THE NEUROSURGEON AS LOCAL ONCOLOGIST: CELLULAR AND MOLECULAR NEUROSURGERY IN MALIGNANT GLIOMA THERAPY

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MALIGNANT GLIOMAS ARE among the most challenging of all cancers to treat successfully, being characterized not only by aggressive proliferation and expansion but also by inexorable tumor invasion into distant brain tissue. Although considerable progress has been made in the treatment of these tumors with combinations of surgery, radiotherapy, and chemotherapy, these efforts have not been curative. Neurosurgeons as oncologists have increasingly turned their attention to therapies on a molecular scale. Of particular interest to neurosurgeons is the ability to deliver therapy locally to the tumor site or to take advantage of existing immunological mediators, enhancing drug concentrations or therapeutic cell numbers while bypassing the blood-brain barrier to maximize efficacy and minimize systemic toxicity. Exciting local-therapy approaches have been proposed for these devastating tumors. In this review, we discuss the potential applications of bioreactors, neural stem cells, immunotherapies, biodegradable polymers, and convection-enhanced drug delivery in the treatment of malignant gliomas. These approaches are at different stages of readiness for application in clinical neurosurgery, and their eventual effects on the morbidity and mortality rates of gliomas among human patients are difficult to ascertain from successes in animal models. Nevertheless, we are entering an exciting era of "nanoneurosurgery," in which molecular therapies such as those discussed here may routinely complement existing surgical, radiological, and chemotherapeutic approaches to the treatment of neuro-oncological disease. The potential to deploy any of a number of eloquently devised molecular therapies may provide renewed hope for neurosurgeons treating malignant gliomas.

KEY WORDS: Cancer therapy, Glioblastoma multiforme, Glioma, Local delivery

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Malignant gliomas are among the most challenging of all cancers to treat successfully. Because these tumors are characterized by rapid invasive growth into much of the surrounding brain parenchyma, it is often impossible to achieve complete surgical resection without risking devastating neurological compromise. Furthermore, despite limited successes, the combined approaches of surgery, radiotherapy, and chemotherapy have not been curative in the treatment of this disease.

Neurosurgeons as oncologists are thus beginning to use a new, and ultimately revolutionary, therapeutic approach for the treatment of malignant gliomas. Because we are beginning to elucidate the precise molecular changes that may render glioma cells distinct from their nontransformed glial cell counterparts, neurosurgeons have begun to consider that the most effective way to treat gliomas would be to specifically target the dysregulated signaling cascades and proteins that impart and maintain the tumorigenic phenotype, rather than relying solely on macro-scale resection approaches. Interventional molecular neuro-oncology may thus signal a paradigm shift in malignant disease treatment as significant as that produced by the introduction of the operating microscope in the development of microneurosurgery.

With a basis in fundamental cancer cell and molecular biology, neurosurgeons are clearly entering a new era that Apuzzo and Liu (4) termed *nanoneurosurgery*, in which neurosurgeons may intervene at the molecular level to complement existing surgical, radiological, and chemotherapeutic approaches to oncological disease. This new age is exemplified by the recent emergence of local-therapy approaches to glioma treatment, in which macro-scale techniques are used to deliver antitumor agents that function inside the tumor cells with

microscopic and submicroscopic precision. The delivery of agents directly to the tumor site enhances drug concentrations or therapeutic cell numbers while bypassing the blood-brain barrier (BBB) to maximize efficacy and minimize systemic toxicity. Importantly, neurosurgeons are ideal "local oncologists," because of our unique ability to use operative interventions to deliver novel molecular therapies directly to the site of disease.

Here we discuss local therapy delivered by neurosurgeons for the treatment of gliomas, although it is clear that molecular interventions with agents delivered locally to diseased brain tissue by neurosurgeons are also applicable to the treatment of other diseases. Several local-therapy approaches to the treatment of malignant gliomas have been proposed. Advances in gene therapy for the treatment of gliomas have been extensively reviewed (3, 60, 127, 128) and are not discussed; here we review other recent exciting advances in the development of adjunctive molecular local-therapy techniques to treat oncological disease of the central nervous system (CNS). We focus specifically on advances in the following areas: "bioreactors," the use of neural stem cells (NSCs) in the treatment of CNS neoplastic disease, locally delivered immunotherapies, biodegradable polymers releasing chemotherapeutic agents, and convection-enhanced drug delivery (CEDD).

BIOREACTORS

Research into how bioreactors may deliver therapeutic proteins for the treatment of neurological diseases continues at a rapid rate and promises to bring into the realm of neurosurgery diseases that previously involved nonsurgical management. Bioreactors are essentially cellular minipumps; they are capsules of approximately 20 to 800 μm in diameter that contain cells engineered to secrete particular proteins (*Fig. 1*). The capsules of the bioreactor permit passage of recombinant proteins into the surrounding environment, allow inward diffusion of oxygen and nutrients and egress of cellular waste (to sustain the encapsulated producer cells), and exclude immunocompetent cells (and are thus considered immunoisolated). Their biocompatibility is determined by the manner in which the cells are encapsulated, cell-capsule interactions, host-capsule interactions, and ultimately reactions between the secreted protein product and its target (125).

Alginate, which is a mixture of L-guluronic acid and D-mannuronic acid, has the best cell attachment profile and least cytotoxicity and is thus the most commonly used capsule in bioreactor technology (87). Frequently used cell types include primary postmitotic cells, immortalized or dividing cells (such as pheochromocytoma PC-12 cells, which are used in Parkinson's disease therapies), and engineered fibroblast lines (such as baby hamster kidney cells). Cell encapsulation technology, like biodegradable-polymer drug release, addresses current obstacles to successful chemotherapy through local drug delivery; it circumvents the BBB, avoids systemic drug toxicity, and achieves high local drug concentrations. Additionally, this technology provides constitutive release of proteins, allows the use of xenogeneic cells without immunosuppression and with negligible immune responses (115, 120), and requires a single stereotactic injection for intracerebral deployment of the bioreactor. Surgeons could also place bioreactors in the tumor bed after resection.

