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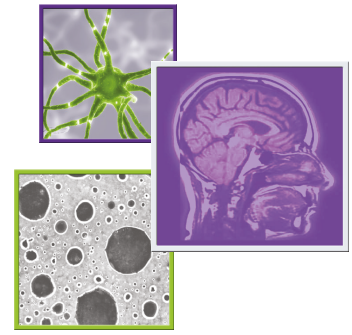

Biological intratumoral therapy for the high-grade glioma part II: vector- and cell-based therapies and radioimmunotherapy

Joshua Loya[†,1], Charlie Zhang[†,2], Emily Cox[3], Achal S Achrol[*,‡,4] & Santosh Kesari[**,‡,4]

[1]Department of Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48202, USA

[2]Buffalo School of Medicine, State University of New York, Buffalo, NY 14202, USA

[3]Providence Medical Research Center, Spokane, WA 99204, USA

[4]John Wayne Cancer Institute, Pacific Neuroscience Institute, Santa Monica, CA 90404, USA

*Author for correspondence: Tel.: +1 310 829 8265; Fax: +1 310 582 7287; achrol@jwci.org

**Author for correspondence: kesaris@jwci.org

† First authorship is equally shared

‡ Senior authorship is equally shared

Management of high-grade gliomas (HGGs) remains a complex challenge with an overall poor prognosis despite aggressive multimodal treatment. New translational research has focused on maximizing tumor cell eradication through improved tumor cell targeting while minimizing collateral systemic side effects. In particular, biological intratumoral therapies have been the focus of novel translational research efforts due to their inherent potential to be both dynamically adaptive and target specific. This two part review will provide an overview of biological intratumoral therapies that have been evaluated in human clinical trials in HGGs, and summarize key advances and remaining challenges in the development of these therapies as a potential new paradigm in the management of HGGs. Part II discusses vector-based therapies, cell-based therapies and radioimmunotherapy.

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Considerable innovations in biological treatment agents for high-grade gliomas (HGGs) have occurred over the preceding decades. Given HGGs inherent invasion of functional neural tissue it is an essential part of a tolerable and effective treatment to minimize collateral effects on normal tissues while effectively targeting neoplastic cells. Therefore, many of these advancements have been in not only enhancing the antineoplastic processes of various agents but also in developing improved agent targeting and specificity. For optimal impact of these agents on the neoplastic cells it is important that they both interact selectively and with high affinity on a cellular level but also that they can permeate the neural tissue in high concentration on a large scale. In this way the blood–brain barrier (BBB) poses a significant challenge to systemically administered therapeutics. Intratumoral administration of therapeutics directly through implanted catheters is one method to effectively bypass the BBB to help achieve optimal exposure and concentration of neoplastic cells to treatment agents far beyond that which could be achieved through systemic treatment while also reducing peripheral side effects [1–3]. This second part of a review of biological intratumoral therapy of the HGG will focus on vector-based therapy, cell-based therapy and radioimmunotherapy (RIT) evaluated in clinical trials.

The use of viruses in treating HGG began in the 1990s when advances in recombinant DNA technology set the stage to allow for the development of tropism toward tumor tissue [4]. These oncolytic viruses began as simple gene deletions attenuating the ability of the virus to replicate unless the host cell had innately high levels of cellular proliferation such as rapidly dividing tumor tissue. These initial studies proved these viruses to be relatively safe; however, the overall benefit on improving survival in patients was minimal at best [5]. Since then, a new wave of

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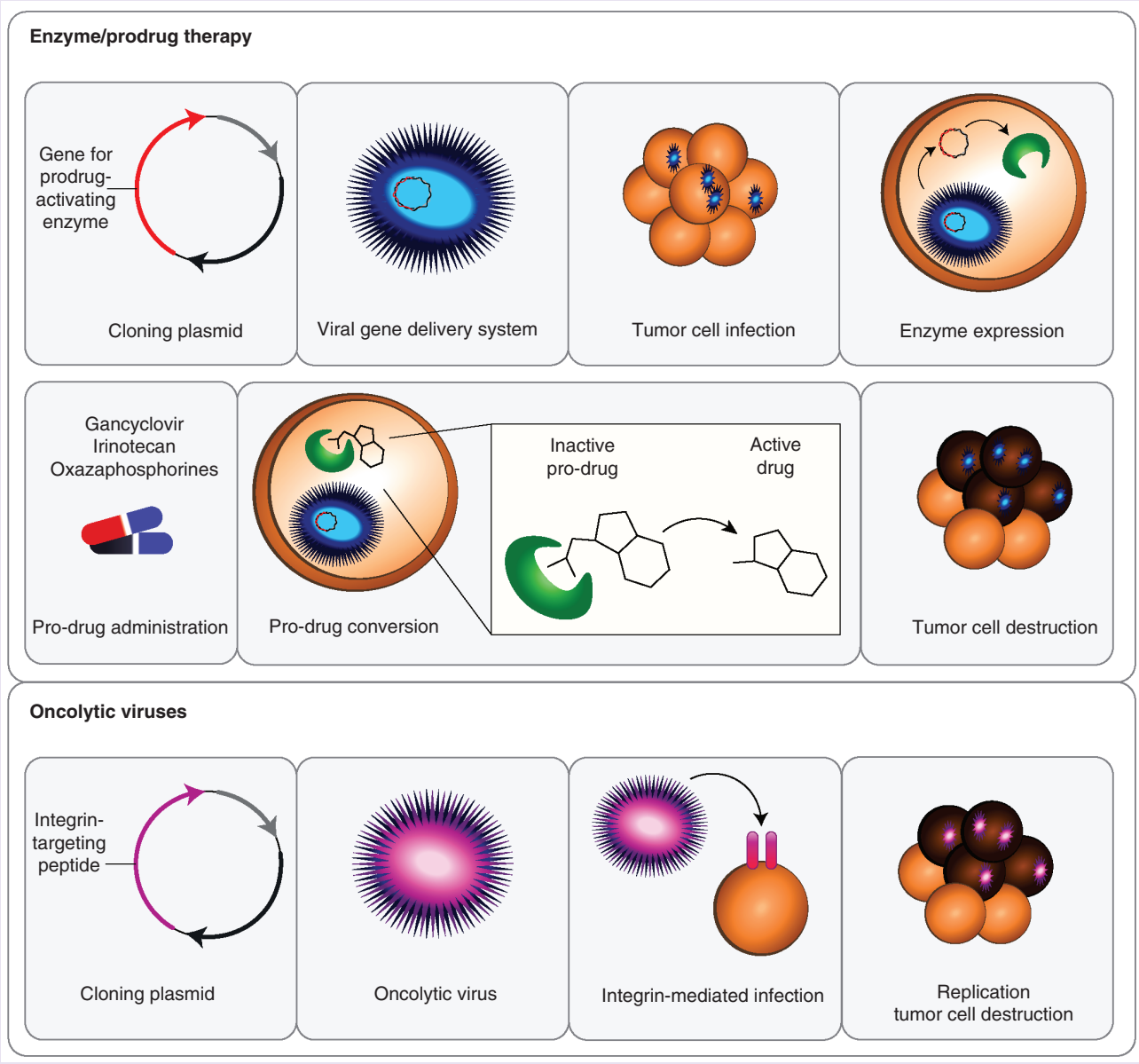

