

Preclinical And Clinical Development Of Oncolytic Adenovirus For The Treatment Of Malignant Glioma

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Abstract: Replication conditional oncolytic human adenovirus has long been considered a promising biological therapeutic to target high-grade gliomas (HGG), a group of essentially lethal primary brain cancer. The last decade has witnessed initiation and some completion of a number of Phase I and II clinical investigations of oncolytic adenovirus for HGG in the US and Europe. Results of these trials in patients are pivotal for not only federal approval but also filling an existing knowledge gap that primarily derives from the stark differences in permissivity to human adenovirus between humans and preclinical mouse models. DNX-2401 (Delta-24-RGD), the current mainstream oncolytic adenovirus with modifications in E1A and the fiber, has been shown to induce impressive objective response and long-term survival (>3 years) in a fraction of patients with recurrent HGG. Responders exhibited initial enlargement of the treated lesions for a few months post treatment, followed by shrinkage and near complete resolution. In accord with preclinical research, post-treatment specimens revealed virus-mediated alteration of the immune tumor microenvironment as evidenced by infiltration of CD8+ T cells and M1-polarized macrophages. These findings are encouraging and together with further information from ongoing studies have a potential to make oncolytic adenovirus a viable option for clinical management of HGG. This review deals with this timely topic; we will describe both preclinical and clinical development of oncolytic adenovirus therapy for HGG, summarize updated knowledge on clinical trials and discuss challenges that the field currently faces.

Keywords: oncolytic adenovirus, high-grade, glioma, glioblastoma, immunotherapy, tumor microenvironment, pseudo-progression

Introduction

Glioma is a group of neoplasms that primarily arise within the central nervous system (CNS). The current 2016 World Health Organization (WHO) classification uses both histology and genetic molecular features to define gliomas into subtypes and malignancy grade: grades I–IV.¹ Grades III and IV are considered high-grade (i.e., malignant) gliomas (HGG). Glioblastoma (GBM), grade IV, is the most common glioma typically affecting adults, and includes GBM, IDH-wildtype and GBM, IDH-mutant that each show distinct clinical characteristics. GBM, in general, is characterized by aggressive and infiltrative growth and resistance to therapy. The prognosis of IDH-wildtype GBM is very poor with the median survival of 15 months despite the current standard treatment consisting of a combination of surgical resection, radiation and chemotherapy.² HGG also affect children, and include diffuse mid-line glioma, histone3 K27M mutant and HGG in the

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cerebral hemispheres. Recent large-scale genetic studies have vastly advanced our understanding of molecular background of both adult and pediatric HGG, and led to clinical testing of numerous molecular-targeted strategies. However, no molecular targeting agents have been shown to extend the overall survival of patients with HGG. The development of novel therapies for HGG is highly needed.

The concept of applying virus to the treatment of cancer is old and was clinically tested in the 1950–1970s.^{3,4} It was in the early 1990s when technology to engineer viral genome first enabled the generation of oncolytic virus (OV) as a cancer-selective therapeutic.⁵ A variety of genetically modified viruses and some naturally occurring viruses have been developed as OV that possesses the ability to replicate in cancer cells and kill them but spare normal cells. The basic OV effect is a direct killing of host cancer cells following its selective replication in tumor cells. Upon cancer cell killing, many OVs induce inflammatory and anti-tumor immune responses that have been increasingly shown to play a major role in overall anti-cancer effects mediated by OV. In addition, genetic modification of OV allows loading of a new function such as therapeutic gene expression or selective infectability to increase efficacy and safety.⁶ A number of virus species, both DNA and RNA viruses, have been studied as OV platforms for cancer therapy, such as herpes simplex virus (HSV), adenovirus, vaccinia virus, reovirus, poliovirus and measles virus. In 2015, FDA approved talimogene laherparepvec (T-VEC), a genetically modified HSV, for advanced melanoma as the first OV therapeutic in the US.⁷

Extensive preclinical and clinical research conducted in the past three decades has accumulated a body of evidence that supports that HGG, in particular GBM, is an appropriate target of OV therapy. Along with oncolytic HSV, oncolytic adenovirus is one of the most studied and promising OVs in the treatment of HGG, with multiple oncolytic adenovirus clinical trials currently ongoing in patients with HGG.⁸ In this review article, we will summarize preclinical and clinical development of oncolytic adenovirus therapies for HGG and discuss challenges and future directions to advance the field.

