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Convection-enhanced delivery of immunomodulatory therapy for high-grade glioma

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Abstract

The prognosis for glioblastoma has remained poor despite multimodal standard of care treatment, including temozolomide, radiation, and surgical resection. Further, the addition of immunotherapies, while promising in a number of other solid tumors, has overwhelmingly failed in the treatment of gliomas, in part due to the immunosuppressive microenvironment and poor drug penetrance to the brain. Local delivery of immunomodulatory therapies circumvents some of these challenges and has led to long-term remission in select patients. Many of these approaches utilize convection-enhanced delivery (CED) for immunological drug delivery, allowing high doses to be delivered directly to the brain parenchyma, avoiding systemic toxicity. Here, we review the literature encompassing immunotherapies delivered via CED—from preclinical model systems to clinical trials—and explore how their unique combination elicits an antitumor response by the immune system, decreases toxicity, and improves survival among select high-grade glioma patients.

Keywords:

convection-enhanced delivery | drug delivery | glioma | immunotherapy

Glioblastoma is the most common primary brain tumor in adults. Despite standard of care (SOC) treatment including surgery, radiation, and alkylating agents, survival after diagnosis is typically fewer than 20 months.[1] SOC for glioblastoma has not changed significantly since 2005, when temozolomide was added. Together with improvements to radiotherapy, imaging and intraoperative mapping, temozolomide increased the median survival from 12 to 16 months.[2,3] However, because SOC treatment remains hindered by poor drug penetration into the central nervous system (CNS), systemic toxicity, and the immunosuppressive glioma microenvironment, outcomes for patients with glioblastoma remain poor despite numerous clinical trials since 2005.[4]

Convection-enhanced delivery (CED) addresses the limitations of systemic drug delivery and bypasses the blood–brain barrier (BBB) by providing a method of local intratumoral delivery. Using a pressure gradient generated by positive pressure, CED optimizes the volume of distribution and uniformly infuses macromolecules into a localized area of the brain via a catheter-connected pump.[5–7] Compared with diffusive delivery, the distribution of macromolecules via CED is unaffected by size and molecular weight.[8–10] Initially, the volume of distribution is linearly related to the volume infused by the catheter. However, preclinical studies have shown that over prolonged infusions this relationship changes as clearance and infusion reach equilibrium.[11–13] Typically, the device delivers a mostly spherical distribution of therapy at a rate of 0.5 to 10 μL/min.[5,14] Compared with a single injection technique, which distributes therapy 5 mm from the catheter tip, CED distributes therapy up to 6 cm from its tip, a 4000-fold increase in the volume of distribution.[5,14,15] By circumventing the BBB, CED allows for far greater concentrations to enter the brain parenchyma, compared with intravenous delivery, while eliminating dose-related toxicity.[16,17]

In addition to the BBB, glioblastoma remains difficult to treat due to its immunosuppressive microenvironment.[18]

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Mechanisms underlying poor immune response within glioblastoma remain partly unclear, but both host response and tumor-intrinsic features play important roles. The presence of immunosuppressive myeloid cells and few infiltrating T cells within the tumor, combined with low tumor mutational burden, hinder immunogenicity.[18–20] Furthermore, typical therapies given to glioblastoma patients, including alkylating chemotherapy, radiotherapy, and steroids, contribute to systemic and local immunosuppression.[21]

Given the challenges associated with generating an effective antitumor response, immunotherapies, such as oncolytic viruses and nucleotide-based therapies, have been proposed as potential methods to help turn the immune system against the tumor. These immunotherapies target local interactions within the glioma microenvironment like tumor–myeloid and tumor–lymphoid interactions. Thus, local delivery of immunotherapies via CED allows for the most direct delivery of immunotherapies to their intended targets.

In addition, by bypassing the BBB, CED allows for delivery of more precise concentrations of drug to the brain.[22] Precision is crucial as certain immunotherapies, like oncolytic therapies, operate within an infectious window as narrow as 10^2 infectious particles, where too large of a dose may lead to life-threatening adverse events.[23] Similarly, CED avoids toxicities associated with systemic delivery of highly inflammatory therapies. For example, systemic delivery of IL-12 transgene, a potentially potent antitumor therapy, is associated with severe adverse events including life-threatening hemodynamic instability.[24] Thus, delivery of IL-12 via CED has been proposed to avoid such toxicity.[25] Lastly, manual placement of the catheter allows for direct targeting of the tumor bed following resection.

In this review, we address 2 major obstacles in glioblastoma—the immunosuppressive tumor microenvironment and poor drug penetrance. We examine the use of CED to deliver immunomodulatory therapies for the treatment of glioma, which include viral therapies, cytokine therapies, nucleotide-based therapies, monoclonal antibodies, and nanoparticles (NPs) (Figure 1). We will discuss the benefits that local delivery of these immunotherapies via CED imparts, as well as its limitations and side effects, and how these therapies can shape the future treatment landscape of glioblastoma.

