Immunotherapy Advances, 2025, **5**, ltaf024
https://doi.org/10.1093/immadv/ltaf024
Advance access publication 25 July 2025
Review

Going viral: targeting glioblastoma using oncolytic viruses

Katie Coates[1], Robert J. Nibbs[1], Alasdair R. Fraser[1,2,*]

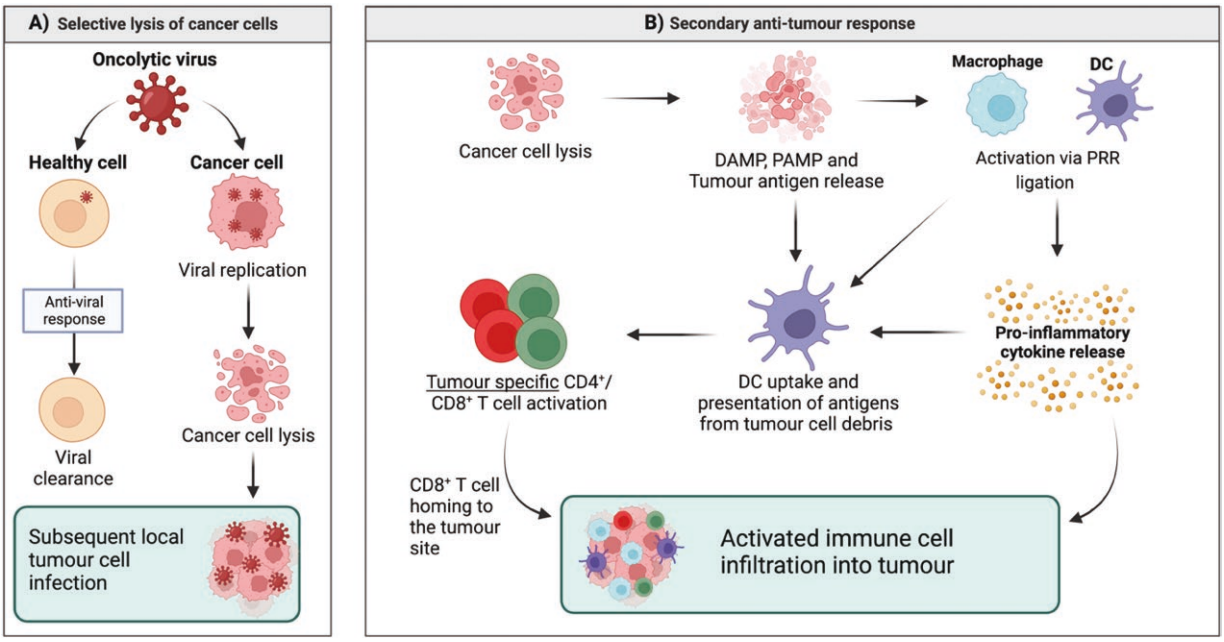
[1]School of Infection and Immunity, University of Glasgow, Sir Graeme Davies Building, Glasgow G12 8TA, United Kingdom
[2]Swarm Oncology Ltd, 15 Stukeley Street, London WC2B 5LT, United Kingdom

[*]Corresponding author. Alasdair R. Fraser, School of Infection and Immunity, University of Glasgow, Sir Graeme Davies Building, Glasgow G12 8TA, United Kingdom. E-mail: alasdair.fraser@glasgow.ac.uk

Abstract

Glioblastoma (GBM) is a devastating malignant disease with a remarkably low 5-year survival rate and invariably poor prognosis. Current conventional treatment options (surgery, targeted radiotherapy, and a limited number of chemotherapies) are rarely entirely effective, leading to the majority of GBM patients experiencing disease recurrence shortly after primary treatment. Thus far, immunotherapeutic approaches towards GBM treatment have been largely unsuccessful due to the tumour site and profoundly immunosuppressive tumour microenvironment (TME). However oncolytic virus therapy (OVT) has been recently developed and licenced for the treatment of cancers and has the potential to switch the TME to become immune-reactive. This has been shown to both directly reduce tumour burden while also enhancing responsiveness to other therapies. In this review, we review the challenges faced by standard immunotherapies in GBM and outline the various approaches to OV treatment of GBM. We highlight the promise of OVT for targeting GBM by critically assessing the outcomes from recent clinical trials.

Graphical Abstract



Keywords: glioblastoma; oncolytic virotherapy; immunomodulatory; early-phase trial

Abbreviations: APC:Antigen-presenting cell,BBB:Blood–brain barrier,BEV:Bevacizumab,CAR:Chimeric antigen receptor,CCL:C-C motif chemokine ligand,CEA:Carcinoembryonic antigen,CNS:Central nervous system,CTLA-4:Cytotoxic T-lymphocyte–associated antigen 4,DC:Dendritic cell,DLDA:Diagonal linear discriminate analysis,DLT:Dose-limiting toxicities,EGFR:Epidermal growth factor receptor,GBM:Glioblastoma,GM-CSF:Granulocyte Macrophage Colony-Stimulating Factor,GSC:Glioma Stem cell,HER2:Human epidermal growth factor receptor 2,HIF:Hypoxia-inducible factor,HSV-1:Herpes simplex virus 1,ICD:Immunogenic cell death,ICI:Immune checkpoint inhibitor,IFN:Interferon,IHC:Immunohistochemistry,IL:Interleukin,ISG:Interferon-stimulated gene,IgG1:Immunoglobulin G1,MDSC:Myeloid-derived suppressor cells,MGMT:O^6-methylguanine-DNA methyltransferase,MOA:Mechanism of action,mOS:Median overall survival,MV:Measles virus,NK:Natural killer,OV:Oncolytic virus,OVT:Oncolytic virus therapy,PD-1:Programmed cell death protein 1,PD-L1:Programmed cell death ligand 1,pGBM:Primary glioblastoma,PFS:Progression free survival,rGBM:Recurrent glioblastoma,SOC:Standard of care,T-VEC:Talimogene laherparepvec,TAM:Tumour-associated macrophage,TIL:Tumour infiltrating lymphocytes,TME:Tumour microenvironment,TMZ:Temozolomide ,TNF:Tumour necrosis factor,TRAE:Treatment-related adverse events,Treg:T-regulatory cell,VEGF:Vascular endothelial growth factor

Received: October 2, 2024; Accepted: June 20, 2025

Introduction

Over 300,000 new cases of Brain and Central Nervous System (CNS) tumours emerge globally each year, contributing to ~ 250,000 deaths across the world in 2020 alone [1]. Glioblastoma (GBM) is the most common and aggressive form of primary malignant brain and CNS tumour in adults and accounts for about 50% of all malignant brain tumours [2]. GBM is a form of high-grade glioma derived from glial cells (the only actively dividing cells in the brain) or possibly neural progenitors [3, 4].

Even with the optimized standard of care (SOC) for GBM (maximal surgical resection followed by adjuvant chemoradiotherapy with temozolomide (TMZ)) [5, 6], outcomes are frequently dismal with a median life expectancy post-diagnosis of < 15 months [5]. Recurrence of GBM usually occurs within 6–8 months post-primary treatment and is the primary cause of death due to the absence of other effective treatment options [7, 8]. This has largely directed research towards finding a suitable therapy for recurrent GBM (rGBM), considered the 'end-stage' of a patient's life with a 1-year survival rate of ~14% and median overall survival (mOS) of 24–44 weeks [9–11]. The revolution of immunotherapy in cancer treatment promised to transform GBM treatment [12]. Various immunotherapeutics including immune checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR)-T cell therapy have reached late phase clinical trials in GBM patients, but have so far yielded disappointing results [13]. The issue primarily is that GBM is an immunologically 'cold' tumour in that it does not induce a strong immune response by virtue of the low level of mutational burden and consequent neoantigen load. This results in poor immune cell infiltration and control of cancer growth, which is exacerbated by the accumulation of immune-suppressive cells in and around the tumour. Coupled with the 'immune-excluded' nature of the brain, this severely limits the effectiveness of many immune-based therapies [14].

Oncolytic virus therapy (OVT) is an immunotherapeutic approach with substantial promise of clinical benefit for GBM patients. OVT relies on the selective infection and lysis of cancer cells by oncolytic viruses (OVs) and a secondary antitumour immune response driven by the resultant cancer cell debris [15]. OVs can also be used as a biological vehicle for transgene payload delivery to the tumour site which has further promising clinical implications [16]. This immunotherapy may improve GBM treatment due to its ability to reprogramme the TME from immunologically 'cold' to immunologically 'hot' (characterized by infiltration of the tumour by immune cells and modulation of the immunosuppressive milieu), thus making the TME more responsive to other immunotherapeutics.

The tumour microenvironment of GBM

The landscape of GBM is intrinsically complex consisting of many features which contribute to immunosuppression, treatment resistance and tumour progression. Hypoxic niches are characteristic of the GBM tumour microenvironment (TME) and support the survival of glioma stem cells (GSCs), a population of undifferentiated cancer cells with the potential for self-renewal [17–19]. GSCs are pro-tumorigenic through activation of hypoxia-inducible factor-1α (HIF-1α), a transcription factor which controls the expression of various growth factors (vascular endothelial growth factor (VEGF), erythropoietin (EPO), and transforming growth factor-β (TGF-β)), adhesion molecules and metabolic proteins contributing to angiogenesis and tumour-cell invasiveness [19–22]. These hypoxic niches are characterized by high acidity and increased interstitial pressure due to elevated anaerobic cell respiration and disruption of the blood–brain barrier (BBB; respectively), factors known to affect the penetration of conventional chemotherapies [9, 23].

At a molecular level, some GBMs harbour epigenetic modifications in the O^6-methylguanine-DNA methyltransferase (*MGMT*) gene which have been linked to clinical response and prognosis [24]. *MGMT* promoter hypermethylation is a prognostic marker of good response to TMZ treatment [25]. Hypermethylation of the *MGMT* promoter silences MGMT expression, thereby enhancing the cytotoxic effects of TMZ [26]. Patients without *MGMT* hypermethylation (~40%–70%) are innately resistant to TMZ, emphasizing that the current SOC therapy is insufficient [27].