History Of The Development Of Conditionally Replicative Adenoviruses (CRAd)

Adenoviruses are non-enveloped viruses with an icosahedral capsid that contains a double-stranded DNA genome of about 36 kb in size. They have a broad range of vertebrate hosts, from

fish to humans, showing species-specificity. In human adenovirus, 57 serotypes have been identified.⁹ Depending on the serotype, human adenoviruses cause various disease conditions such as respiratory disease, conjunctivitis, gastroenteritis and myocarditis. The well-understood adenovirus biology allows opportunities for genetic manipulation and tailored gene modifications to generate oncolytic adenoviruses, i.e. conditionally replicative adenovirus (CRAd). With regard to manufacturing, CRAd can be produced at high titer and particles are of excellent physicochemical stability.^{10,11}

1st Generation Of CRAd: E1B-55kD Deleted Onyx-015

dl1520 (Onyx-015) is the first genome-modified CRAd that is based on human adenovirus type 2/5 chimera.¹² Onyx-015 has a large deletion at the E1B locus, and does not express E1B-55kD. When wild-type adenovirus infects cells and forces entry into the S phase triggering p53-mediated apoptosis, the E1B-55kD protein binds and inactivates p53, abrogating p53-mediated apoptosis and enabling the virus to replicate. Onyx-015 is unable to replicate in normal cells due to the deletion of this E1B-55kD, and was initially shown to replicate only in tumor cells lacking p53. However, subsequent studies revealed that Onyx-015 was not selective to p53-defective cells as it replicated in cancer cells having wild-type p53.^{13–15} On the other hand, tumor cells exist that do not support replication of Onyx-015. Multi-functional E1B-55kD mediates export of late adenoviral mRNA and tumor cells resistant to Onyx-015 do not provide the RNA export functions of E1B-55kD.¹⁶ Heat shock at 39.4°C rescued the mRNA export function of E1B-55kD and enabled efficient replication of E1B-55kD-deletion mutants.¹⁷

Onyx-015 clinical trials have been conducted in several types of cancer, and a Phase I trial for malignant glioma was reported in 2004.¹⁸ In this trial, Onyx-015 was injected into the brain that surrounded a surgically resected glioma. None of a total of 24 patients experienced serious adverse events related to Onyx-015. Although lymphocytic and plasma cell infiltrates were found within tumors histologically, no definite anti-tumor efficacy was demonstrated in this trial. After an unsuccessful Phase III trial of combination therapy of chemotherapy and Onyx-015 in head and neck cancer patients (NCT00006106),^{19,20} clinical development of Onyx-015 discontinued in the United States. In China, combination therapy of intratumoral injection of H101, a CRAd very similar to Onyx-015,

and chemotherapy demonstrated promising efficacy for head and neck cancer.²¹ In 2005, H101 (Oncorine) was approved as the world's first OV in China.²² Currently, H101 trials for hepatocellular carcinoma are underway in China (NCT03790059, NCT03780049).

2nd Generation CRAd: Delta-24-RGD (DNX-2401)

The presence of multiple adenoviral genes that target cellular cell cycle regulators provided the opportunity to develop oncolytic adenoviruses targeting new pathways. Since many cancers including gliomas have deficiencies in the Rb (retinoblastoma) tumor suppressor pathway, virus therapy targeting the Rb pathway was developed as the second generation of CRAd.²³ E1A-delta24 (Delta-24) was based on human adenovirus type 5 and had a 24-base pair deletion in the Rb-binding domain of the E1A gene. The major function of E1A protein is to bind to Rb protein and release transcription factor E2F from the Rb-E2F complex, promoting E2F-driven transcription of genes associated with the S phase (e.g. DNA metabolism) and thereby viral replication.²⁴ Delta-24 cannot replicate in cells with intact Rb due to the inability of the mutant E1A to bind to Rb protein. On the other hand, Delta-24 can replicate in tumor cells in which Rb is deleted, since E2F that exists in a free state promotes gene transcription and transition to the S phase. At about the same time, dl922-947, another oncolytic adenovirus containing a 24-base pair deletion within the Rb-binding region of E1A, was developed and showed better in vivo efficacy than dl1520 (Onyx-015).²⁵

Human adenovirus type 5 binds to Coxsackievirus and adenovirus receptor (CAR) on the cell surface and enters the host cells. Although CAR is expressed in a variety of normal cells,²⁶ several cancers including glioma express lower levels of CAR,^{27,28} causing poor infectivity of CRAd in cancer cell lines. In order to overcome this problem, Suzuki et al developed the approach that incorporates a sequence encoding an RGD (Arg-Gly-Asp) peptide in a cyclic conformation (RGD-4C) into the HI loop of the fiber knob.²⁹ The RGD sequence interacts with α_v integrins on the cell surface³⁰ that many cancer cells express, including HGG known to highly express integrin $\alpha_v\beta_3$.³¹ The fiber modification with RGD improved the infection rate of CRAd in cancer cell lines and enabled CAR independent infection. The resulting CRAd, Delta-24-RGD (DNX-2401) having a 24-bp deletion in the E1A gene and an RGD motif in fiber protein, became the prototype of a newer generation of oncolytic adenoviruses.