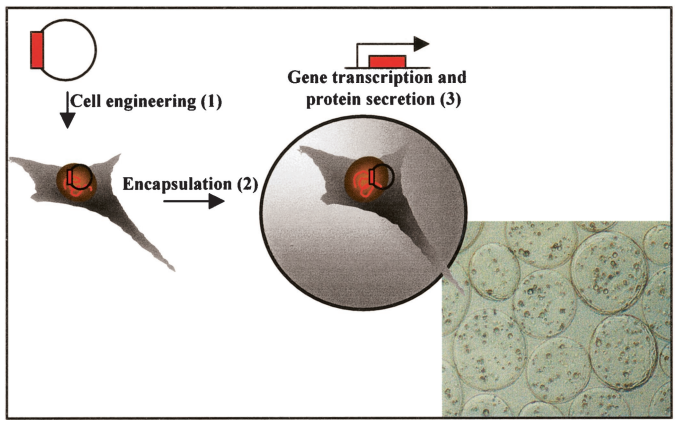

FIGURE 1. *Engineering of a bioreactor. A stable cell line is first engineered to secrete a protein of interest (1). After stable protein expression has been established, producer cells are encapsulated (2); encapsulation allows secretion of the therapeutic gene product (3) and excretion of cellular waste while permitting nutrient and oxygen delivery to producer cells and excluding immune cells. Bioreactors may then be implanted either stereotactically or during craniotomy for tumor resection.* Inset, *phase-contrast micrograph, showing cell-loaded bioreactors with an average microcapsule diameter of 700 μm (24).*

Although considerable work on the use of bioreactors as novel treatment strategies for Parkinson's disease, Huntington's disease, Alzheimer's disease, chronic pain, spinal cord injuries, and seizures has been performed (2, 5, 20, 28, 32–35, 53, 63, 75, 105, 106, 112), investigators are just beginning to study their use for the treatment of malignant gliomas. Initial efforts, which were only recently reported (57, 98), assessed the efficacy of bioreactor-delivered endostatin, a potent anti-angiogenic protein, in rat and mouse glioma models. It has become well accepted that, to grow appreciably, solid tumors must create a vascular system for nutrient delivery and waste removal (42). This process (angiogenesis) is critical for the progression of gliomas, with vascular changes accompanying the advancement of these tumors. The cascade of events in this process of blood vessel formation involves complex interactions between growth factors and tumor cells, endothelial cells, and their surrounding basement membranes, in which enzymatic degradation of surrounding ground substance and subsequent endothelial cell migration and proliferation and tube formation occur. Growth factors, such as vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, and transforming growth factor (TGF), influence glioma angiogenesis by directly stimulating endothelial cell proliferation, by mediating the expression of key proteases on endothelial cells that are necessary for angiogenesis, or by regulating the expression of each other (23, 30).

It is likely that a host of growth factors are responsible for mediating these key events, and therapeutic strategies have been devised accordingly (61, 95). A role for vascular endothelial growth factor in glioma angiogenesis has been convincingly demonstrated, and potential therapies exploiting its role include direct receptor blockade with antibody and interruption of the downstream signal transduction cascade with molecules such as SU5416 or SU6668 (44, 67). Other approaches have focused on inhibiting key proteases necessary for endothelial cell migration through basement membranes (114).

Our laboratory and others have focused on the inhibition of glioma angiogenesis with the endogenous collagen fragments endostatin and angiostatin (57, 85, 86, 98). Our laboratory previously demonstrated that systemic delivery of angiostatin inhibited glioma growth in the Swiss nude mouse model in a dose-dependent manner, with growth inhibition to 11% of control values ($P < 0.01$), without detectable signs of toxicity (62).

Recently, two groups reported elegant approaches to the treatment of malignant gliomas with endostatin, combining bioreactor delivery with antiangiogenic therapy (57, 98). Both groups engineered cell lines to secrete endostatin, encapsulated the cells in alginate formulations, and implanted the bioreactors at the tumor site. Our laboratory stably transfected baby hamster kidney-21 cells with a human endostatin expression vector and encapsulated the cells in alginate-poly-L-lysine microcapsules for long-term delivery of endostatin. We demonstrated that a single local injection of encapsulated endostatin-secreting cells resulted in a 72.3% reduction in subcutaneous U87 glioma xenograft weight 21 days after treatment, in a nude mouse model. Read et al. (98) used a similar approach, encapsulating human 293 cells engineered to secrete human endostatin and assessing the effect of bioreactor-delivered endostatin on tumor growth in an intracerebral B4TC rat glioma model. Rats treated with locally released endostatin survived 84% longer than did control animals, and intracapsular cell viability for at least 4 months was confirmed.

Those studies demonstrated that continuous local delivery of endostatin might offer an effective therapeutic approach to the treatment of a variety of tumor types. This novel approach has a number of advantages. Bioreactors can deliver high local concentrations of proteins for prolonged periods of time while avoiding potential systemic effects by bypassing the BBB, they are not antigenic, and they can be implanted at the site of or distant from a craniotomy for primary tumor resection. As with each of the novel approaches discussed here, refinement of bioreactor technology is contingent on continued discovery of appropriate molecular targets. This approach can be used for any molecule that can be secreted after transfection into bioreactor producer cells. An intriguing candidate molecule that might be highly effective in glioma therapy when expressed locally at high concentrations is PEX, a naturally occurring fragment derived from the autocatalytic digestion of matrix metalloproteinase-2 (18). We demonstrated that PEX acts simultaneously as an inhibitor of glioma angiogenesis, cell proliferation, and migration. Administration of PEX to subcutaneous and intracranial human glioma xenografts resulted in 99% suppression of tumor growth, with no signs of toxicity (8). It is likely that an even more effective approach would involve a locally delivered cocktail of proteins, each specifically aimed at a different process critical to glioma progression. Issues to be more fully addressed in the continued development of bioreactor therapy include assessment of the adequacy of protein delivery with time, further analysis of the distributive properties of bioreactor-secreted agents in the intracranial space, and the continued search for candidate secreted proteins.

NSC THERAPY

One of the insidious biological features of malignant gliomas is the potential of single cells to invade normal brain tissue, blurring tumor margins and establishing numerous "micro-tumors" at a distance from the primary tumor, which makes surgical resection palliative and not curative. Indeed, the ultimate goal of brain tumor research is the discovery of a therapeutic approach in which even single invading glioma cells are engaged and destroyed. An intriguing aspect of adoptive immunotherapy (see below) is that implanted or incited tumoricidal T cells have the potential to migrate from their initial sites of implantation and track down dispersing glioma cells. Another therapeutic approach that holds great promise for the treatment of malignant gliomas is the use of engineered human NSCs as therapeutic vehicles. NSCs are migratory cells that can generate neural tissue, have some capacity for self-renewal, and can give rise to other cells (45), whose potential applications in the treatment of neurological disease have been well reported (see References 88, 89, and 108 for reviews).

