# Oncolytic viruses as anticancer agents: clinical progress and remaining challenges

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**Immunotherapy has transformed the treatment of cancer, yet many patients do not have response or lasting benefit. Strategies to overcome resistance remain of crucial importance. Oncolytic viruses offer a promising approach, with the unique ability to selectively replicate within (and to destroy) cancer cells, remodel the immunosuppressive tumour microenvironment, and stimulate antitumour immunity. Interest in the potential of oncolytic viruses has grown steadily over the past two decades, fuelled by advances in cancer immunology and viral engineering. However, clinical translation has not kept pace, and although a plethora of promising new constructs have entered clinical testing, several barriers continue to restrict widespread clinical implementation. This Therapeutics paper highlights key milestones in oncolytic virus clinical development, discusses the challenges that remain, and, through clinical reflection, considers how future research might be streamlined to achieve meaningful benefit for patients.**

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## Introduction

Oncolytic viruses are naturally occurring or genetically modified viruses that selectively infect and replicate within cancer cells, with the potential to induce oncolysis, immunogenic cell death, and systemic anticancer immunity. Although often described as a novel therapeutic class, oncolytic viruses are now decades old. The 2015 US Food and Drug Administration (FDA) approval of the oncolytic herpes simplex virus type 1 (HSV-1) talimogene laherparepvec (T-VEC) for treatment of advanced melanoma was a milestone for the field, as it was the first FDA-approved oncolytic virus. However, this has been followed up with disappointing results in late-stage trials.[1–3] Oncolytic viruses are, therefore, still striving to make a substantial impact in clinical practice, despite considerable preclinical and early-phase promise.

Optimism about the potential clinical impact of oncolytic viruses persists, supported by a surge in registered clinical trials, high-impact publications, and promising new strategies. Clinically, extensive trial and real-world data have reinforced that oncolytic viruses are safe and tolerable, with a subset of patients reaching dramatic and durable responses with manageable side-effects.[4,5] Distinct mechanisms and largely non-overlapping toxicities with pillars of cancer therapy (including immune checkpoint blockade, chemotherapy, and radiotherapy) make oncolytic viruses appealing therapeutic adjuncts, and oncolytic viruses are gaining traction in focused clinical contexts outside advanced melanoma. Scientifically, oncolytic viruses are challenging people who are sceptical about the usefulness of oncolytic viruses, with an unmatched flexibility to evolve with the immuno-oncology landscape. They can encode diverse immunomodulatory transgenes for expression within tumour tissue, and be used synergistically in combination with other therapeutics, including with immune checkpoint inhibitors and adoptive cell transfer therapy.

The adaptable constructs and diverse combinations of oncolytic viruses is a clear benefit, yet this presents a challenge in streamlining clinical translation. The rate of preclinical development far outstrips the ability to test concepts clinically, and the question remains as to why oncolytic viruses have not yet substantially disrupted the landscape, despite such prolonged and wide-ranging preclinical and clinical testing. As we approach 10 years from the approval of T-VEC,[6] we reflect on clinical translation, including comprehensive appraisal of the application of oncolytic viruses and outstanding questions. Through this discussion, we will begin to elucidate how oncolytic viruses might truly translate into improved outcomes for patients.

## Oncolytic viruses: scientific and mechanistic considerations

### Design of oncoviral agents

Oncolytic viruses are commonly generated from native viral strains, selected based on their capacity for tumour selectivity and oncolysis. Genetic modifications, including deletion of viral genes or insertion of transgene payloads, can enhance safety or therapeutic effects. Tumour cells are commonly inherently vulnerable to viral infection in comparison to normal tissue, through mechanisms including interferon signalling defects,[7] tumour-intrinsic

#### Search strategy and selection criteria

References for this work were selected from a search of PubMed from Jan 1, 1998, to Dec 12, 2024, for peer-reviewed, published manuscripts in English using combinations of search terms including "oncolytic viruses", "viroimmunotherapy", "checkpoint inhibitors", or "immunotherapy". Searches were refined by additional filtering for "clinical trials" or "randomized clinical trials". Publications were selected based on their clinical relevance, with criteria including number of study participants, clinical novelty and impact. Additional searches were performed for published abstracts from international meetings from 2022 to 2025 for updated results from clinical trials. The references quoted here were reviewed by the authors.