The homeostatic brain is frequently considered immune-privileged due to the limited infiltration of immune cells [28]. Under healthy conditions, microglia are the only immune cells present in the brain, responsible for maintaining brain homeostasis [29, 30]. During GBM tumorigenesis, immune cells are recruited via the release of cytokines such as IL-6 and IL-8, chemokines including CCL2 and CXCL10 and growth factors produced by both GBM and infiltrating cells within the nascent TME [31]. These mediators, along with direct cell-to-cell contact signalling (through immune checkpoints, for example) are primarily responsible for immune cell reprogramming towards a pro-tumorigenic phenotype following recruitment. Tumour-associated macrophages (TAMs; both resident microglia and peripheral macrophages) constitute the largest immune cell population within the GBM TME. These cells, along with GBM cells, abundantly express programmed death ligand-1 (PD-L1) which interacts with the immune checkpoint protein programmed death protein-1 (PD-1) expressed on activated tumour-infiltrating lymphocytes (TILs) to inhibit their effector functions, contribute to exhaustion and fuel TME immunosuppression [32, 33]. Consequently, the fractional population of TILs in GBM have a predominantly exhausted phenotype [34]. Other leukocytes in the TME include T regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), and dendritic cells (DCs) [13, 35]. TAMs, Tregs, and MDSCs are the main producers of immunosuppressive cytokines such as IL-10, TGF-β, and IL-4 which drive differentiation of $CD4^+$ T cells to Tregs, inhibit apoptosis-inducing proteins (e.g. granzyme) and dampen the immune response [14, 35]. Additionally, DCs and TAMs produce indoleamine 2,3-dioxygenase which further fuels T-cell exhaustion and promotes Treg expansion by depleting key metabolites such as tryptophan [35]. Collectively, these immune cell populations contribute to a highly immunosuppressive TME. Immune profiles of patients with primary GBM (pGBM) or rGBM differ, though clinical data are complicated by patient heterogeneity [7]. Some studies report an increase in exhausted $CD8^+$ T cells or TAMs in rGBM [36, 37], whereas others report that immune cell infiltration varies from patient to patient, independent of pGBM or rGBM status [7, 38]. Consequently, rGBM remains one of the most poorly understood tumours with limited treatment options.

Table 1. Current and completed CAR-T cell clinical trials in GB

CAR target antigen	Disease	Status	Clinicaltrials.gov ID	References
HER2	HER2-positive rGB	Completed	NCT01109095	Ahmed et al. (2017) [54]
EGFRvIII	EGFRvIII, MGMT-unmethylated rGB	Completed	NCT02209376	O'Rourke et al. (2017) [55]
	EGFRvIII-positive rGB	Completed	NCT01454596	Goff et al. (2019) [56]
	rGB	Recruiting	NCT05868083	N/A
	EGFRvIII-positive GB	Recruiting	NCT06186401	N/A
IL13Rα2	rGB	Completed	NCT00730613	Brown et al. (2015) [57]
	rGB/ rfGB	Recruiting	NCT04003649	N/A
	Leptomeningeal GB	Recruiting	NCT04661384	N/A
Dual targeting: IL13Rα2/ EGFR	EGFR-overexpressing rGB	Recruiting	NCT05168423	N/A

Information accessed May 2024 from Clinicaltrials.gov. All trials are phase I.
Abbreviations: HER2—human epidermal growth factor receptor-2, EGFRvIII—Epidermal growth factor receptor variant III, IL13Rα2—interleukin-13 receptor α2, rfGB—refractory glioblastoma, pGB—progressive glioblastoma.

Immunotherapy of glioblastoma: a false dawn?

Immunotherapy has had substantial benefits in many cancers, particularly through the use of targeted cell therapies (CAR-T) and checkpoint inhibitors (ICI) [34]. ICIs are therapeutic antibodies that block immune checkpoint receptors/ligands, boosting T cell activation. PD-1/PD-L1 inhibitors such as nivolumab and pembrolizumab have proved particularly effective in solid tumour types such as melanoma and lung cancer [39]. The PD-1/PD-L1 axis is a key pathway in GBM immunosuppression and models of GBM have demonstrated that targeting this in GBM could be effective [40–42]. This led to Checkmate 143 [43] and Checkmate 498 [44], both phase III trials of nivolumab in rGBM treatment where efficacy was compared to current SOC (bevacizumab with radiotherapy versus TMZ and radiotherapy). Primary endpoints were not reached for either trial—nivolumab did not improve overall survival (OS) compared to SOC treatments [45]. Patient responses were also poor in a phase I multicohort clinical trial (KEYNOTE-028) evaluating the safety and efficacy of pembrolizumab in 20 PD-L1 positive rGBM patients [46].

ICIs targeting cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) have also been utilized for GBM treatment. CTLA-4 is a T cell receptor which when bound to its ligands (CD80/86) expressed on antigen-presenting cells (APCs) prevents co-stimulation, inhibiting T cell activation and driving robust antitumour immune responses [13, 47]. CTLA-4 expression is thought to be dependent on the tumour subtype [48], but high levels of CTLA-4 on $CD4^+$ and $CD8^+$ T cells have been associated with a poorer prognosis [49, 50].

Inhibition of CTLA-4/CD86 ligation has shown varying levels of survival benefit in mouse models of GBM [41, 50, 51]. In a phase I clinical trial, GBM patients received systemic administration of ipilimumab [52] with nivolumab which resulted in increased occurrence of serious treatment-related adverse events (TRAEs) compared to nivolumab monotherapy (70% vs 20%, respectively), whilst not significantly improving patient outcomes (mOS 9.2 versus 10.4 months, respectively) [53]. Intracerebral administration of lower doses of nivolumab and ipilimumab to rGBM patients post-resection was tolerated with limited TRAEs, but still did not improve survival [54].

There are multiple factors which could be attributed to the failure of ICIs in GBM. The BBB presents a structural barrier for drugs with a molecular weight greater than 400–600 Da—nivolumab and ipilimumab are > 140 kDa [45, 55]. Therefore, ICIeffects focus primarily on secondary lymphoid organs to reactivate anergized or exhausted T cells primed against tumour antigens. However, a proportion of checkpoint-expressing immune cells have likely already infiltrated the tumour where they are inaccessible to the antibodies [45]. Although intra-tumoral drug administration could address this issue, this alone is insufficient to overcome the immunosuppressive TME. PD-1/PD-L1 inhibitors have been shown to improve the trafficking of activated T cells to tumour sites, but soon became exhausted due to pro-tumorigenic myeloid cells (particularly TAMs) action in the TME [56]. Despite these failures, ICIs may still be useful in combination therapies.

CAR-T therapy

Another treatment that has revolutionized cancer immunotherapy in recent years is CAR-T cell therapy. CAR-T cells are generated either via viral or non-viral vectors which carry the gene encoding a chimeric antigen receptor (CAR) which recognizes a cancer-specific target. Patient T cells are collected and transformed with the CAR vector which permanently integrates into the genome resulting in cell-surface CAR expression [57, 58]. CD19-targeting CAR-T therapies have been highly effective in the treatment of B cell-derived haematological malignancies [59] but have shown limited clinical success in the targeting of solid tumours [57]. There are various clinical trials ongoing for CAR-T in rGBM (Table 1) but all have so far failed, primarily due to a lack of CAR-T cell expansion due to TME immunosuppression and downregulation/loss of target antigens [7]. It is possible that multiple antigen-targeting CARs/neoadjuvant treatment with an immune-stimulating therapy may boost the effectiveness of CAR-T therapy [60].

Although efforts are continuing, ICIs and CAR-T cells have been largely unsuccessful in the treatment of GBM. The immunosuppressive TME is a major factor in this failure and research has shifted towards developing treatments that can switch the GBM TME from immunosuppressive to immune-reactive, improving the efficacy of immunotherapies.

Response to viral infections is rapid and acute due to the recognition of viral products by Toll-like receptors after cell lysis and harnessing this mechanism offers a novel way to modulate the TME in solid cancers. As such, OVT is at the forefront of this particular approach.

Oncolytic virus therapy: an overview

OVT relies on replication-competent viruses to preferentially lyse cancer cells and induce an antitumour immune response, while leaving healthy cells unharmed [61]. OVT is highly promising due to its typically low toxicity profile and high compatibility with other cancer drugs as its mechanism of action (MOA) is distinct (Fig. 1) [16]. Although the exact MOA depends on the OV/tumour, the basic principle is that OVs selectively infect cancer cells, undergo viral replication resulting in oncolysis, releasing virions into the TME and driving subsequent infection of other tumour cells (Fig. 1A). The viral products and dead cell debris drive a secondary antitumour immune response (Fig. 1B) [62].

Many viral strains are suitable for OVT based on natural oncolytic properties and have potential as therapeutics via genetic engineering. Ideal candidates are pro-immunogenic, capable of inducing oncolysis and suitable for large-scale clinical production [62]. DNA and RNA viruses have both shown success in OVT clinical trials, and each viral strain has unique benefits [63]. OVs must have natural or genetically engineered oncotropism to avoid damage to normal cells ('off-targeting'). Recent advances in genetic engineering approaches, such as CRISPR-based gene editing mean that most OVs have been genetically modified to ensure tumour cell selectivity regardless of their natural tropism [64]. OVs can exploit various factors to ensure tumour-specific viral entry/replication including overexpression of extracellular receptors, immune evasion mechanisms, and dysregulated intracellular pathways. For example, CD46 is commonly overexpressed on tumour cells to prevent recognition by the complement system but is also the receptor for measles virus (MV), so MV-based OVs can exploit CD46 for enhanced natural tumour selectivity [64, 65]. Alternatively, oncotropism can be genetically engineered into a virus. The RGD protein motif has been inserted into the genomes of various OVs [66, 67] to facilitate recognition of cell surface α-integrins ($\alpha_v\beta_3$ [68] and $\alpha_v\beta_5$ [69]) which are often upregulated in cancers. Finally, genetic alterations can be made to enhance the anti-viral response in infected healthy cells to prevent viral spread in normal tissue. For example, adenovirus E1B downregulates anti-viral gene expression such as interferon-stimulated genes (ISGs) [70]: its deletion from the virus results in attenuated viral replication in healthy cells but not in cancer cells as they downregulate cytokines involved in the anti-viral immune response (such type I IFNs) during the transformation process [62, 71].