Since the late 2000s, a number of clinical trials started testing DNX-2401 for HGG (Table 1), and the first report of a Phase I trial was published in 2018,³² as detailed below.

3rd Generation CRAds

Continued efforts have been made to create CRAd that are more efficacious and cancer-selective. Many novel CRAds have been developed based on DNX-2401 and tested for their effects on HGGs in translational research. To express mutant E1A in a cancer-selective manner, Alonso et al generated the oncolytic adenovirus ICOVIR5 which contains an insulated E2F1-responsive promoter and a Kozak sequence preceding the E1A start codon.³³ DeltaE1A expression driven by a promoter consisting of four palindromic E2F-binding sites and one Sp-1 binding site in ICOVIR15 increased cytotoxicity and antitumor activity.³⁴ ICOVIR17 is a modified version of ICOVIR15 and is armed with cDNA for human hyaluronidase PH20.³⁵ Hyaluronidase-mediated degradation of hyaluronic acid in the tumor extracellular matrix enabled ICOVIR17 to spread better within orthotopic HGG xenografts and extended animal survival.^{35,36} Furthermore, VCN-01, in which RGD was inserted in the fiber shaft instead of fiber knob to improve the infectivity of ICOVIR17, similarly produced a significant survival prolonging effect in an HGG xenograft model.^{37,38} Although these viruses have not entered clinical trials for HGG, Phase I trials of VCN-01 are active for retinoblastoma (EudraCT 2016-001060-11) and pancreatic adenocarcinoma (NCT02045589 and NCT02045602) in Spain.

Delta-24-RGDOX was generated by arming DNX-2401 with mouse OX40L (OX40 ligand) cDNA to activate T cells through the activating receptor OX40 on the surface of T cells.³⁹ Intratumoral injection of Delta-24-RGDOX recruited more CD4+ and CD8+ T cells in tumor sites than Delta-24-RGD, and significantly prolonged survival time in an immuno-competent mouse GBM model. Combination therapy with anti-PD-L1 antibody further increased the therapeutic effect on survival. A Phase I trial of DNX-2440, which is armed with human OX40L, is currently ongoing for patients with recurrent glioblastoma (NCT03714334).

The genomic structures of 2nd and 3rd generations of CRAd are illustrated in Figure 1.

Induction Of Anti-Tumor Immune Response By CRAd

The CNS has long been considered the “immunologically privileged site” representing an immunologically distinct

Table I Current Clinical Trials Studying Oncolytic Adenovirus In High-Grade Glioma

Trial Number	Phase	Virus	Other Treatments	Target	Status	Start Date	Country	Report
NCT03896568	I	DNX-240I	Surgery	Recurrent high-grade glioma	Recruiting	Feb. 2019	USA	
NCT03178032 2016-001577-33 (D24-DIPG)	I	DNX-240I		Naïve DIPG	Recruiting	May. 2017	Spain	Martinez-Velez 2019
NCT02798406 (CAPTIVE)	II	DNX-240I	Pembrolizumab	Recurrent glioblastoma	Active, not recruiting	Jun. 2016	USA	Zadeh 2018
NCT02197169 (TARGET-I)	I	DNX-240I	IFN γ	Recurrent glioblastoma and gliosarcoma	Completed	Sep. 2014	USA	Tufaro 2016
NCT01956734 (D24GBM)	I	DNX-240I	TMZ	Recurrent glioblastoma	Completed	Sep. 2013	Spain	
NCT01582516	I, II	d24-RGD (DNX-240I)		Recurrent glioblastoma	Completed	Jun. 2010	Netherlands	
NCT00805376	I	DNX-240I		Recurrent malignant glioma	Completed	Feb. 2009	USA	Lang 2018
2007-001104-21	I, II	DNX-240I		Recurrent glioblastoma	Ongoing	Jan. 2009	Netherlands	
2016-001600-40 (D2401GBM2)	II	DNX-240I	Standard care	Newly diagnosed glioblastoma	Ongoing	Sep. 2016	Spain	
NCT03714334	I	DNX-2440		Recurrent glioblastoma	Recruiting	Nov. 2018	Spain	
NCT03072134	I	NSC-CRAAd-Survivin-pk7		Newly diagnosed malignant glioma	Recruiting	Apr. 2017	USA	