immunosuppression, and resistance to apoptosis. Tumour selectivity can also be engineered, by viral attenuation or targeting replication to tumour cells.[8]

Large DNA viruses such as HSV-1, adenovirus, and vaccinia virus are most heavily represented in oncolytic virus research, due to their stability and large, adaptable genomes,[9] which allow incorporation of various transgenes. RNA viruses such as reovirus, vesicular stomatitis virus, coxsackievirus, and measles virus are also undergoing clinical evaluation in a variety of contexts;[10] their advantages include steadfast replication, potential for more potent immunogenicity, and smaller size.[11]

Most oncoviral agents in clinical development retain replication competence, presenting unique challenges around logistics, biosafety, and pharmacokinetics. Oncolytic viruses have an exceptionally validated safety profile, with no documented cases of transmission or reversion to pathogenic strains in clinical trials or preclinical models.[8] However, a careful balance should be preserved between viral attenuation for safety, and maintenance of enough virulence to maximise oncolytic effects. Emerging strategies for balancing attenuation and efficacy include restoration of virulence factors under the control of tumour-specific promoters.[12] Genetic switches can also be incorporated into the viral genome, as either suicide genes to cease viral replication, or to enable inducible transgene expression.[13] Virtually any component can now be engineered, presenting a vast capacity for creativity and growth within the field, although continued close regulation and allegiance to virological principles remains of paramount importance.

## Mechanisms of action

Initially, researchers thought that oncolytic viruses exert their anticancer effects primarily through direct viral oncolysis,[14] with efficacy most heavily influenced by how effectively an oncolytic virus could propagate through the tumour microenvironment to infect and kill cancer cells. Although direct oncolysis is clearly a mechanism of action of oncolytic viruses, building evidence now implicates cohesive involvement of the innate and adaptive immune system in robust and durable oncolytic virus-mediated anticancer effects. Oncolytic virus infection has been shown to lead to diverse inflammatory reprogramming of the tumour microenvironment,[15] including immune infiltration, stromal breakdown, IFNγ expression, and upregulation of the PD-1–PD-L1 axis.[16,17] This reprogramming is often termed as increasing the heat of the tumour microenvironment, defined as switching from an immunosuppressive (cold) tumour microenvironment to an immuno-infiltrated (hot) inflammatory state that could favour immunotherapy responsiveness (figure 1). Oncolytic destruction of tumour cells might also facilitate priming of antitumour T cells, and the mechanistic model has now evolved to one in which tumour-selective oncolytic virus infection leads to oncolysis and immunogenic cell death, releasing stress signals and tumour antigens. This induces innate immunity, with recruitment and maturation of antigen-presenting cells facilitating subsequent activation and priming of antitumour (and antiviral) T cells. Primed T cells can then infiltrate the oncolytic virus-inflamed tumour microenvironment through oncolytic virus-enhanced cytokine and chemokine signalling, potentiating cytotoxic T lymphocyte-mediated immunogenic cell death, and systemic antitumour immunity.[18]

The evidence for this model in patients, which is one of in situ vaccination, primarily stems from the observation that locally delivered oncolytic viruses can induce durable regression both of tumours treated with oncolytic viruses by intratumoural injection, and of distant non-injected tumours in a subset of patients (average ~20%). In the case of T-VEC, regression of non-injected lesions was not associated with viral presence in non-injected tumours,[19] pointing to systemic, cancer-specific, immune effects. However, detailed validation of oncolytic virus-induced antigenicity and the frequency of induction of a robust antitumour T-cell response remains to be elucidated.

## The juxtaposition between antiviral and antitumour immunity

The term increasing the heat is widely used in the field of oncolytic viruses. However, what constitutes beneficial immunological heat remains incompletely understood, particularly in the context of viral agents, which face a complex juxtaposition of antiviral and antitumour immunity (figure 2). Pre-existing immunity can lead to a rapid antiviral response, with viral clearance and neutralisation by viral antibodies before any effects become established. Equally, rapid seroconversion, (evidenced by development of viral antibodies) on first treatment is largely inevitable, and has been widely shown in patients who were previously seronegative. The challenge of balancing antiviral and antitumour immunity is a particular limitation of systemic delivery, and most oncolytic viruses in clinical development are therefore delivered by intratumoural injection, to maximise viral delivery to the tumour microenvironment.