The use of NSCs for the treatment of brain tumors, however, is just beginning to be explored. In theory, migrating cells that are capable of tracking down glioma cells and that have been engineered to deliver a therapeutic molecule represent an ideal solution to the seemingly insurmountable problem of glioma cells invading normal brain tissue. Pioneering work by Snyder and coauthors (41, 52, 109) and other laboratories (45) demonstrated that the migratory capacity of NSCs is ideally suited to therapy in neurodegenerative disease models that require brain-wide cell replacement and gene expression. On the basis of those and other studies, it was hypothesized that NSCs may specifically home to sites of disease within the brain. Further studies from that laboratory yielded the intriguing observation that transplanted NSCs were able to home to a primary tumor mass when injected at a distance from the tumor itself (1); furthermore, NSCs were observed to distribute themselves throughout the tumor bed, even migrating in juxtaposition to advancing single tumor cells, as if "chasing them down" (*Fig. 2*). NSCs also seemed to track to tumor when injected intravascularly. The authors further demonstrated that NSCs engineered to produce cytosine deaminase, which converts 5-fluorocytosine to the oncolytic drug

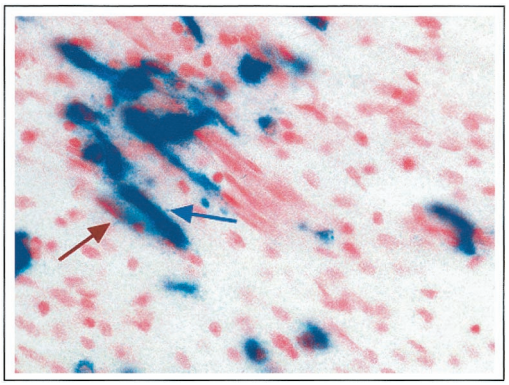

FIGURE 2. *High-power view of adult rat brain with established glioma after NSC injection. The slide was processed with X-Gal to detect blue-staining β-gal-producing NSCs (*blue arrow*) and counterstained with neutral red to show dark red tumor cells (*red arrow*). NSCs are observed to infiltrate red tumor cells in a rat model of CNS-1 tumor (46).*

5-fluorouracil, yielded tumor mass reductions after administration of 5-fluorocytosine (1). If engineered NSCs can indeed track and destroy advancing glioma cells, then this therapeutic approach would be an attractive adjunct to surgical resection, with tumor debulking being complemented by the administration of engineered NSCs in the tumor bed to track and destroy remaining cells.

If human NSCs can indeed migrate to human gliomas, then another technical question is which particular genetic modifications of NSCs would prove most effective for killing glioma cells. Benedetti et al. (10) retrovirally transfected NSCs with the interleukin (IL)-4 immunogene and implanted them into the brains of mice with GL261 tumors, with notable effect. The authors reported progressive disappearance of large tumors on magnetic resonance imaging scans and increased survival rates for treated groups. Cytosine deaminase and IL-4, however, represent two of many candidate genes with which NSCs could be engineered as therapeutic vehicles. Genes encoding the following might be appropriate for transfer into NSCs: proteins inducing tumor cell differentiation, apoptosis-promoting agents, antiangiogenesis factors, immunogenic agents, and oncolytic agents (1), among others.

Therefore, it seems that the migratory capacity of NSCs is retained in the tumor environment and that, as suggested by the studies of Benedetti et al. (10) and Aboody et al. (1), NSCs might be used as novel drug delivery vehicles for both primary tumors and cells at a distance from the central tumor mass. An additional benefit of using NSCs as therapeutic delivery agents is that multipotent cells might be able to repair damage caused by the tumor, surgery, or radiotherapy, while retaining their capacities to migrate and express therapeutic transgenes (82).

The therapeutic potential of this novel application seems, at the moment, almost endless; like immunotherapy, stem cell therapy has the potential for tracking and destroying invasive single tumor cells. Investigators have been quick to temper our enthusiasm, however, by noting potential complications of human NSC deployment (83). Concerns include the following: will migratory capacity be maintained if therapeutic NSCs differentiate? Can the biological features of stem cells be controlled, to ensure that dividing stem cells do not themselves form masses or tumors? Can stem cells keep pace with rapidly dividing glioma cells? Can we control the delivery of therapeutic molecules by NSCs after the cells are deployed? Further in vivo studies should clarify other technical issues related to NSC therapy. We look forward to the refinement of this therapy in animal models and to its future application in the treatment of human brain tumors.

IMMUNOTHERAPY

A potential weapon against gliomas is the body's own immune system, whose role in antiglioma therapy has been extensively investigated. Immunotherapy is an attractive proposition if we can harness a therapeutic component already in place, particularly if invasive glioma cells can be tracked by tumor-specific migratory lymphocytes. There are two main types of immune responses: i.e., humoral, mediated by B cells secreting antibodies, and cell-mediated, comprising direct interactions between T cells and target cells bearing antigens. Immunotherapy delivered locally would involve stimulation of peritumoral immune cells, administration of antibodies, or introduction of cellular immune effectors. A clear understanding of how these responses occur in the CNS, resulting in B cell-mediated antibody-dependent cellular cytotoxicity and T cell-mediated direct cytotoxicity, has been complicated by three factors. The first factor is the BBB. If appropriate molecular signals are expressed on endothelial cells or if the BBB is disrupted, then immune effector cells can enter the brain. Otherwise, the BBB may serve as a potential barrier to the entry of lymphocytes into the CNS. Local-therapy strategies involving either stimulation of the brain's immune system or local deployment of immune agents would circumvent this obstacle.

The second factor is the absence of lymphatic vessels in the CNS. This has raised questions regarding how foreign antigens in the CNS are delivered to lymph nodes. However, evidence does suggest that there is interaction between cerebrospinal fluid and cervical lymphatic vessels (26). Once again, a local-therapy approach in which immune effectors are introduced may overcome some of the problems of antigen delivery.

The third factor involves the expression and distribution of major histocompatibility complex (MHC) proteins in the CNS. T cell activation involves MHC-restricted antigen presentation, in which antigens associated with particular cells (such as tumor cells) are presented to would-be effector T cells as antigen-MHC complexes. However, the MHC proteins that are critical for this process have not been consistently detected in cells of normal brain tissue or brain tumors. More sensitive detection methods such as flow cytometry were recently used to demonstrate low levels of MHC Class I molecules in 16 of 16 early-passage human glioma cultures (90). In addition,

interferon-γ could dramatically up-regulate MHC Class II expression on tumor-adjacent, antigen-presenting cells in vivo, despite still-undetectable levels of MHC proteins (as assessed in immunohistochemical assays) on tumor cells (68, 118).