environment from other organs. Glioma in the brain induces angiogenesis and disruption of the blood brain barrier (BBB), which allows various drugs and immune cells to enter the tumor. However, the microenvironment of GBM is highly immunosuppressive characterized by secretion of immunosuppressive cytokines (TGF- β , IL-10, etc.), and the dominance of immuno-suppressive immune cells such as regulatory T cells, functionally exhausted T cells, myeloid-derived suppressor cells and M2-polarized tumor-associated macrophages.^{40–43} In recent years, immune checkpoint blockade has emerged as a novel immune-therapeutic modality for cancer, but unfortunately, the Phase III CheckMate-143 study (NCT02017717) investigating the anti-PD-1 nivolumab monotherapy in recurrent GBM patients did not extend overall survival compared with bevacizumab (Reardon et al, WFNOS2017⁴⁴). This result may be attributable to the

immunosuppressive microenvironment of GBM and suggest that combination with other therapy that stimulates immune responses may enhance the efficacy of immune checkpoint inhibitors in GBM patients.⁴⁵

CRAAd has been shown to alter the immune microenvironment in experimental GBM in mice.^{46–48} Delta-24-RGD (DNX-2401) treatment of GL261 murine GBM recruited F4/80+ macrophages, NK cells and CD4+ and CD8+ T cells to the tumor site in C57/BL6 mice, and induced T cells to react to the virus and tumor cells.^{46,47} These immune effects mediated the therapeutic efficacy of DNX-2401 as co-treatment with dexamethasone, an immunosuppressive steroid, abrogated the survival benefit in mice.⁴⁷ Thus, adenoviral modification of the immunological landscape within HGG can set a stage for enhancing the efficacy of immunotherapy for HGG. Indeed, Jiang et al showed that the combination therapy with

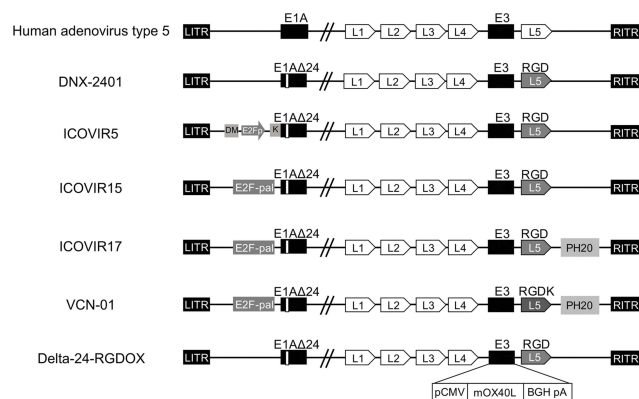


Figure 1 Genomic structure of oncolytic adenoviruses currently used in pre-clinical and clinical studies on high-grade glioma. ITR, inverted terminal repeat. E1AΔ24: a deletion of 24 base pairs within the E1A region. DM: insulator DM-I. E2Fp: E2F-responsive promoter. K: a Kozak sequence. E2F-pal: E2F-responsive palindromes (8 E2F-binding sites). PH20: human sperm PH20 hyaluronidase cDNA. RGD: an RGD integrin-binding motif in the HI loop of the fiber. RGDK: RGDK in the putative heparin sulfate-glycosaminoglycans binding domain KKTK in the fiber shaft. pCMV: the cytomegalovirus promoter. mOX40L: mouse OX40L cDNA. BGH pA: bovine growth hormone poly-adenylation signal. The mOX40L expression cassette replaces the E3 region in Delta-24-RGDOX.

Delta-24-RGDOX and anti-PD-L1 antibody, both administered intratumorally twice, effectively prolonged the survival of murine GL261 GBM model,³⁹ providing a rationale for clinical testing of this immunotherapeutic strategy.

Clinical Trial Of DNX-2401 For Malignant Gliomas

Table 1 summarizes clinical trials of CRAd for HGGs that recently completed or are currently active.