The effect of previous serostatus on oncolytic virus response has not been clearly defined. Notably, pre-existing immunity has not been reliably associated with an absence of response to oncolytic viruses in clinical studies (although some have excluded patients with high amounts of pre-existing neutralising antibodies).[20,21] A recent study by Ling and colleagues reported improved median overall survival (14·2 months [95% CI 9·5–15·7] in the treatment group *vs* 7·8 months [3·0 to not reached] in the control group) in patients with high-grade gliomas who were HSV-1 seropositive when treated with oncolytic HSV-1 CAN-3110 (rQnestin34.5v.2; Candel Therapeutics, Worcester, MA, USA), with the authors suggesting that pre-existing HSV-1 seropositivity led to a stronger

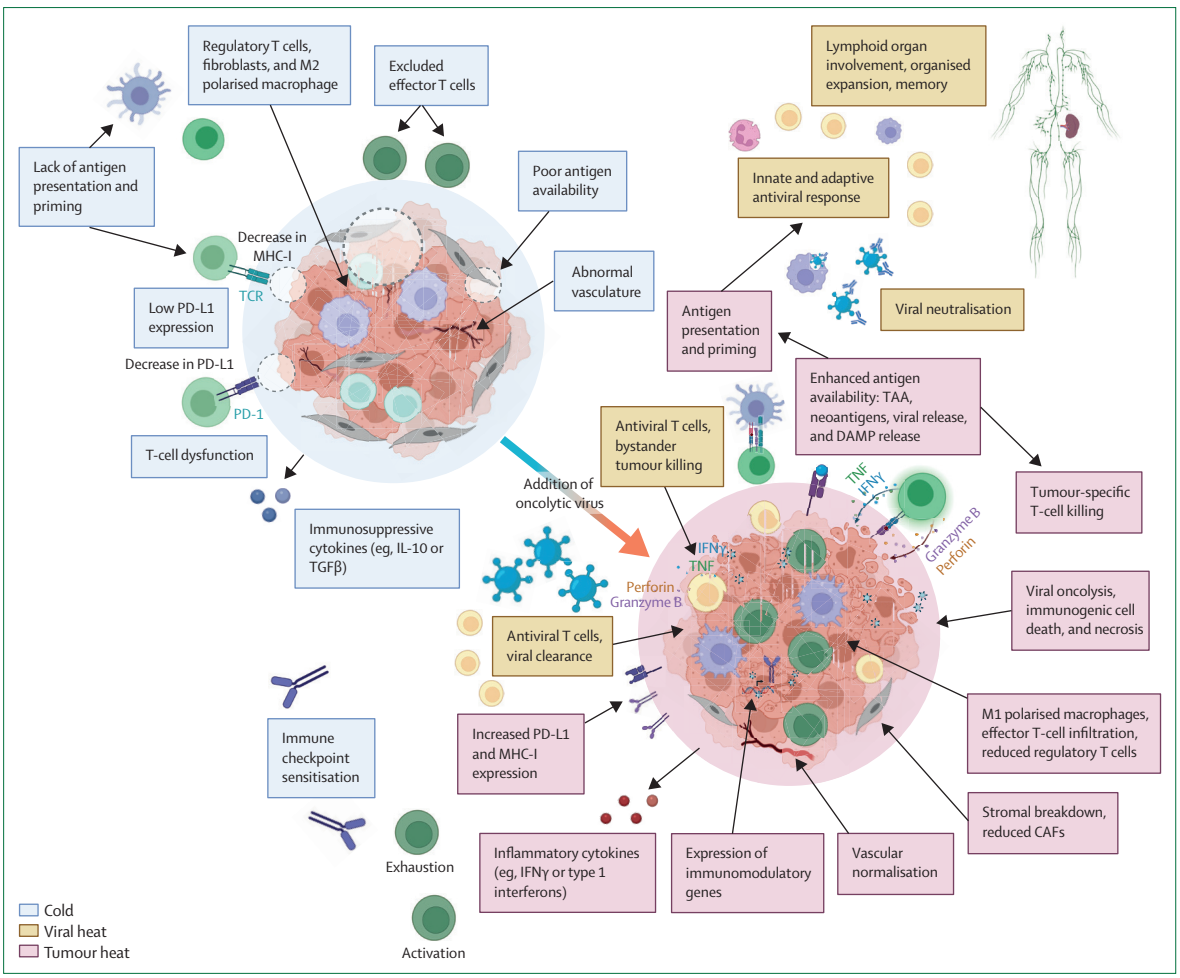


***Figure 1:*** **Oncolytic viruses—increasing the heat of the tumour microenvironment**
Cold refers to low immune infiltration. Viral heat is the enrichment of components of the viral response including antiviral T cells. Tumour heat is the enrichment of components of an antitumour immune response including antitumour T cells. Oncolytic viruses can exert anticancer effects through direct infection and viral oncolysis, and by stimulation of an anticancer immune response through inflammatory modification of the tumour microenvironment, and priming of T cells against tumour antigens released through oncolytic virus-mediated immunogenic cell death. Delivery of oncolytic viruses, either via intratumoural or intravenous injection has been shown to lead to diverse inflammatory changes within the tumour microenvironment, including T-cell infiltration, inflammatory cytokine production, dendritic maturation, enhanced antigen presentation and priming, stromal remodelling, and vascular normalisation. However, there is complex interaction and competition within an oncolytic virus-treated