a result, cerebral capillaries are much less permeable to ions and small molecules and virtually impermeable to peptides and macromolecules. Moreover, unlike systemic endothelium, cerebral endothelial cells have a marked deficiency of pinocytic molecules. The transport of

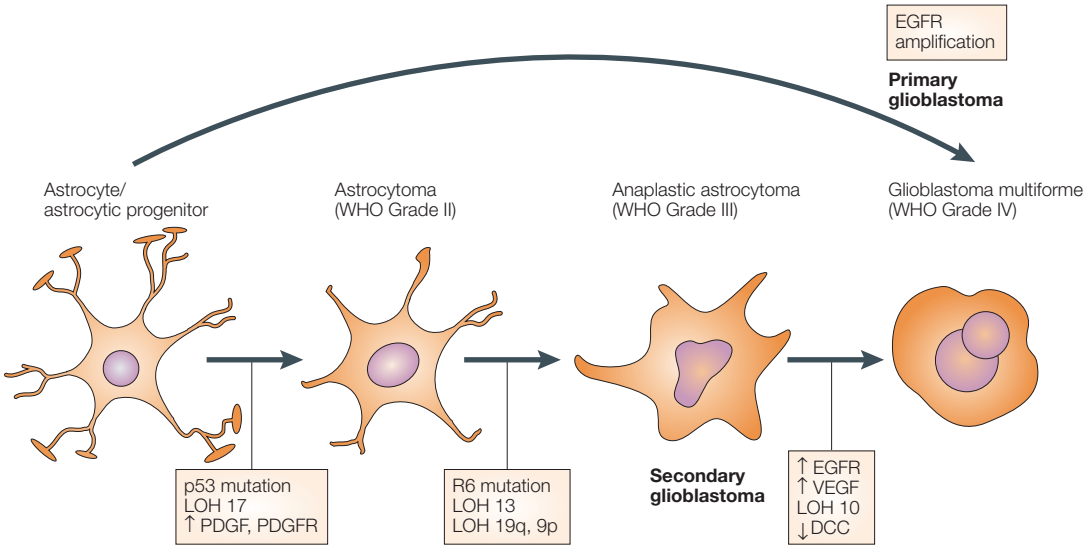


Figure 1 | **Development and progression of astrocytic brain tumours.** Malignant brain tumours can arise in one of two ways. On the one hand, astrocytes undergo genetic changes accompanied by upregulation of certain receptors, such as the platelet-derived growth factor (PDGF), endothelial growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF). These progressive changes culminate in the formation of a glioblastoma. On the other hand, most primary glioblastomas arise de novo, without the need for gradual progression from an astrocytoma to a high-grade astrocytoma to a glioblastoma multiforme.

molecules, which depends on cellular TRANSCYTOSIS, is therefore severely compromised, further contributing to the selectivity of the BBB (FIG. 2).

Two other features of the BBB affect the capacity of a drug to penetrate the capillary endothelium. First, the tightly fused junctions of the cerebral endothelium essentially form a continuous lipid layer. Consequently, only small, electrically neutral, lipid-soluble molecules can penetrate the BBB, and most chemotherapeutic agents do not fall in this category. Second, the identification of ATP-binding cassette C1 (ABCC1, also known as multiple organic anion transporter) and ABCB1 (also known as P-glycoprotein), an active drug-efflux transporter protein, on the luminal membrane of the cerebral endothelium, significantly affects the transport of molecules across the BBB. This efflux transporter actively removes a broad range of drug molecules before they can cross into the brain PARENCHYMA, effectively excluding drugs from entering the CNS.

In addition to the BBB, the blood–cerebrospinal fluid barrier (BCB) forms the second line of defence against drug delivery to the CNS. This barrier is formed by the tightly bound choroid epithelial cells, which are responsible for the production of cerebrospinal fluid (CSF) within the ventricles. Because the BCB closely regulates the exchange of molecules between the blood and CSF, it can control the penetration of molecules within the interstitial fluid of the brain parenchyma. Furthermore, because the BCB is fortified by an active organic-acid transport system, it can actively remove from the CSF a number of agents, such as methotrexate, and therefore actively prevent the diffusion of chemotherapeutic agents directly into the brain parenchyma.

Last, the microvasculature of the brain tumour contributes to the underlying problems associated with drug delivery to the CNS by essentially forming a third barrier, the blood–tumour barrier (BTB). Tumour microvessels tend to be abnormal, and are frequently dilated and tortuous. It is not uncommon to see features such as ARTERIOVENOUS SHUNTS or VENOUS ANASTOMOSES, both of which affect the pattern of blood flow and interstitial pressure. In general, as the leakiness of the tumour vasculature increases, the intratumoural interstitial pressure increases. This high pressure results in a net flow of fluid from the centre to the periphery of the tumour and the surrounding tissue, known as peritumoural oedema. In this setting, most tumour microvessels are partially or completely collapsed, a feature that further limits flow and tissue penetration of pharmacological agents from the bloodstream to the tumour parenchyma[3].

Together, the BBB, the BCB and the BTB work to maintain the homeostasis of the CNS. In so doing, however, they effectively prevent the systemic delivery of agents which might otherwise be beneficial in the treatment of malignant brain tumours.

Delivery systems for brain tumour therapy

The limitations presented by the natural barriers of the CNS have led to the development of novel methods of drug delivery to tumours of the CNS. At present, five potential approaches exist for achieving high intratumoural drug concentrations without the associated systemic toxicities: enhancing drug permeability through the BBB; temporary disruption of the BBB; the interstitial deliver of drugs via catheters; convection-enhanced delivery of drugs to the CNS; and the use of polymers or microchips to directly deliver medical therapy.

TRANSCYTOSIS
The transport of material across an epithelium by uptake on one face into a coated vesicle, which then can be transported to the opposite face in another vesicle.

PARENCHYMA
Tissue that constitutes the essential part of an organ, as contrasted with connective tissue and blood vessels.

ARTERIOVENOUS SHUNTS
A passage by which blood is directly diverted from the arterial side to the venous side.

VENOUS ANASTOMOSES
Communication between two or more veins forming a network of vessels.