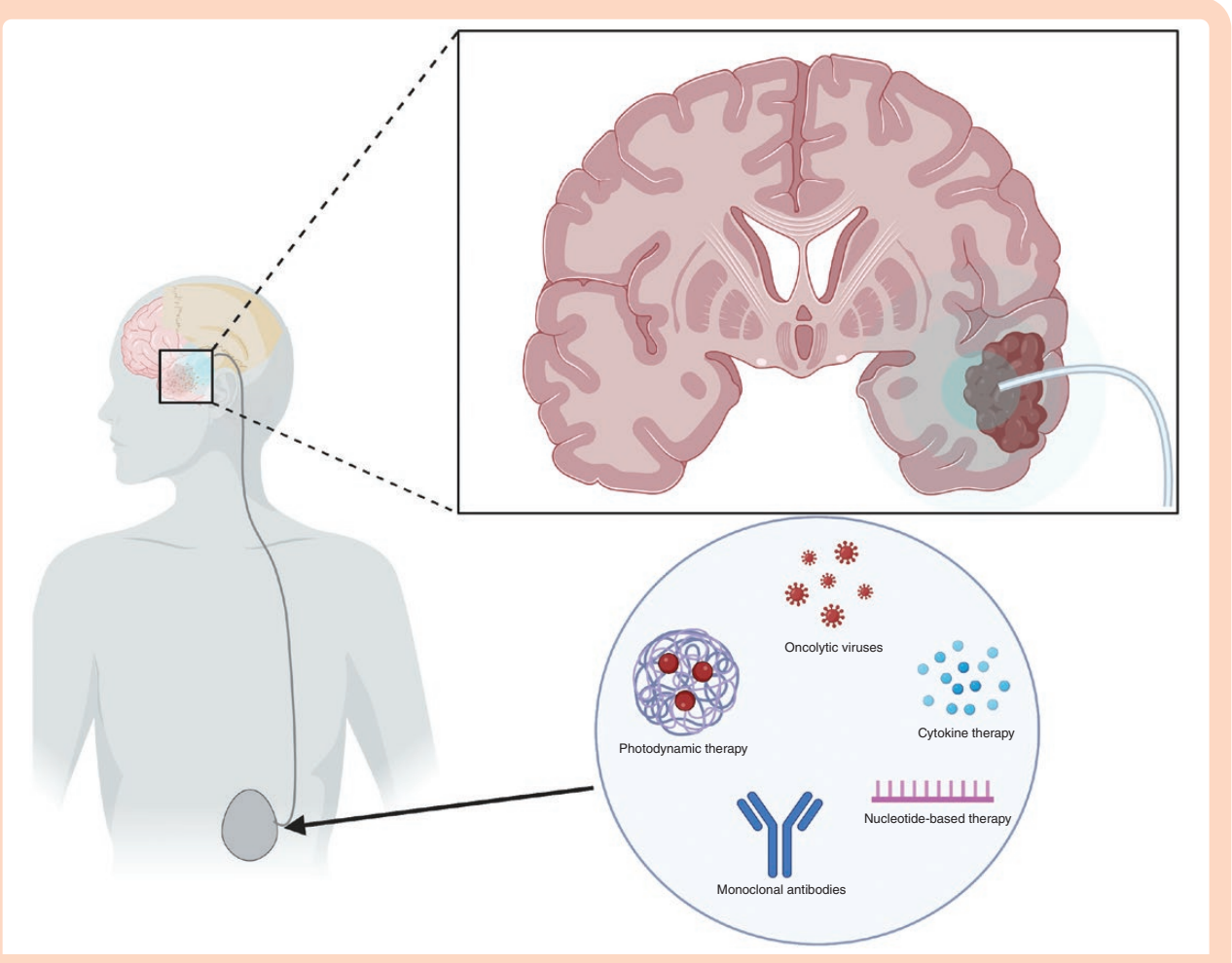

Figure 1. Diversity of immunotherapies delivered via CED in high-grade glioma.

Oncolytic Viruses

Viral therapies may be used in a multitude of ways to treat glioblastoma.[23,26–31] First, viruses incapable of replication can be used to deliver transgenes that augment the antitumor immune response via expression of cytokines, receptors, and other immune-stimulatory molecules.[31] Second, oncolytic viruses with intact replication capabilities can enter tumor cells via neoplastic cell surface markers and undergo robust viral replication that leads to tumor cell lysis.[23] In this way, oncolytic viruses can both generate a direct antitumor cytotoxic effect and serve as a proinflammatory modulator, via the tumor cell lysis-induced extracellular release of immunogenic tumor-associated antigens, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns.[32–34] Systemic delivery of oncolytic viruses faces multiple hurdles. Following intravenous injection, viruses infect off-target cells like blood cells, become neutralized by preexisting antibodies, and activate complement.[35–37] They are also sequestered in other organs, leading to toxicity, and later become cleared by specialized cells in lungs, liver, and the spleen.[36] Intratumoral injection avoids many of these challenges. However, deep tumors, like glioma, can be difficult to access, and repeated dosing via intratumoral injection is also challenging.[38] Further, large viral loads, leading to robust replication in a specific area, can lead to adverse responses carried out by the host immune system such as tumor swelling, fever, headache, and vomiting.[39] However, using CED, access to a deep tumor bed is accessible via a catheter. CED avoids the need for repeat procedures as delivery is continuous over a set period of time, and lastly, continual dosing avoids large viral boluses that may lead to adverse reactions.[38]