tumour microenvironment between antitumour and antiviral immunity. Elements of antiviral immunity might be beneficial, including bystander killing, trafficking of immune cells to or from lymphoid organs, inflammatory cytokine secretion and cross-priming; however, viral antigens are commonly immunodominant, and might eclipse true antitumour immunity. CAF=cancer associated fibroblasts. DAMP=damage-associated molecular patterns. MHC-I=major histocompatibility complex class 1 molecules. TAA=tumour associated antigens. TCR=T-cell receptor.

antiviral immune response, facilitating more potent antitumour immunity.[22]

From a T-cell perspective, although oncolytic virus infection might be highly immunogenic, many recruited effectors will be primed against immunologically dominant viral epitopes, as opposed to being primed to tumour epitopes.[23] Antiviral T cells could facilitate antitumour immunity, through bystander activation, and epitope spreading (ie, where an antigen-specific immune response expands to include other epitopes or antigens). Infiltration of both antiviral and antitumour CD8 T cells has been associated with oncolytic virus-mediated tumour regression in mice.[24] However, antiviral T cells have also been shown to abolish antitumour T-cell populations, restricting response to immune checkpoint inhibitor therapy.[23] The extent to which antitumour priming can occur in the presence of dominant viral immunity remains a huge hurdle and is yet to be robustly quantified in patients. Emerging strategies to overcome this hurdle include efforts to embrace the antiviral response and repurpose or redirect oncolytic virus-induced antiviral T cells (figure 2).

For example, incorporation of a tumour-derived cDNA library into a vesicular stomatitis virus genetic backbone was shown to enhance priming of antitumour T cells.[23] Delivery of oncolytic vesicular stomatitis virus with