These encouraging data on MHC expression in tumor cells emphasize the fundamental issue in glioma immunotherapy. How can specific recognition of tumor cells by effector cells of the immune system be achieved? It is understandable why the CNS has traditionally been considered a site of immune privilege, but this view has been marginalized by accumulating evidence to the contrary. Although it seems that immune reactions can occur in the CNS as in the rest of the body, particular aspects of the CNS environment, especially the intricacies of efficient antigen presentation, require further elucidation. Nevertheless, evidence of a functioning immune system in the CNS, as reported by Lampson and coauthors (68, 118), has led to promising studies suggesting that we may be able to use this system to target brain tumors. We review current approaches to tumor therapy that specifically entail locally delivered immunotherapy, including nonspecific activation of the local peritumoral immune system, local adoptive immunotherapy, and locally delivered passive immunotherapy.

Nonspecific Activation of the Local Immune System: Cytokine Therapy

Secreted cytokines are central to the regulation of the immune system, binding to receptors on a variety of immune cell types and influencing cellular responses. Therefore, their potential to stimulate the immune system in antitumor therapy has been studied extensively. They typically exert their effects in a paracrine manner, acting at relatively high concentrations at the site of a particular immune-mediated event. The neurosurgeon's role in their clinical application would thus be to deliver cytokine therapy to the site of the tumor with a stereotactic device, either at the time of primary tumor resection or pre- or postoperatively, if local application was desired to maximize peritumoral cytokine concentrations. To date, studies have focused on the potential of tumor necrosis factor-α, IL-2, IL-4, and interferon- α, -β, and -γ in antiglioma immunotherapy (15, 40, 76, 79, 80, 93, 116, 121, 122, 124). A number of clinical trials have been conducted and were extensively reviewed in Zeltzer et al. (126). Although isolated successes have been reported, no clear trend in survival rates has been observed with cytokine administration alone. Considerable toxicities in trials of IL-2 and interferon-α have been reported (21, 101).

Novel ways of delivering cytokines, in addition to combining cytokine delivery with other methods of enhancing the immune response, currently seem more promising. IL-2 therapy of gliomas has been extensively studied; although IL-2 is systemically toxic, it may be amenable to local delivery. Glick and Lichtor and coauthors (47, 71) demonstrated that allogeneic H2K fibroblast producer cells engineered to secrete IL-2 and delivered intratumorally exhibited potent activity against glioma growth in in vivo models. Brem and coauthors (49) experimented with the novel delivery of IL-2 to the tumor bed via biopolymers. Further work with local administration of IL-2 alone might be curtailed by recent reports that suggested that IL-2 might actually promote the growth of malignant gliomas (22).

IL-4 has also been demonstrated to have activity against gliomas in in vivo models (102, 123). IL-4 is secreted by activated T helper cells to enhance humoral immunity. In one study, Benedetti et al. (9) demonstrated that intracerebral injection of retrovirus-producing cells yielding increased amounts of IL-4 dramatically increased survival rates and induced tumor regression in rat C6 glioblastoma multiforme and 9L gliosarcoma models. Interestingly, immunohistological assessments demonstrated inflammatory infiltrates in IL-4-treated tumors in which $CD8^+$ T lymphocytes were more abundant, although $CD4^+$ T lymphocytes, B lymphocytes, and macrophages were also present (9). Those authors also demonstrated promising results with IL-4 delivered in a novel manner by mouse primary neural progenitor cells (see above). It was also demonstrated that IL-4 enhances the antitumor response of the immune system not only by enhancing the function of existing tumor-adjacent cells but also by increasing the numbers of recruitable T cell precursors (38).

Interestingly, recent work investigating a potential role for IL-10, which is typically an immunosuppressive cytokine, in tumor rejection demonstrated that glioma-specific $CD4^+$ T cells produced IL-10 but neither IL-4 nor interferon-γ and glioma rejection was compromised in IL-10-(−/−) hosts (107). Taken together, these findings indicate that, although it is a potentially practical adjunct to other means of immunotherapy, cytokine administration alone is unlikely to provide effective therapy, principally because this approach is nonspecific and seems unlikely to address the central question posed above—namely, how can we specifically enhance tumor recognition by immune effector cells?

Local Adoptive Immunotherapy

Local adoptive immunotherapy describes the intratumoral administration of autologous lymphocytes that have been harvested from peripheral blood or from the tumor bed, stimulated and/or modified ex vivo in an attempt to enhance tumoricidal activity, and subsequently reimplanted into the tumor bed. Variations include the following techniques.

The first technique involves in vitro IL-2 stimulation of autologous lymphocytes harvested from peripheral blood to produce lymphokine-activated killer cells, with subsequent delivery of cells to the tumor bed during resection and/or postoperatively (via an intratumoral catheter) (7, 65, 121). Clinical trials have demonstrated isolated successes but no clear evidence of an increase in long-term survival rates (74, 121). The toxicity of IL-2 is well known.

The second technique involves expansion and reimplantation of tumor-infiltrating lymphocytes (TILs). This approach entails harvesting of TILs at the time of resection, stimulation with IL-2, and reintroduction of the expanded TILs at the tumor site, on the premise that tumor-adjacent lymphocytes may be more tumor-specific (96, 104). It was initially thought

that the peritumoral location of the harvested lymphocytes indicated a certain degree of specificity in these TILs, but studies of the use of TILs in animal models have not demonstrated effectiveness (96). In fact, recent work demonstrated that TILs might have diminished proliferative capacity and might actually be in the initial stages of cell death, compared with native $CD8^+$ T cells, which could partly explain the lack of efficacy of this reintroduction therapy (94).

The third technique is the use of human, non-MHC-restricted, cytotoxic T cell leukemic cell lines, such as TALL-104 (24, 46, 66). This immortal cell line isolated from human acute T cell leukemia displays cytotoxic activity against a broad range of human tumors while sparing normal tissue. Initial studies demonstrated safety in animal models; the published tumoricidal activity of TALL-104, coupled with the sparing of normal brain tissue, in studies reported to date makes this an intriguing therapeutic prospect.

The fourth technique involves stimulation of autologous lymphocytes with the patient's own tumor cells or with allogeneic donor lymphocytes, to generate a more specific MHC Class I-tumor antigen interaction (92), for reimplantation at the tumor site. This approach aims to confer a higher degree of specificity to implanted lymphocytes by exposing harvested lymphocytes to either resected tumor (64) or allogeneic lymphocytes from donor patients before readministration (51). It is hoped that the former method will lead to the generation of tumor antigen-specific T lymphocytes, whereas the latter method is based on the premise that tumor cells expressing MHC Class I will be more specifically recognized if reimplanted lymphocytes are primed by allogeneic lymphocytes.

Studies using these forms of local adoptive immunotherapy have reported enough isolated successes to prompt further work in this area. Furthermore, the concept that a sufficient quantity of ex vivo-expanded cytotoxic lymphocytes primed specifically for autologous tumor and delivered directly to the resection cavity could kill individual tumor cells will continue to foster active research in this area.