Figure 1. Vector-based biological therapies used the treatment of high-grade glioma. Enzyme/prodrug combination therapies are a form of vector-based therapies include co-administration of antiviral prodrugs, and modified viruses expressing prodrug-activating enzymes. Oncolytic viruses modified to express cell surface receptors (e.g., integrins) are also used to target cytotoxic viruses to tumor cells.

oncolytic viruses with greater tumor virulence but also greater specificity through targeting of cell surface molecules specific to HGG cells has begun testing in clinical trials with promising results [6].

Gene therapy through insertion of genes through viral vectors has undergone a variety of approaches in targeting HGG. Initial studies primarily consisted of administration of a prodrug along with a virus that specifically targets tumor tissue to insert the gene that activates the drug such as the herpes virus tyrosine kinase along with ganciclovir (Figure 1) [7]. Further investigation involved specifically targeting the unique properties of HGG such as the ability to resist natural immunity and apoptosis by inserting immune activating genes [8] and tumor suppressor genes [9], respectively.

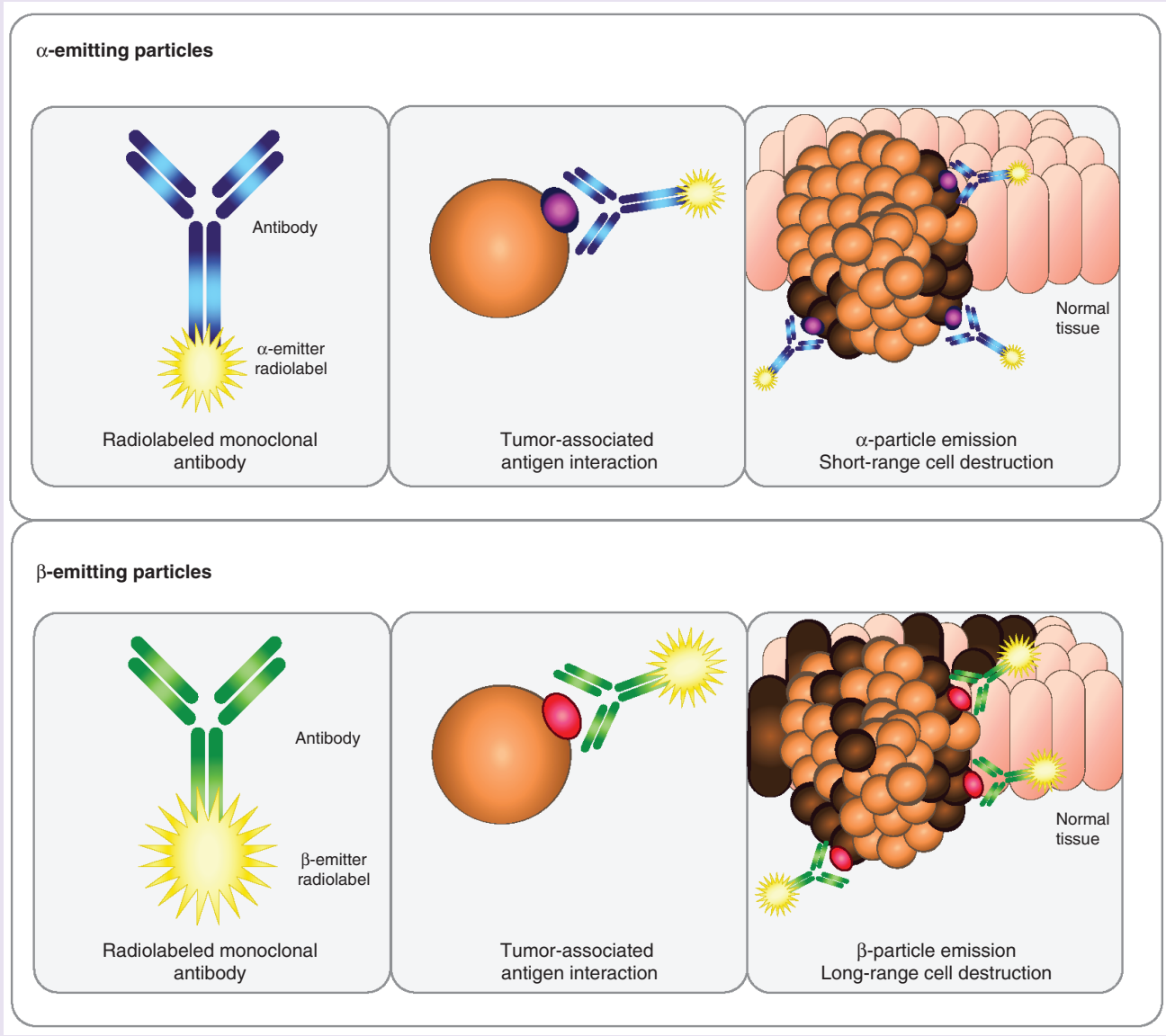

Figure 2. Radioimmunotherapy-based therapies used in the treatment of high-grade glioma. Recombinant monoclonal antibodies fused to α- and β-particle emitters form the basis of intratumorally infused radioimmunotherapies used in the treatment of high-grade gliomas. Targeting the radioligand via an antibody raised against a tumor-specific antigen permits specific destruction of tumor cells. While α-emitting radioligands