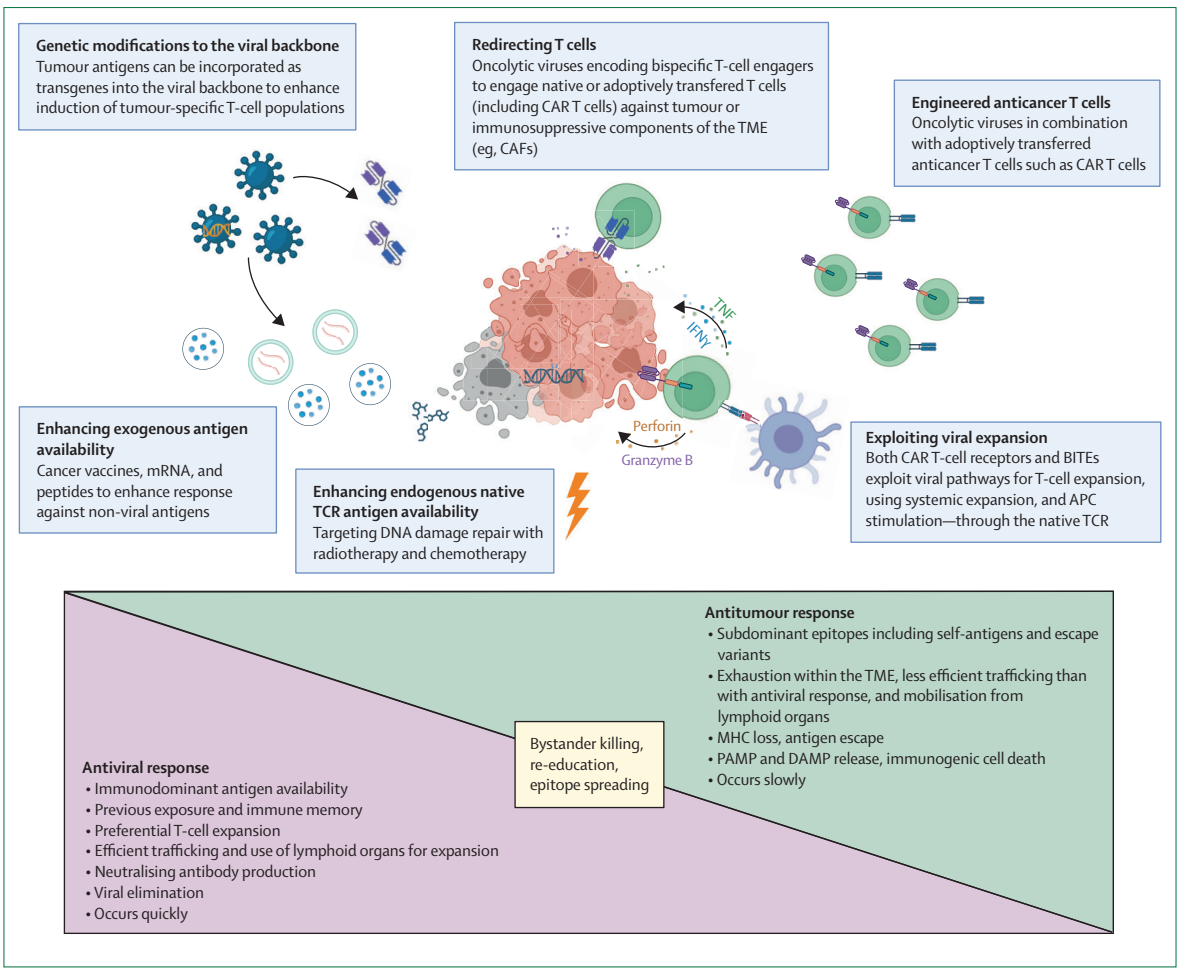


***Figure 2*: Antiviral versus antitumour immunity—redirecting the antiviral immune response**
Oncolytic viruses are, primarily, viruses rather than inert immunotherapeutic agents. To generate a response within a tumour there is a delicate balance between the adaptive immune response against the virus, and the tumour. The antiviral response is heavily evolved and efficient and often occurs quickly to facilitate pathogen clearance. It is associated with often immunodominant viral antigens, rapid expansion of CD4 and CD8 T cells and initiation of a B-cell or antibody response. Antitumour immunity classically occurs more slowly, is often associated with subdominant tumour antigens (many of which are tolerised, self-antigens) and escape variants. There is frequent T-cell exhaustion within the tumour microenvironment, and trafficking and systemic expansion might be less efficient than viral immunity. There are elements of overlap, where an antiviral immune response might promote expansion of tumour-specific T cells, such as bystander killing of tumour cells and T-cell re-education and epitope spreading. Several strategies aim to exploit the oncolytic virus-induced dominant antiviral response to promote more effective tumour-cell killing. These strategies include redirection of T cells (such as with engineered CAR T-cell receptors, or BITEs) enhancement of antigen availability in vaccine strategies, that use the oncolytic virus-inflamed tumour microenvironment for more effective priming. APC=antigen-presenting cells. BITE=bispecific T-cell engager. CAF=cancer associated fibroblasts. CAR=chimeric antigen receptors. DAMP=damage-associated molecular patterns. MHC=major histocompatibility complex. PAMP=pathogen-associated molecular patterns. TCR=T-cell receptor. TME=tumour microenvironment.

chimeric antigen receptor (CAR) T cells was also shown to enhance therapy, through induction of a population of CAR T cells with viral specificity of their native T-cell receptor.[25] As oncolytic viruses progress in the clinic, a deeper understanding of where antiviral heat is helpful, and where it could distract from the generation of true, systemic antitumour immunity will be crucial.