Passive Immunotherapy

This form of immunotherapy entails the intratumoral administration of antibodies directed at specific tumor antigens, which leads to cell demise via antibody-dependent cellular cytotoxicity or the effects of toxin or radioisotope conjugated to the antibody. Candidate target antigens should ideally be expressed only on tumor cells and not in normal brain tissue; however, because such glioma-specific molecules have not yet been identified, studies have focused on molecules that are expressed at higher concentrations than in surrounding tissue, including tenascin and the epidermal growth factor receptor (EGFR) (77), or molecules that have been demonstrated to be expressed in some malignant gliomas, such as the EGFR mutant EGFRvIII (31, 81, 110). A substantial volume of preclinical data exists, with results promising enough to prompt clinical trials. Encouraging data from Phase II trials of the ^{131}I-labeled murine antitenascin monoclonal antibody 81C6 were recently published and warrant a Phase III trial (12, 99). Phase I studies of anti-EGFR demonstrated minimal toxicity and substantial binding in vivo (39). Further clinical trials will surely follow, especially in light of the excellent preclinical results achieved by targeting EGFR and EGFRvIII (103) and the increasing sophistication of antibody specificity, such as that of Mab 806, which recognizes EGFRvIII and multiply amplified EGFR but not wild-type EGFR (56, 103). Variations of receptor-targeted immunotherapy have included receptor targeting with ligand-toxin conjugates. The IL-4 receptor on glioma cells has been targeted in vitro with IL-4 conjugated to a truncated form of *Pseudomonas* exotoxin; preclinical studies demonstrated substantial tumor regression, and a recent Phase I trial demonstrated the safety of this approach (54, 97). Antibody and receptor-specific therapies merit further investigation, in the form of randomized controlled trials, given the promising early results in clinical trials. Whether antibodies can be delivered more effectively by one of the local delivery methods described in this review has not yet been investigated.

It is clear that the most successful local-immunotherapy approach will be the technique that best addresses the issue of how efficient recognition of tumor cells by effector cells of the immune system can be achieved. It is possible to consider a combination of local-immunotherapy approaches, such as a pairing of passive immunotherapy with locally delivered tumoricidal T effector cells, perhaps in combination with more systemic immunotherapeutic approaches not discussed here, such as active immunotherapy and dendritic cell therapy.

BIODEGRADABLE-POLYMER DRUG RELEASE

Exciting advances in the refinement of biodegradable polymers designed for slow release of chemotherapeutic agents for the treatment of neurological diseases have been made in the past 10 years. This approach entails the implantation of drug-impregnated polymers (commonly referred to as wafers) into the tumor resection cavity, with subsequent long-term release of drug. Well-publicized efforts have been directed at the treatment of malignant gliomas with local delivery of chemotherapeutic agents using biodegradable polymers (16, 17, 50); these approaches take advantage of the observation that most malignant brain tumors recur within 2 cm of the original tumor resection site, making diffusion of agents from drug-impregnated wafers a practical therapeutic approach. Additionally, systemic toxicity of chemotherapeutic agents is minimized by bypassing the BBB altogether, whereas drug concentrations at the tumor site are maximized with the local-therapy approach.

Folkman and Long (43) first demonstrated the potential of slow-release drug delivery systems in 1964. Since then, a substantial body of literature on the topic has been published, with particular emphasis on the applications of this technique to CNS malignancies. Most notably, Brem and coauthors (36) studied the feasibility of polymer-mediated drug delivery by using the standard chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and demonstrated that lo-

cal treatment of gliomas with this method was effective in animal models of intracranial tumors. Initial Phase I and II trials of this technique paved the way for further testing.

A randomized, double-blind, placebo-controlled study of patients with high-grade recurrent gliomas treated with carmustine-impregnated polymers demonstrated the promise of this novel technology. In the trial, polymer-delivered chemotherapy significantly improved survival rates, inasmuch as 56% of the patients with glioblastoma multiforme survived, compared with 36% of the placebo-treated group, with clinically insignificant side effects (113). Phase III results from a more recent European trial demonstrated mean survival times of 60 weeks for the group treated with Gliadel (Rhône-Poulenc Rorer Pharmaceuticals, Collegeville, PA), compared with 50 weeks for the placebo-treated group (119). Those studies led to further evaluation of this novel treatment strategy, not only for primary brain tumors but also for metastatic intracranial neoplasms. Encouraging data in animal models and proven safety led to a National Institutes of Health-funded clinical trial assessing the effect of Gliadel wafers on metastatic intracranial neoplasms; this study is now under way (36, 37). However, there were reports of a lack of efficacy of BCNU-impregnated wafers in the treatment of recurrent high-grade gliomas at one center, with that group also reporting a higher incidence of postoperative complications for their experimental group (111).

Recent efforts to potentiate the local effects of BCNU have focused on inhibiting a deoxyribonucleic acid-repair enzyme, namely, O^6-alkylguanine-deoxyribonucleic acid alkyltransferase, which confers resistance to nitrosoureas such as BCNU in human brain tumors. Rhines et al. (100) recently demonstrated that O^6-benzylguanine, a potent O^6-alkylguanine-deoxyribonucleic acid alkyltransferase inhibitor, could potentiate the activity of BCNU delivered intracranially via polymers, in a rat F98 glioma model. National Institutes of Health-funded Phase I trials are currently under way to explore the coupling of Gliadel therapy with O^6-benzylguanine administration. Although local delivery of BCNU via polymer release has been studied most extensively, other agents delivered in a similar manner have also demonstrated promising results. Other chemotherapeutic agents used in sustained-release polymers for the treatment of malignant gliomas in animal models, with encouraging results, include paclitaxel, cyclophosphamide, carboplatin, and cisplatin, among others (58, 73, 84, 117).

Variations of this technology include microsphere release of chemotherapeutic agents (11). Current polymer release vehicles are dime-sized wafers that are not amenable to stereotactic delivery, whereas microspheres, as the name suggests, are on the order of micrometers in size. Microsphere polymers could easily be implanted stereotactically, eliminating the requirement for open surgery for implantation. Menei et al. (78) demonstrated the safety and distribution of sustained delivery of the antimetabolite and radiosensitizer 5-fluorouracil in poly-D,L-lactide-co-glycolide microspheres for patients with glioblastoma multiforme, which suggested future possibilities for collaborative adjunctive therapy administered by neurosurgeons and radiation oncologists in a multimodal molecular approach.

The clinical introduction of Gliadel represents the most successful introduction of a novel local-therapy approach to glioma treatment to date. The technology has been demonstrated, and the search should continue for candidate therapeutic agents to be released via polymer delivery, with appropriate release profiles and areas of distribution.