exhibit high specificity to tumor cells, the relatively low penetrance of these emitters restricts their use to small tumors. In contrast, β-emitting radioligands permit a longer range of cell destruction but may penetrate the parenchymal tissue. GBM: Human glioblastoma.

Similar to using antigen specific vectors to direct therapeutic agents at a cellular level, RIT uses radio-labeled antibodies to selectively deliver adjuvant radiation locally to tumor cells based on tumor-associated antigens (Figure 2). Intratumoral radio-labeled antibody therapies include ^{131}iodine and astatine 211-labeled antitenascin, and ^{131}iodine-labeled tumor necrosis factor (TNF) antigen [2,10–13] and others that are detailed below.

Another class of immunotherapy, cell-based therapy, seeks to utilize both the innate and the adaptive immune system to combat malignancy (Figure 3). The adaptive immune system is bridged to the innate immune system by cells such as natural killer (NK) and dendritic cells (DCs) that present abnormal antigens on their surface after phagocytosis and destruction of the pathological cell [14,15]. Antigen-presenting cells (APCs) such as these then stimulate an adaptive immune response by directly activating T cells and initiating a cascade involving a

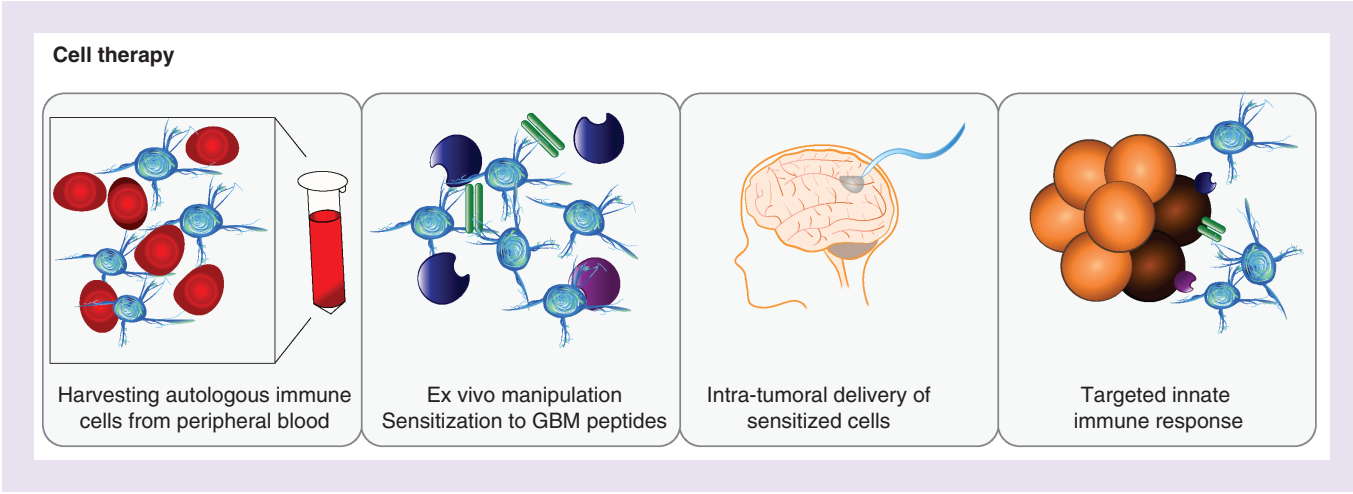

Figure 3. Cell therapy approach used in the treatment of high-grade glioma. Cell therapy approaches sensitize autologous dendritic cells to glioblastoma peptides *ex vivo*. Sensitized cells are re-infused intratumorally to excite an innate immune response to tumor cells. GBM: Human glioblastoma.

Table 1. Ongoing vector-based trials for high-grade gliomas.