## Oncolytic viruses in the clinic: finding space in a rapidly evolving field

To date, oncolytic viruses have most extensively targeted advanced melanoma; at the time of T-VEC approval, immune checkpoint inhibitors and targeted agents against the *BRAF* and *MEK* oncogenes were in their infancy.[26] Over the past 10 years there have been unprecedented advances, including the implementation of immune checkpoint inhibitor immunotherapies targeting key negative regulators of T-cell mediated immunity, PD-1, CTLA-4, and PD-L1.[27] This presents a challenging landscape for oncolytic viruses to gain clinical traction in the wake of such remarkable success.

Despite this seismic shift, response rates to immune checkpoint inhibitors across all solid tumours remain suboptimal at between 15% and 30%,[17] with frequent toxicity and treatment interruptions. Even within

| | Delivery | Backbone | Modifications |
|---|---|---|---|
| T-VEC | Intratumoural | HSV-1 | *ICP34.5* deletion; *ICP47* deletion; *GM-CSF* insertion |
| Teserpaturev | Intratumoural | HSV-1 | *ICP34.5* deletion; *ICP47* deletion; *ICP6* inactivation |
| RP1 | Intratumoural | HSV-1 | *ICP34.5* deletion; *ICP47* deletion; *GM-CSF* insertion; *GALV* insertion |
| RP2 | Intratumoural | HSV-1 | *ICP34.5* deletion; *ICP47* deletion; *GM-CSF* insertion; *GALV* insertion; encodes *CTLA-4* |
| CAN-3110 | Intratumoural | HSV-1 | Retains one copy of *ICP34.5* under control of nestin promoter |
| T3011 | Intratumoural | HSV-1 | One copy of *ICP34.5* deleted; retains *ICP47*; *IL12* and anti-PD-1 monoclonal antibody insertion |
| CAN-2409 | Intratumoural | Replication-defective adenovirus | Encodes HSV-thymidine kinase |
| Nadofaragene firadenovec | Intravesical | Replication-defective adenovirus | Delivers *IFNα2b* |
| Cretostimogene grenadorepvec | Intravesical | Adenovirus (Ad5) | Promoter for viral replication under control of *E2F*; *GM-CSF* insertion |
| Tasadenoturev | Intratumoural | Adenovirus (Ad5) | *RGD* insertion; *E1A* deletion |
| Igrelimogene litadenorepvec | Intratumoural or intravenous | Adenovirus (chimeric Ad5–Ad3) | Modified fibre protein to avoid neutralisation; encodes TNF and IL-2 |
| CAdVEC | Intratumoural | Adenovirus and HD-Ad | Encodes IL-12 and anti-PD-L1 antibody |
| Vocimagene amiretropvec | Intratumoural | Replication-competent retrovirus | Encodes yeast cytosine deaminase (prodrug activating) |
| Pelareorep | Intratumoural | Reovirus | Unmodified |
| Pexastimogene devacirepvec | Intratumoural | Vaccinia virus | Thymidine kinase gene-inactivated; *GM-CSF* insertion |
| H-1 parvovirus | Intratumoural or intravenous | Parvovirus | Unmodified |

Ad3=adenovirus serotype 3. Ad5=adenovirus serotype 5. HD-Ad=helper-dependent adenoviral vectors. HSV-1=herpes simplex virus type 1. T-VEC=talimogene laherparepvec.

***Table 1:*** **Examples of viral agents under clinical development**

melanoma, refractory disease and resistant subtypes, such as uveal melanoma, remain a considerable challenge. In some cancer sites, there has been little movement in 20 years despite major efforts to incorporate immunotherapy into treatment framework. For example, treatment of high-grade glioma or glioblastoma and pancreatic cancer have remained largely static, with several recent negative phase 2 and phase 3 immune checkpoint inhibitor trials.[28,29] The immunosuppressive tumour microenvironment remains one of the major barriers to effective immunotherapy, and a rational hypothesis is that oncolytic viruses could serve as valuable adjuncts for immune checkpoint inhibitors and other immunotherapeutic approaches. With the number of constructs in development, there is a sense that oncolytic viruses might now be finding their place both as single agents and within increasingly sophisticated therapeutic combinations.