CEDD

A final exciting technique for drug delivery that bypasses the BBB while maximizing local drug concentrations is CEDD. Oldfield and coauthors (14) pioneered this technique for intracranial drug delivery, which is known by a variety of terms but is most commonly referred to as CEDD. CEDD attempts to overcome the limitations of simple diffusion by using a positive-pressure infusion mechanism to distribute therapeutic agents through brain interstitium down a pressure gradient, without any structural or functional damage to the brain (*Fig. 3*). As therapeutic molecules are infused with the pump, drug distribution can be controlled by varying the infusion volume or rate. The application of this technique to the treatment of human disease is similar to that of other novel mechanisms of drug delivery, such as drug-impregnated polymers (see above). The infusion apparatus, involving a catheter placed into the brain or tumor and connected to a minipump, would be implanted at the time of primary tumor resection. This technology also has applications in the delivery of therapeutic agents to tumors that are unresectable because of inaccessibility or locations in eloquent brain tissue (14, 72).

Early studies focused on optimizing conditions for intracranial drug delivery. Chen et al. (25) studied the delivery of [^{14}C]albumin to the rat striatum and demonstrated that changes in the cannula size and infusion rate actually decreased the volume of distribution by increasing the amount

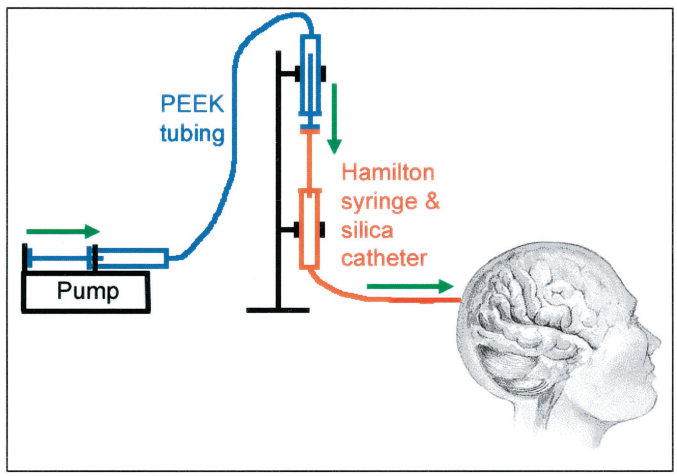

FIGURE 3. *CEDD. Infusate is delivered directly into the brain via high-flow infusion. With this continuous high-flow infusion technique, bulk flow is increased, producing interstitial convection and thus efficiently and homogeneously delivering drugs to large regions of the brain, without significant functional or structural damage.* PEEK, *polyetheretherketone.*

of back-leakage through the cannula. However, varying the actual concentration of the infusion had little effect on the volume of distribution or regional distribution. Groothuis et al. (48) indicated the superiority of CEDD, compared with intravenous administration, demonstrating a 10,000-fold increase in the concentration of [^{14}C]sucrose in rat brain and demonstrating that the distribution pattern after chronic CEDD infusion delivered a central component resulting from convention and a peripheral component in gray matter resulting from diffusion.

Oldfield and coauthors (69) demonstrated the feasibility and effectiveness of long-term interstitial brain infusion for the delivery of drugs on a multicentimeter scale in the primate brain, with more than one-third of the white matter receiving the infused compound. Accompanying perfusion studies demonstrated that the pressure gradient required for convective flow decreased blood flow by less than 5%, compared with noninfused regions of the brain. More recently, Bruce et al. (19) developed a rat glioma model of CEDD (which in their studies was referred to intracerebral clysis) to simplify their preclinical studies of potential therapeutic compounds amenable to delivery via high-flow infusion and to demonstrate the utility of convection-enhanced delivery of BCNU. Those investigators also recorded compelling data with topotecan administered via intracerebral clysis in their rat model (59). Topotecan, a camptothecin, is associated with dose-limiting side effects when administered intravenously (13), which makes it an ideal drug for local administration at high concentrations. Those authors demonstrated increased survival rates after intracerebral clysis-administered topotecan in their rat C6 glioma model, indicating the efficacy of a chemotherapeutic agent when toxicity is avoided with adequate local delivery methods (59).

One clinical trial of convection-enhanced delivery of chemotherapeutic agents has been reported. Oldfield and coauthors (70) reported on a series of patients with malignant gliomas for whom CEDD was used. In that trial, the authors investigated the efficacy of high-flow infusion of transferrin-CRM107, a conjugate of transferrin and a mutant diptheria toxin, for the treatment of 15 patients with malignant gliomas. Encouragingly, at least a 50% reduction in tumor volume was documented, with two complete responses during the study period. No systemic toxicity was noted, suggesting that this method of local high-flow infusion of chemotherapeutic agents may be used successfully as adjunctive therapy for the treatment of malignant gliomas (70).

Another application of direct intratumoral delivery is represented by intriguing work involving the administration of conjugated *Pseudomonas* toxins specifically designed to target glioma cells, as previously mentioned. One approach has taken advantage of previously reported selective expression of high-affinity IL-13 receptors on established glioma cell lines and primary glioblastoma cell cultures (29). In one study, a chimeric protein composed of human IL-13 and a mutated form of *Pseudomonas* exotoxin (termed IL-13-PE38QQR) was delivered intratumorally, yielding considerably higher local concentrations of cytotoxin than achieved with intravenous or intraperitoneal administration (55). Furthermore, in the U251 subcutaneous xenograft model, once-daily intratumoral injection of IL-13-PE38QQR led to complete elimination of established tumors for extended periods, with no associated toxicity. In that study, the novel chimeric protein was delivered via intratumoral injection and not convection. However, the observation that IL-13-PE38QQR acts with biological specificity, with few or no observed adverse effects, suggests that it could be ideally delivered via convection, which would sustain its delivery, maximize its diffusion throughout the tumor, and perhaps even improve on the results obtained with simple intratumoral injection.

The superiority of intratumoral delivery, compared with systemic delivery, of a novel exotoxin fusion protein was also demonstrated by Brem and coauthors (91), who also used a novel fusion protein whose efficacy might be improved with CEDD. They took advantage of differences in receptor expression between normal brain tissue and malignant glioma, noting the amplification and/or overexpression of EGFR. This differential expression was exploited for therapeutic purposes by using the well-known EGFR ligand TGF-α fused to the *Pseudomonas* exotoxin (designated TGF-α-PE38). In a nude mouse model with glioblastoma or medulloblastoma xenografts, a single intratumoral injection of TGF-α-PE38 led to increased survival rates for all xenografts tested, compared with intraperitoneal injection, with which notable increases in median survival times were observed only for tumors with the highest EGFR expression. The selective targeting of malignant glioma cells with recombinant toxin therapy, such as IL-13-PE38QQR or TGF-α-PE38, clearly represents an eloquent nontoxic therapeutic approach to these tumors. It can be imagined that a delivery modality such as CEDD, which has been proven to distribute molecules on a multicentimeter scale and at high concentrations via intratumorally implanted catheters, could optimize the efficacy of novel therapies such as these.