Virus	Cancer type	Phase	Trial
Delta 24 RGD	Recurrent malignant glioma	Phase I	NCT00805376
Delta 24 RGD	Recurrent glioblastoma	Phase I/II	NCT01582516
MV-CEA	Recurrent glioblastoma	Phase I	NCT00390299
H1-PV	Recurrent glioblastoma	Phase I/IIa	NCT01301430
Delta 24 RGD with IFN-γ	Recurrent malignant glioma	Phase Ib	NCT02197169
Reovirus with sargramostim	Recurrent pediatric brain tumors	Phase I	NCT02444546
DNX-2401 with pembrolizumab	Recurrent malignant glioma	Phase II	NCT02798406
NSC-CRAd-Survivin-pk7	Newly diagnosed malignant glioma	Phase I	NCT03072134
PVSRIPO with lomustine	Recurrent malignant glioma	Phase II	NCT02986178
Toca 511/Toca FC	Recurrent malignant glioma	Phase II/III	NCT02414165

series of costimulatory and checkpoint responses leading to activated cytotoxic T cells, antibody production and immune memory [16,17]. The general principle behind cell-based immunotherapy for HGGs is to harvest autologous immune cells, such as DCs, NKs or T cells, which are then modified or sensitized to target specific tumor-associated neoantigens and can then be re-infused into the resection cavity [18–21]. These immune cells are optimized to attack tumor cells directly and trigger an immune response with the goal of eradication of residual disease and enhancing long term control, a process often colloquially termed with the lay public as a 'tumor vaccine.'

Vector-based intratumoral therapies

Oncolytic viral therapy

Oncolytic viruses are live genetically modified viruses designed to target tumor cells (Figure 1). These viruses selectively infect malignant cells and propagate through their lytic cell cycle to resulting in selective tumor cell destruction. They were first developed in the 1990s when recombinant DNA technology provided a means of selective gene elimination to allow for tropism toward tumor tissue [4]. While clinic trials for vector-based therapies are reviewed in detail elsewhere [22], Table 1 summarizes the ongoing clinical trials for the vector-based therapies discussed below. The first oncolytic virus designed against glioblastoma was a modified herpes simplex virus (HSV) with a thymidine kinase deletion that rendered it incapable of replicating in post-mitotic cells such as neurons [23]. The first Phase I clinical trial of an oncolytic was a variant HSV strain with an infected cell protein (ICP) 34.5 deletion (HSV 1716) that can only replicate in cells with high levels of proliferating cell nuclear antigen such as glioblastoma cells [5]. The results demonstrated the safety and specificity of the oncolytic model as no encephalitis,

adverse clinical symptoms or reactivation of HSV were found; however, four of the nine patients expired by 5 months.

One of the major feared complications of oncolytic viral therapy is the risk of potentially reverting to the wild-type pathogenic form with subsequent encephalitis of nonmalignant tissue. The HSV G207 virus was a double attenuated virus created to address this issue and significantly decreased the risk of conversion back to wild-type through the deletion of the *ICP6* gene in addition to *ICP34.5* [24]. The initial clinical trial of HSV G207 found no adverse clinical symptoms or encephalitis; however mean time to progression was 3.5 months [25].

In addition to HSV, other viral vectors have been modified for use in oncolytic viral therapy in the treatment of glioblastoma. ONYX-015 is a modified adenovirus (AV) designed to replicate in cells lacking wild-type p53. A Phase I trial of 24 patients with ONYX-015 demonstrated no significant adverse effects; however, median progression-free survival (PFS) was found to be 46 days [26]. MTH-68/H is an oncolytic strain of Newcastle disease virus (NCV) that was initially found to result in no effect on seven individuals, 1 year survival in three individuals and 5–9 years survival in four individuals [27]. However, when a slightly modified strain of the virus was assessed in a controlled Phase I/II trial, the median PFS was only found to be 12 weeks, which is comparable to other vectors [28]. Finally, wild-type reovirus, which functions as an oncolytic virus due to its dependence on activated Ras signaling for propagation [29], was evaluated in a Phase I clinical trial with 12 patients achieving a median PFS of 4.3 weeks [30]. The results of these initial trials of oncolytic viruses demonstrated safety across most of the techniques and no dose limiting toxicities were observed; however, the ability of these viruses to delay progression of glioblastoma was underwhelming. The limited efficacy of oncolytic viral therapy observed in these trials was made further evident by a study involving a retrovirus or AV expressing a β-galactosidase marker gene where these vectors achieved only 4 and 11% infection efficiency, respectively [31]. Nevertheless, as these were Phase I studies aimed in evaluating the safety of these therapies, they were instrumental in demonstrating proof of concept and safety, and thereby opening the door for the development of the next generation of oncolytic therapies currently in research and development pipelines.

New advancements in the understanding of viral entry and the surface receptors unique to glioma cells has resulted in development of viruses with enhanced infectivity of glioblastoma cells. The delta-24-RGD virus is an AV with a modified surface receptor that targets integrin receptors highly expressed in glioma cells in order to increase infectivity [32]. A Phase I study is currently ongoing for this virus, though no longer recruiting (NCT00805376). A similar virus aimed to enhance delivery to glioma cells is the measles virus, which enters cells through the cluster of differentiation 46 (CD46) complement receptor, which is often overexpressed on gliomas [33]. A Phase I study of the measles virus modified with a reporter soluble carcinoembryonic antigen marker (MV-CEA) is currently being tested (NCT00390299). Similarly, a modified polio/rhinovirus virus (PVS-RIPO) preferentially targets glioblastoma cells through the CD155/Necl-5 polio virus receptor, for which a Phase I trial has been completed [34]. The results of this Phase I trial, in which PVS-RIPO was delivered by convection-enhanced delivery (CED), reported a mild increased median survival of 12.5 months versus historical untreated controls of 11.3 months; although most notably, the survival rate at 24 (21%) and 36 (21%) months were markedly higher than historical control rates of 14 and 4%, respectively [6]. The authors also discuss clinical, radiographic and cellular findings suggestive of a potential benefit of single, rather than multicycle chemotherapy. These findings provide the rationale for the Phase II trial of PVS-RIPO in combination with single cycle lomustine (NCT02986178).