As of January, 2025, a search of clinicaltrials.gov reveals over 200 ongoing clinical trials of oncolytic viruses alone or in combination with other therapies, with many more in the pipeline. Although a comprehensive preclinical evaluation of emerging oncoviral agents and combinations is addressed elsewhere,[30,31] this Therapeutics paper will focus on oncolytic viruses that have been most widely tested in the clinic (table 1), to evaluate how emerging clinical data are shaping the field moving forward.

## Insights from the approval of T-VEC

The oncolytic HSV-1 T-VEC remains the only oncolytic virus to reach worldwide approval status to date.[32] T-VEC is modified by deletion of viral neurovirulence factor ICP34.5 and immune regulator protein ICP47, with insertion of immune stimulant GM-CSF. Intratumoural T-VEC was shown to be tumour selective and associated with regression of injected and non-injected (including visceral) lesions in a subset of patients.[33] A phase 2 single-arm study of 50 patients with stage III to stage IV melanoma reported an overall response rate (ORR) of 26%, with durability in 92% of patients with response (response maintained for 7–31 months).[34] Treatment was shown to be well tolerated, with side-effects including local inflammation and mild influenza-like symptoms, as is common to the majority of oncolytic viruses.

The phase 3 OPTiM study subsequently compared T-VEC with subcutaneous GM-CSF in 436 patients with stage IIIb to stage IVM1c melanoma, reporting a durable response rate (DRR) of 19·0% for T-VEC versus 1·4% for GM-CSF.[20,35,36] T-VEC was approved by the US Food and Drug Administration in 2015 for the treatment of advanced melanoma, followed up by approval in Europe, Australia, and Israel.[32]

The OPTiM study, and approval of T-VEC at a time when there were so few treatment options for melanoma, has provided valuable insight into the potential benefit of oncolytic viruses for carefully selected patient cohorts.

For **clinicaltrials** see https://clinicaltrials.gov

***Panel 1:*** **Clinical considerations—lessons learned from the approval of talimogene laherparepvec and beyond**

**Treatment earlier in the course of disease**

Subgroup analysis of the OPTiM phase 3 study evaluating talimogene laherparepvec (T-VEC) in advanced melanoma showed that 92% of patients with complete response had earlier stage disease (stage IIIb to stage IVM1a *vs* stage IVM1b or IVM1c), providing rationale for the use of oncolytic viruses earlier in the disease course.[20,21] Clinically, adenoviral vector nadofaragene firadenovec (Adstiladrin; Ferring Pharmaceuticals, Bridgewater, NJ, USA) has been approved for the treatment of early non-muscle invasive bladder cancer, and neoadjuvant T-VEC has shown promise in melanoma and triple negative breast cancer, with several other neoadjuvant oncolytic virus studies underway. With well documented safety and tolerability, including in combination with chemotherapy and immunotherapy (which are now established pillars of neoadjuvant therapy), further evaluation of the efficacy of oncolytic viruses in early stage disease is warranted.

**Treatment for patients at high risk of therapeutic morbidity**

The approval of T-VEC has enabled real-word studies evaluating oncolytic virotherapy in patients who would conventionally not be represented in trial cohorts (eg, advanced age or significant comorbidities). Therapeutic regimens such as dual immune checkpoint inhibitors are associated with substantial morbidity, and older age (age >75 years), or poor Eastern Cooperative Oncology Group performance status (>2) are negative prognostic indicators. Real-world studies of T-VEC have shown safety in older cohorts (eg, median age 83)[37] with low rates of adverse events, and case reports have shown safety of T-VEC in patients with heart and renal transplants. Oncolytic herpes simplex virus-1 RP1 was also shown to be well tolerated in recipients of solid organ transplants with advanced cutaneous malignancies. Oncolytic viruses could therefore be an appealing therapeutic option for those with comorbidities, older age, or poor performance status.

**Consideration of dose scheduling and choice of combination**

T-VEC did not show clinical benefit in combination with anti-PD-1 agent pembrolizumab in the phase 3 MASTERKEY-265 study, despite promise in early-phase studies.[1] This (and other trials of immune checkpoint inhibitors in combination with oncolytic viruses) highlighted the potential importance of dose scheduling. In this study, agents were delivered concomitantly, whereas previous early-phase trials used a sequential approach. Few preclinical studies (and no clinical studies) have evaluated scheduling comparisons; however, concomitant therapy could favour a dominant viral response, or lead to inhibitory inflammation. Optimal scheduling is also a consideration for incorporation of immunomodulatory transgenes, leading to expression at the same time in the same biological space. Further, MASTERKEY-265 opted for a combination with pembrolizumab (anti-PD-1), while T-VEC in combination with ipilimumab had also shown promise in a comparative phase 2 study versus ipilimumab alone. The right immune checkpoint inhibitor in the right context remains to be elucidated, and a focus on deeper translational understanding of complex combinations within early phase studies will be imperative to streamline effective clinical translation.