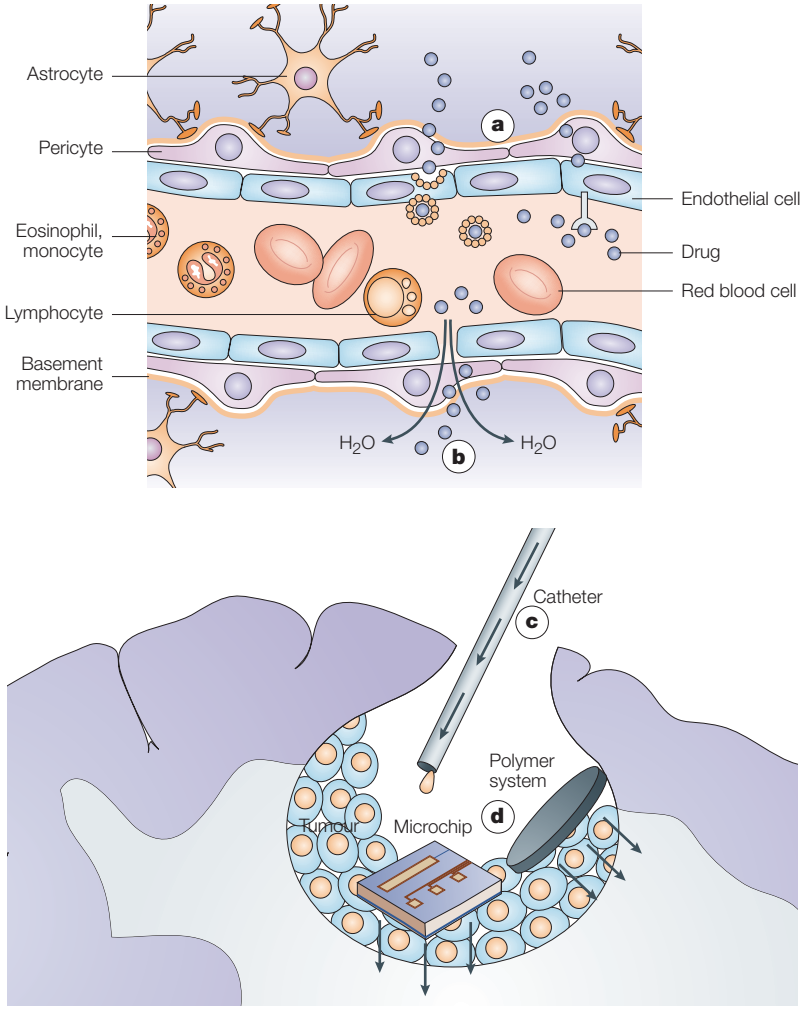


Figure 2 | **The blood–brain barrier.** The blood–brain barrier (BBB) is composed of tight junctions of cerebral capillary endothelium. These tight junctions do not permit the exchange of ions or molecules between systemic circulation and the central nervous system. As a result, efforts aimed at improving drug delivery to the central nervous system focus on enhancing drug permeability through the BBB (**a**); temporary disruption of the BBB (**b**); interstitial delivery of drugs via catheters (**c**); and the use of polymers or microchips (**d**). Modified from original by Ian Suk.

The first of these methods involves chemical and/or structural alteration of a drug. Most chemotherapeutic agents are large, positively charged, hydrophilic molecules, and are therefore effectively excluded from the brain parenchyma. Lomustine (CCNU) and semustine (methyl-CCNU) are two more lipophilic variants of carmustine (BCNU), an agent previously shown to be of some benefit against malignant brain tumours (FIG. 3). Unfortunately, clinical trials have not shown improved efficacy of these drugs over BCNU[4]. It would now seem that increased lipophilicity of BCNU is indirectly proportional to the efficacy of alkylating agents. In other words, by making the drug more BBB-traversable, one also makes it less efficacious. Furthermore, when administered intravenously, lipophilic agents readily bind to plasma proteins, leading to lower concentrations within the CNS. The experience with lomustine and semustine has therefore hampered the development of structurally altered pharmacological agents.

LIPOSOMES
Synthetic, uniform, bilayer lipid membrane vesicles formed by emulsification of cell membranes in dilute salt solutions. Liposomes are being developed as an approach for drug delivery in which toxic drugs are 'wrapped' inside a liposome and tagged with an organ-specific antibody.

An alternative approach to changing the lipophilicity of the drug involves encapsulating it in a sphere of lipids. Such LIPOSOMES can then theoretically carry the drug across the cerebral endothelium and deliver it to the brain parenchyma. Recent work in this area using doxorubicin (FIG. 3) has indeed shown that encapsulation increases the delivery of the drug in experimental brain tumours[5,6]. Furthermore, in a recent Phase I/II clinical trial involving brain-tumour patients, liposomal doxorubicin was found to stabilize the disease and contribute to prolonged survival[7,8]. Although it might be premature to speculate on the future potential of liposomal doxorubicin, it clearly represents an attractive and potentially promising approach that obviates the need to pharmacologically manipulate the drug. Furthermore, liposomal drug delivery has opened a potentially new and powerful method for the delivery of genes, the details of which will be discussed under biologic therapy.

Carrier- and receptor-mediated transport of drugs across the BBB deserves further attention. The attraction of this approach is obvious: by linking a drug to a carrier and/or a receptor capable of traversing the BBB, one can achieve therapeutic concentrations within a tumour. This is particularly true in the case of nanoparticles. Drugs that have successfully been transported into the brain using this carrier include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the *N*-methyl-D-aspartate receptor antagonist MRZ 2/576 and doxorubicin[9–14] (FIG. 3). In experimental models, intravenously injected doxorubicin-loaded polysorbate-80-coated nanoparticles led to a 40% cure in rats with intracranially transplanted glioblastomas. It would seem that nanoparticles mimic low-density-lipoprotein (LDL) particles and interact with the LDL receptor, which leads to their uptake by the endothelial cells. After this uptake, the drug can be released in these cells and diffuse into the brain interior, or the particles can be transcytosed. Although this methodology has demonstrated promise in the laboratory models, the evaluation of clinical efficacy in neurological patients is awaited with interest.

The second approach to enhancing the delivery of drugs to the CNS involves direct disruption of the BBB. Certain hyperosmolar agents, such as mannitol, draw water out of endothelial cells, thereby shrinking them and opening the gaps between cerebral endothelial cells. Other compounds, such as the bradykinin agonist RMP-7, directly disrupt the BBB. In both cases, experimental studies have clearly shown an increased penetration of drug into the brain parenchyma[15–18]. However, this has not translated into clinical efficacy. For instance, although intravenous etoposide has a low level of activity in the treatment of recurrent low-grade astrocytomas, the efficacy of this agent was not enhanced by the coincident intravenous administration of mannitol[19]. Similarly, in a recent randomized, double-blind, controlled Phase II trial, the use of RMP-7 did not improve the efficacy of carboplatin in patients with recurrent malignant glioma[20]. These studies point to an important pitfall: whereas the osmotic BBB disruption

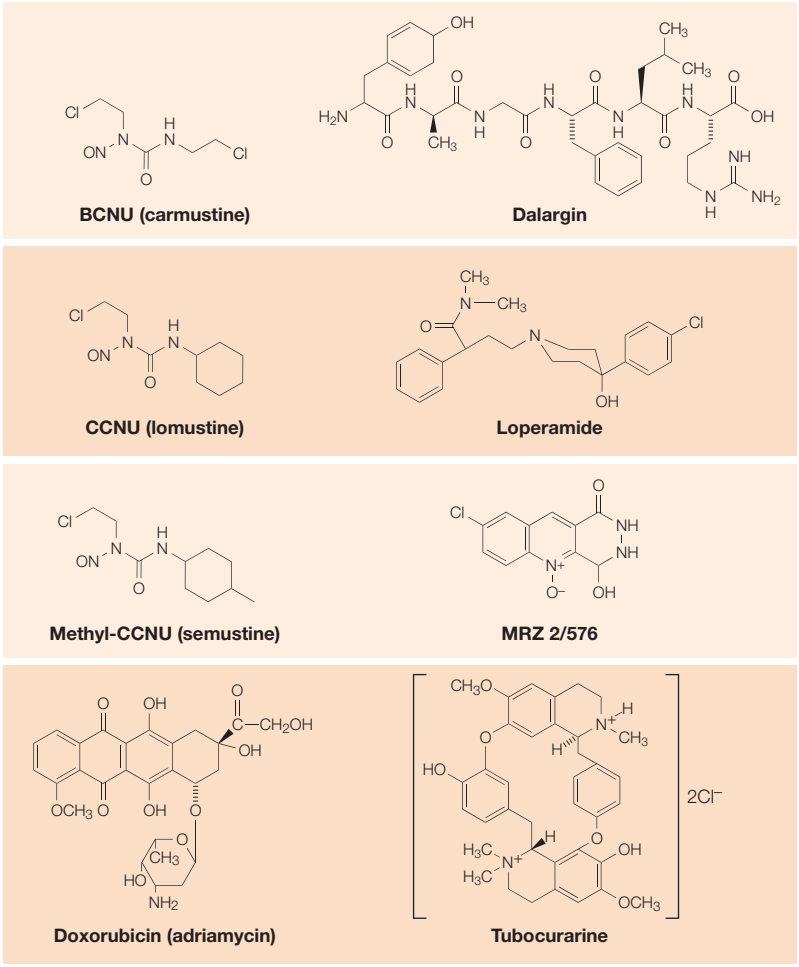


Figure 3 | **Chemotherapeutic drugs and experimental neurological agents.** In general, only small, electrically neutral, lipid-soluble molecules can penetrate the blood–brain barrier, and most chemotherapeutic agents do not fall into this category. Both lomustine and semustine represent two more lipophilic variants of carmustine. The other drugs presented in the figure have been successfully delivered to the central nervous system via nanoparticle-mediated transport.

probably increases the passage of intravascular hydrophilic substances into the brain parenchyma, it does not improve transfer into the actual tumour.