Treatment With DNX-2401 In Recurrent High-Grade Gliomas

Lang et al reported the results of a Phase I trial of DNX-2401 for recurrent malignant glioma³² (NCT00805376). Thirty-seven patients were enrolled and divided into Group A (n = 25) and Group B (n = 12). In Group A, DNX-2401 at escalating doses (1×10^7 to 3×10^{10} viral particles) was stereotactically injected into the tumor after biopsy confirming tumor recurrence. Following treatment, 20% of patients (5 of 25) survived more than 3 years, and dramatic tumor reduction (95% or more, CR) was seen in 3 patients, whose progression-free survival (PFS) was over 3 years. To investigate the mechanism of virus treatment, tumors in Group B were injected with DNX-2401 (1×10^7 to 3×10^8 viral particles) through catheters inserted into the tumors and the tumors were resected 14 days later, along with a second DNX-2401 injection to the wall of the resection cavity. Interestingly, MRI of the 3 complete

responders showed an initial increase in contrast enhancement within 4 months after injection of DNX-2401, suggesting an immune or inflammatory response, followed by regression over 1–1.5 years. Immunohistochemical analysis showed evidence of DNX-2401 replication within the tumor in 6 of 11 Group B tumors (55%) 2 weeks post-virus treatment. Furthermore, intratumoral infiltration of CD4⁺ T cells and T-bet⁺ cells, suggestive of a helper 1 (Th1) response, was observed. Among immune checkpoint proteins, expression of TIM3, but not PD-L1, was decreased after treatment with DNX-2401. Histological examination of a lesion that developed distant from the treated tumor and was resected in one of the complete responders revealed inflammatory cells, with no evidence of tumor cells, suggesting a response driven by adaptive immune memory. Thus, this clinical study for the first time showed direct oncolytic effects in human brain tumors and provided evidence for elicitation of anti-glioma immune responses. No dose-limiting toxicities were observed in this trial. Adverse effects were reported in 15% of patients, but no serious events of grade 3 or higher were noted that were considered to be related to the virus, supporting the safety.

In the Netherlands, a Phase I/II clinical trial was initiated in 2010 testing DNX-2401 for recurrent glioblastoma (NCT01582516). The virus is administered using convection-enhanced delivery via catheters targeting tumor mass as well as the surrounding infiltrated brain. Using tumor tissues and cerebrospinal fluid (CSF) collected from the study participants, van den Bossche et al studied the impact of DNX-2401 injection on tumor macrophages.⁴⁹ In some patients, DNX-2401 treatment increased the CSF concentrations of cytokines such as TNF, IL6 and IFN γ , and such CSF was able to increase the levels of CD64, a marker of M1-polarization, on macrophages in vitro. A tumor that re-recurred and was resected 26 months after DNX-2401 therapy provided a valuable research material that suggested a prolonged shift of a macrophage phenotype from M2 to M1. The feasibility and efficacy results of the trial have not been published.

DNX-2401 And Immunotherapy

Because of the ability of CRAd to alter the immune status by turning the cold tumor microenvironment to hot (inflamed), combining CRAd with immune-modulating therapies is a rational approach, which is supported by preclinical studies for GBM³⁹ and other cancers.^{50–52}

Currently, a Phase II multicenter study is investigating a combination therapy of DNX-2401 and Pembrolizumab (anti-PD-1 antibody) for recurrent malignant glioma (CAPTIVE, NCT02798406). In this trial, a single intratumoral dose of DNX-2401 (5×10^8 , 5×10^9 and 5×10^{10} virus particles) is injected via cannula inserted into the tumor, and followed 7 days later by intravenous injection of 200 mg pembrolizumab every 3 weeks for up to 24 months or until confirmed progression, intolerable toxicity or study withdrawal. Interim outcome was reported at the SNO 2018 annual meeting that 23 patients have been treated and DNX-2401 followed by pembrolizumab has been well tolerated (Zadeh et al,⁵³ SNO2018). Preliminary efficacy is encouraging as two partial responses and 100% 9-month survival for the first 7 patients treated were noted. Publication of longer follow-up data is eagerly awaited.

Addition Of IFN γ To DNX-2401 Did Not Improve Survival

Lang et al reported at ASCO 2017 the Phase Ib trial of DNX-2401 with or without interferon gamma (IFN γ) for recurrent glioblastoma (TARGET-I⁵⁴). Twenty-seven patients were enrolled and randomized to intratumoral DNX-2401 followed by subcutaneous IFN γ (n = 18) or to DNX-2401 alone (n = 9). Due to the poor tolerability of IFN γ , the median duration of treatment was only 6 weeks. Overall survival of 12 months and 18 months for all patients enrolled was 33% and 22%, respectively, regardless of treatment assignment. Three patients (DNX-2401, n=1; DNX-2401 with IFN γ , n=2) remain alive over 1.5 years after the treatments.