PVSRIPO is a live attenuated poliovirus vaccine with its internal ribosomal entry site replaced with that of human rhinovirus. PVSRIPO has been studied as a potential treatment for glioblastoma.[23] The benefits of PVSRIPO are 2-fold—they lyse tumor cells, while activating dendritic cells (DCs). Infection requires presence of CD155, which is broadly expressed in both tumor and antigen-presenting cells (APCs).[40,41] Infection of tumor cells results in cytotoxicity, release of tumor-associated antigens and activation of antiviral interferon signaling via release of dsRNA.[42] Meanwhile, infection in DCs leads to activation, expression of costimulatory molecules, type I IFN response, and proinflammatory cytokine production. This DC phenotype eventually promotes stimulation of T cells in vitro.[42] Furthermore, PVSRIPO is incapable of replicating in neurons, and thus spares healthy brain tissue.[23,43]

Desjardins et al. conducted a Phase I dose-escalation study of PVSRIPO via CED in 61 patients with recurrent glioblastoma (Table 1).[23] Their primary goal was to assess the toxicity profile and determine appropriate dosing for a Phase II trial.[23] Only 1 patient experienced a dose-limiting adverse event. The median overall survival (OS) of those who received PVSRIPO was 12.5 months (95% CI 9.9–15.2) compared with 11.3 months (CI 9.8–12.5) among the historical control group.[23] Long-term survivors in this study lived as long as 57 months post-treatment.[23] Preliminary studies examining lymphocytes in the peripheral blood of those long-term survivors demonstrated a significant reduction in immunosuppressive regulatory T cells. However, specific T cell markers among this immunosuppressive population were not reported. One patient, who received lomustine after recurrence of disease following PVSRIPO, experienced reconstitution of effector T cells 4 weeks following lomustine administration. Typically, continual cycles of chemotherapy can lead to significant lymphodepletion and dampened immune responses.[23] Thus, this patient's experience unveiled a benefit of combining single-cycle chemotherapy and PVSRIPO vaccination that may have otherwise been lost with multiple cycles of chemotherapy.[23] This

Table 1. Completed Clinical Trials of Immunotherapies Delivered via CED in High-Grade Glioma

Author	Year	Agent	Phase	Identifier	WHO Grade, Tumor Type	Number of Patients	Number of Catheters	Flow Rate	Volume of Infusion (mL)	Duration	Median OS (Weeks)
van Putten et al.	2022	Delta24-RGD	I	N/A	Recurrent Gr IV GBM	20	4	0.2–0.3 mL/h	N/A	2–3 days	18.4
Friedman et al.	2021	G207	I	NCT02457845	Recurrent Pediatric HGG	12	3–4	N/A	N/A	6 hours	48.8
Desjardins et al.	2018	PVSRIPO	I	NCT01491893	Recurrent Gr IV GBM	61	1	0.5 mL/h	3.25	6.5 days	50
Bogdahn et al.	2010	TGFB2 inhibitor	IIb	NCT00431561	Recurrent Gr IV GBM (103) and Gr III AA (42)	145	1	0.24 mL/h	40	7 days	36.4 (GBM)
Carpentier et al.	2010	CpG-ODN	II	NCT00190424	Recurrent Gr IV GBM	34	2	3.3 µm/h	2	6 hours	28
Hau et al.	2007	TGFB2 inhibitor	I/II	N/A	Recurrent Gr IV GBM (20) and Gr III AA (5)	25	1	0.5 mL/h	N/A	7 days	44 (GBM)
Carpentier et al.	2006	CpG-ODN	I	N/A	Recurrent Gr IV GBM	24	1–2	0.2 mL/h	N/A	6 hours	28.8

CED, convection-enhanced delivery; OS, overall survival.

Table 2. Clinical Trials of Immunotherapies Delivered via CED in High-Grade Glioma Currently Underway

Principal Investigator(s)	Agent	Phase	Identifier	Status	WHO Grade, Tumor Type	Number of Patients	Start Date	Completion Date
Vogelbaum	Anti-CD29	I	NCT04608812	Recruiting	Recurrent Gr IV GBM and Gr III AA	24	March 2021	April 2024
Landi	Anti-CD40	I	NCT04547777	Recruiting	Recurrent Gr IV GBM and Gr III AA	30	July 2021	December 2025
Landi, Thompson	PVSRIPO	Ib	NCT03043391	Active	Recurrent Pediatric HGG	12	December 2017	March 2022
Randazzo	PVSRIPO	II	NCT02986178	Active	Recurrent Gr IV GBM	122	June 2017	December 2023
Istari Oncology	PVSRIPO	II	NCT04479241	Active	Recurrent Gr IV GBM	30	October 2020	March 2023