Another method of improving the efficacy of oncolytic treatment involves adaptation of viruses that activate cell death pathways to bypass the resistance mechanisms to apoptosis, which are frequently found in glioma cells. One such virus is the Parvovirus H-1, which is shown to activate a cathepsin-mediated death pathway that is effective in killing apoptosis resistant glioma cells [35]. This virus is being evaluated in a Phase I/IIa trial (NCT01301430).

In addition to their oncolytic mechanisms of tumor destruction, several clinical studies have indicated that the oncolytic viruses induce an antitumor cytotoxic adaptive immune system response. This effect was initially observed in preclinical models when intratumorally injected G207 virus caused regression of remote established tumors. This mechanism was also demonstrated in a clinical trial using the talimogene laherparepvec HSV virus, which replaces the *ICP34.5* gene with one coding for granulocyte macrophage colony-stimulating factor. In this study, aimed at metastatic melanoma, the oncolytic virus not only led to a higher complete response rate, but also led to regression of uninjected tumors, demonstrating the effectiveness of oncolytics in triggering an adaptive endogenous vaccine effect [36]. The results of the Phase I trial of the Delta-24-RGD oncolytic virus in glioblastoma suggests that this particular strain may be capable of similar enhancement of the adaptive immune response in primary brain tumors [37], leading to a new trial that combines intratumoral injection if the virus with INF-γ to enhance the

immune response (NCT02197169). An alternative trial using the Reovirus in combination of the immunostimulant sargramostim is also underway (NCT02444546) as well as the modified oncolytic AV DNX-2401 in combination with pembrolizumab checkpoint inhibitor therapy (NCT02798406).

In addition to genetic modifications of oncolytic viruses, there has also been research focused on advancing the intratumoral delivery method of these vectors. Most clinical trials in the past used magnetic resonance imaging guided insertion of biopsy needles or silicone catheters to inject the viruses. These methods result in suboptimal delivery of the virus due to backflow of solution as well as human gliomas possessing multiple heterogenous barriers to viral spread such as necrosis, hemorrhage, cysts and edema [38]. As discussed in prior sections, the development of CED delivery as an infusion technique characterized by consistent low pressure infusion over time to deliver the vector under convection current may achieve better volume distributions compared with previous methods. Utilization of CED in viral oncolytic therapy treatment has shown promise in preclinical studies, including improved efficacy in preclinical mouse models implanted with human glioblastoma (GBM) xenografts with improved delivery and survival in animals treated with CED of HSV as well as other viral particles in adenovirus (AV) and adeno-associated virus models [39–41]. On-going trials of CED with the oncolytic viruses Delta24-RGD (NCT01582516) and PVS-RIPO are being evaluated (NCT01582516).

Future development of oncolytic therapeutic delivery may involve the use of neuronal stem cells (NSC) as a vector. Pilot studies demonstrated that NSCs show tropism for glioma cells and self-distribute to infiltrative tumor cells outside of the primary tumor mass [42]. This led to trials that demonstrated the efficacy of transgenic NSCs as a form of glioma targeted therapy and gene delivery (discussed in the gene therapy section). More recently, the production of the NSC that produces a modified oncolytic AV CRad-survivin-pk7 (NSC-CRAd-Survivin-pk7) was developed and found to be successful in intracranial glioma mouse models [43] and is now currently undergoing a Phase I trial (NCT03072134).

Gene therapy

Gene therapy was originally proposed as early as the 1970s when it was hypothesized that selective transfer of functional versions of genes in genetic diseases could have therapeutic benefit [44]. Since then, multiple advances have been made in recombinant DNA technology that have allowed for *in vitro* modification of genes. The ability to manipulate genes has become a vital component of cancer therapeutics as new pathways of tumorigenesis and tumor lysis are being uncovered. Gene therapy in glioblastoma has evolved into three major therapeutic arms – suicide genes, immunomodulation and tumor suppressors.

The primary form of suicide gene therapy for glioblastoma is through induction of tumor lysis through delivery of pro-drug activating genes in combination with prodrug systemic therapy (Figure 1). The earliest of these was the use of the combination of HSV-thymidine kinase (*HSV-tk*) gene with the prodrugs ganciclovir or valacyclovir. By inserting the gene specifically in tumor cells only the malignant cells expressed the *HSV-tk* gene. Unlike wild type cells, these cells would be rendered susceptible to the pro-apoptotic effects of the drugs ganciclovir and valacyclovir that are dependent on *HSV-tk* to be activated within the cell. Another well studied enzyme/prodrug combination is the selective expression of cytosine deaminase in combination with 5-fluorocytosine (5-FU), which works on the same premise that cytosine deaminase is permissive to the pro-apoptotic effects of 5-FU.

The first Phase l trial with *HSV-tk* used an AV vector (AV-tk) and found that median survival after treatment was 4 months [7]. The demonstrated safety profile in subsequent Phase I/II trials resulted in a large Phase III study that randomized 248 patients to either standard chemotherapy and radiotherapy or with adjuvant *HSV-tk* and ganciclovir. Although the gene therapy was well tolerated, no significant difference was found in the 1-year survival between the two groups [45]. A Phase III trial also found no significant difference in overall survival between groups but detected a significant hazard ratio reduction [46]. A Phase IB trial involved injections of the AV-tk particles at the time of surgery followed by valacyclovir and the chemotherapeutic temozolomide 14 days after surgery resulting in 25% of the participants surviving 3 years [47]. Post-treatment histological specimens demonstrated enhanced lymphocyte infiltration at 22 months suggesting that this regimen promotes adaptive immunity against the tumor and is currently undergoing Phase II trial (NCT000589875). Considering these findings, a separate trial involving combination therapy with immunomodulation through human cytomegalovirus fms-like tyrosine kinase 3 ligand (hCMV-Flt3L) is discussed below (NCT01811992).