The third strategy to defeat the BBB is to bypass it with local delivery to the tumour site. The simplest method involves direct infusion of a drug via a catheter directly to the tumour tissue. The OMMAYA RESERVOIR was developed with this idea in mind and has been utilized over the years to intermittently deliver a number of chemotherapeutic agents (including BCNU, methotrexate, adriamycin, bleomycin and cisplatin), as well as biological agents (interleukin-2 (IL-2) and interferon-γ (IFN-γ)) to brain tumours[21–26]. Although the success of these therapies has been clearly documented in individual cases, the overall survival benefit has not been tested in large-scale clinical trials. Most recently, however, several implantable pumps have been developed that possess certain advantages over the Ommaya reservoir. These pumps — such as the Infusaid pump (Infusaid), MiniMed PIMS pump (MiniMed), and the Medtronic SynchroMed system (Medtronic) — deliver drugs at a constant rate and over an extended period of time. Such pumps have been shown to be clearly beneficial in experimental brain-tumour models[27] and are currently used in a number of Phase III brain-tumour trials in the United States.

Finally, convection-enhanced delivery represents a potentially powerful method of drug delivery to the CNS, particularly in areas such as the brainstem, in which surgical intervention is not always feasible. Convection, unlike diffusion, results from a simple pressure gradient and it is independent of molecular weight. This pressure gradient can be used to deliver high concentrations of drugs to large regions of the brain without functional or structural damage. Convection has been used to deliver immunotoxins, such as IL-13 and *Pseudomonas* exotoxin (a form of recombinant fusion protein) to malignant brain tumors[28,29]. In addition, certain chemotherapeutic agents, such as Taxol, are now under active investigation using convection-enhanced drug delivery[30,31].

Polymer systems

Sustained controlled-release polymers for macromolecules were first described in 1976 by Langer and Folkman[32], who reported the sustained and predictable release of macromolecules from an ethylene vinyl acetate copolymer (EVAc). This polymer is inert and therefore non-biodegradable. It releases its agent by diffusion through the micropores of its matrix. The rate of diffusion depends on the chemical properties of the drug, including water solubility, charge and molecular mass. Although not specifically FDA-approved for use in the brain, EVAc has found application in the treatment of glaucoma, diabetes, asthma and contraceptive therapy (BOX 1). Its primary limitation is due to its non-biodegradable nature: once the drug diffuses and is completely released, the matrix remains in place permanently as a foreign body.

A major step in the improvement of polymer technology and its clinical application was the development of a new generation of biodegradable polymers. The polyanhydride poly[bis(p-carboxyphenoxy)propane–sebacic acid] (PCPP–SA) polymer is an example of a biodegradable polymer that breaks down to dicarboxylic acids by spontaneous reaction with water[33]. These polymers therefore release a drug by means of both polymer degradation and drug diffusion. The hydrophobic nature of PCPP–SA is important not only because any incorporated drug is protected from interaction with the surrounding aqueous environment, but also because the breakdown of the polymer is limited to its surface. This process, known as surface erosion, leads to a constant rate of drug delivery. Moreover, the breakdown of the PCPP–SA polymer can be altered to occur over a range of days to years, simply by altering the ratio of the CPP to SA. For example, a 1-mm polymer composed of 100% CPP would require three years to degrade, compared with three weeks when SA is added to reach 80% of the molecular mass. Finally, another significant advantage of PCPP–SA over the EVAc system is that PCPP–SA can be fabricated in an endless variety of

OMMAYA RESERVOIR
A device with a fluid reservoir implanted under the scalp with a catheter inside a ventricle. It allows for medication to be given directly to the cerebrospinal fluid and into the brain.

Box 1 | **Application of polymers in ophthalmology**

Glaucoma is caused by a number of different diseases, which in most cases produce increased pressure within the eye. The increase in pressure causes damage of the optic nerve. The death of the nerve cells results in permanent visual loss. Nearly three million people in the US have glaucoma, a leading cause of blindness in the United States.

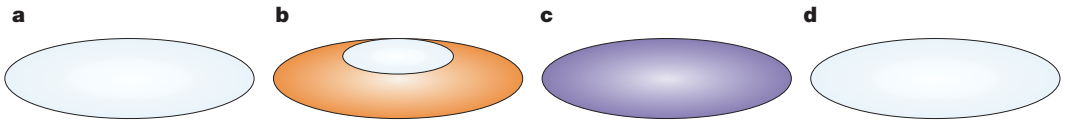
The Ocusert system, developed by Alza Corp., contains a core reservoir consisting of pilocarpine and alginic acid. The core is surrounded by a hydrophobic ethylene/vinyl acetate (EVA) copolymer membrane, which controls the diffusion of pilocarpine from the insert. Pilocarpine is released as soon as it is placed in contact with conjunctival surfaces and acts as a direct parasympathomimetic drug that produces papillary constriction, stimulates the ciliary muscle and increases aqueous humour outflow. Due to the increase in aqueous humour outflow, there is a decrease in intraocular pressure.

By system design, pilocarpine molecules are released at a rate of 20–40 μg per hour over a period of seven days. During the first few hours of the seven-day course, the release rate is higher than during the remainder of the time. The insert releases drug at three times the rated value in the first hours and drops to the rated value in approximately six hours. A total of 0.7 mg of pilocarpine is released during this initial six-hour period. During the remainder of the seven-day period, the release rate is within ± 20% of the rated value.

The ocular hypotensive effect is fully developed within 1–2 hours after placement of the insert. A satisfactory ocular hypotensive response is maintained around the clock. Most importantly, intraocular pressure reduction for an entire week is achieved with the Ocusert insert from 6.7 mg pilocarpine (40 μg per hour, 24 hours a day, for seven days) as compared with 28 mg administered as a 2% ophthalmic solution four times a day.

Another promising technology utilizes implantable pellets, designed in layers like an onion. Slowly biodegradable polymers gradually expose and release microscopically thin wafers of medication into the vitreous cavity of the eye, providing 6–26 months of therapy. This system has already been FDA-approved for viral retinitis, and shows promise for uveitis, macular degeneration, diabetic retinopathy, glaucoma and optic-nerve damage.

The figure shows the Ocusert ocular insert system for treatment of glaucoma. **a** | Transparent polymer membrane. **b** | Opaque annular ring. **c** | Pilocarpine insert. **d** | Transparent polymer membrane.



shapes, including rods, wafers or microspheres, for the delivery of many different compounds, including hormones, neurotransmitters, enzymes and antineoplastics. In the end, there is no foreign body left behind and the breakdown products are nonmutagenic, noncytotoxic and nonteratogenic[34].

Since the development of the first biodegradable matrices, several new polymers have come under active investigation. These ongoing efforts to improve polymer technology were spurred, in part, by the fact that PCPP–SA did not release a high enough percentage of many hydrophilic agents. In addition, certain hydrolytically unstable compounds, such as carboplatin, did not follow constant-release kinetics[35]. In view of these limitations, the fatty-acid dimer–sebacic acid (FAD–SA) copolymer was developed. As with PCPP–SA, the FAD–SA is biodegradable and can be fabricated into any shape. Moreover, the degradation rate and subsequent release profile can be manipulated by altering the ratio of FAD to SA. Both proteins and chemotherapeutic agents have been effectively released from FAD–SA[36,37]. Other advances in polymer technology include the development of poly(lactide-co-glycolide) polymer, which can be used to deliver the radiosensitizer 5-fluorouracil (5-FU) to malignant brain tumours; the polyethyleneglycol-coated liposomes which encapsulate anthracyclines; and the gelatin–chondroitin-sulphate-coated microspheres, which have been shown to release cytokines *in vivo*[38–42] (TABLE 1).