Transgene insertion into the OV genome allows the OV to act as a biological vehicle to deliver antitumorigenic factors alongside the primary MOA. This is a way of further 'arming' the OV to enhance their cytolytic functions. Many transgenes have exhibited their efficacy in OVT for various tumour types [72]. T-VEC is a recombinant Herpes

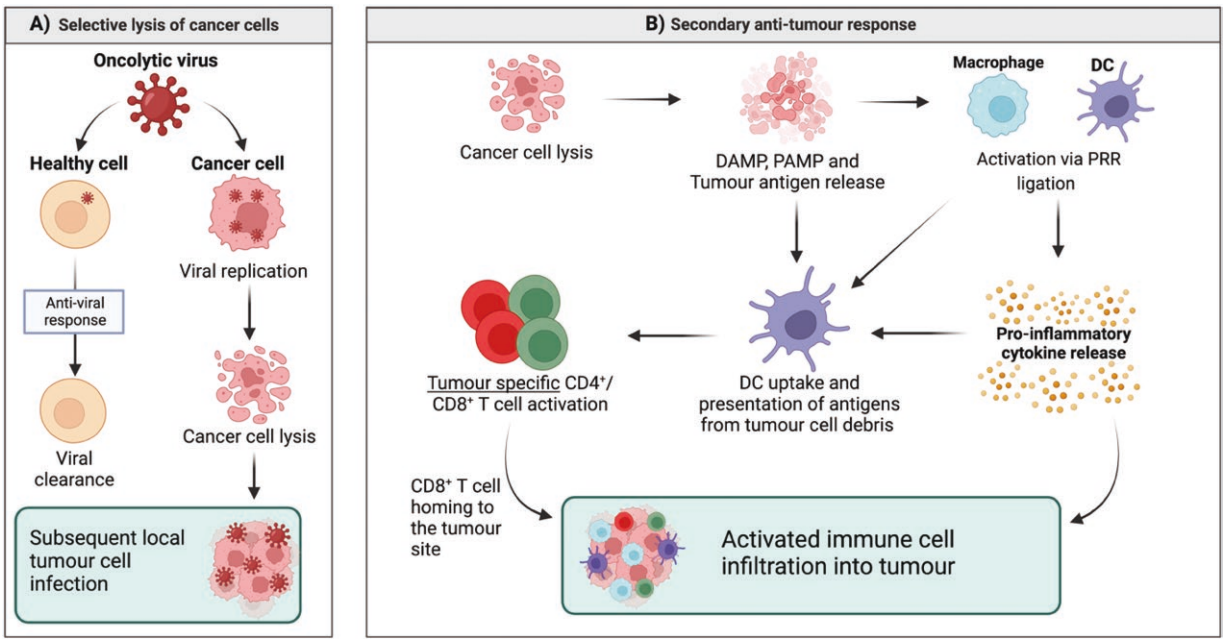


Figure 1. The dual mechanism of action of oncolytic virus therapy. (A) OVs cause direct cell lysis through selective infection of cancer cells, ultimately resulting in cell lysis. OVs exploit the defective anti-viral pathways in tumour cells for uncontrolled replication. Cell lysis allows release of virions into TME, infecting other tumour cells and driving further cytolysis. In healthy cells, the anti-viral response pathways are intact, thus viral clearance is achieved rapidly after viral entry. (B) Cell death releases tumour antigens to be picked up by APCs and various immune-stimulating factors such as DAMPs and PAMPs which activate PRRs, primarily in DCs and macrophages. This stimulates pro-inflammatory cytokine release, recruiting immune cells to the tumour site. Activation of DCs via PRR allows DCs to mature and present tumour antigens via MHC to naïve $CD4^+$/$CD8^+$ T cells in the draining lymph node. T cells specific for tumour antigens will undergo activation, clonal expansion, differentiation and then relocation via chemokine interactions to the tumour site. Illustration created using BioRender.com. Abbreviations: DAMP—Damage-Associated Molecular Pattern; PAMP—Pathogen-Associated Molecular Pattern; PRR—Pattern Recognition Receptor. Created in BioRender. Coates, K. (2025) https://BioRender.com/gs1vlz6

Simplex Virus 1 (HSV-1) licenced for clinical use which was genetically engineered to express granulocyte-macrophage colony-stimulating factor (GM-CSF) [16, 73] which enhances DC recruitment and subsequent activation after tumour antigen uptake, driving a stronger adaptive immune response [74]. T-VEC demonstrated marked clinical success prior to its FDA approval in 2015 for the treatment of metastatic melanoma. T-VEC is now one of the first-line treatments for metastatic, unresectable melanoma [74]. The OVs H101 and ECHO-7 are also clinically approved for their indications (Table 2) [16]. Preclinical and clinical studies of OVTs have indicated that the therapy is safe and effective, with some even being implemented as first-line therapies for solid tumours.

OVT: A transformative therapy for GBM?

The first OV licenced for clinical use was ECHO-7, which substantially reduced the risk of progression in melanoma [64] though this has been joined by several other virus candidates.

HSV-1 as an oncolytic virus

Todo *et al.* developed G47Δ, a triple-mutated oncolytic HSV-1 created from a predecessor oncolytic virus (G207) by a further 312 base pair (bp) deletion within the *α47/ICP47* gene (Fig. 2A) [75]. G47Δ was found to reduce tumour size significantly in thr U87MG xenograft glioma model in athymic mice [75]. However, these xenografts were subcutaneous and not intracerebral, limiting the applicability of these results to the actual human disease. Both G47Δ and G207 were safe when injected intratumourally into U87MG mice. Further studies found that G47Δ was capable of infecting and killing GSCs *in vitro* either alone [76] or synergistically with TMZ [77]. As GSCs are drivers of SOC resistance and tumour recurrence, these findings have promising clinical implications.

Following its preclinical success, G47Δ (Dalytect® or teserpaturev, Daiichi Sankyo Co. Ltd.) progressed to clinical trial. A phase I, single-arm, dose-escalation trial of G47Δ, injected via stereotactic intra-tumoral injection, examined its safety and efficacy in recurrent/progressive GBM treatment (UMIN000002661; Fig. 2B). The primary endpoint of safety was achieved as all adverse events were considered non-severe (grade 1–grade 3) [10]. The secondary endpoint of efficacy (tumour shrinkage, improved overall survival/progression-free survival (OS/PFS) measured by the complete response (CR) and partial response (PR)) was harder to confirm as successful due to the occurrence of 'pseudo-progression', a phenomenon where there is an enlargement of a contrast-enhanced lesion driven by local inflammation and cellular infiltration [78]. However, data from 2 years post-G47Δ treatment revealed CR in one patient, PR in one patient, stable disease (SD) in six patients, and progressive disease in five patients. On the date the study was released (March 2022), 12/13 patients had died, with the patient who experienced PR surviving > 11 years from the last G47Δ administration. Additionally, 2/12 patients who died had experienced long-term benefits from G47Δ treatment surviving 46 months and 47 months from the final dose. Post-treatment data analysis revealed a mOS from the last G47Δ injection of 7.3 months and a 1-year survival rate of 38.5%. The predefined historical control for this trial (obtained from meta-analysis of other clinical trials of various novel rGBM treatments) was mOS of 5 months and a 1-year survival rate of 14% in rGBM patients so G47Δ successfully achieved its secondary endpoint [10].

Immunohistochemistry confirmed viral presence at the tumour site and showed enhanced infiltration of $CD4^+$ /$CD8^+$ lymphocytes in post-treatment biopsies compared to pre-treatment biopsies. Biopsies taken at later time-points (>13 months) indicated the persistence of antitumour $CD4^+$ and $CD8^+$ T cell infiltration, even after viral clearance, suggesting G47Δ was capable of inciting a long-lasting, tumour-specific immune response [10]. Todo *et al.* observed lymphocyte aggregation at areas with remaining tumour cells rather than areas of HSV-1 positivity, once again suggesting a tumour-specific adaptive immune response. Collectively, these data provided a rationale for further investigation in the phase II clinical trial.

The phase II clinical trial evaluated the efficacy of G47Δ delivered intratumorally (up to six doses) in 19 patients with residual/recurrent GBM, with the primary endpoint of improved 1-year survival rate (UMIN000015995; Fig. 2B) [79]. All patients had previously received SOC. The study reached its primary endpoint as the 1-year survival rate following G47Δ administration was 84.2% compared with the historical control of 15% (from chemo-radiotherapy). Secondary endpoints of the trial were defined as improved OS and PFS and mOS and PFS were 20.2 and 4.7 months, respectively, following G47Δ initiation which compared favourably with pooled data from 16 chemotherapy trials in rGBM (mOS and PFS of 5 months and 1.8 months, respectively) [79]. The safety of G47Δ was similar to that of the previous trial, with most adverse events such as fever and headache associated with immune responses.

Analysis of tumour biopsies following OV administration aligned with the phase I trial findings showing infiltration of $CD4^+$/$CD8^+$ T cells which increased with each G47Δ injection. However, it was observed that it took ~4 months for these immune cells to cause tumour shrinkage, but this could be due to the occurrence of 'pseudo-progression'. It was also

Table 2. Clinically approved OVs

Oncolytic virus	Viral strain	Genetic modifications	Route of administration	Indication	Country, year of approval
T-VEC	HSV-1	GM-CSF insertion, both ICP34.5 gene deletion, ICP47 gene deletion [62].	Intratumoural injection	Metastatic melanoma	USA, 2015
H101 (Oncorine)	Adenovirus	Total (55 kD) deletion of E1B gene, partial deletion (78.3–85.8μm) of E3 region [63].	Intratumoural injection	Nasopharyngeal carcinoma	China, 2005
ECHO-7 (Rigvir)	Enterovirus, ECHO group [62]	No genetic modifications [64]	Intramuscular injection local to tumour site	Early-stage melanoma	Latvia, 2004

Use approved by clinical regulators in the listed countries.