DNX-2401 Combination With TMZ

The alkylating agent Temozolomide (TMZ) is part of the standard treatment for newly diagnosed GBM.² TMZ chemosensitivity is known to depend on epigenetic silencing of O⁶-methylguanine-DNA methyltransferase (MGMT) by promoter methylation.⁵⁵ Pre-clinically, DNX-2401 infection was shown to reduce MGMT levels in human GBM cell lines (U87 and T98G) in vitro and decrease IC₅₀ of TMZ.⁵⁶ In vivo, combination therapy of DNX-2401 and TMZ significantly extended survival in a GBM xenograft model in immunodeficient mouse.⁵⁶ Kleijn et al subsequently showed that combination was also efficacious in immunocompetent mouse GBM models.⁵⁷ These studies provided a rationale for the Phase I trial of the combination therapy of DNX-2401 and TMZ that is ongoing in Spain for recurrent glioblastoma (NCT01956734).

DNX-2440 – Oncolytic Adenovirus Armed With T Cell Stimulator

A Phase I trial of intratumoral injection of DNX-2440, a modified version of DNX-2401 armed with OX40L, for recurrent glioblastoma is ongoing in Spain (NCT03714334). This is the first clinical trial testing a 3rd generation CRAAd for malignant glioma.

DNX-2401 In Pediatric Glioma

Pediatric high-grade gliomas (pHGG) are distinct from adult HGG as pHGG harbor genetic drivers that adult HGGs do not and arise at different CNS locations. Diffuse intrinsic pontine glioma (DIPG) is an aggressive pHGG that arises in the brainstem and is characterized by histone H3 K27M mutation that is also frequent in pediatric gliomas in the midline structures such as the thalamus. pHGG of the cerebral cortex typically harbors pG34R/V histone H3 mutations. In contrast, H3 mutations are extremely rare in adult HGGs.^{58,59} Because of the vital functions of the brainstem, resection is not a therapeutic option for DIPG. Radiotherapy is the standard of care for DIPG, but this only improves the quality of life for several months and is not curative.⁶⁰

Preclinically, Martinez-Velez et al reported that DNX-2401 viral infection and replication were observed in several human and murine DIPG cell lines. Intratumoral administration of DNX-2401 was safe in mice and resulted in a significant increase of survival rate in both immunodeficient and immunocompetent models of pHGG and DIPG.⁶¹ The same group further showed that DNX-2401 treatment downregulated DNA damage repair proteins and when combined with radiotherapy mediated a synergistic anti-glioma effect in mouse models of pHGG and DIPG.⁶² Currently, a Phase I trial of DNX-2401 for newly diagnosed DIPG is ongoing in Spain (NCT03178032⁶³). And a case report in this trial showed the tolerability of DNX-2401 in an 8-year-old DIPG patient. DNX-2401 was injected via a catheter into the tumor within the pons following biopsy and the procedure was not associated with safety problems or new neurological deficit.⁶⁴ After 3–4 weeks of virus administration, patients will start receiving standard radiation therapy.

Stem Cell Delivery Of CRAAd To HGG

Because of their ability to migrate preferentially toward tumor cells, stem cells have been explored as vehicles to deliver antitumor agents including OV to brain tumors.^{65–67}

Preclinically, combination of different types of stem cells and CRAd have been studied as experimental treatments of glioma; mesenchymal stem cell MSC-Delta24-RGD,⁶⁸ and MSC-ICOVIR17,³⁶ MSC-CRAd-CXCR4-5/3,⁶⁹ neural stem cells (NSC)-CRAd-survivin-pk7⁶⁹⁻⁷² demonstrated anti-glioma activity. CRAd-CXCR4-5/3 and CRAd survivin-pk7 are transcriptionally targeted CRAd using tumor-specific C-X-C chemokine receptor 4 (CXCR4) and survivin promoter, respectively, to control E1A expression and target the viruses to GBM.^{73,74} CRAd-CXCR4-5/3 has a chimeric Ad 5/3 fiber (chimera of the shaft of human adenovirus type 5 and the knob of human adenovirus type 3) that targets CD46 or CD80/86 cellular receptors and exhibits increased transduction of malignant glioma compared with wild-type Ad5.^{73,75} CRAd-Survivin-pk7 has fiber modification with pk7 encoding polylysine that was designed to increase transduction of malignant glioma by direct binding to heparan sulfate and polyanionic cellular receptors.^{74,76,77} Currently, a Phase I trial is ongoing in the US (NCT03072134) in which NSC loaded with CRAd-survivin-pk7 are injected into the resection cavity of newly diagnosed GBM.