Gliadel polymers

The choice of carmustine, or BCNU, for the development of polymer-based brain-tumour chemotherapy was based on the favourable activity of nitrosoureas against malignant brain tumours. This low-molecular-mass alkylating agent is relatively lipid soluble, penetrates the BBB and modestly prolongs the survival of patients with brain tumours[43–45]. On the other hand, the relatively short half-life (about 15 minutes) when given intravenously, combined with severe toxicity such as myelosuppression and pulmonary fibrosis, have precluded the widespread use of systemic BCNU. Given its potential efficacy against brain tumours and unacceptable systemic toxicity, BCNU was a natural choice for incorporation into the new polymer technology and its clinical application in brain-tumour therapy.

The first preclinical studies of BCNU–polymer preparations assessed the pharmacokinetics of active drug release *in vitro* and *in vivo*. All confirmed that a prolonged, controlled and sustained release of intact BCNU can be achieved with the polymer system[46–48]. Second, the efficacy of BCNU–polymers was tested against a rat intracranial glioma model. The results clearly showed that local delivery of BCNU by polymer was superior to systemic administration and led to significant prolongation of survival in animals with malignant glioma[49]. Third, toxicity studies performed in primates showed that BCNU–polymers were well-tolerated and that concomitant external beam radiotherapy did not increase toxicity[50]. Cumulatively, these

Table 1 | **Clinical use of polymer technology**

Polymer	Chemical composition	Biodegradable	Clinical condition	Drug–polymer formulation
EVA	Ethylene/vinyl acetate	No	Glaucoma	Ocusert (EVA with Pilocarpine)
PCPP-SA	Poly[bis(p-carboxyphenoxy) propane–sebacic acid]	Yes	Malignant glioma	Gliadel (PCPP–SA with Carmustine)
FAD-SA	Fatty acid dimer-sebacic acid	Yes	–	–
PLG	Poly(lactide-co-glycolide)	Yes	Schizophrenia	Risperal Consta (PLG with Risperidone)
PEG	Polyethyleneglycol	Yes	SCID ALL	Adagen (PEG with adenosine deaminase) Oncaspar (PEG with L-asparginase)
Microspheres	Gelatin/chondroitin-sulphate-coated microspheres	Yes	Periodontitis	Arestin (minocycline with microspheres)

ALL, acute lymphocytic leukaemia; SCID, severe combined immunodeficiency syndrome.

studies proved the safety and efficacy of the polymer technology and set the stage for clinical trials.

In a Phase I/II clinical trial, 21 patients were treated with three different doses of BCNU loaded in PCPP–SA polymers (1.93%, 3.85% and 6.35% BCNU by polymer weight)[51]. Up to eight BCNU-loaded polymer wafers were implanted within the tumour cavity (FIG. 4). The treatment was well tolerated and no patient experienced any signs of local or systemic toxicity. On the basis of this work, a Phase III prospective, randomized, double-blind, placebo-controlled clinical trial of PCPP–SA polymer containing 3.8% BCNU by weight was conducted in 222 patients with recurrent malignant gliomas at 27 medical centres in the United States and Canada[52]. Although the BCNU–polymer treatment group had a median survival of 31 weeks, the median survival of the control group was 23 weeks (HAZARD RATIO = 0.69, $P = 0.005$). The results were even more striking in the GBM group, with 50% greater survival at six months in patients treated with BCNU–polymers than with placebo alone ($P = 0.02$). There were no significant toxicities observed in the BCNU–polymer group. Consequently, this study established that BCNU–polymers are safe and effective in the treatment of recurrent malignant gliomas. In 1996, the FDA approved Gliadel as the first new treatment against malignant brain tumours in 23 years.

Most recently, Westphal *et al.* published the results of a large-scale, Phase III, placebo-controlled trial involving patients with newly diagnosed malignant glioma[53]: 240 patients were randomized to receive either BCNU or placebo wafers at the time of primary surgical resection; both groups were treated with external beam radiation post-operatively. The two groups were similar for age, sex, KARNOFSKY PERFORMANCE STATUS (KPS) and tumour histology. Median survival in the intent-to-treat group was 13.9 months for the BCNU wafer-treated group and 11.6 months for the placebo-treated group (log-rank P-value stratified by country = 0.03), with a 29% reduction in the risk of death in the treatment group. When adjusted for factors affecting survival, the treatment effect remained positive, with a risk reduction of 28% ($P = 0.03$). Time to decline in KPS and in ten out of eleven neuro-performance measures was statistically significantly prolonged in the BCNU wafer-treated group ($P \leq 0.05$). Furthermore, at three-year follow-up, 11 (9.2%) of the patients in the Gliadel group versus only 2 (1.7%) patients in the control group were long-term survivors. This study confirmed that local chemotherapy with BCNU wafers is well tolerated and offers a survival benefit to patients with newly diagnosed malignant glioma. In February 2003, and on the basis of this study, the FDA approved Gliadel for use in initial resection of malignant glioma.

Efforts to improve the efficacy of Gliadel have focused on two different areas. The first involves the use of higher doses of BCNU. In the original studies, the dose of BCNU in Gliadel was restricted to 3.8%. It was proposed, therefore, that increasing doses of BCNU might have beneficial effects on the treatment of malignant brain tumours. In a recently completed Phase I study, Olivi *et al.* showed that doses containing up to 20% of BCNU by weight are well tolerated without any significant systemic side effects[54]. Plans are now underway for a Phase III trial with PCPP–SA wafers containing up to 20% of BCNU. The second strategy aims to combine BCNU with O^6-benzylguanine (O^6-BG), an inhibitor of the DNA-repair protein O^6-methylguanine-DNA methyltransferase. As the majority of brain tumours possess alkylguanine-DNA alkyltransferase (AGT) activity, which repairs BCNU-induced DNA damage and O^6-BG is a substrate inhibitor of AGT, the two agents represent an attractive combination. Indeed, in an experimental glioma model, O^6-BG in conjunction with locally delivered BCNU has been shown to potentiate the effects of BCNU alone[55]. In tumours with significant AGT activity, O^6-BG-mediated suppression of AGT could therefore be useful for BCNU to provide therapeutic benefit.

Although Gliadel has been approved and used for patients with malignant brain tumours, its clinical use has expanded to both benign, though locally aggressive tumours, as well as metastatic brain tumours. Although some tumours, such as pituitary adenomas and craniopharyngiomas, are benign, their location — in terms of their proximity to the pituitary gland and optic nerve, respectively — can sometimes prevent total resection. In those instances in which the tumours recur with high

HAZARD RATIO
A summary of the difference between two survival curves, representing the reduction in the risk of death on treatment compared with control, during the period of follow up.

KARNOFSKY PERFORMANCE STATUS
A standard way of measuring the ability of cancer patients to perform ordinary tasks.

frequency despite surgical intervention and radiation therapy, Gliadel has been shown to be useful in preventing new growth of aggressive pituitary adenomas and craniopharyngiomas[56]. Similarly, preclinical studies have indicated that local delivery of chemotherapy from polymers in combination with radiotherapy might demonstrate enhanced efficacy over standard therapies for patients with metastatic brain tumours. This hypothesis has been tested in a series of animal models involving intracranial metastases from melanoma, renal-cell carcinoma, colon adenocarcinoma and lung cancer[57]. Preliminary results from human studies are equally encouraging[58]; however, further studies involving a larger number of patients will be needed before reaching any meaningful and long-term conclusions.