CED, convection-enhanced delivery.

same patient remained disease free for 20 months after completion of chemotherapy and remained alive as long as 57.5 months after PVRIPO infusion. Potential benefits of PVSRIPO plus lomustine among a larger cohort are not immediately obvious, and expectations of efficacy should be tempered given that the response was only seen in 1 patient. However, this finding from the single responder did inspire the Phase II trial (NCT02986178), involving PVSRIPO alone and in combination with a single cycle of lomustine, which is expected to be completed in 2023.[23] Adverse inflammatory responses were common among patients. Over half of those treated with PVSRIPO required bevacizumab to reduce peritumoral inflammation.[23] Additionally, a Phase I trial in recurrent pediatric high-grade gliomas (NCT03043391) has been completed with results submitted as of June 2022 (Table 2).

Delta24-RGD (DNX-2401) is another replication-competent oncolytic adenovirus tested in recurrent glioblastoma.[27,44] This virus lacks 24 base pairs in the E1A region, disabling it from replicating in normal cells, while enabling it to replicate in cells with a dysfunctional Rb pathway.[45] Over 90% of gliomas have a dysfunctional p16/Rb pathway, which makes DNX-2401 a compelling therapy for glioma.[46,47] Further, the typical primary attachment site, Coxsackie Adenovirus Receptor, is substituted for avb5 and avb3 integrin receptors, which are both expressed highly on glioma cells.[48,49] Similar to other oncolytic viruses, infection results in direct oncolysis, proinflammatory signaling, and T cell activation.[50]

In a Phase 1 study by Lang et al., 25 patients with recurrent glioblastoma received DNX-2401 via single intratumoral injection, and 5 patients survived longer than 3 years.[27] Expanding upon this, van Putten et al. administered DNX-2401 via CED to 19 patients with recurrent glioblastoma.[44] The goal of this study was to assess safety, and infusion of DNX-2401 via CED was shown to be safe.[44] 14 of the 19 patients experienced serious adverse events (SAEs) due to increased pressure from either edema or viral meningitis, though these symptoms were temporary.[44] Meningitis was thought to be due to suboptimal catheter placement causing backflow. At the time of the study, improved catheters—dedicated to reducing backflow—were not yet available.[44] Two patients in this study experienced long-term survival with 1 patient remaining tumor free for 8 years without secondary treatment. Among those patients who survived more than 6 months following viral infusion, the majority had increasing levels of viral DNA in their cerebrospinal fluid (CSF) up to 4 weeks following infusion.[44] Immunostimulatory chemokines and cytokines were present in the CSF of most patients following treatment, indicating the immune system was eliciting an antitumor response.[44] To study the effects of inflammation on survival, patients were split into 2 groups based on CSF IFN-gamma levels 4 weeks after treatment. High CSF IFN-gamma levels significantly correlated with almost all measured cytokines and chemokines as well as the presence of CD8+ T cells in the CSF. Lastly, patients within the high IFN-gamma subgroup survived longer than those within the low IFN-gamma subgroup.[44]

Similarly, G207, a genetically engineered herpes simplex virus type 1 (HSV-1), is another oncolytic virus designed to replicate selectively within tumor cells. This selectivity is due to deletion of the diploid γ134.5 neurovirulence gene and inactivation of the ribonucleotide reductase via insertion of *Escherichia coli lacZ*.[29] In addition to its cytotoxic effects, G207 has been shown to increase antigen cross-presentation and induce antitumor immunity.[51,52] A previous Phase I trial in adults with glioblastoma demonstrated that a stereotactic injection of G207 was shown to be safe and led to a partial response or stable disease in a majority of patients with a median OS of 2.5 months.[28,53,54] A Phase I trial in 12 pediatric patients with high-grade glioma delivered G207 treatment via CED. The primary objective was to asses safety, and G207 therapy via CED in this pediatric population was determined to be safe.[29] Median OS was 12.2 months, and 4 patients remained alive 18 months after treatment.[29] Clinical, neuropathological, and radiographic responses were noted in 11 patients. There were no attributable SAEs or dose-limiting effects. Furthermore, no neurological effects due to the surgical procedure were observed.[29]

In the DNX-2401, PVSRIPO, and G207 trials, select groups of patients demonstrated a long-term benefit. However, these studies were uncontrolled, and thus lack

the analysis that well-designed randomized controlled trials offer. Given these early successes, it is paramount that future studies center upon rigorous controlled trials with these virotherapies. Further, more in-depth tissue-based analyses of these responders' tumors are required to better understand the potential antitumor response levied by the immune system.

Nucleotide-Based Therapies

Increased TGFB-SMAD signaling has been proposed as an important contributor to the immunosuppressive microenvironment as well as progression in glioblastoma.[55–57] It is hypothesized the TGFB2 is a key contributor to T cell hyporesponsiveness, especially having an effect during later tumor stages.[58] Inhibition of TGFB2 reverses tumor-induced immunosuppression and inhibits growth and invasion.[59,60] Trabedersen, also known as OT101 or 12900, is an anti-TGFB2 RNA oligodeoxynucleotide designed to block the effects of TGFB2.[61]

Preclinical data using human-derived glioma cell lines revealed that treatment with trabedersen resulted in decreased secretion of TGFB2, decreased tumor cell proliferation, decreased migration, and increased cytotoxic activity among autologous immune cells.[62] Nevertheless, these studies were solely in vitro, and further studies, for example demonstrating therapeutic efficacy using an in vivo animal model, were never published.