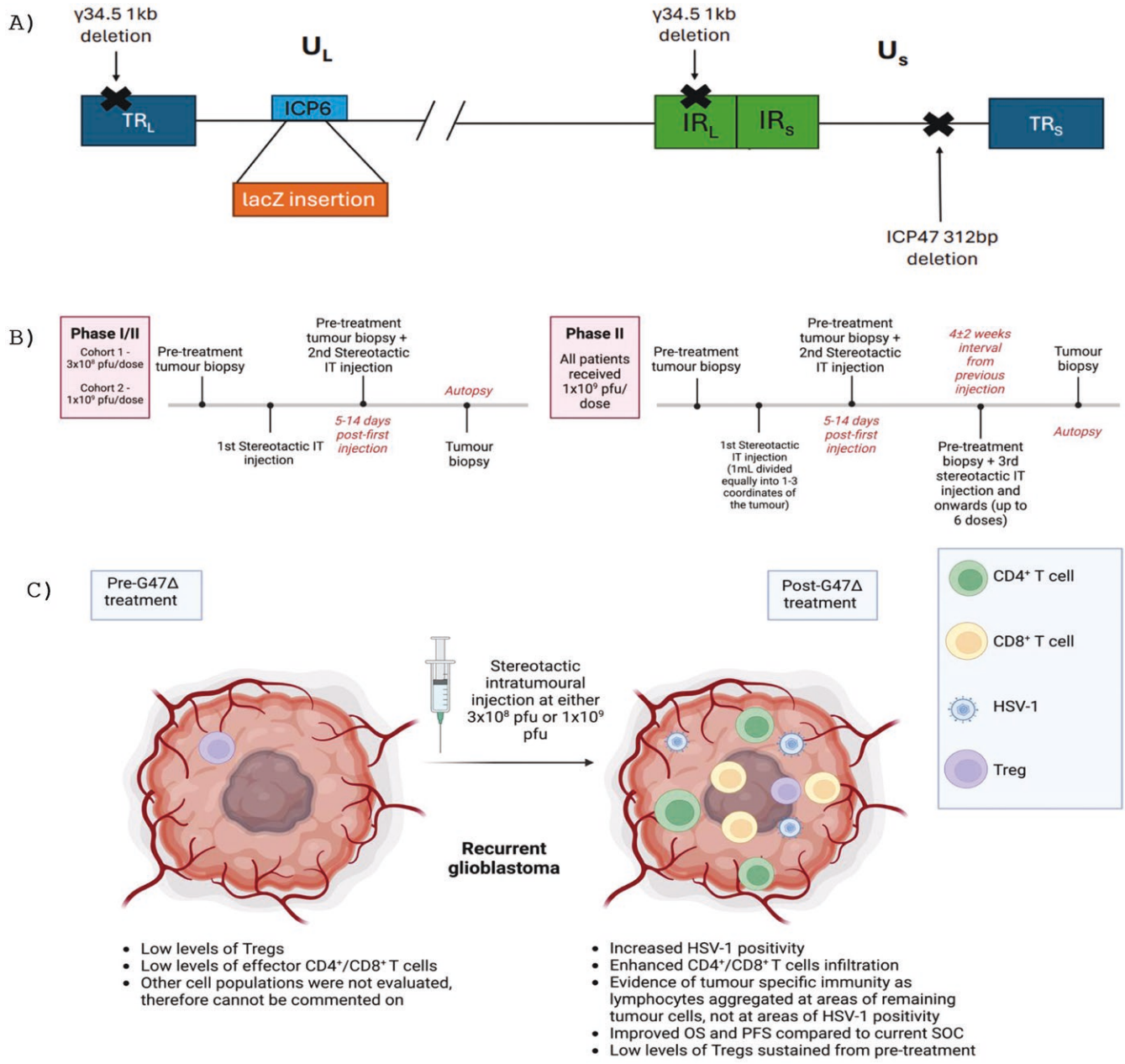


Figure 2. An overview of G47Δ. (A) Genetic modifications used to create G47Δ from HSV-1. The HSV-1 genome consists of two unique strands—long (U_L) and short (U_S). Each contains a terminal repeat region (TR_L and TR_S) and an internal repeat region (IR_L and IR_S). G47Δ contains a 1kb deletion of both γ*34.5* genes in the TR_L and IR_L, inactivation of the *ICP6* gene through insertion of the *Escherichia coli lacZ* gene and a 312bp deletion in the *ICP47* gene. These deletions/inactivation increase immune recognition of viral infection in healthy cells by upregulating viral protein expression. Adapted from Todo et al. (2001) and Fukuhara et al. (2016) [65, 70]. (B) Trial designs of the phase I/II and phase II clinical trial of G47Δ in rGB led by Todo et al. (C) Cellular changes in recurrent GB post-G47Δ treatment. Biopsies were taken from patient tumours pre- and post-G47Δ and analysed by IHC. It was found that there was HSV-1 positivity in the G47Δ-treated tumours, confirming viral presence, along with significant increase in CD4+/CD8+ T cell infiltration, but low levels of Tregs in pre- and post-treatment biopsies. Figures 2b and c created using information gathered from both G47Δ phase I and phase II trials led by Todo et al. [10, 69]. Abbreviations: pfu—plaque forming units; HSV-1—herpes simplex virus-1; Treg—regulatory T cell; TAM—tumour-associated macrophage; OS—overall survival; PFS—progression free survival; SOC—standard of care; GB—glioblastoma; kb—kilobase; bp—base pair; MHC—major histocompatibility complex; WT—wild type; rGB—recurrent glioblastoma. (B) Created in BioRender. Coates, K. (2025) https://BioRender.com/ki2ooo0; (C) Created in BioRender. Coates, K. (2025) https://BioRender.com/soknn4o

found that there were low levels of Tregs in the tumour site pre-treatment and this was sustained over the period of G47Δ treatment/post-treatment [79]. Autopsy biopsies revealed post-treatment recurrent lesions had greater Treg infiltration than was observed in pre-treatment biopsies and over the course of G47Δ treatment, but still had evidence of IT adaptive immunity (CD4⁺/CD8⁺ T cells) [79]. Therefore, although there is evidence of sustained adaptive immunity long after treatment, the recurrence of Treg-infiltrated GBM suggests G47Δ is not sufficient for long-term tumour remission by itself. The cellular changes likely to have occurred post-G47Δ are summarized in Fig. 2C.

As a result of these findings, G47Δ was approved for the treatment of recurrent/progressive GBM in Japan. G47Δ is the first and only OV to be approved for GBM and provides a pathway for future GBM therapies.

These studies highlighted a key limitation of contrast-enhanced magnetic resonance imaging (MRI), a common diagnostic tool in GBM clinical trials used to evaluate PFS by monitoring tumour size [80]. MRIs rely on gadolinium (a contrast-enhancing agent) leak due to BBB breakdown characteristic of GBM, but inflammation also causes the breakdown of the BBB. As OVs produce inflammation at the tumour site, this leads to pseudo-progression [80]. More clinically informative imaging techniques are needed for inflammation-inducing therapies such as OVT. Alternatively, the development of separate treatment response criteria for OVT is needed as the current World Health Organization (WHO) gold standard is not applicable [10]. Additionally, the patient cohorts for both trials were small suggesting a lack of representation of tumour heterogeneity. Due to this, Japanese legislation gave time-limited approval due to a lack of robust clinical evidence. Full approval may be achieved following justification of clinical benefit in a post-marketing report [81]. Finally, there were no transgenes inserted into G47Δ which could be an area of investigation in the future to improve efficacy.

CAN-3110 is another HSV-based oncolytic virus which has shown clinical benefit in rGBM patients [82]. A phase I clinical trial of CAN-3110 in rGBM patients met the primary endpoints with no dose-limiting toxicities observed at any dose level and improved mOS compared to historical data from SOC treatment (11.6 months vs 6–9 months), and furthermore, an important connection was identified between the serology status of patients and subsequent response. Pretreatment HSV-1 seropositivity was associated with significantly prolonged survival compared with seronegative counterparts (mOS of 14.2 months vs 7.8 months, respectively). A follow-up phase I clinical trial is underway for CAN-3110 in rGBM (ClinicalTrials.gov ID: NCT03152318) in which they are primarily investigating the maximum tolerated dose with and without cyclophosphamide (chemotherapy) but also assessing the suitability of MRIs in OVT through measurement of the rate of pseudoprogression. This OV has now received fast-track designation for Candel Therapeutics by the FDA for the treatment of rGBM.

Adenovirus-based OVs

DNX-2401/Delta-24-RGD is an oncolytic human adenovirus type 5 with two genetic alterations—a 24 bp deletion in the early 1A adenovirus (E1A) gene within the retinoblastoma (Rb) protein binding region and insertion of a peptide (RGD-4c) which acts as an anchor for adenoviral attachment to integrins on cancer cells (Fig. 3) [67, 83]. Athymic U87MG xenograft mice showed improved survival ($P < .001$) when treated with an intra-tumoral injection of DNX-2401 (mean survival of 131 days) compared with controls [83]. DNX-2401 had low toxicity and immunohistochemistry from another study showed increased T-bet expression in the tumour sites of DNX-2401-treated animals compared to controls, indicative of a Th1-skewed immune response with high IFN-γ expression [84].

A Phase I clinical trial of DNX-2401 in patients with malignant glioma in 37 patients was initiated (GBM (89%), gliosarcoma (5%) or anaplastic astrocytoma (5%) in two groups—study design summarized in Fig. 4A) [85]. Group A was designed to evaluate safety and response at different doses while group B was to evaluate the MOA. Pre-treatment biopsies were taken from all patients.

Intra-tumour administration of DNX-2401 was safe with few AEs (only grade 1–2 events). There were no DLTs encountered in this study and the maximal achieved dose was 3×10 [11] virus particles (vp). Results were promising with 72% of patients in group A experiencing tumour regression and 5 out of the 25 patients surviving more than 3 years post-treatment, three of which had > 95% tumour regression with no further tumour progression for over 3 years. One patient with CR had a recurrence 2.5 years post-treatment, but pathologic analysis of the lesion after resection revealed only necrosis and inflammation with no evidence of viable GBM cells. This suggests that DNX-2401 generated tumour-specific immune memory which was activated upon lesion recurrence [85]. As patients in group B only had resection at day 14 post-treatment with no subsequent biopsies/MRIs, they were only evaluable for overall survival—there was a 2-year survival rate of 17% in this group. The mOS for both groups was 13 months which, when compared to historical data (mOS of 6 months), suggested that DNX-2401 substantially improved survival.

In group A, MRI showed that contrast-enhanced lesions increased in size by 4 months post-treatment in the three CR patients, consistent with the incidence of an inflammatory immune response [85]. Following this, there was a 12- to 18-month period of lesion size reduction similar to results seen with G47Δ. Using the specimens derived from group B resections, IHC found that 6 out of 11 resected tumours had evidence of adenoviral replication 14 days post-treatment. The resected tumours consisted of three zones—a central necrotic core (presumably virus-induced), active viral replication in tumour cells, and uninfected peripheral tumour cells.

Of the 11 patients in group B, 9 patient tumours were evaluated via IHC for CD3, CD4, CD8, and T-bet. All samples contained $CD3^+$ cells (and primarily CD8+) which were either distributed throughout the tumour or perivascular. These lymphocytes were positive for T-bet, indicative of a T_H1 response seen in preclinical studies. Furthermore, DNX-2401 appeared to ameliorate the exhaustion of T-cells in the glioma TME, as there was downregulation of TIM-3 (a cell surface marker of T-cell exhaustion) expression post-treatment [85]. Notably, there was a significant increase in $CD4^+$ but not $CD8^+$ T cells in post-treatment biopsies compared to pre-treated. Collectively, these data indicate that IT injection of DNX-2401 was capable of inducing infiltration of T cells into gliomas. The postulated changes induced by DNX-2401 are shown in Fig. 4B. This study quantified the effects of DNX-2401 on T cell infiltration but other leukocytes were assessed via IHC qualitatively. The study was limited in using a single dose of DNX-2401: recent findings indicate that multiple doses of OVs greatly increase treatment success [67]. These positive results led to subsequent trials investigating the potential of transgene insertion and combination therapy with other therapeutics [67].