Beyond Gliadel

The choice of BCNU for the initial polymer–drug combination was based on the demonstrated efficacy of alkylating agents against brain tumours. The success of Gliadel, however, has led to a plethora of studies utilizing a variety of different antineoplastic agents. In the following section, we will highlight some of the current research involving a spectrum of chemotherapeutic agents.

Taxol. One of the most promising agents for brain-tumour therapy is taxol. Originally isolated from the bark of Pacific yew tree (*Taxus brevifolia*), taxol is a microtubule-binding agent that promotes microtubule assembly and stability, thereby leading to arrested mitosis and modulation of apoptosis-regulating proteins. Taxol is already FDA-approved for the treatment of ovarian, breast and lung cancers; however, it has failed several clinical trials against malignant glioma[59–61]. In each trial, the concentrations measured within the CNS were low to undetectable at the rate-limiting systemic dose of the drug. However, following interstitial delivery by biodegradable polymers or by means of convection, taxol has been shown to be safe and effective in different animal models of malignant glioma[30,62,63].

Camptothecin. Another potential drug for use in the CNS is camptothecin. Camptothecin is an inhibitor of DNA-replicating enzyme topoisomerase I. This naturally occurring alkaloid was first isolated from the tree *Camptotheca acuminata* in China. Although camptothecin appeared as a highly promising new antineoplastic agent, clinical trials involving this drug were stopped because of unexpected systemic toxicity. In a series of *in vivo* experiments, local delivery of camptothecin via polymers was shown to be safe and highly effective against malignant glioma[64–66]. In fact, up to 60% of treated animals were long-term survivors showing no evidence of systemic or local side effects related to local delivery. Camptothecin is a drug that illustrates a proof of principle: local delivery is superior to systemic chemotherapy.

Minocycline. Anti-angiogenic drugs could also benefit from sustained local delivery. For example, minocycline, a broad-spectrum antibiotic, has demonstrated anti-angiogenic properties. The anti-angiogenic potential of minocycline was first predicted using the rabbit cornea angiogenesis assay in 1991 (REF. 67). Since then, polymers loaded with minocycline have been extensively studied and shown to inhibit the growth of glioma tumours, both in the flank and intracranially[68]. Most recently, treatment with a combination of minocycline delivered locally in a controlled-release polymer and systemic BCNU has exhibited synergistic activity in the treatment of intracranial glioma[68].

The use of non-chemotherapeutic agents along with chemotherapeutic agents might therefore one day further expand the repertoire of agents used in the fight against malignant brain tumours.

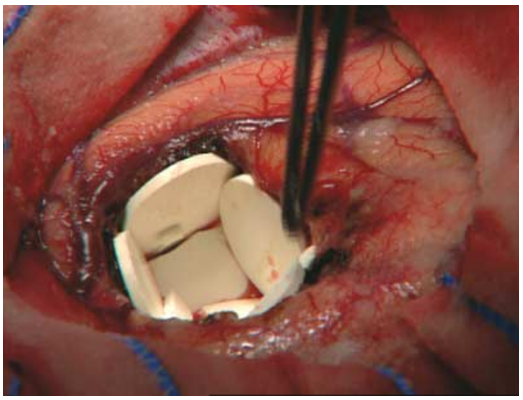


Figure 4 | **Implantation of Gliadel.** Following resection of a malignant glioma, up to eight carmustine (BCNU)–polymer wafers are placed within the tumour cavity. As the wafers dissolve, they release BCNU locally and provide direct delivery of chemotherapy to the tumour cavity.

Microchip biotechnology

A novel and potentially powerful method of drug delivery involves the use of microchips. Such microchips can be engineered to deliver drugs in a pulsatile fashion using micro-fabrication technology. The first solid-state silicon drug delivery microchip was developed by Santini, Cima and Langer at the Massachusetts Institute of Technology. These microchips are capable of providing controlled release of a single or several chemical substances on demand[69]. The release mechanism is based on the electrochemical dissolution of thin anode membranes covering micro-reservoirs filled with chemicals in solid, liquid or gel form. A microbattery, multiplexing circuitry and memory can be integrated into the device, allowing it to be mounted on a tip of a small probe, implanted or swallowed.

Most recently, biodegradable polymeric microchips were fabricated that significantly improved on the solid-state silicon microchip. In preliminary studies, these new biodegradable chips released four pulses of radiolabelled dextran, human growth hormone or heparin *in vitro*[70]. Heparin that was released over 142 days retained on average 96% (+/– 12%) of its bioactivity. The microchips were 1.2 cm in diameter, 480–560 μm thick and had 36 reservoirs that could each be filled with a different chemical. The devices were fabricated from

Table 2 | **Representative new clinical trials for brain tumours**

Generic name	Product name	Company/institution	Status	Mode of action
O^6-benzylguanine (with BCNU)	Alkylade	Pacific Pharmaceuticals	Phase I	Inhibits the repair of DNA damaged by BCNU
BG00001	N/A	Biogen	Phase I	IFN-β gene therapy
Adv-HSV-tk	N/A	Mount Sinai	Phase I	Viral gene therapy
Oxaliplatin	N/A	NABTT	Phase I	Alkylating agent
Arsenic trioxide	N/A	NABTT	Phase I	Interferes with growth of cancer cells and radiotherapy
EMD 121974	Cilengitide	EMD	Phase I	Inhibitor of $\alpha_V\beta_3$ integrin receptor
Erlotinib	Tarceva	Genentech	Phase II	Inhibitor of epidermal growth factor
Human reovirus	Reolysin	Oncolytics Biotech	Phase II	Virus infects tumour cells with activated RAS
Topotecan	N/A	Columbia University, New York	Phase II	Topoisomerase I inhibitor
Dendritic cell therapy	N/A	Cedars-Sinai	Phase II	Vaccine of tumour pulsed dendritic cells
IL-13-PE38QQR	N/A	Neopharm	Phase III	Immunotoxin
Edotecarin	N/A	Florida Cancer Institute	Phase III	Topoisomerase I inhibitor
Temozolomide + RT	N/A	SWOG	Phase III	Alkylating agent

BCNU, carmustine; EMD, (EMD 121974/Cilengitide; Merck); IFN, interferon; IL-13, interleukin-13; N/A, not available; NABTT, New Approaches to Brain Tumor Therapy; RT, radiotherapy; SWOG, Southwestern Oncology Group.

poly(L-lactic acid) and had poly(D,L-lactic-co-glycolic acid) membranes of different molecular masses covering the reservoirs. A drug delivery system can therefore be designed with the potential to release pulses of different drugs at intervals after implantation in a patient by using different molecular masses or materials for the membrane. Such 'pharmacy-on-a-chip' could be used to deliver up to 1,000 different drug dosages or drugs on demand, and is likely to find significant applications in the field of neuro-oncology.

Targeted gene therapy

The concept of gene therapy arose from the observation that certain human diseases are caused by the inheritance of a single non-functional gene. The ability to replace the defective or missing gene with a normal, functional copy emerged as an alternative treatment strategy. Early work focused on diseases caused by these single-gene defects, such as severe combined immunodeficiency syndrome and cystic fibrosis. However, the more recent detection of common genetic alterations shared by a variety of human neoplasms has fostered interest in the application of gene transfer techniques to the development of novel therapies for cancer. In fact, cancer gene therapy has become one of the most rapidly evolving areas in preclinical and clinical cancer research. The idea that human neoplasms are the result of accumulated genetic lesions that culminate in a transformed phenotype has increased optimism that gene therapy techniques might provide a rational basis for therapeutic intervention. In this setting, gene therapy is intended to broaden the spectrum of available therapies. However, with more than 200 cancer gene therapy trials approved worldwide since the early 1990s, it has become increasingly clear that several issues remain to be addressed before the full potential of gene therapy in the care of cancer patients can be realized.