Despite the limited preclinical evidence, a number of clinical trials investigated the effects of trabedersen via CED in glioblastoma and anaplastic astrocytoma.[62,63] In the largest trabedersen study, a Phase IIb trial enrolled 145 patients with recurrent glioblastoma or anaplastic astrocytoma. The primary goal of this study was to identify the appropriate dose for future trials, while also comparing its safety and efficacy to standard chemotherapy. 10 μM trabedersen was determined to be safe.[63] However, trabedersen failed to demonstrate a significant difference in the primary endpoint, tumor control rate, after 6-month treatment of trabedersen via CED when compared with 6 months of standard chemotherapy.[63] However, assessment of tumor response early after treatment may not be the most appropriate endpoint as immunotherapies, which rely on building immune response over time, may have a different time-course of response as compared with fast-acting chemotherapies.[64] Again, among the entire population at 2 years, 10 μM trabedersen (39%) conferred a nonsignificant survival benefit compared with standard chemotherapy (22%). Previous research has shown that the immune response driven by trabedersen continues to improve months to years after trabedersen treatment has concluded.[59,60] Thus, it is unsurprising that any potential survival benefit the authors appreciate would occur at later time points. Further, the authors performed exploratory, un-prespecified, post hoc subgroup analyses, which revealed that glioblastoma patients ≤55 years old with a Karnofsky performance status >80% had a 2-year survival rate of 40% compared with 13% among those who received standard chemotherapy.[63] Notably, the authors perform no tissue-based analyses on post-treatment tumor to assess immunogenic response of trabedersen. It is also important to note that the patients in the control group underwent *systemic* delivery of the conventional chemotherapy, and therefore did not have a CED catheter implanted. Neurological adverse events (AEs) were more common among those who received trabedersen, and it was speculated that the mode of administration, CED, may have played a role. However, these AEs were manageable, and may be further reduced by improved training of the investigators.[63]

Further post hoc analysis of the same study (NCT00431561) was later completed.[65] The authors found 26 of 89 patients treated with trabedersen experienced a favorable response, defined by complete response, partial response, or stable disease ≥6 months.[65] Positive outcomes were slow to develop. Robust size reduction required a median time of 11.7 months, and, among the 19 complete or partial responders, response required a median of 287 days.[65] Lastly, among those with a favorable response, over one-third experienced >3-year progression-free survival (PFS) and >3.5-year OS.[65] Again, it is also worth noting that the authors failed to include any pharmacodynamic data found in humans. Their final conclusion aligns with findings similar to other immunomodulatory therapies delivered via CED—that trabedersen leads to a durable response among a subgroup of patients. Given that these analyses were exploratory and post hoc, and therefore not powered sufficiently, it is critical to take these results, as well as any conclusions drawn from them, with degrees of caution and skepticism.

Oligodeoxynucleotides containing unmethylated cytosine-guanosine motifs (CpG-ODN) have been designed to stimulate Toll-like receptor 9 (TLR9). Toll-like receptors (TLRs) are receptors encoded to recognize DAMPs shared by foreign microbes as well as endogenous molecules released during inflammation.[66] TLR9 is mainly expressed by APCs, including microglia.[67] CpG motifs have been demonstrated to induce secretion of IL-2, IL-12, and IFN-gamma.[68] In preclinical studies of glioma, treatment with CpG-ODNs increased markers of antigen presentation on microglia, shifted the immune system toward CD8+ T cells, and decreased the number of regulatory T cells. Further, treatment with CpG-ODNs induced caspase-3-dependent apoptosis among tumor cells (Table 1).[69]

An early Phase I trial delivering CpG-ODNs via CED to recurrent glioma patients demonstrated safety.[70] The following Phase II trial was designed to assess efficacy. Among their cohort of 31 patients treated with CpG-ODNs via CED, the PFS at 6 months was a modest 19%.[71] Multiple theories may explain this study's underwhelming results. Investigators speculated poor immune cell infiltration and an associated low level of TLR9 expression within the tumor.[71] Further, they did not see the hypothesized downregulation of regulatory T cells in those patients who had frozen leukocyte samples.[71] The overwhelming majority of patients received steroids during their treatment, which may have contributed to increased regulatory T cells and created an immunosuppressive tumor environment.[71,72] The final explanation, and probably the simplest, is that, despite increased antigen presentation in the setting of CpG-ODN treatment, these antigens failed to be recognized as foreign by T cells.[73]

Despite disappointing results in both CpG-ODN and trabedersen studies, CED provides unique benefits to the delivery of nucleotide-based therapies. Intratumoral injection is the most common application among solid tumors.[74] Compared with systemic delivery, intratumoral injection most obviously bypasses the BBB and reduces chances of off-target effects. However, nucleotides can be unstable and have poor cellular uptake, requiring multiple injections that can be difficult for the surgeon and dangerous for the patient.[75] Thus, CED, with its continual delivery over a set period of time, provides a steady stream of stable nucleotides, increasing the likelihood of sufficient cellular uptake.