Another use of HSV as an oncolytic includes NSC-CRAd-S-pk7, an engineered HSV delivered via a human neural stem cell line as an allogeneic product for rGBM (City of Hope / Calidi Biotherapeutics). In a phase I dose-escalation

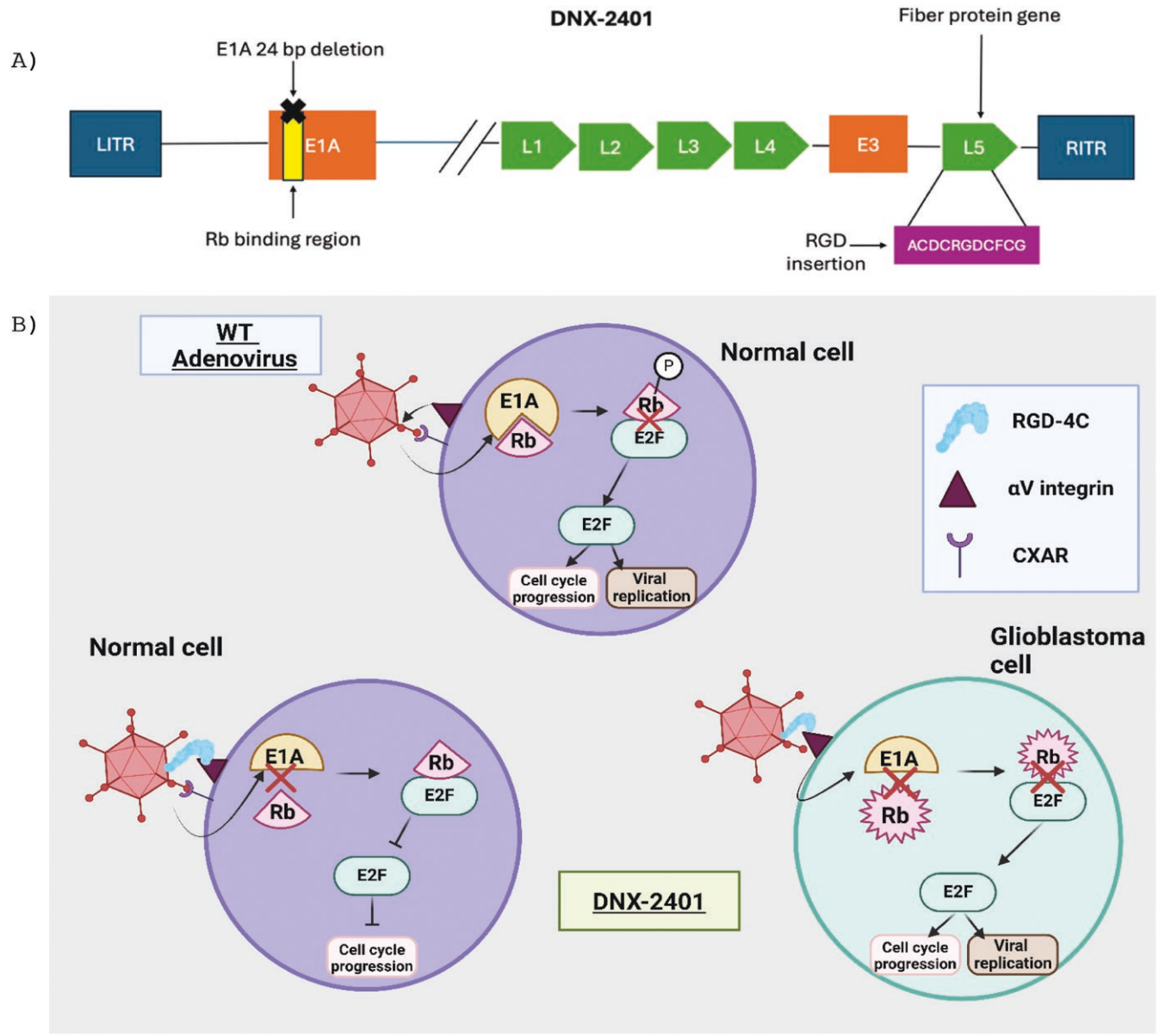


Figure 3. The design and mechanism of DNX-2401. (A) Genetic manipulations used to create DNX-2401 from human adenovirus type 5. DNX-2401 was created via a 24 bp deletion in the E1A gene with a RGD-4c motif insertion. These alterations increase viral protein expression to allow immune clearance in healthy cells and improve viral uptake in cancer cells, respectively. Figure created on Microsoft PowerPoint and adapted from Kiyokawa et al. (2019); Zhou et al. (2014); Kulanayake and Tikoo (2021) [77–79]. (B) Mechanism of tumour selectivity in DNX-2401. In normal cells both WT adenovirus and DNX-2401 viral entry is mediated by the binding of the adenovirus fibre knob with CXAR and subsequent association of the penton base with αv integrins (αvβ3 and αvβ5), both on the surface of the target cell. Virus is then internalized by the target cell via clathrin-mediated endocytosis. GB cells lack CXAR, thus the RGD-4c peptide insertion provides an alternative entry route via αvβ3/αvβ5 integrins. E1A binds and inactivates Rb by phosphorylation. Inactive Rb cannot form a complex with E2F resulting in high levels of free E2F, pushing the cell cycle from G1 to S phase and permitting viral replication. DNX-2401 lacks the Rb binding region of E1A therefore it cannot bind and inactivate Rb, arresting cell cycle and preventing DNX-2401 replication. In GB cells, Rb is already inactivated, therefore it cannot bind to E2F, thus there will be cell cycle progression and viral replication. Figure created using BioRender.com and adapted from Vecil et al., 2007 [80]. Abbreviations: LITR—left inverted terminal repeat; E(1A, 3)—early transcription genes; L(1,2,3…)—late transcription genes; RITR—right inverted terminal repeat; bp—base pairs; CXAR—coxsackie and adenovirus receptor; WT—wild type; CXAR—coxsackie and adenovirus receptor; Rb—retinoblastoma; E1A—early 1A adenovirus gene; GB—glioblastoma. (B) Created in BioRender. Coates, K. (2025) https://BioRender.com/vrulopj

clinical trial, NSC-CRAd-S-pk7 was safe in newly diagnosed glioma patients when administered into the post-resection cavity [86]. The mOS of NSC-CRAd-S-pk7-treated patients was 18.4 months compared to the historical control of 14.6 months in SOC-treated patients, demonstrating some efficacy.

Measles virus-based OV

Measles-based OVs have the advantage that they can invade through CD46 on tumour cells [65]. MV-CEA is an oncolytic measles virus (MV) expressing a tumour-associated antigen CEA (carcinoembryonic antigen) developed by the Mayo Clinic for the treatment of GBM [12]. Phuong et al.

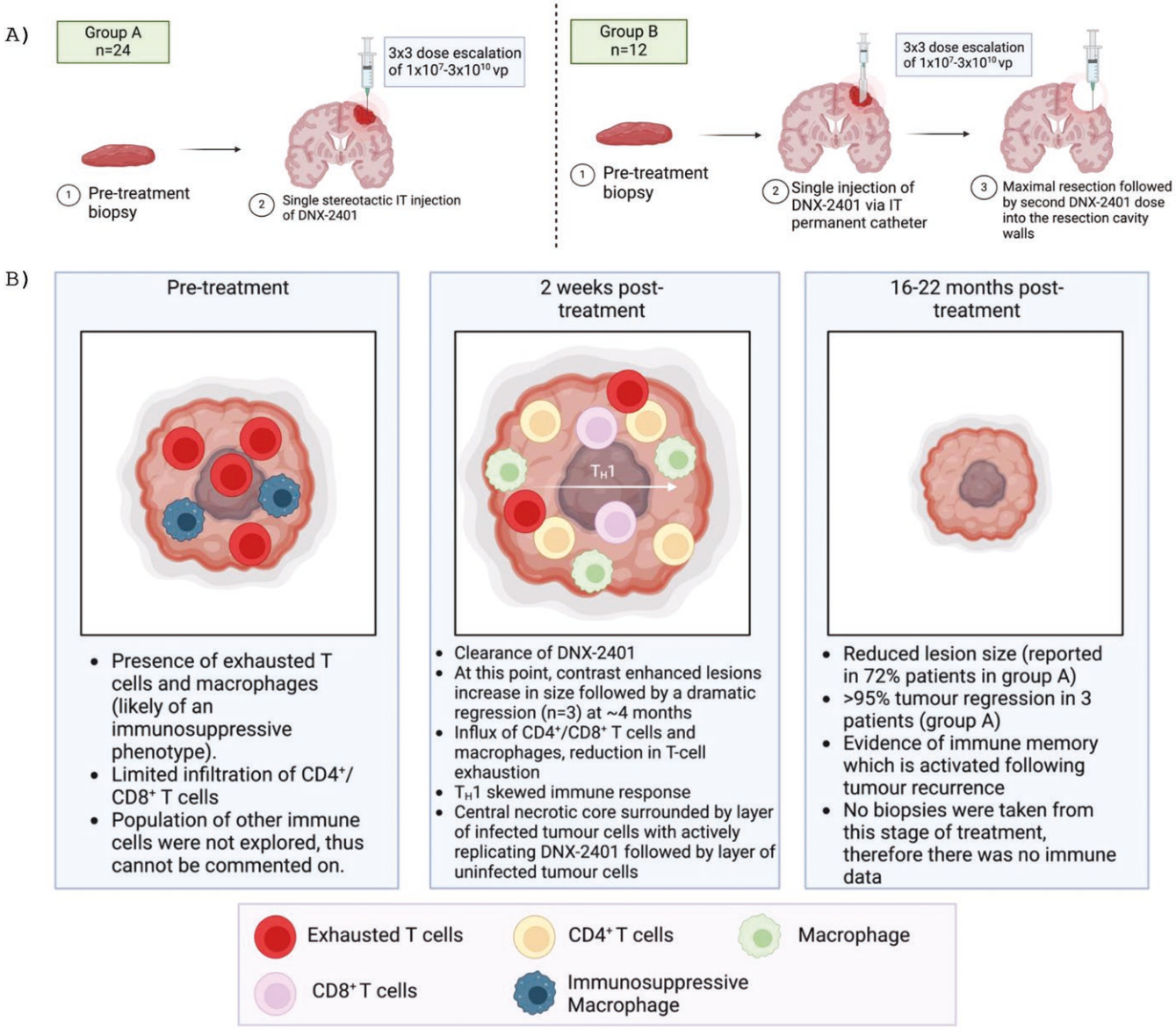


Figure 4. The trial design and cellular changes induced by DNX-2401. (A) Group A (n = 25) were treated with a single IT injection of DNX-2401, while group B (n = 12) received an injection of DNX-2401 via permanent IT catheter, resection at day 14 post-treatment and then a final DNX-2401 injection into the walls of the resection cavity. (B) Data derived from both groups A and B in the clinical trial. Figure created using BioRender.com. Abbreviations: IT—intra-tumour. (A) Created in BioRender. Coates, K. (2025) https://BioRender.com/gnjrg1r; (B) Created in BioRender. Coates, K. (2025) https://BioRender.com/4p90eok

found that MV-CEA infection of GBM cell lines (U87, U251, and U118) *in vitro* resulted in abundant syncytia formation (a key mechanism of MV-mediated apoptosis) and subsequent cell death in > 90% of cells 72 hours post-infection at an MOI of 1.0 [87]. Following this, an in vivo experiment of IT-administered MV-CEA (1.8×10^6 pfu) in U87 xenograft BALB/c nude mouse models found that MV-CEA significantly improved tumour regression (P = .0028) compared to control groups. There were no neurological toxicities observed after treatment.