The two most important obstacles in gene therapy are the low efficiency of gene transfer achieved by presently available gene-delivery vectors and the lack of selectivity of these vectors to specifically target cancer cells. At present, the delivery of DNA to target cells can be achieved through one of three distinct modalities. In the first method, tumour cells are removed from a patient, manipulated *in vitro* and subsequently transferred back to the patient. Alternatively, DNA can be delivered directly into the target tissue, provided this tissue is localized and accessible to manipulation. An example of this approach includes injection of a tumour mass with a vector carrying a gene encoding a cytokine or toxin. The third method is *in vivo* gene therapy, in which a vector is administered systemically, yet the gene is delivered locally to cells of interest. The realization of such targeted vector therapies that can be safely administered intravenously will represent a major breakthrough in the field of gene therapy, given the systemic nature of most malignancies.

At present, two major vectors are available for the delivery of gene therapy: viruses and non-viral vectors. Each one offers certain advantages as well as disadvantages; however, one major benefit of viral vectors is the ability to manipulate viral DNA to target specific tissue or tumour. A major area of current research in our laboratories involves genetic retargeting of adenoviral vectors to a set of surface markers called integrins, surface membrane proteins involved in angiogenesis. Malignant brain tumours are among the most angiogenic of all human solid tumours. The principal angiogenic factors produced by glioblastomas are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). One known mechanism by which VEGF and bFGF promote angiogenesis is by stimulating the activity of integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ on endothelial cells[71,72]. Integrin activation and ligand binding result in the propagation of intracellular signals that maintain endothelial

cell survival and enhance proliferation, motility and capillary sprouting[73,74]. Preventing $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins from binding to their specific extracellular matrix ligands results in endothelial cell apoptosis and arrest of angiogenesis[75,76]. We have recently engineered a new retargeted adenoviral vector that selectively binds to the $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins, and *in vivo* studies are now under way to assess its clinical efficacy[77].

Immunotherapy

The goal of gene therapy is to replace a defective gene or augment a specific response to a tumour. Although most of the work involving the delivery of deficient genes to brain tumours has met with limited success[78,79], immunotherapy has emerged as a potentially powerful adjuvant for the treatment of brain tumour patients. The identification and cloning of cytokines has provided one important tool for manipulation of the immunological response to tumours. The generation of an effective immune response requires both the presentation of a foreign antigen to lymphocytes and an appropriate co-stimulatory molecule such as a cytokine. The rationale for using cytokines is based on their ability to produce a strong, local inflammatory response that is specific to a particular cytokine. High local concentrations closely mimic the natural biology of cytokine action. In the context of this paracrine physiology, it has been proposed that local delivery of a cytokine can alter the immunological environment and thereby enhance either antigen presentation or the activation of tumour-specific lymphocytes.

To date, several studies have attempted to exploit the ability of cytokine-gene-transduced tumour cells in the immunotherapy of brain tumours. For instance, Glick and colleagues demonstrated a significant increase in survival in the mouse glioma model when tumour cells mixed with IL-2-secreting allogeneic fibroblasts were injected intracerebrally[80,81]. Subsequently, IL-2-secreting fibroblasts were shown to be effective not only in the treatment of primary but also metastatic brain tumours[82,83]. This work has been further corroborated by reports from our laboratory, in which IL-2 was shown to have potent antitumour activity against both primary and metastatic tumour models, utilizing both cells as well as microspheres for the delivery of IL-2 (REFS 84–87). Moreover, when combined with biodegradable chemotherapy-impregnated polymer, IL-2 seems to exert a synergistic effect in the treatment of experimental brain tumours[88,89]. On the basis of the results obtained in these studies, IL-2 represents an important cytokine for gene therapy using viral and non-viral vectors.

Future directions

The past decade has seen unprecedented advances in the therapy of brain tumours. New imaging and surgical techniques have shifted treatments towards earlier and safer interventions. Biotechnology has revolutionized the delivery of drugs to the CNS and led to the development of new pharmacological agents. The challenge ahead is to choose the most promising biological therapies for further development and application. These therapies can be divided into two different though overlapping interest areas: new vectors for local delivery, and new agents suitable for the emerging vectors. Some of this work is already finding its way into new clinical trials for patients with malignant brain tumours (TABLE 2). In the next decade, we are likely to see significant progress with biodegradable microchip technology, allowing physicians to dose several drugs at varying time points and in different quantities. Nanoparticle-mediated drug transport to the brain will provide further refinement to the delivery of drugs across the BBB. New viruses, specifically targeted to tumour tissue, will selectively infect tumour cells while leaving normal and healthy tissue intact. These new vectors are likely to deliver powerful and locally active chemo- and biotherapy that will exert its effect locally, with minimal systemic side effects. Powerful new ways to stimulate the body's own natural immune system will further augment natural treatment strategies. Cumulatively, these new therapies will further refine our treatment of patients with malignant brain tumours, offering new hope and possibly cure against one of the most devastating human diseases.

1. Mohan, D. S. *et al*. Outcome in elderly patients undergoing definitive surgery and radiation therapy for supratentorial glioblastoma multiforme at a tertiary care institution. *Int. J. Radiat. Oncol. Biol. Phys.* **42**, 981–987 (1998).
2. Barker, F. G. *et al*. Survival and functional status after resection of recurrent glioblastoma multiforme. *Neurosurgery* **42**, 709–920; discussion 720–723 (1998).
3. Roose, T., Netti, P. A., Munn, L. L., Boucher, Y. & Jain, R. K. Solid stress generated by spheroid growth estimated using a linear poroelasticity model small star, filled. *Microvasc. Res.* **66**, 204–212 (2003).
4. Kornblith, P. L. & Walker, M. Chemotherapy for malignant gliomas. *J. Neurosurg.* **68**, 1–17 (1988).
5. Albrecht, K. W. *et al*. High concentration of Daunorubicin and Daunorubicinol in human malignant astrocytomas after systemic administration of liposomal Daunorubicin. *J. Neurooncol.* **53**, 267–271 (2001).
6. Koukourakis, M. I. *et al*. High intratumoural accumulation of stealth liposomal doxorubicin (Caelyx) in glioblastomas and in metastatic brain tumours. *Br. J. Cancer* **83**, 1281–1286 (2000).
7. Fabel, K. *et al*. Long-term stabilization in patients with malignant glioma after treatment with liposomal doxorubicin. *Cancer* **92**, 1936–1942 (2001).
8. Lippens, R. J. Liposomal daunorubicin (DaunoXome) in children with recurrent or progressive brain tumors. *Pediatr. Hematol. Oncol.* **16**, 131–139 (1999).
9. Kreuter, J., Alyautdin, R. N., Kharkevich, D. A. & Ivanov, A. A. Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles). *Brain Res.* **674**, 171–174 (1995).
10. Schroeder, U., Sommerfeld, P., Ulrich, S. & Sabel, B. A. Nanoparticle technology for delivery of drugs across the blood–brain barrier. *J. Pharm. Sci.* **87**, 1305–1307 (1998).
11. Alyautdin, R. N. *et al*. Delivery of loperamide across the blood–brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharm. Res.* **14**, 325–328 (1997).
12. Alyautdin, R. N. *et al*. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an *in situ* brain perfusion study. *J. Microencapsul.* **15**, 67–74 (1998).
13. Friese, A., Seiller, E., Quack, G., Lorenz, B. & Kreuter, J. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. *Eur. J. Pharm. Biopharm.* **49**, 103–109 (2000).
14. Gulyaev, A. E. *et al*. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharm. Res.* **16**, 1564–1569 (1999).
15. Rapoport, S. I. Advances in osmotic opening of the blood-brain barrier to enhance CNS chemotherapy. *Expert Opin. Investig. Drugs.* **10**, 1809–1818 (2001).
16. Zylber-Katz, E. *et al*. Pharmacokinetics of methotrexate in cerebrospinal fluid and serum after osmotic blood-brain barrier disruption in patients with brain lymphoma. *Clin. Pharmacol. Ther.* **67**, 631–641 (2000).
17. Rapoport, S. I. Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications. *Cell. Mol. Neurobiol.* **20**, 217–230 (2000).
18. Morikawa, N. *et al*. Dose-related increases in cerebrospinal fluid concentrations of methotrexate in a postoperative patient with glioblastoma. *Ann. Pharmacother.* **33**, 952–956 (1999).
19. Kobrinsky, N. L. *et al*. Etoposide with or without mannitol for the treatment of recurrent or primarily unresponsive brain tumors: a Children's Cancer Group Study, CCG-9881. *J. Neurooncol.* **45**, 47–54 (1999).
20. Prados, M. D. *et al*. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro-oncology* **5**, 96–103 (2003).