Monoclonal Antibodies

Monoclonal antibodies are the most commonly used and approved immunotherapies in the treatment of cancer.[76,77] They have 3 typical mechanisms of action (1) inhibition of receptors or factors that activate tumorigenic signaling; (2) antibody-mediated cytotoxicity; (3) complement-mediated cytotoxicity.[78] Their efficacy is primarily based on 2 components: the affinity of the Fv region for the target and the ability of the Fc region to bind to the host immune system.[78]

Similar to systemic delivery of most therapies to the brain, monoclonal antibody-based therapies have poor penetrance of the BBB and off-target effects when delivered systemically.[79] Intratumoral injection eludes these challenges. However, intratumoral injection is still limited by poor diffusion and distribution of both small and large molecules, including monoclonal antibodies. Conversely, CED improves both diffusion and distribution by maintenance of a constant pressure gradient.[5]

CD40, a tumor necrosis factor family member, is found on glioma cells in vivo and in vitro, as well as on APCs and DCs.[80,81] CD40 ligand (CD40L) is expressed on activated T cells, macrophages and platelets. Crosslinking between CD40 and CD40L has been reported to induce activation of B cells and DCs.[82] This early work generated interest in CD40 as a target for immunotherapy. Anti-CD40 agonistic mAb, FGK45, has been shown to induce activation of APCs and antitumor T cells while vaccination with FGK45 prolonged survival in a mouse glioma model.[81]

Shoji et al. demonstrated that delivery of CD40 mAb via CED was efficacious when treating 2 distinct glioma stem cell tumor models in mice. These models, NSCL61 and bRiTS-G3, are typically more resistant to treatment as they are stem cell like. In these models, both apoptosis and infiltration by CD4+/CD8+ T cells were noted, indicating the cytotoxic and immunostimulatory effects of anti-CD40 therapy.[83] Using immunocompromised mice, there was no benefit with anti-CD40 compared with IgG, highlighting the importance immunomodulatory effect.[83] Previous research has shown significant cytokine-release syndrome with systemic administration of anti-CD40, limiting the maximum tolerated dose and leading to poor efficacy.[84–86] However, in the Shoji et al. in vivo CED model, the maximal dose of refinement, 1 μg/μL, was associated with minimal tissue toxicity and neurological side effects (Table 2).[83]

Photodynamic Therapy

Photodynamic therapy (PDT) is a novel technique by which diseased cells are damaged by irradiation of a photosensitizing drug, causing generation of superoxide anion radicals and reactive oxygen species.[87] This reaction leads to cell death, via apoptosis or necrosis, and also immunogenic cell death (ICD), releasing tumor-derived protein antigens, tumor-associated antigens, and DAMPs.[87,88] Signals released in the setting of ICD stimulate both a local innate inflammatory response and recruit local and peripheral immune cells. Further, they promote engagement through phagocytosis and antigen presentation, eventually polarizing these immune cells toward an antitumor phenotype.[89] Thus, PDT induces both cytotoxic and immunogenic effects. However, PDT, when treating glioblastoma, is plagued by 2 issues. First, PDT is reliant on the presence of oxygen to produce oxygen radicals. However, advanced solid tumors are inherently hypoxic.[90] Second, there is poor delivery efficiency of PDT to the brain.[91,92]

Sunil et al. set out to design an NP that directly addresses these 2 issues. First, they incorporated an oxygen-generating enzyme to allow for the production of reactive oxygen species (ROS) by Protoporphyrin IX (PpIX), their photosensitizing drug. Second, they delivered this NP via CED, which allows for 1000-fold greater concentrations compared with intravenous delivery and supplies an even volume of distribution without leakage or reflux compared with wafers or intraneoplastic injection.[17,88,93] In addition to PpIX, they included Nutlin-3a, which induces apoptosis, cell-cycle arrest, and promotes antitumor immunity.[94] Lastly, their payload featured an amphiphilic polymer brush composed of phosphatidyl ethanolamine and polyethylene glycol to retain tumor antigens, improving the likelihood of antigen uptake.[94] Their early in vitro work demonstrates in situ oxygen generation, cell death, antigen retention, and lymphocyte activation, but their work remains in the in vitro stage as they continue to optimize for CED duration.[94]