A phase I clinical trial (NCT00390299) of MV-CEA was initiated for safety/toxicity (primary endpoint) and markers of efficacy (secondary endpoint) of MV-CEA in the treatment of human rGBM [12]. There were two treatment groups (summarized in Fig. 5) and the safety profile was good with only grade 1/2 adverse events observed and no DLTs. Preliminary efficacy was evaluated by the mOS and 1-year survival rate of 11.6 months and 45.5%, respectively. When compared with contemporary controls defined prior to the trial (mOS around 6–8.5 months after gross total resection followed by bevacizumab), the secondary endpoint was also reached [12].

Interestingly, there was an association between baseline ISG expression and treatment response. An algorithm (diagonal linear discriminate analysis—DLDA) was utilized to analyse the expression of 22 ISGs from each patient, indicating that the DLDA score was inversely correlated (P = 0.04, R = –0.06) with viral replication and treatment efficacy. Moreover, tumour RNA analysis revealed that the most enriched pathways in responsive tumours were involved in

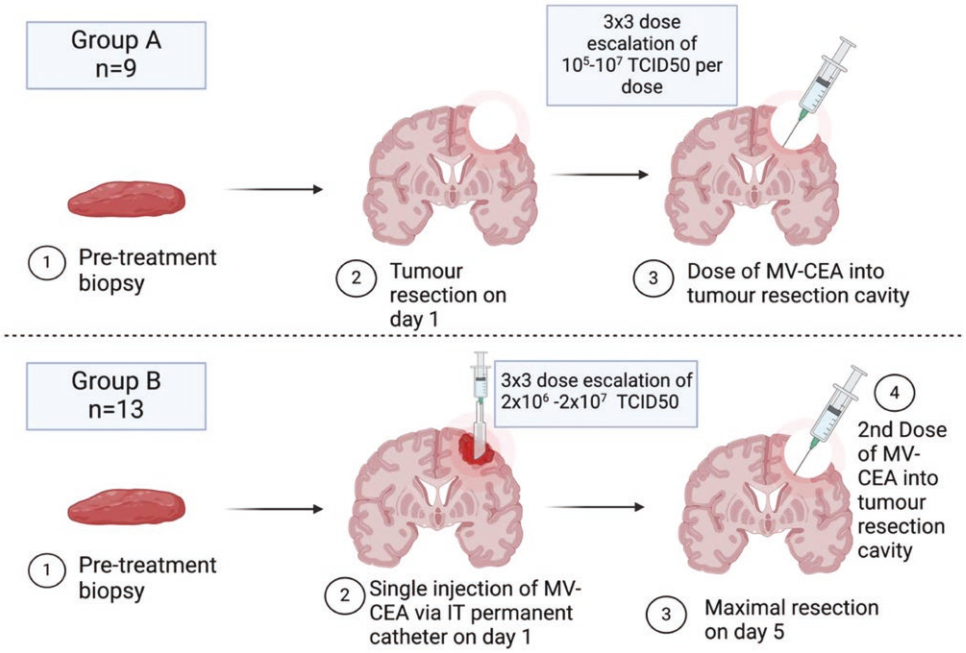


Figure 5. Trial design of the phase I clinical trial of MV-CEA in rGB. Group A (n = 9) underwent tumour resection followed by a MV-CEA injection in the resection cavity walls, while group B (n = 13) received IT injection of MV-CEA followed by tumour resection (day 5) and a second dose post-resection in the cavity walls. Figure created using BioRender.com. Abbreviations: TCID50—50% tissue culture infectious dose. Created in BioRender. Coates, K. (2025) https://BioRender.com/aaq7bs1

inflammatory responses including innate/adaptive immune cell chemotaxis and response to pro-inflammatory cytokines (TNF-α, IL-1, and IFN-γ) [12]. Due to the importance of ISGs in the efficacy of most other OVs, this finding emphasizes the benefit of RNA-sequencing for patient stratification [12]. This suggests that ~1 in 5 GBM patients will have the optimal ISG profile for OVT efficacy. Although this is promising for a small subset of patients, it presents a future issue for later-stage phase III trials of OVs with a much larger, unselected patient population diluting the outcome [12]. By performing IHC on matched pre- and post-treatment (day 5) biopsies from group B patients it was found that there was a significant increase in $CD8^+$ and $CD68^+$ cells in the post-treatment biopsies while $CD4^+$ T cells were unchanged. Interestingly, CD8+ T cell infiltration was inversely correlated with DLDA score.

Collectively, these data highlight the importance of patient stratification for OVT. However, the effect of multiple injections at different dose levels should be investigated in future, and, since this trial was small, progression to a larger phase III trial would give a better representation of MV-CEA efficacy.

The trials outlined here provide promising data for the clinical use of OVT in rGBM. Other oncolytic virus types have also shown success, such as the oncolytic reovirus developed by Samson et al (Oncolytics Biotech Ltd) which induced promising immune-related intra-tumoral changes in a window-of-opportunity clinical study, which are predictive of a good combinatorial response with PD-1/PD-L1 blockade [88]. Additionally, there are a number of ongoing clinical trials of OVT in GBM (Table 3) primarily focused on OVT as a monotherapy, but its potential in combination with other therapies is also being explored.

The promise of combination therapy: OVT and immunotherapy

Monotherapy of GBM using OVT is still relatively nascent, so many of the obvious combinatory approaches with other immunotherapies are still at the preclinical stage. However, it is expected that a combinatory approach to treatment will have a positive synergistic effect [64].

Previous failure of ICIs and CAR-T cells have largely been attributed to the immunosuppressive environment but OVT clearly creates a TME that is more responsive to these immunotherapies [89]. ICIs have shown benefits in preclinical and clinical studies when used in combination with OVT. Hardcastle et al (2017) found that combination therapy of anti-PD-1 and oncolytic MV enhanced survival in glioma mouse models [90]. CAR-T cell therapy in combination with OVT has also shown preclinical success. An oncolytic adenovirus genetically engineered to express CCL5 and IL-15 showed an increased ability to promote survival and proliferation of CAR-T cells at the tumour site, which significantly increased OS in preclinical models of neuroblastoma [89, 91].

Nassiri et al (2023) described a phase I clinical trial which showed favourable mOS (12.5 months) with the combination of DNX-2401 and pembrolizumab (anti-PD-1) [67]. This study met its primary (safety) and secondary (efficacy) endpoints as this combination was deemed tolerable and the 12-month survival rate (52.7%) surpassed the pre-specified threshold of 20% survival. Overall, five patients experienced objective responses and two patients had CR sustained more than 45 months. This study was promising but did not investigate either treatment as a monotherapy, so could not provide direct comparisons to understand the full benefit of their combination.

Table 3. Recent and ongoing clinical trials for various novel OVTs in GB

OV name	Virus type	Indication	Combination therapy	Trial stage, *n*	Clinicaltrials.gov ID
DNX-2440	Adenovirus	1st/2nd rGB	N/A	Phase I (terminated), *n* = 16	NCT03714334
YSCH-01	Adenovirus	rGB	N/A	Recruiting, *n* = 6 (estimated)	NCT05914935
TG6002	Vaccinia virus	rGB	Flucytosine	Phase I/II, *n* = 78	NCT03294486
G207	HSV-1	GB/Anaplastic astrocytoma	N/A	Phase I/II (complete), *n* = 21	NCT00036699
H-1PV	Parovirus	Progessive pGB or rGB	N/A	Phase I/II (complete), *n* = 18	NCT01301430
DNX-2401	Adenovirus	rGB or Gliosarcoma	IFN-γ	Phase I (complete), *n* = 37	NCT02197169
NSC-CRAd-S-p7	Adenovirus	Malignant glioma	SOC	Phase I (complete), *n* = 12	NCT03072134
PVSRIPO	Polio/Rhinovirus	GB	N/A	Phase I (complete), *n* = 61	NCT01491893
M032	HSV-1	Malignant glioma	Pembrolizumab	Recruiting, *n* = 28 (estimated)	NCT05084430
DNX-2401	Adenovrius	Recurrent malignant glioma	N/A	Recruiting, *n* = 36 (estimated)	NCT03896568
C134	HSV-1	Recurrent malignant glioma	N/A	Phase I (enrolling by invitation), *n* = 12 (estimated)	NCT06193174
rQNestin34.5v.2	HSV-1	Recurrent/progressive brain tumour	CPX	Phase I (recruiting)	NCT03152318
Reolysin®	Reovirus	Recurrent/progressive brain tumour	N/A	Phase I/II (complete), *n* = 18	NCT00528684

Data collected by searching 'Glioblastoma' and 'Oncolytic virus' on Clinicaltrials.gov. Where trials have not finished recruiting patients, they have provided an 'estimated' number of patients expected to be enrolled in the trial.
Abbreviations: N/A—no answer, CPX—cyclophosphamide, HSV-1—herpes simplex virus-1, rGB—recurrent glioblastoma, pGB—primary glioblastoma, SOC—standard of care, IFN-γ—interferon-γ.

Conclusions, challenges, and future perspectives

OVT has the potential to revolutionize the treatment of GBM. Pre-clinical and clinical data have been very promising and have spurred further novel GBM treatment approaches. Importantly, virus positivity at the tumour site was confirmed in each trial with limited reports of 'off-targeting'. Each trial demonstrated that OVT could achieve a complete response in certain patients via reprogramming of the immunosuppressive TME via infiltration of innate and adaptive immune cells. Associations between baseline IFN-γ expression, treatment response and the indication of tumour-specific immune memory should be used in the future to tailor and boost the effectiveness of OVTs.

Although these trials are very promising, some challenges for general OV use need to be considered. OVs are live viruses with replicative potential and despite their genetic alterations, they may still hold the risk of viral shedding and off-targeting [16]. Consequently, they require unique clinical considerations/monitoring following administration. However, dose-limiting toxicities (DLT) and neurological effects have not been encountered so far. Due to their actively replicating status, OVT manufacturers need consistent monitoring for pathogen contamination, viral purity and sustained replicative ability [72]. These quality control measures are costly and time-consuming as there is currently no technology which accurately measures them. Development of these tests would make OVTs more clinically accessible for both manufacturing and from a regulatory perspective.

Looking to the future, there are questions surrounding OVT which are yet to be addressed by clinical trials. Many trials have focused on the effects OVTs have on intra-tumoral adaptive immune cell infiltration, however, investigations into how these therapies affect established populations of innate immune cells such as TAMs, MDSCs,s and DCs are necessary to understand the complete MoA. Secondly, Galanis et al (2024) have highlighted the importance of RNA sequencing for patient stratification and maximum clinical benefit [12]. This is supported by the incidence of CR in the clinical trials. In terms of assessing tumour changes after OVT, there may be issues with pseudo-progression (an increase in tumour size measured by RECIST due to infiltration of new immune cells) which can mask the decrease in tumour size. To understand which patients should be eligible for OVT, future trials must explore immunophenotyping and genetic sequencing to provide a degree of patient screening and stratification. Thirdly, there is a lack of experimental dosing schedules in OVT clinical trials. Understanding the optimal dosing window for OVT needs to be prioritized in order to understand whether efficacy is improved when administered prior to resection and immunotherapy or after resection followed by other conventional/immunotherapies. Is there a period post-OVT where ICIs would be most successful? Finally, further clinical trials involving different combinations of immunotherapy and OVs could unlock maximal potential for both these treatments in GBM. The results of ongoing combination trials are eagerly anticipated: they will undoubtedly inform future research and will hopefully establish OVs as an effective weapon in the armamentarium required for the effective treatment of GBM.