21. Boiardi, A. *et al*. Locally delivered chemotherapy and repeated surgery can improve survival in glioblastoma patients. *Ital. J. Neurol. Sci.* **20**, 43–48 (1999).
22. Morantz, R. A., Kimler, B. F., Vats, T. S. & Henderson, S. D. Bleomycin and brain tumors. A review. *J. Neurooncol*. **1**, 249–255 (1983).
23. Patchell, R. A. *et al*. A phase I trial of continuously infused intratumoral bleomycin for the treatment of recurrent glioblastoma multiforme. *J. Neurooncol.* **60**, 37–42 (2002).
24. Voulgaris, S. *et al*. Intratumoral doxorubicin in patients with malignant brain gliomas. *Am. J. Clin. Oncol.* **25**, 60–64 (2002).
25. Huang, Y., Hayes, R. L., Wertheim, S., Arbit, E. & Scheff, R. Treatment of refractory recurrent malignant glioma with adoptive cellular immunotherapy: a case report. *Crit. Rev. Oncol. Hematol.* **39**, 17–23 (2001).
26. Boiardi, A. *et al*. Local immunotherapy (β-IFN) and systemic chemotherapy in primary glial tumors. *Ital. J. Neurol. Sci.* **12**, 163–168 (1991).
27. Giussani, C. *et al*. Local intracerebral delivery of endogenous inhibitors by osmotic minipumps effectively suppresses glioma growth *in vivo*. *Cancer Res.* **63**, 2499–2505 (2003).
28. Husain, S. R. & Puri, R. K. Interleukin-13 receptor-directed cytotoxin for malignant glioma therapy: from bench to bedside. *J. Neurooncol*. **65**, 37–48 (2003).
29. 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.* **88**, 105–111 (2003).
30. Mardor, Y. *et al*. Monitoring response to convection-enhanced taxol delivery in brain tumor patients using diffusion-weighted magnetic resonance imaging. *Cancer Res.* **61**, 4971–4973 (2001).
31. Lidar, Z. *et al*. Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II study. *J. Neurosurg.* **100**, 472–479 (2004).
32. Langer, R. & Folkman, J. Polymers for the sustained release of proteins and other macromolecules. *Nature* **263**, 797–800 (1976).
The original article describing polymers and their potential use in clinical applications.
33. Leong, K. W., Brott, B. C. & Langer, R. Bioerodible polyanhydrides as drug-carrier matrices. I: Characterization, degradation, and release characteristics. *J. Biomed. Mater. Res.* **19**, 941–955 (1985).
34. Leong, K. W., D'Amore, P. D., Marletta, M. & Langer, R. Bioerodible polyanhydrides as drug-carrier matrices. II. Biocompatibility and chemical reactivity. *J. Biomed. Mater. Res*. **20**, 51–64 (1986).
35. Domb, A., Bogdansky, S. & A., O. Controlled delivery of water soluble and hydrolytically unstable anti-cancer drugs for polymeric implants. *Polymer Prepr.* **32**, 219–222 (1991).
36. Tabata, Y., Gutta, S. & Langer, R. Controlled delivery systems for proteins using polyanhydride microspheres. *Pharm. Res.* **10**, 487–496 (1993).
37. Olivi, A. *et al*. Interstitial delivery of carboplatin via biodegradable polymers is effective against experimental glioma in the rat. *Cancer Chemother. Pharmacol.* **39**, 90–96 (1996).
38. Menei, P. *et al*. Drug targeting into the central nervous system by stereotactic implantation of biodegradable microspheres. *Neurosurgery* **34**, 1058–1064; discussion 1064 (1994).
39. Gref, R. *et al*. Biodegradable long-circulating polymeric nanospheres. *Science* **263**, 1600–1603 (1994).
40. Gabizon, A. A. Liposomal anthracyclines. *Hematol. Oncol. Clin. North. Am.* **8**, 431–450 (1994).
41. Golumbek, P. T. *et al*. Controlled release, biodegradable cytokine depots: a new approach in cancer vaccine design. *Cancer Res.* **53**, 5841–5844 (1993).
42. Menei, P. *et al*. Stereotaxic implantation of 5-fluorouracil-releasing microspheres in malignant glioma. *Cancer* **100**, 405–410 (2004).
43. Loo, T. L., Dion, R. L., Dixon, R. L. & Rall, D. P. The antitumor agent, 1,3-bis(2-choloethyl)-1-nitrosourea. *J. Pharm. Sci.* **55**, 492–497 (1966).
44. Green, S. B. *et al*. Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. *Cancer Treat. Rep.* **67**, 121–132 (1983).
45. Walker, M. D. *et al*. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N. Engl. J. Med.* **303**, 1323–1329 (1980).
46. Grossman, S. A. *et al*. The intracerebral distribution of BCNU delivered by surgically implanted biodegradable polymers. *J. Neurosurg.* **76**, 640–647 (1992).
47. Yang, M. B., Tamargo, R. J. & Brem, H. Controlled delivery of 1,3-bis(2-chloroethyl)-1-nitrosourea from ethylene-vinyl acetate copolymer. *Cancer Res.* **49**, 5103–5107 (1989).
48. Wu, M. P., Tamada, J. A., Brem, H. & Langer, R. *In vivo* versus *in vitro* degradation of controlled release polymers for intracranial surgical therapy. *J. Biomed Mater. Res.* **28**, 387–395 (1994).
49. Tamargo, R. J. *et al*. Interstitial chemotherapy of the 9L gliosarcoma: controlled release polymers for drug delivery in the brain. *Cancer Res.* **53**, 329–333 (1993).
50. Brem, H. *et al*. Biodegradable polymers for controlled delivery of chemotherapy with and without radiation therapy in the monkey brain. *J. Neurosurg.* **80**, 283–290 (1994).
51. Brem, H. *et al*. Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas. *J. Neurosurg.* **74**, 441–446 (1991).
One of the first papers to describe the efficacy of Gliadel against recurrent maligant brain tumors.
52. Brem, H. *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* **345**, 1008–1012 (1995).
53. Westphal, M. *et al*. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-oncol.* **5**, 79–88 (2003).
54. Olivi, A. *et al*. Dose escalation of carmustine in surgically implanted polymers in patients with recurrent malignant glioma: a New Approaches to Brain Tumor Therapy CNS Consortium trial. *J. Clin. Oncol.* **21**, 1845–1849 (2003).
55. Rhines, L. D. *et al*. O^6-benzylguanine potentiates the antitumor effect of locally delivered carmustine against an intracranial rat glioma. *Cancer Res.* **60**, 6307–6310 (2000).
56. Laws, E. R., Jr., Morris, A. M. & Maartens, N. Gliadel for pituitary adenomas and craniopharyngiomas. *Neurosurgery* **53**, 255–269; discussion 259–260 (2003).
57. Ewend, M. G. *et al*. Local delivery of chemotherapy and concurrent external beam radiotherapy prolongs survival in metastatic brain tumor models. *Cancer Res.* **56**, 5217–5223 (1996).
58. Ewend, M. G., Brem, S., Gilbert, M., Goodkin, R. & Penar, P. Treating single brain metastasis with resection, placement of BCNU-polymer wafers, and radiation therapy. *Am. Assoc. Neurol. Surgeons* Toronto, Canada, 24–26 April 2001.