Gene-Mediated Cytotoxic Immunotherapy Using Adenoviral Vectors

Adenovirus vectors that deliver the thymidine kinase gene of HSV (HSV-tk) have been long studied as a therapeutic strategy for GBM. Adenoviral transduction of HSV-tk in tumor cells followed by systemic administration of a nucleoside analog prodrug such as ganciclovir induces the phosphorylation of the prodrug, termination of DNA replication and death in dividing cells, as well as neighboring cells through the bystander effect.^{78,79} The death in cancer cells results in release of tumor neoantigens and elicitation of anti-tumor cellular immune responses.^{78,80} Although this modality, so-called gene-mediated cytotoxic immunotherapy (GMCI), is outside the field of OV therapy due to use of replication-defective vectors, induction of tumor-selective immunogenic cell death may underlie a mechanism-of-action common in GMCI and CRAd therapies.

In neuro-oncology, Ad-HSV-tk (AdV-tk) was first tested clinically in a Phase I study in patients with recurrent malignant glioma.⁸¹ A subsequent Phase Ib dose-escalation study for newly diagnosed GBM⁸² confirmed the tolerability and safety of the local injection of the vector and oral valacyclovir administration. A Phase II study conducted in parallel showed an increase in overall survival of patients receiving GMCI, as compared with those treated with the

standard of care, with prominent efficacy observed when gross total resection was followed by GMCI. Recently, a potential efficacy of GMCI was reported in pediatric patients with glioma (NCT00634231).⁸³ Currently, a Phase I trial is investigating the combination of GMCI and immune checkpoint blockade with nivolumab for newly diagnosed high-grade gliomas (NCT03576612). Another interesting clinical trial for newly diagnosed malignant gliomas involves a rational combination of two adenoviral vectors that express HSV-tk or Flt3L, a cytokine that differentiates precursors into dendritic cells and recruits those to the brain tumor environment (NCT01811992).⁸⁴

Discussion – Challenges And Future Directions

During the last 10 years, research on CRAd has been rapidly evolving as a potential novel treatment for patients with HGG. Extensive efforts to translate findings gained from preclinical research using animal models have resulted in a number of early-stage (Phase I and II) clinical trials that have completed or are active in the US and Europe (Table 1). The results of these clinical investigations, regarding feasibility, safety and efficacy, have largely been unpublished yet. As shown by the report by Lang et al,³² survival and radiographic (MRI) follow-up as well as analysis of biospecimens including tumors, CSF, serum, sputum and urine all provide valuable information that helps us understand the complex biological processes and mechanisms-of-action of oncolytic adenovirus therapy (Figure 2). Detailed reports of trials are therefore awaited by the large research community and patients alike. DNX-2401 is currently the mainstream CRAd that is under vigorous testing in multiple trials, and may become the first FDA-approved CRAd. That 20% of patients with recurrent HGGs (mostly IDH wildtype GBM) survived over 3 years is encouraging and such a long survival tail in a fraction of patients is consistent with the results with PVS-RIPO (poliovirus).⁸⁵

Since the clinical benefits of DNX-2401 were very variable between patients,³² search of biomarkers of response is one of the critical tasks. One of the intriguing findings that Lang et al reported with the DNX-2401 trial was that, in all three patients who responded extremely well to DNX-2401 treatment, contrast enhancement of treated lesions showed an increase on MRI between 1 and 6 months after therapy, which then subsided and almost disappeared (Figure 2). This initial enlargement of