Acknowledgements

This work is a review of current literature prepared by the first author as part of their academic degree and as such does not need ethical approval and does not have raw data associated with it for subsequent availability. The Editor-in-Chief, Tim Elliott, and handling editor, Stephanie Dougan, would

like to thank reviewers Daniel Hoces and an anonymous reviewer, for their contribution to the publication of this article.

Author Contributions

Katie Coates (Investigation, Writing—original draft), Robert Nibbs (Conceptualization, Supervision, Writing—review & editing), and Alasdair Fraser (Project administration, Supervision, Writing—review & editing)

Conflict of interest:

The authors declare no conflicts of interest.

Funding

None declared.

References

1. Sung H, Ferlay J, Siegel RL et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71:209–49. https://doi.org/10.3322/caac.21660
2. Ostrom QT, Price M, Neff C et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2016–2020. *Neuro-Oncology* 2023; 25:iv1–iv99. https://doi.org/10.1093/neuonc/noad149
3. Ah-Pine F, Khettab M, Bedoui Y et al. On the origin and development of glioblastoma: multifaceted role of perivascular mesenchymal stromal cells. *Acta Neuropathol Commun* 2023; 11:104. https://doi.org/10.1186/s40478-023-01605-x
4. Alcantara Llaguno SR, Parada LF. Cell of origin of glioma: biological and clinical implications. *Br J Cancer* 2016; 115:1445–50. https://doi.org/10.1038/bjc.2016.354
5. Fernandes C, Costa A, Osório L et al. Current standards of care in glioblastoma therapy. In: De Vleeschouwer S (ed.), *Glioblastoma [Internet]*. Brisbane (AU): Codon Publications, 2017 [cited 2024 Mar 20]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK469987/
6. Stupp R, Mason WP, van den Bent MJ et al; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352:987–96. https://doi.org/10.1056/NEJMoa043330
7. Widodo SS, Hutchinson RA, Fang Y et al. Toward precision immunotherapy using multiplex immunohistochemistry and in silico methods to define the tumor immune microenvironment. *Cancer Immunol Immunother.* 2021; 70:1811–20. https://doi.org/10.1007/s00262-020-02801-7
8. Chen W, Wang Y, Zhao B et al. Optimal therapies for recurrent glioblastoma: a bayesian network meta-analysis. *Front Oncol [Internet]* 2021; 11. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2021.641878/full
9. Hills KE, Kostarelos K, Wykes RC. Converging mechanisms of epileptogenesis and their insight in glioblastoma. *Front Mol Neurosci* 2022; 15:903115. https://doi.org/10.3389/fnmol.2022.903115
10. Todo T, Ino Y, Ohtsu H et al. A phase I/II study of triple-mutated oncolytic herpes virus G47Δ in patients with progressive glioblastoma. *Nat Commun* 2022; 13:4119. https://doi.org/10.1038/s41467-022-31262-y
11. Cloughesy TF, Mochizuki AY, Orpilla JR et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat Med* 2019; 25:477–86. https://doi.org/10.1038/s41591-018-0337-7
12. Galanis E, Dooley KE, Keith Anderson S et al. Carcinoembryonic antigen-expressing oncolytic measles virus derivative in recurrent glioblastoma: a phase 1 trial. *Nat Commun* 2024; 15:493. https://doi.org/10.1038/s41467-023-43076-7
13. Zhang X, Zhao L, Zhang H et al. The immunosuppressive microenvironment and immunotherapy in human glioblastoma. *Front Immunol* 2022; 13:1–16. https://doi.org/10.3389/fimmu.2022.1003651
14. Rocha Pinheiro SL, Lemos FFB, Marques HS et al. Immunotherapy in glioblastoma treatment: current state and future prospects. *World J Clin Oncol* 2023; 14:138–59.
15. Asija S, Chatterjee A, Goda JS et al. Oncolytic immunovirotherapy for high-grade gliomas: a novel and an evolving therapeutic option. *Front Immunol* 2023; 14:1118246. https://doi.org/10.3389/fimmu.2023.1118246
16. Shalhout SZ, Miller DM, Emerick KS et al. Therapy with oncolytic viruses: progress and challenges. *Nat Rev Clin Oncol* 2023; 20:160–77. https://doi.org/10.1038/s41571-022-00719-w
17. Janiszewska M, Suvà ML, Riggi N et al. Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. *Genes Dev* 2012; 26:1926–44. https://doi.org/10.1101/gad.188292.112
18. Schiffer D, Annovazzi L, Casalone C et al. Glioblastoma: microenvironment and niche concept. *Cancers* 2018; 11:5. https://doi.org/10.3390/cancers11010005
19. Boyd NH, Tran AN, Bernstock JD et al. Glioma stem cells and their roles within the hypoxic tumor microenvironment. *Theranostics* 2021; 11:665–83. https://doi.org/10.7150/thno.41692
20. Auffinger B, Spencer D, Pytel P et al. The role of glioma stem cells in chemotherapy resistance and glioblastoma multiforme recurrence. *Expert Rev Neurother* 2015; 15:741–52. https://doi.org/10.1586/14737175.2015.1051968
21. Ahir BK, Engelhard HH, Lakka SS. Tumor development and angiogenesis in adult brain tumor: glioblastoma. *Mol Neurobiol* 2020; 57:2461–78. https://doi.org/10.1007/s12035-020-01892-8
22. Méndez O, Zavadil J, Esencay M et al. Knock down of HIF-1α in glioma cells reduces migration in vitro and invasion in vivo and impairs their ability to form tumor spheres. *Mol Cancer* 2010; 9:133. https://doi.org/10.1186/1476-4598-9-133
23. Damgaci S, Ibrahim-Hashim A, Enriquez-Navas PM et al. Hypoxia and acidosis: immune suppressors and therapeutic targets. *Immunology* 2018; 154:354–62. https://doi.org/10.1111/imm.12917
24. Della Monica R, Cuomo M, Buonaiuto M et al. MGMT and whole-genome DNA methylation impacts on diagnosis, prognosis and therapy of glioblastoma multiforme. *Int J Mol Sci* 2022; 23:7148. https://doi.org/10.3390/ijms23137148
25. Szylberg M, Sokal P, Śledzińska P et al. MGMT promoter methylation as a prognostic factor in primary glioblastoma: a single-institution observational study. *Biomedicines* 2022; 10:2030. https://doi.org/10.3390/biomedicines10082030
26. Leske H, Camenisch Gross U, Hofer S et al. MGMT methylation pattern of long-term and short-term survivors of glioblastoma reveals CpGs of the enhancer region to be of high prognostic value. *Acta Neuropathol Commun* 2023; 11:139. https://doi.org/10.1186/s40478-023-01622-w
27. Butler M, Pongor L, Su YT et al. MGMT status as a clinical biomarker in glioblastoma. *Trends Cancer* 2020; 6:380–91. https://doi.org/10.1016/j.trecan.2020.02.010
28. Ransohoff RM, Kivisäkk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* 2003; 3:569–81. https://doi.org/10.1038/nri1130
29. Norris GT, Kipnis J. Immune cells and CNS physiology: microglia and beyond. *J Exp Med* 2019; 216:60–70. https://doi.org/10.1084/jem.20180199
30. Bausart M, Préat V, Malfanti A. Immunotherapy for glioblastoma: the promise of combination strategies. *J Experimental Clin Cancer Res* 2022; 41:35. https://doi.org/10.1186/s13046-022-02251-2