Use of the cytosine deaminase/5-FU system was investigated using the Toca 511 vector, a recombinant nonlytic retrovirus that can only infect dividing cells. The advantage of this system is the enhancement of the bystander effect whereby the activated chemotherapeutic product 5-FU is diffusible through cell membranes that can kill

adjacent noninfected tumor cells [48]. The two initial Phase I trials which are still ongoing (NCT01156584 and NCT01470794) have shown promising results [49]. Of the 68 participants, the treatment was well tolerated, and higher survival rates were found at 6 and 12 months. Additionally, histological analysis demonstrated immune activation against residual tumor suggesting that future trials may enhance efficacy when combined with immunomodulation therapy. The success of these initial studies has spurred a Phase II/III clinical trial of Toca 511 against the investigator's choice of single agent chemotherapy standard of care (NCT02414165).

Immunomodulation gene therapy became of interest due to growing evidence that gliomas actively evade the immune system. The enhancement of the immune system has demonstrated efficacy in the treatment of both hematological and solid malignancies in the clinical setting; however, due to the difficulty of immune cells in penetrating the BBB this avenue of investigation has been suggested [50]. The first *in vivo* model demonstrating the efficacy of this approach, through combination of IL-12 with blockade of cytotoxic T-lymphocyte (CTL) associated antigen 4, resulted in significant survival in preclinical mice glioma models [8]. This resulted in a Phase I trial using a novel *in vivo* regulated expression system involving an AV vector with a human IL-12 transgene that is under control of a promoter activated by the exogenous ligand veledimex (NCT02026271).

An alternative arm of investigation involving immunomodulation has used a modified AV expressing the FMS-like tyrosine kinase 3 ligand (Flt3L), a growth factor for immune cells under the human cytomegalovirus promoter (AV-hCMV-Flt3L), which is used in combination with the a modified AV-tk virus with the gene under the *hCMV* gene (AV-hCMV-tk) [51]. A Phase I study using the combination of these vectors in combination with radiation and temozolomide has demonstrated the induction of enhanced immune surveillance in preclinical glioma models [52] and is currently undergoing Phase I trial (NCT01811992).

Tumor suppressor genes in functional cells aim to preserve DNA stability and prevent disorganized cell growth. It has been demonstrated that all glioblastomas have at least 1 tumor-suppressor gene mutated or deleted, and that in 91% of cases more than two are non-functional [53]. Restoring the function of the most important of these – p53 which detects DNA damage and controls the transition of cells from the G1 to S phase – has shown tremendous promise in animal models of glioblastoma [54]. However, the first clinical trial to use an AV to transfer the *p53* gene to tumor cells found limited success, which may have been due to the inability of the viral particles to infect cells more than a few mm from the injection site [55] a limitation that might be addressed using improved neurosurgical delivery such as CED.

A subsequent attempt of tumor suppression gene transfer was done through use of an AV encoding beta-interferon – a protein product that is anti-proliferative and pro-apoptotic in malignant cells [56]. Unlike tumor protein p53, IFN-β that is expressed in infected cells is secreted into the surrounding tissue causing a bystander effect. In addition, studies in animal models of glioma showed that beta interferon induced antitumor immunity [57]. Phase I trials of the beta interferon AV found that it was well tolerated, but the gliomas progressed in all patients by 4 months [58].

The initial studies in gene therapy were as proof of concept experiments of the validity of the method; however, it was obvious that better delivery systems of genes were necessary to advance the field. Most of the initial viruses were developed from the human AV serotype 5 which relies on the coxsackievirus/AV receptor which is poorly expressed in glioblastoma cells [59]. The infectivity of the AV model was enhanced by incorporation of a polylysine residue onto the receptor binding motif to enhance glioma infection efficiency [60]. The use of this modified strain is currently being used in the trial mentioned above with AV-hCMV-Flt3L and AV-hCMV-tk (NCT01811992).

Advancements in technology also allowed for nonviral means of gene delivery primarily in the forms of nanoparticles and liposomes. Nanoparticles are defined as small synthetic objects <100 nm in size that are most commonly composed of silver or gold which have the benefit of being inert and nonimmunogenic [61] or multiwalled carbon nanotubes which can be modified with polyethylene glycol chains to allow preferential accumulation with tumors [62]. To date, no clinical trials of these vectors have been attempted, but trials are likely to occur in the near future. Liposomes and micelles have also been established as potential vehicles for gene delivery that can be enhanced with tumor specificity through PEGylation of the lipid surface [63]. The initial trials of these particles involving HSV-tk in combination with ganciclovir [64] and interferon-beta [65] showed limited therapeutic efficacy; however, a combination of the liposome nanocomplex that delivers wild-type p53 to confer sensitivity to temozolomide has shown promise in animal models [60] and is currently undergoing Phase II clinical trial (NCT02340156).

Finally, advances in the usage of NSCs and mesenchymal stem cells has provided an alternative method of targeted gene delivery. With their natural tropism toward brain tumor tissue, NSCs are ideal cellular carriers for anti-tumor genes [66]. NSC have been modified to express the CD/5-FU system described above and showed

significant reduction of glioma size in mouse models and underwent a pilot study in humans the result of which have yet to be published (NCT01172964).