Both Sunil et al. and Atik et al. employ NPs in order to improve delivery of their drug of interest. NPs were first brought into the adult-type diffuse glioma armamentarium in the 1990s in an effort to develop drugs with better efficiency in crossing the BBB, avoiding degradation and prolonging circulation.[95] However, a wide-array of varying NPs have been reported in recent literature.[88,96–110] Loosely, NPs are defined as inorganic or organic carries ranging from 1 to 1000 nm in size. Ideally, these substances are nontoxic, biodegradable, compatible with tissue biology, less than 100 nm in size and positively charged in order to cross the BBB. The molecule of interest is then associated in some way—whether its dissolved, attached, dispersed within, encapsulated—with the NP. NPs play an important role in augmenting CED in the treatment of adult-type diffuse glioma. A major setback in the use of CED is the short half-life of drugs, most commonly chemotherapies, as they are infused and often quickly cleared.[110] However, association with NPs can lead to a controlled release of these drugs, increasing their half-life. These NPs may also be designed to limit release specifically to diseased tissue, saving healthy brain.[110] Several studies have shown enhanced

potential of CED when delivered therapies incorporate NPs to treat adult-type diffuse glioma.[111–113] Although still working to develop preclinical efficacy, Sunil et al. and Atik et al. capitalize on the compounded benefit of 2 powerful delivery systems—CED and NPs.[88,109]

Cytokine Therapy

Cytokine therapy may be a potent inducer of the immune system, but when given systemically, it poses a large risk for AEs.[114] Using a rat glioma model, Frewert et al. continuously infused IFN-gamma or IL-1B for 48 hours via CED followed by sacrifice. IFN-gamma has been shown to increase expression of major histocompatibility complex I (MHC I), activate NK and T cells, and decrease tumor cell growth.[115,116] Stains of rat brains showed statistically significant increases in markers of macrophages, APCs, CD4+ and CD8+ T cells at the tumor margin compared with vehicle treatment.[117] The presence of lymphocytes and APCs at the margin suggests that tumor antigen is being recognized and presented by invading microglia.[117]

Gene therapy, and specifically cytokine therapy, likely require sustained expression to induce tumor regression.[118] Viral vectors have been shown to promote sustained expression with just a single administration.[119–121] However, viral vectors have limited therapeutic potential, mainly due to limitations in large-scale manufacturing.[118] In contrast, nonviral vectors typically require multiple administrations to overcome their associated transient expression.[122,123] Wu et al. sought to improve sustained expression of gene therapy in a nonviral vector. Their previous work revealed that the Sleeping Beauty (SB) transposable element integrates into the host chromosome and facilitates prolonged expression in human glioblastoma xenografts.[124] Mice bearing GL261 glioma were treated with vectors containing IFN-gamma cDNA with and without an SB-transposon element via CED. Mice treated with IFN-gamma cDNA and the SB element exhibited expression for 3 weeks, while those mice treated with IFN-gamma cDNA and without the SB element did not express IFN-gamma past 1 week. Further, only SB-treated mice displayed tumor regression, and survived significantly longer than non-SB-treated mice.[118] Interestingly, PCR data revealed increased endogenous expression of IFN-gamma. This may be explained by SB-mediated IFN-gamma-induced activation of APCs, phagocytosis, antigen presentation and T cell activation.[118] This was further supported by lymphocyte infiltration in IFN-gamma-treated mice.[118] Thus, SB-mediated IFN-gamma expression may create a positive feedback loop promoting activation of the adaptive immune system in in vivo models.[118]

Limitations and Alternatives to CED

CED has several limitations that require further development. Backflow remains 1 major challenge of CED.[8] Backflow results in exit of drug from the target tissue and spread to locations such as normal brain and CSF spaces, potentially leading to severe immune stimulation in unintended areas of the CNS and limited dose delivery to the intended site. Improvements to catheter design such as tapered tips and soft or porous membrane constructs, along with improved placement guidelines have demonstrated decreased backflow.[8,9,125,126] These guidelines include reducing trauma associated with insertion, delayed infusion initiation, and slow increases in infusion rates.[127,128] Further, most studies suggest placing catheters at least 2 cm from the resection cavity and pial surfaces.[8] Tumor-specific factors may also limit the potential of CED. Tumors with increased interstitial pressure impede the positive pressure gradient that drives drug flow to the tissue site, limiting potential for homogenous drug distribution.[129] Similarly, especially vascular tumors may present with heterogenous networks of vasculature, and this too may alter homogenous drug distribution as infusate will preferentially flow to perivascular spaces.[130,131] Lastly, although volume of distribution and pump placement can be carefully designed to limit infusate outside of the tumor, off-target effects on the normal brain remain a possibility.

Given these challenges, a variety of alternative local delivery strategies exist. Direct intratumoral injection allows for often singular, precise drug delivery at the tumor cavity, while bypassing the BBB and avoiding systemic toxicity. Furthermore, direct injection is amenable delivery of cellular therapies. Conveniently, it is often completed during resection.[26,27,31,132] However, repeat dosing is often required for optimal results. Further, direct injection typically lacks a large, homogenous volume of distribution. Focused ultrasound (FUS) is an exciting new frontier for local drug delivery aimed at disrupting the BBB. Previously, FUS has been used to augment certain chemotherapy treatments that normally fail to efficiently cross the BBB.[133] Most importantly, FUS is an noninvasive method of BBB disruption and recent improvements allow for tightly controlled and precise delivery.[134] Combined with intra-arterial delivery, FUS may allow for focused, effective penetrance of immunotherapies across the BBB. Lastly, biodegradable reservoirs or wafers may play a future role in local delivery of immunotherapies. So far restricted to aiding chemotherapy delivery, these locally situated modalities deliver high doses of drug at the resection cavity over long periods of time.[135] Recent literature is quite critical of implantable reservoirs and wafers mostly because of significantly limited volumes of distribution, and CED currently is more effective at uniformly diffusing drug directly into tissue.[136] Although promising, each of these strategies require further development.