31. Elguindy M, Young JS, Mondal I et al. Glioma–immune cell crosstalk in tumor progression. *Cancers*. 2024; 16:308. https://doi.org/10.3390/cancers16020308
32. Bloch O, Crane CA, Kaur R et al. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. *Clin Cancer Res* 2013; 19:3165–75. https://doi.org/10.1158/1078-0432.CCR-12-3314
33. Nduom EK, Wei J, Yaghi NK et al. PD-L1 expression and prognostic impact in glioblastoma. *Neuro-Oncology* 2016; 18:195–205. https://doi.org/10.1093/neuonc/nov172
34. Woroniecka K, Chongsathidkiet P, Rhodin K et al. T cell exhaustion signatures vary with tumor type and are severe in glioblastoma. *Clin Cancer Res* 2018; 24:4175–86.
35. Sharma P, Aaroe A, Liang J et al. Tumor microenvironment in glioblastoma: current and emerging concepts. *Neurooncol. Adv* 2023; 5:vdad009. https://doi.org/10.1093/noajnl/vdad009
36. Mohme M, Schliffke S, Maire CL et al. Immunophenotyping of newly diagnosed and recurrent glioblastoma defines distinct immune exhaustion profiles in peripheral and tumor-infiltrating lymphocytes. *Clin Cancer Res* 2018; 24:4187–200. https://doi.org/10.1158/1078-0432.CCR-17-2617
37. Lin H, Liu C, Hu A et al. Understanding the immunosuppressive microenvironment of glioma: mechanistic insights and clinical perspectives. *J Hematol Oncol* 2024; 17:31. https://doi.org/10.1186/s13045-024-01544-7
38. Rahman M, Kresak J, Yang C et al. Analysis of immunobiologic markers in primary and recurrent glioblastoma. *J Neurooncol* 2018; 137:249–57. https://doi.org/10.1007/s11060-017-2732-1
39. Robert CA. decade of immune-checkpoint inhibitors in cancer therapy. *Nat Commun* 2020; 11:3801.
40. Zeng J, See AP, Phallen J et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys* 2013; 86:343–9. https://doi.org/10.1016/j.ijrobp.2012.12.025
41. Reardon DA, Gokhale PC, Klein SR et al. Glioblastoma eradication following immune checkpoint blockade in an orthotopic, immunocompetent model. *Cancer Immunol Res* 2016; 4:124–35. https://doi.org/10.1158/2326-6066.CIR-15-0151
42. Wainwright DA, Chang AL, Dey M et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4 and PD-L1 in mice with brain tumors. *Clin Cancer Res* 2014; 20:5290–301. https://doi.org/10.1158/1078-0432.CCR-14-0514
43. Reardon DA, Brandes AA, Omuro A et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol* 2020; 6:1003–10. https://doi.org/10.1001/jamaoncol.2020.1024
44. Omuro A, Brandes AA, Carpentier AF et al. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: an international randomized phase III trial. *Neuro-Oncology* 2023; 25:123–34. https://doi.org/10.1093/neuonc/noac099
45. Filley AC, Henriquez M, Dey M. Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. *Oncotarget* 2017; 8:91779–94. https://doi.org/10.18632/oncotarget.21586
46. Reardon DA, Kim TM, Frenel JS et al. Treatment with pembrolizumab in programmed death ligand 1–positive recurrent glioblastoma: results from the multicohort phase 1 KEYNOTE-028 trial. *Cancer* 2021; 127:1620–9. https://doi.org/10.1002/cncr.33378
47. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. *Blood* 2018; 131:58–67. https://doi.org/10.1182/blood-2017-06-741033
48. Garg AD, Vandenberk L, Van Woensel M et al. Preclinical efficacy of immune-checkpoint monotherapy does not recapitulate corresponding biomarkers-based clinical predictions in glioblastoma. *Oncoimmunology* 2017; 6:e1295903. https://doi.org/10.1080/2162402X.2017.1295903
49. Fong B, Jin R, Wang X et al. Monitoring of regulatory T cell frequencies and expression of CTLA-4 on T cells, before and after DC vaccination, can predict survival in GBM patients. *PLoS One* 2012; 7:e32614. https://doi.org/10.1371/journal.pone.0032614
50. Wang H, Yang J, Li X et al. Current state of immune checkpoints therapy for glioblastoma. *Heliyon* 2024; 10:e24729. https://doi.org/10.1016/j.heliyon.2024.e24729
51. Fecci PE, Ochiai H, Mitchell DA et al. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell function. *Clin Cancer Res* 2007; 13:2158–67. https://doi.org/10.1158/1078-0432.CCR-06-2070
52. Shiravand Y, Khodadadi F, Kashani SMA et al. Immune checkpoint inhibitors in cancer therapy. *Curr Oncol* 2022; 29:3044–60. https://doi.org/10.3390/curroncol29050247
53. Omuro A, Vlahovic G, Lim M et al. Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. *Neuro-Oncology* 2018; 20:674–86. https://doi.org/10.1093/neuonc/nox208
54. Duerinck J, Schwarze JK, Awada G et al. Intracerebral administration of CTLA-4 and PD-1 immune checkpoint blocking monoclonal antibodies in patients with recurrent glioblastoma: a phase I clinical trial. *J ImmunoTher Cancer* 2021; 9:e002296. https://doi.org/10.1136/jitc-2020-002296
55. Patel SP, Woodman SE. Profile of ipilimumab and its role in the treatment of metastatic melanoma. *Drug Des Devel Ther* 2011; 5:489–95. https://doi.org/10.2147/DDDT.S10945
56. Lee AH, Sun L, Mochizuki AY et al. Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to overcome the immunosuppressive tumor associated macrophages in recurrent glioblastoma. *Nat Commun* 2021; 12:6938. https://doi.org/10.1038/s41467-021-26940-2
57. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 2021; 11:69. https://doi.org/10.1038/s41408-021-00459-7
58. Zhang C, Liu J, Zhong JF et al. Engineering CAR-T cells. *Biomark Res* 2017; 5:22. https://doi.org/10.1186/s40364-017-0102-y
59. Montagna E, de Campos NSP, Porto VA et al. CD19 CAR T cells for B cell malignancies: a systematic review and meta-analysis focused on clinical impacts of CAR structural domains, manufacturing conditions, cellular product, doses, patient's age, and tumor types. *BMC Cancer* 2024; 24:1037. https://doi.org/10.1186/s12885-024-12651-6
60. Choi Bryan D, Gerstner Elizabeth R, Frigault Matthew J et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. *N Engl J Med* 2024; 390 :1. https://doi.org/10.1056/NEJMoa2314390
61. Todo T, Rabkin SD. Development of oncolytic replication-competent herpes simplex virus vectors. In: Curiel DT, Douglas JT (ed.),. *Cancer Gene Therapy [Internet]*. Totowa, NJ: Humana Press, 2005, 199–210. https://doi.org/10.1007/978-1-59259-785-7_13
62. Rahman MM, McFadden G. Oncolytic viruses: newest frontier for cancer immunotherapy. *Cancers* 2021; 13:5452. https://doi.org/10.3390/cancers13215452
63. Lin D, Shen Y, Liang T. Oncolytic virotherapy: basic principles, recent advances and future directions. *Signal Transduct Target Ther* 2023; 8:1–29. https://doi.org/10.1038/s41392-023-01407-6
64. Jhawar SR, Thandoni A, Bommareddy PK et al. Oncolytic viruses—natural and genetically engineered cancer immunotherapies. *Front Oncol* 2017; 7:202. https://doi.org/10.3389/fonc.2017.00202
65. Ong HT, Timm MM, Greipp PR et al. Oncolytic measles virus targets high CD46 expression on multiple myeloma cells. *Exp Hematol* 2006; 34:713–20. https://doi.org/10.1016/j.exphem.2006.03.002
66. Dmitriev I, Krasnykh V, Miller CR et al. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *J Virol* 1998; 72:9706–13. https://doi.org/10.1128/JVI.72.12.9706-9713.1998
67. Nassiri F, Patil V, Yefet LS et al. Oncolytic DNX-2401 virotherapy plus pembrolizumab in recurrent glioblastoma: a phase 1/2 trial.

Nat Med 2023; 29:1370–8. https://doi.org/10.1038/s41591-023-02347-y
68. Schnell O, Krebs B, Wagner E et al. Expression of integrin αvβ3 in gliomas correlates with tumor grade and is not restricted to tumor vasculature. *Brain Pathol* 2008; 18:378–86. https://doi.org/10.1111/j.1750-3639.2008.00137.x
69. Roth P, Silginer M, Goodman SL et al. Integrin control of the transforming growth factor-β pathway in glioblastoma. *Brain* 2013; 136:564–76. https://doi.org/10.1093/brain/aws351
70. Miller DL, Rickards B, Mashiba M et al. The adenoviral E1B 55-kilodalton protein controls expression of immune response genes but not p53-dependent transcription. *J Virol* 2009; 83:3591–603. https://doi.org/10.1128/JVI.02269-08
71. Zhan X, Guo S, Li Y et al. Glioma stem-like cells evade interferon suppression through MBD3/NuRD complex–mediated STAT1 downregulation. *J Exp Med* 2020; 217:e20191340. https://doi.org/10.1084/jem.20191340
72. Yang L, Gu X, Yu J et al. Oncolytic virotherapy: from bench to bedside. *Front Cell Dev Biol* 2021; 9:790150. https://doi.org/10.3389/fcell.2021.790150
73. Liu BL, Robinson M, Han ZQ et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther* 2003; 10:292–303. https://doi.org/10.1038/sj.gt.3301885
74. Kaufman HL, Shalhout SZ, Iodice G. Talimogene laherparepvec: moving from first-in-class to best-in-class. *Front Mol Biosci* 2022; 9:834841. https://doi.org/10.3389/fmolb.2022.834841
75. Todo T, Martuza RL, Rabkin SD et al. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. *Proc Natl Acad Sci U S A* 2001; 98:6396–401. https://doi.org/10.1073/pnas.101136398
76. Sgubin D, Wakimoto H, Kanai R et al. Oncolytic herpes simplex virus counteracts the hypoxia-induced modulation of glioblastoma stem-like cells. *Stem Cells Transl Med* 2012; 1:322–32. https://doi.org/10.5966/sctm.2011-0035
77. Kanai R, Rabkin SD, Yip S et al. Oncolytic virus-mediated manipulation of DNA damage responses: synergy with chemotherapy in killing glioblastoma stem cells. *J Natl Cancer Inst* 2012; 104:42–55. https://doi.org/10.1093/jnci/djr509
78. Thust SC, van den Bent MJ, Smits M. Pseudoprogression of brain tumors. *J Magn Reson Imaging* 2018; 48:571–89. https://doi.org/10.1002/jmri.26171
79. Todo T, Ito H, Ino Y et al. Intratumoral oncolytic herpes virus G47Δ for residual or recurrent glioblastoma: a phase 2 trial. *Nat Med* 2022; 28:1630–9. https://doi.org/10.1038/s41591-022-01897-x
80. Christie JD, Chiocca EA. Treat and repeat: oncolytic virus therapy for brain cancer. *Nat Med* 2022; 28:1540–2. https://doi.org/10.1038/s41591-022-01901-4
81. Frampton JE. Teserpaturev/G47Δ: first approval. *BioDrugs* 2022; 36:667–72. https://doi.org/10.1007/s40259-022-00553-7
82. Ling AL, Solomon IH, Landivar AM et al. Clinical trial links oncolytic immunoactivation to survival in glioblastoma. *Nature* 2023; 623:157–66. https://doi.org/10.1038/s41586-023-06623-2
83. Fueyo J, Alemany R, Gomez-Manzano C et al. Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. *J Natl Cancer Inst* 2003; 95:652–60. https://doi.org/10.1093/jnci/95.9.652
84. Jiang H, Clise-Dwyer K, Ruisaard KE et al. Delta-24-RGD oncolytic adenovirus elicits anti-glioma immunity in an immunocompetent mouse model. *PLoS One* 2014; 9:e97407. https://doi.org/10.1371/journal.pone.0097407
85. Lang FF, Conrad C, Gomez-Manzano C et al. Phase I study of DNX-2401 (delta-24-RGD) oncolytic adenovirus: replication and immunotherapeutic effects in recurrent malignant glioma. *J Clin Oncol* 2018; 36:1419–27. https://doi.org/10.1200/JCO.2017.75.8219
86. Fares J, Ahmed AU, Ulasov IV et al. Neural stem cell delivery of an oncolytic adenovirus in newly diagnosedmalignant glioma: a first-in-human, phase 1 clinical trial. *Lancet Oncol* 2021; 22:1103–14. https://doi.org/10.1016/S1470-2045(21)00245-X
87. Phuong LK, Allen C, Peng KW et al. Use of a vaccine strain of measles virus genetically engineered to produce carcinoembryonic antigen as a novel therapeutic agent against glioblastoma multiforme. *Cancer Res* 2003; 63:2462–9.
88. Samson A, Scott KJ, Taggart D et al. Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. *Sci Transl Med* 2018; 10:eaam7577. https://doi.org/10.1126/scitranslmed.aam7577
89. Oh CM, Chon HJ, Kim C. Combination immunotherapy using oncolytic virus for the treatment of advanced solid tumors. *Int J Mol Sci* 2020; 21:7743. https://doi.org/10.3390/ijms21207743
90. Hardcastle J, Mills L, Malo CS et al. Immunovirotherapy with measles virus strains in combination with anti–PD-1 antibody blockade enhances antitumor activity in glioblastoma treatment. *Neuro-Oncol* 2017; 19:now179–502. https://doi.org/10.1093/neuonc/now179
91. Nishio N, Diaconu I, Liu H et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors. *Cancer Res* 2014; 74:5195–205. https://doi.org/10.1158/0008-5472.CAN-14-0697