Radioimmunotherapy

Another unique intratumoral therapy for HGGs is RIT that seeks to deliver focal radiation at a cellular level (Figure 2). This technique uses monoclonal antibodies (mAbs) against tumor-associated antigens as molecular vehicles to deliver radionuclides selectively to tumor cells. Radioimmunotherapeutics consist of two main conjugated components: the vehicle, which consists of a mAbs targeting a specific tumor associated antigen, and the radionuclide, which is the source of the therapeutic radiation.

Radionuclides can be generally categorized based on the type of energy they emit, of which there are two main types that have been evaluated clinically: α particles and β particles. Iodine-131 (^{131}I) is one of the most commonly utilized radionuclides in glioma treatment. It emits β particles that deposit 95% of their energy (R_{95}) within 0.992 mm which can be beneficial for localized delivery of radiation while helping to minimize normal brain exposure; however, ^{131}I also emits a fraction of high energy γ rays, which can increase dose exposure of surrounding tissues to a degree but can also be utilized for dosimetry. Another β particle emitter that has been frequently used clinically is yttrium-90 (^{90}Y) which has the advantage of being a pure high energy β particle emitter but with significantly larger R_{95} at 5.94 mm [67]. A third β particle radionuclide that has been investigated is rhenium-188 (^{188}Re) that has a maximum tissue penetration of 10 mm and averaging 3.1 mm [68]. Conversely, α particle emitting radionuclides such as Astatine-211 (^{211}At) have a tissue range of only a few cell bodies resulting in significantly less cross fire effect than β emitters but as a result rely heavily on tumor cell proximity for effect [69]. These radionuclides have been conjugated to mAb vehicles targeting various tumor associated antigens to evaluate their therapeutic potential in treating HGGs.

One of the most widely utilized mAb targets for clinical studies is tenascin, a glycoprotein of the extracellular matrix that is over-expressed in over 90% of glioblastoma tumors and to a lesser but significant extent in WHO grade II and III lesions [70]. Other tumor associated antigen targets that have been clinically evaluated include EGFRvIII, extradomain B of fibronectin, integrin $\alpha_v\beta_3$, histone H1 complexed to deoxyribonucleic acid, IL-13 and podoplanin.

With a sound theoretical concept, RIT in HGGs has been heavily studied with over sixty publications over the last 15 years. While utilization of RIT in HGGs is reviewed in detail elsewhere [69,71,72], mixed results and underwhelming and inconsistent impacts on survival has resulted in waning interest in utilizing RIT for HGGs, as evidenced by no active clinical trials at time of this writing. DeBonis and colleagues [69] found that RIT in gliomas face multiple challenges including dynamic genetic heterogeneity of tumor cells which may limit treatment success, difficulty comparing published clinical studies due to the use of differing agents, and importantly, studies have targets chosen from active tumor cells, which may represent a separate antigen pattern compared with the 'quiescent' cancer cells present in adjacent brain tissue that may be sources of recurrence [73]. Additionally, a persistent problem in RIT with α-emitters is that of recoil energy, which cleaves the daughter nuclide from the fused mAb after particle emission [74,75]. One method for preventing damage to healthy tissue caused by these freed daughter nuclides is enclosing the nuclides in liposome or polymersome nanocarriers [75–78].

Cell-based therapy

Cell-based immunotherapies offer the advantage of the potential for high tumor specific cytotoxicity (Figure 3). One branch involves DC vaccines which generally consists of *ex vivo* manipulation to elicit a directed endogenous adaptive immune response once implanted. It has been hypothesized that part of the reason for the immunological resistance of CNS tumors is lower mutational load and neoantigen expression relative to other more immunologic tumors such as melanoma, thereby creating a poor overall substrate for APCs [79], as well as direct tumor and tumor microenvironment production of immunosuppressive cytokines such as TGF-β [80] and IL-10 [81].

The first approaches in DC vaccines involved peripheral blood DCs that were then pulsed with surface peptides or autologous tumor lysates followed by implantation subcutaneously or intratumoral injection directly [21,82]. These studies found that there was indeed induction of glioma reactive $CD8^+$ T cells in 4/7 and 7/17, respectively as well as detection of infiltration of $CD8^+$ and memory T cells in 2/4 and 2/2 patients who underwent reoperation after vaccination, respectively. While these trials demonstrated that there was indeed stimulation of a delayed cell-mediated response leading to an increase in survival of 455 versus 257 days in the Yu trial and 480 versus 400 days

in the Yamanaka trial as well as limited adverse effects of the therapy, there was room to improve the efficacy of the immune response as well as potential increase the specificity of antigenic targets.

To enhance the specificity and reproducibility of immune activation, a vaccine was developed using specific purified GBM peptides including IL-13Ralpha2 known as ICT-107. The Phase I trial demonstrated median PFS increase of 2 months and that multiple vaccine inductions demonstrated prolonged survival and is currently undergoing Phase III trial [83]. There has also been development of a vaccine known as ICT-121 that specifically targets CD133 currently in Phase I clinical trial (NCT02049489).

A different branch of cell-based immunotherapies involves the expansion and/or modification of antitumor lymphocytes *ex vivo*. One cell employed in this approach is the lymphokine-activated killer (LAK) cell that functions such as NK cells but have demonstrated greater effectiveness in killing tumor cells [84]. The initial Phase I trials involving LAK with IL-2 stimulation showed some promise in advanced GBM with a mean survival time of 20.5 months [19]. Additionally, there was a trial to increase the potency specificity of LAKs through use of a combination bispecific antibody against EGFR and Fc receptors found on LAKs; however, no additional benefit was found (NCT00005813).