The tumor microenvironment in glioblastoma is profoundly immunosuppressive, making the effects of any immunotherapy somewhat limited. Additionally, immunosuppression may be further enhanced by SOC treatments, including steroids, radiation and chemotherapy.[137] Necrosis, a central hallmark of glioblastoma, results in the release of extracellular potassium, which has been demonstrated to reduce activity of infiltrating T cells.[138] Thus, even high concentrations of immunotherapies delivered to the tumor via CED may be limited by intrinsic tumor microenvironment characteristics. Novel combination treatments that tackle some of these immunosuppressive pathways, like potassium release in the setting of hypoxic cell death, may be key for realizing the full potential of immunotherapies in glioblastoma.

Conclusions and Future Directions

Although among limited populations, delivery of immunotherapies via CED leads to dramatic treatment responses. This is especially prevalent among those patients treated with oncolytic viruses and TFGB2 inhibitors.[23,29,44,63] However, only a minority of these patients experience a sustained response, and it is unclear why most tumors are not as susceptible to immunomodulatory treatment. Age and treatment history (eg, steroids, multicycle chemotherapy) may hinder the strength of the immune system, which would prohibit a strong adaptive antitumor response. While these early clinical trials are promising, larger studies may provide clarity as to why some tumors are more responsive than others.[23,29,44,63] Phase III trials, with larger cohorts and comparison to SOC, are required to fully assess the benefits of these innovative treatments. Furthermore, there is a need to better analyze the local and systemic immune responses to these therapies over time.

A number of preclinical trials also show promise. Cytokine and monoclonal antibody studies, when delivered via CED, show robust immune responses within mice.[83,117,118] These results provide strong evidence to transition to the clinical setting. Additionally, several more complex, pioneering technologies, within intentions to deliver via CED, remain in the in vitro stage. Sunil et al., following successful preliminary studies, continue to validate durations of CED delivery for their oxygen-generating photodynamic payload.[88]

Directly targeting the tumor or resection cavity with CED offers many benefits. Its most obvious strength is the minimization of systemic toxicity. However, recurrence most often occurs within the resection cavity, and thus directing therapies at this site may provide the greatest defense against further recurrence.[139] Looking ahead, delivery of these immunotherapies via CED may 1 day become a suitable alternative for patients who are poor candidates for surgery.

Another limiting factor among these clinical trials is the duration of treatment. Duration of immunotherapy delivery for the treatment of solid tumors is debated, but Phase III trials of cancers responsive to immunotherapies, such as NSCLC and melanoma, often treat until severe toxicity or progression of disease.[140]

As mentioned previously, in trials using CED immunotherapies, small subsets of patients experience durable responses long after discontinuation, but longer treatments may benefit a greater number of patients for 2 reasons. A longer duration of therapy allows for a longer period of cytotoxic effects or inhibition of key signaling molecules (eg, TGFB2), and a longer duration allows for more opportunities for the adaptative immune system to develop an antitumor response. Previously, investigators interested in CED have been unable to provide chronic treatment as they have been limited by risks of infection due to external catheters and bedside pumps. However, recent work by Spinazzi et al. demonstrated the feasibility of chronic delivery in glioblastoma patients via CED.[15] Following designs of a similar system used in the treatment of Parkinson's disease, they engineered a refillable subcutaneous catheter-pump system placed within the abdomen, where they were able to deliver multiple cycles of topotecan.[15,141] Their success delivering sustained chronic cycles of topotecan may serve as a foundation for future studies involving the chronic delivery of immunotherapies alone or in combination with chemotherapies. More interestingly, this trial provided a novel clinical trial framework in which MRI-localized biopsies were taken immediately before and after therapy in order to study the effects of the drug at the tissue level. With these samples, they studied the effects of chronic treatment on not only tumor cell populations, but also of the immune microenvironment. This "window-of-opportunity" trial approach is critical to assess patient-specific responses to immunotherapy and improve future therapies.

Despite slow advances in the treatment of glioblastoma since the turn of the 21st century, numerous novel therapies have shown promising results at both preclinical and clinical stages. In order to defend against treatment recurrence, a sustained antitumor response must be invoked. Immunotherapy, locally delivered via CED at high doses, allows for a robust and sustainable antitumor response in a subset of patients. Further studies are required not only to better understand the spectrum of susceptibility to these therapies, but more importantly to demonstrate improved survival and quality of life for glioblastoma patients.

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Conflict of interest statement

J.N.B. has a consulting agreement with Theracle, Inc. No other author has any decelerations of interest.

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