59. Forsyth, P. *et al*. Phase II trial of docetaxel in patients with recurrent malignant glioma: a study of the National Cancer Institute of Canada Clinical Trials Group. *Invest. New Drugs* **14**, 203–206 (1996).
60. Freilich, R. J., Seidman, A. D. & DeAngelis, L. M. Central nervous system progression of metastatic breast cancer in patients treated with paclitaxel. *Cancer* **76**, 232–236 (1995).
61. Glantz, M. J., Chamberlain, M. C., Chang, S. M., Prados, M. D. & Cole, B. F. The role of paclitaxel in the treatment of primary and metastatic brain tumors. *Semin. Radiat. Oncol.* **9**, 27–33 (1999).
62. Walter, K. A. *et al*. Interstitial taxol delivered from a biodegradable polymer implant against experimental malignant glioma. *Cancer Res.* **54**, 2207–2212 (1994).
63. Li, K. W. *et al*. Polilactofate microspheres for Paclitaxel delivery to central nervous system malignancies. *Clin. Cancer Res.* **9**, 3441–3447 (2003).
64. Sampath, P. *et al*. Camptothecin analogs in malignant gliomas: comparative analysis and characterization. *J. Neurosurg.* **98**, 570–577 (2003).
65. Storm, P. B. *et al*. Polymer delivery of camptothecin against 9L gliosarcoma: release, distribution, and efficacy. *J. Neurooncol.* **56**, 209–217 (2002).
66. Weingart, J. D., Thompson, R. C., Tyler, B., Colvin, O. M. & Brem, H. Local delivery of the topoisomerase I inhibitor camptothecin sodium prolongs survival in the rat intracranial 9L gliosarcoma model. *Int. J. Cancer.* **62**, 605–609 (1995).
67. Tamargo, R. J., Bok, R. A. & Brem, H. Angiogenesis inhibition by minocycline. *Cancer Res* **51**, 672–675 (1991).
68. Weingart, J. D., Sipos, E. P. & Brem, H. The role of minocycline in the treatment of intracranial 9L glioma. *J. Neurosurg.* **82**, 635–640 (1995).
69. Santini, J. T., Jr. Cima, M. J. & Langer, R. A controlled-release microchip. *Nature* **397**, 335–8 (1999).
This article shows how a solid-state silicon chip could have the potential to deliver several drugs at different time points.
70. Richards Grayson, A. C. *et al*. Multi-pulse drug delivery from a resorbable polymeric microchip device. *Nature Mater.* **2**, 767–772 (2003).
Describes a biodegradable polymeric microchip that allows release of several drugs *in vivo*.
71. Brooks, P. C., Clark, R. A. & Cheresh, D. A. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. *Science* **264**, 569–571 (1994).
72. Hynes, R. O. Integrins: a family of cell surface receptors. *Cell* **48**, 549–554 (1987).
73. Howe, A., Aplin, A. E., Alahari, S. K. & Juliano, R. L. Integrin signaling and cell growth control. *Curr. Opin. Cell Biol.* **10**, 220–231 (1988).
74. Longhurst, C. M. & Jennings, L. K. Integrin-mediated signal transduction. *Cell. Mol. Life Sci.* **54**, 514–526 (1998).
75. Malik, R. K. Regulation of apoptosis by integrin receptors. *J. Pediatr. Hematol. Oncol.* **19**, 541–545 (1997).
76. Scatena, M. *et al*. NF-κB mediates $\alpha_v\beta_3$ integrin-induced endothelial cell survival. *J. Cell Biol.* **141**, 1083–1093 (1998).
77. Lesniak, M. S., Pai, S. I., Johns, D. & Pardoll, D. M. Targeted adenoviral gene delivery for gliomas. *Am. Assoc. Neurol. Surgeons* Chicago, Illinois, 3–5 April 2002.
78. Lang, F. F. *et al*. Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. *J. Clin. Oncol.* **21**, 2508–2518 (2003).
79. Vecil, G. G. & Lang, F. F. Clinical trials of adenoviruses in brain tumors: a review of Ad-p53 and oncolytic adenoviruses. *J. Neurooncol.* **65**, 237–246 (2003).
80. Glick, R. P., Lichtor, T., Kim, T. S., Ilangovan, S. & Cohen, E. P. Fibroblasts genetically engineered to secrete cytokines suppress tumor growth and induce antitumor immunity to a murine glioma *in vivo*. *Neurosurgery* **36**, 548–555 (1995).
81. Glick, R. P., Lichtor, T., de Zoeten, E., Deshmukh, P. & Cohen, E. P. Prolongation of survival of mice with glioma treated with semiallogeneic fibroblasts secreting interleukin-2. *Neurosurgery* **45**, 867–874 (1999).
82. Lichtor, T. *et al*. Application of interleukin-2-secreting syngeneic/allogeneic fibroblasts in the treatment of primary and metastatic brain tumors. *Cancer Gene Ther.* **9**, 464–469 (2002).
83. Deshmukh, P., Glick, R. P., Lichtor, T., Moser, R. & Cohen, E. P. Immunogene therapy with interleukin-2-secreting fibroblasts for intracerebrally metastasizing breast cancer in mice. *J. Neurosurg.* **94**, 287–292 (2001).
84. Thompson, R. C. *et al*. Systemic and local paracrine cytokine therapies using transduced tumor cells are synergistic in treating intracranial tumors. *J. Immunother. Emphasis Tumor Immunol.* **19**, 405–413 (1996).
85. Lesniak, M. *et al*. Comparative analysis of paracrine immunotherapy in experimental brain tumors. *Neurosurgical Focus* **9**, 1–6 (2000).
86. Lesniak, M. S., Tyler, B. M., Pardoll, D. M. & Brem, H. Gene therapy for experimental brain tumors using a xenogenic cell line engineered to secrete hIL-2. *J. Neurooncol.* **64**, 155–160 (2003).
87. Hanes, J. *et al*. Controlled local delivery of interleukin-2 by biodegradable polymers protects animals from experimental brain tumors and liver tumors. *Pharm. Res.* **18**, 899–906 (2001).
88. Sampath, P. *et al*. Paracrine immunotherapy with interleukin-2 and local chemotherapy is synergistic in the treatment of experimental brain tumors. *Cancer Res.* **59**, 2107–2114 (1999).
89. Rhines, L. D. *et al*. Local immunotherapy with interleukin-2 delivered from biodegradable polymer microspheres combined with interstitial chemotherapy: a novel treatment for experimental malignant glioma. *Neurosurgery* **52**, 872–879; discussion 879–880 (2003).

Acknowledgements

The research presented in this work has been supported in part by the National Cooperative Drug Discovery Group of the National Cancer Institute.

Competing interests statement

The authors declare that they have competing financial interests; see Web version for details.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ABCB1 | ATP-binding cassette C1 | bFGF | O^6-methylguanine-DNA methyltransferase | VEGF

FURTHER INFORMATION

American Brain Tumor Association: www.abta.org
Controlled Release Society: http://www.controlledrelease.org/
MIT Program in Polymer Science and Technology: http://web.mit.edu/PPST/index.html
National Academy of Engineering: http://www.nae.edu/
Neurosurgery: www.neurosurgery.org
Society for Biomaterials: http://www.biomaterials.org/
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