The limited success of LAK trials branched into genetic manipulation of autologous T cells to specifically target unique tumor antigens. This has primarily been performed through formation of chimeric antigen receptors (CARs) – fusion of an antibody or ligand binding domain with the ζ chain of the T-cell receptor – directing isolated autologous T cells against tissues expressing the antigen or ligand of interest. The first of these was using CTL expressing IL13Ralpha2 CARs in a single patient that demonstrated the effectiveness and specificity of the approach [85]. The CAR was further modified to improve antitumor potency and T-cell persistence is undergoing clinical trial (NCT02208362). Another promising target for CARs is anti-EGFRvIII which is in Phase I/II trial (NCT02209376 and NCT01454596).

One final branch of cell-based therapies that does not involve autologous transplant has been a report using allogeneic cytotoxic T lymphocytes (alloCTLs) sensitized against recipient human leukocyte antigens [86]. The basis of this therapy is that only tumor cells in the brain express appreciable levels of human leukocyte antigens, limiting the off-target effects [87]. The results of the trial demonstrated the feasibility of this approach of having minimal side effects, although median survival was 8.8 months [88].

Conclusion

Intratumoral biological therapies are an emerging and diverse set of treatment modalities for HGGs. While many intratumoral biological therapies show enough promise to raise hope as potential future options in the management of HGG, none has yet emerged to the point of US FDA approval. As these therapies evolve and progress they may 1 day represent a reliable adjuvant treatment alongside the current standard of care (resection, radiation and chemotherapy). While most studies discussed here have been on recurrent gliomas with limited treatment options, as therapies continue to advance in availability, reliability and effectiveness we may see a select subset of the intratumoral therapies discussed above be assimilated into primary treatment algorithms.

The technical challenge common to intratumoral therapies is achieving optimal distribution throughout normal tissue such that even infiltrative tumor cells within functional tissue, a hallmark of the HGG, are exposed to the treating agent even if several centimeters from the delivery origin. This has been largely ameliorated by the utilization of convection enhanced delivery, however technical hurdles remain in consistently achieving ideal catheter placement while avoiding regions that could rob volume distribution such as the resection cavity, ventricles, or subarachnoid space. Equally as important to intratumoral therapies is the ability for the therapeutic agent to interact with tumor cells with high affinity once exposed while bypassing the much vaster number of normal cells that may be encountered. This specificity has been advanced through multiple means including modifying vectors to selectively infect highly metabolic cells, using mAbs that target antigens nearly exclusively expressed on tumor cells, and sensitizing immune cells to target tumor specific peptides among other engineered molecular changes.

The key to advancing treatment options beyond the current standard of care and overall poor prognosis in HGG lies in the continued push for new treatment paradigms such as intratumoral therapies. In addition, and perhaps more importantly, current and future studies must work to understand what underlies the subset of long-term survivors reported in prior studies, and the mechanisms behind variable responses in these heterogeneic cohorts.

Future perspective

Biological intratumoral therapy is an advancing class of adjuvant treatment for HGGs that continues to show promise and progress as techniques are refined and therapeutic mechanisms optimized. They maintain the distinct therapeutic advantage of high specificity by exploiting discrete tumor cell biology through precision engineering on the molecular level all while bypassing the formidable BBB that can limit efficacy of systemic treatments. Despite advances, technical delivery of various intratumoral therapeutics remains a challenge with the ultimate goal of reliably and reproducibly exposing every remaining tumor cell residing within normal tissue to the targeted therapeutic. However, as delivery methodology advances, striving closer to achieving this, so too does our understanding of the complex and heterogeneous tumor biology of the HGG. Looking to the future, as these tumors and their variegated response to therapeutics is better understood, treatments that are patient specific may be designed harmonizing patient selection with treatment choices, guided by tumor genetic expression profiles and individual patient characteristics.

Executive summary

Background

- Therapies for high-grade glioma (HGG) must precisely target cytotoxic agents to tumor cells in order to minimize damage to the brain parenchyma.
- Intratumoral administration of biological therapies, which cannot pass the blood–brain barrier, allows selective targeting of anti-neoplastic therapies to HGG tumor cells.
- Viral vectors, conjugated antibodies and sensitized autologous immune cells are currently under study as targeting strategies.

Vector-based intratumoral therapies & gene therapies

- Infection of tumor cells with viral gene delivery systems allows tumor cell-specific expression of prodrug converting enzymes. These enzymes convert prodrugs to active cytotoxic drugs in of tumor cells only, minimizing systemic exposure to the active drug.
- Oncolytic viruses modified to express integrin-targeting peptides can be used to target cyotoxic viral infections to tumor cells, thus selectively destroying tumor cells while sparing the brain parenchyma.

Radioimmunotherapy

- In radioimmunotherapy, monoclonal antibodies targeted to tumor cell surface markers can be fused to α- (short-range) or β-particle-emitting (longer-range) radioligands, which destroy cells in the vicinity of the neoplasm.

Cell-based therapy

- In cell-based therapies, autologous immune cells are harvested, sensitized *ex vivo* to HGG peptides, and re-introduced to intratumorally in order to stimulate an innate immune response against HGG tumor cells.

Conclusion

- Phase I and II trials show that several biological therapies permit precise targeting of HGG tumor cells and have reasonable safety and efficacy profiles.
- With refinement, these biological therapies will likely constitute a safe and effective adjunct to resection, radiation and chemotherapy.

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