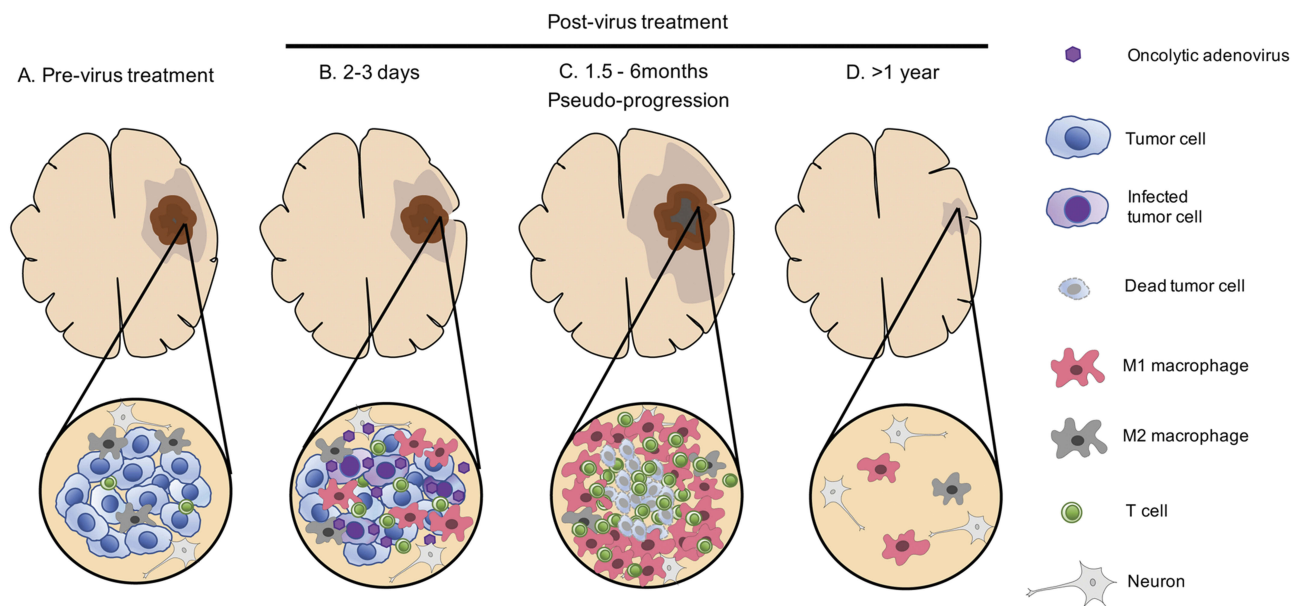


Figure 2 Dynamic changes that occur over time within the tumor microenvironment after oncolytic adenovirus therapy of glioblastoma. Upper, macroscopic and radiographic changes. Lower, microscopic-level changes. **(A)** Before treatment, the GBM tumor microenvironment is immune-suppressive as characterized by M2-polarized macrophages and few effector T cells. **(B)** A few days after virus administration, oncolytic adenovirus is actively replicating within the tumor, starting to induce the recruitment of effector T cells and macrophages with a shift from an M2 to M1 phenotype. **(C)** A few months later, the tumor exhibits an increase in size on magnetic resonance imaging (MRI). At this stage, virus has been cleared, but an active inflammatory reaction persists with the infiltration of immune cells such as T cells and M1-polarized macrophages. Tumor cells are essentially absent (necrosis), despite the worsening of imaging (pseudo-progression). **(D)** During the follow-up, inflammation gradually subsides and MRI shows progressive regression of the lesion and associated edema, which could take over a year.

the lesions in responders is considered pseudo-progression (therapy-mediated tumor enhancement), a phenomenon frequently reported in OV therapy for other cancers³ and explained as inflammatory responses triggered by the virus. The identification of pseudo-progression after clinical CRAd therapy for HGGs raises several questions. First, how can pseudo-progression and recurrence/progression be distinguished, and for this purpose if biospecimens useful or imaging modalities sufficient? Second, is pseudo-progression a reliable biomarker of response and clinical benefit? The Lang report seems to be supportive, but did not provide definitive yes to this question. Third, when pseudo-progression directly causes local brain symptoms or elevation of intracranial pressure, will the use of glucocorticosteroid be appropriate to noninvasively manage the conditions without negatively impacting anti-tumor immune responses and ultimate outcomes? Accumulation of clinical data may be able to address these questions.

The DNX-2401 trial validated prior preclinical research that injection of CRAd elicits the recruitment of immune cells, particularly T cells, to the tumor site. This is an indication that immunocompetent mouse model of HGG^{39,46,47,49,57,61,86} was useful to predict, at least, some biological changes evoked in patients by CRAd therapy. However, all current CRAds are based on human

adenovirus that replicates poorly in mouse cells.⁸⁷ To overcome this problem, multiple administration of high-dose CRAd has been the commonly used approach to mimic the situation of good virus replication.^{39,51} Alternative animal models such as Syrian hamster,⁸⁸ pig⁸⁹ or use of humanized mouse models^{50,90} are being developed and could be shown in the future to better represent clinical scenarios of CRAd therapy.

The field of CRAd is rapidly evolving. The 3rd generation of CRAd, designed to be more potent and maintain safety, are entering several cancer clinical trials as monotherapy or combination,⁶⁷ including a DNX-2440 trial for GBM (NCT03714334). We expect that knowledge on efficacy, feasibility, safety and mechanisms-of-action that will be gained from the current CRAd trials in neuro-oncology will boost efforts of developing newer generations of CRAd and help accelerate transition to their clinical evaluation.

Disclosure

The authors report no conflicts of interest in this work.

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