Interestingly, high-flow infusion delivery has not been limited to molecular delivery but has been applied to the delivery of gene therapy vectors (6, 27). The ability of CEDD technology to deliver viral vectors to brain tissue in sufficient quantities, compared with simple intracranial injection, was demonstrated in studies of adeno-associated virus delivery to monkey brain in a Parkinson's disease model (6). The potential applications of this technique for the distribution of viral vectors in the treatment of gliomas are clear.

CEDD not only circumvents the BBB but also is superior to simple diffusion in the distribution of agents throughout the brain, with apparently no significant side effects of high-flow infusion. The development of this technique thus centers on the identification of tumoricidal substances or glioma-specific molecules that can engage these tumors and compromise their malignant behavior while sparing normal brain tissue.

CONCLUSION

It seems that conventional modes of treatment for malignant gliomas have perhaps reached their asymptotic potential, inas-

much as the prognosis for these devastating tumors remains among the poorest for human oncological disease. Attempts at effective systemic adjuvant chemotherapy have been frustrated by difficulties in gaining access to target intracranial lesions because of the BBB, by the lack of tumor specificity of agents beyond the BBB, by the inability to achieve high intratumoral concentrations of administered agents, and by systemic toxicity. Therefore, therapeutic approaches involving direct delivery to the tumor site, which can circumvent the BBB, deliver agents directly to the tumor, achieve high local concentrations, and avoid damaging systemic toxicities, are ideal adjuvants in the treatment of brain tumors. Recent advances in a wide range of highly creative local-therapy approaches are destined to provide neurosurgeons with renewed hope regarding the treatment of malignant gliomas. We reviewed new advances in local-therapy approaches to the treatment of malignant gliomas, focusing on five therapeutic approaches whose common element is local delivery or, in some forms of immunotherapy, harnessing of the local peritumoral environment. Mechanistically, however, these approaches are considerably different. Bioreactor delivery, polymer release, and CEDD seek to creatively increase concentrations of antitumor drugs or molecules at the tumor site. Immunotherapy seeks to take advantage of humoral or cell-mediated immunity in the targeting of brain tumor cells; the compelling advantage of cell-mediated immunity is the potential of immune cells to track and destroy tumor cells. The immense potential of NSCs as delivery vehicles, with migratory and reparative capacities, for the treatment of malignant gliomas has been elegantly demonstrated, and further work should refine this exciting therapeutic approach. The numerous intriguing approaches with obvious local-therapy potential that are not discussed here include the use of small ribonucleic acid inhibitors.

Progress in adjunctive molecular therapies for malignant gliomas is likely to be rapid, introducing a true age of nanoneurosurgery, in which attacking gliomas on a molecular level will be just as important as surgical debulking. This new era should be just as influential as the age of microneurosurgery heralded by the introduction of the operating microscope. This review has focused on local glioma therapy delivered by neurosurgeons, but it is clear that fighting disease on a molecular level with agents delivered locally to diseased brain tissue by neurosurgeons is a broadly applicable concept. This approach is facilitated by the extensive participation of neurosurgical scientists in molecular biological and experimental therapeutic research on brain tumors and deserves to be a significant focus of neurosurgical oncology in the next decade. Which of these specific approaches provides the most flexibility in therapy, the greatest reversibility if necessary, and the greatest efficacy, while minimizing toxicity, should be clarified in the coming years. It also remains to be determined whether it will be possible to interest drug companies in these limited applications.

Of course, continuing refinement of molecular therapy is contingent on increasing our understanding of glioma pathogenesis, whose key molecular participants may then be targeted appropriately. We look forward to the day when we will complement our conventional therapeutic approaches with a cocktail of locally deployed therapeutic options. Perhaps one or a combination of these innovative techniques will begin to narrow the gap between our diagnostic and therapeutic capabilities.

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COMMENTS

This review by Dunn and Black elaborates on a very important aspect of modern treatment of malignant gliomas. During the past decade, a multitude of locoregional treatments have been offered to neurosurgeons to enhance the effect of cytoreductive surgery. Whereas neurosurgeons used to be restricted to resection, which was then followed by radiation and any other therapies the neuro-oncologists would administer, the neurosurgeon can now assume a much more active role in the local control of the disease. It has been recognized that during or at the end of resection, many modalities can potentially be brought into action, even if the first attempts at some, as in gene therapy, did not go beyond proof of principle, with still elusive efficacy. Others, like intracavitary chemotherapy, have been proved effective in three Phase III trials. Neurosurgeons are now, more than ever, therapists in the area of translational research. Unhampered by the many restrictions associated with the traditional oral or intravenous approach, we have to weigh new compounds by their prop-

erties, intracerebral toxicity, target selectivity, permeability through extracellular spaces, immunogenicity, and ease of handling. New interdisciplinary challenges such as the imaging of convection or the calculation of distribution volumes warrant close collaboration with neuroradiologists, specialists in the area of tumor physiology, and above all, neuropathologists, who can assess the neuronal and glial toxicity of our local targeted therapies. The capacity for single-cell invasion throughout the whole brain will still be the limiting factor for all our local therapies for a long time, but a sufficient number of studies indicate that improvement of local control should improve the outcome for patients with malignant glioma. Conversely, we have to realize that the invasive single malignant glioma cell clearly has a separate biology from the proliferating cell in the center of the tumor, and its biology will eventually offer therapeutic opportunities that most likely will be very separate from the treatment of the main neoplastic mass.

The authors of this review have summarized the current locoregional therapies for malignant glioma that are in the hands of neurosurgeons and that demand from the neurosurgeon who deals with malignant gliomas that he or she acquire more highly specialized knowledge to make these therapies available to the individual patient. From the exceedingly rapid developments in this field, it is to be expected that within the next decade, each patient will receive an individually selected additional locoregional therapy, either intracavitary, perilesional, or intratumoral, at some point during the resection of a primary tumor or a recurrence or during a stereotactic biopsy.

Manfred Westphal
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Dunn and Black provide an interesting look at some of the novel ways in which neurosurgeons are approaching the treatment of gliomas. It is refreshing to review some of the molecular techniques that are being applied to brain tumor therapy. One of the messages from this report is that science is joining the clinic to incorporate glioma-specific targets in the planning of future clinical trials.

This is a relatively new concept. Most of us have spent our careers learning how to remove lesions from the brain. One of the major accomplishments in neuro-oncology is the dramatic improvement in removing tumors with greater safety. Many tumors that in the past were considered inoperable are now candidates for aggressive surgical treatment. Moreover, surgeons can now learn how to put something back into the brain to address the infiltrative part of the lesion that previously was not accessible in the operating room. This is an exciting time in neuroscience and in neuro-oncology. This report demonstrates why.

Joseph M. Piepmeier
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In the past, neurosurgeons remained on the periphery of therapeutic neuro-oncology. We watched as our colleagues manipulated various drugs and combination-type protocols to attempt control of malignant glioma progression. As we all know, this has met with very little success. During the past decade, we have turned our attention to overcoming the limitations of systemic administration by delivering various compounds or constructs locally into the tumor cavity itself. The dawn of this era began with local implantation of chemotherapy polymers, which to date has had only a very modest effect on the outcome of patients with malignant gliomas. Also, at the beginning of the use of local therapeutic strategies, we became clever enough to administer gene therapy constructs into tumor cavities, either by direct injection or through Ommaya reservoirs. This, too, has offered very little benefit, except in sporadic cases.

As we move forward in this effort, in which neurosurgeons become more involved with locoregional control of malignant gliomas, we have developed new strategies to embark upon. Perhaps the most promising is convection-enhanced delivery, in which surgeons insert small delivery catheters into the periphery or center of a tumor and attempt to infuse interstitially small molecules that selectively target key growth-regulatory pathways. One of the early trials in this approach involved the application of immunotoxin therapy, in which the *Pseudomonas* exotoxin was coupled to the commonly expressed interleukin-4 and interleukin-13 receptors seen on gliomas. This has actually met with more than minimal success, and the ability to deliver these toxins not only to the tumor but also to the periphery has been quite good. This strategy will take on a new dimension as we begin to test the feasibility of direct delivery of small molecules that block aberrant signaling pathways. This also leads the way for other creative local delivery strategies, including placement of alginate bioreactors and liposomes into the tumor cavity. Modulation of stem cells can be used to potentially deliver effector molecules that can once again either destroy tumor cells through apoptosis or immune modulation or regulate key pathways that block invasion, proliferation, and differentiation. My expectation is that as we advance in our understanding of the key pathways that regulate cell growth in gliomas, we will learn that perhaps the only way to modulate those pathways is to directly deliver a small molecule that blocks the pathway when administered locally as opposed to systemically. Perhaps this strategy, in combination with some of the other delivery methods described in this article, will give us the "magic bullet" that we have all desperately hoped for but have not achieved to date.

Mitchel S. Berger
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Dunn and Black have written a review on "nanoneurosurgery," a term coined by Dr. Michael Apuzzo. In their review, the authors focus primarily on the perceived utility of nanoneurosurgery in the arena of the surgical neuro-oncologist. This excellent review focuses on novel technologies as they are evolving, including bioreactors, neural stem cell therapy, immunotherapy, biodegradable polymer drug release, and convection-enhanced drug delivery. In this way,

neurosurgeons can easily identify with rapidly evolving technologies that predict that the scalpel will one day be placed in the archives while these newer instruments are being wielded for the benefit of our patients. It is not inconceivable that some 50 years from now, a craniotomy for a malignant tumor will be a procedure of the past. In fact, I look forward to that day! This review by Dunn and Black gives much food for thought in this regard.

James T. Rutka
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Exploring the potential of local treatments is very appealing for gliomas, which are aggressive, infiltrative, but nonmetastatic neoplasms. In their review, Dunn and Black provide the reader with an impressive amount of updated information concerning the primary strategies currently being developed to deliver novel therapeutic tools locally. However, we would like to open some points of controversy.

The concept that tumors need to generate their own vessels to promote their growth and the seminal initial observations made by Folkman's team have opened a new avenue to attack tumors (4). Gliomas are certainly an appropriate target for this novel strategy, considering the definitive importance of unbalance between proangiogenic and antiangiogenic factors in their development (5, 7). This is further supported by the interesting results obtained by the authors in their animal models using capsules releasing endostatin (3). However, this enthusiasm should be tempered by the recent disappointing results obtained in the first Phase I studies (6). Perhaps we should keep in mind that the manipulation of the immune system (even with nonspecific tools such as interleukin-2) is very efficient in animal models, whereas the real impact for patients is low, despite several decades of clinical research. The extrapolation from animal models to the human setting is a long and difficult way.

Another point of controversy is the role of the blood-brain barrier (BBB), which is presented by Dunn and Black as a strong argument favoring local delivery of "immune tools." However, the BBB is no longer seen as a static, impermeable barrier but rather as a filter that is subtly regulated, allowing the fine-tuning of the recruitment of immune cells (2). Indeed, there is now cumulative experimental and clinical evidence that activated lymphocytes may enter the brain across the BBB and that antigen-specific T cells elicited in the periphery (i.e., out of the central nervous system) may exert their cytolytic functions against tumors located in the brain (9, 10). The BBB is not an obstacle to systemic immunotherapy. The main challenge, which is not discussed by the authors, is to obtain a sufficient level of specificity to generate maximal antitumor effects without collateral damage to the normal cells (9).

Finally, we do not share the optimistic view of the authors about carmustine-impregnated wafers. They write that ". . . Gliadel represents the most successful introduction of a novel local-therapy approach," although on close scrutiny, the data are rather weak. Indeed, there are only two randomized studies that have been published as full-length articles. The first one was reported in the *Lancet*, claiming a survival advantage for carmustine wafers compared with placebo for patients with relapsing brain tumors (1). However, one should be aware of the perfectly overlapping survival curves for both treatment groups shown in *Figure 1* of this article. Because of an imbalance of prognostic factors in the two groups, a new analysis was performed to "adjust" the results in a second step that was not planned in the initial design of the study and that artificially allowed achievement of a statistical significance with an approach that was obviously not an intent-to-treat analysis. In the second study (8), designed to include 100 patients with glioma at the time of primary operation, only 32 patients were actually included (because the drug was unobtainable). A statistical analysis performed under such conditions is not valid. Furthermore, the inclusion criteria allowed the recruitment of patients with different diagnoses, and the placebo group included 16 glioblastoma patients, whereas the carmustine wafer group included 11 glioblastoma patients but also 2 patients with anaplastic astrocytomas, 2 with oligodendrogliomas, and 1 with an ependymoma (the survival of whom is obviously not identical to that of glioblastoma patients). Considering the low number of events in both groups, such an imbalance renders any interpretation of the data unacceptable. Thus, we believe that there is no correct evidence supporting the use of carmustine wafers. However, we agree with the authors that the search should continue to find more powerful candidate therapeutic agents that should take advantage of previous errors.

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