Such cohorts include treatment in early-stage disease (including neoadjuvant therapy) or outside conventional trial demographics (ie, patients who are commonly either excluded, or not the main participants within clinical trials such as older people or people who have received organ transplants; panel 1). Patients with stage IIIb to stage IVM1a disease accounted for 47 (92%) of 50 patients with complete response in OPTiM and had the most significant benefit in DRR (28·8% [47 of 163 patients] in patients with stage IIIb to stage IVM1a disease had durable response *vs* 6·3% [four of 64 patients] in patients with stage IVM1b disease) and estimated 5-year survival (48·9% [95% CI 40·6–56·7] in patients with stage IIIb to stage IVM1a disease *vs* 15·1% [9·3–22·2] in patients with stage IVM1b disease).[36] The median time to complete response in OPTiM was 8·6 months (range 2·1–42·3), highlighting that oncolytic viruses could be more suitable for patients with a low disease burden, particularly of visceral metastases. Patients with head and neck melanoma also had better outcomes (DRR 36·1%; 22 of 61 patients),[38] as did those receiving T-VEC as first-line therapy (DRR 23·9%, 95% CI 14·3 to 23·1), compared to those receiving T-VEC as second-line therapy or greater (DRR 9·6%, 95% CI –3·2 to 12·3).[18] Further studies have reproduced these findings,[39,40,41] including a meta-analysis of 642 patients across eight studies which showed higher complete response rates in patients with stage IIIb to stage IVM1a disease (41%; 144 of 346 patients) than in patients with stage IVM1b to IVM1c disease (4%; five of 151 patients).[42] These results rationalise evaluation in the neoadjuvant setting; a study published in 2021, evaluating T-VEC in 150 patients with resectable stage IIIB or stage IVM1a melanoma, reported an estimated 25% risk reduction, with benefit maintained at 3 years.[43] Neoadjuvant oncolytic viruses have also been investigated in other cancers, including breast[44] and gastrointestinal cancer,[45] and the neoadjuvant setting presents a notable opportunity for intravenous delivery of agents such as vaccinia virus, which are limited by rapid neutralisation upon repeat systemic administration (which is likely to be required for treatment of later-stage disease). For example, a biological endpoint study published in 2022, evaluating intravenous vaccinia virus pexastimogene devacirepvec (Pexa-Vec [JX-594]; SillaJen, San Francisco, CA, USA) in metastatic colorectal carcinoma or melanoma liver lesions before resection,[46] showed tumour-specific natural killer and T-cell responses and evidence of tumour necrosis warranting further exploration.

The OPTiM study had a median age of 63; however, two retrospective studies have reported clinical responses with no treatment-limiting toxic effects in older patients (median age 75 years [range 51–94] and 83 years [range 75–89]).[37,47] These data present an attractive prospect when current systemic therapies are associated with substantial risk of toxic effects, particularly in older patients with borderline performance status. Patients who have received organ transplants are also at risk of allograft rejection with immune checkpoint inhibitor regimens, yet have high risk of malignancy. Case reports have shown safety of T-VEC in patients with heart[48] and renal transplants,[49] and a phase 1b/2 study of next-generation oncolytic HSV-1 RP1 for the treatment of cutaneous malignancies in transplant patients is currently recruiting (NCT04349436).

With such biologically complex therapies, evaluation of effects across diverse cohorts of heavily pretreated

| | Viral agent | Approval location (year) | Approval context | Registry studies |
|---|---|---|---|---|
| Andtbacka et al (2015);[20] Andtbacka et al (2019)[36] | Talimogene laherparepvec (T-VEC) | USA (2015); Europe (2015); and Israel (2017) | Unresectable stage IIIb to stage IV melanoma | Phase 3; DRR 19·3% (57 of 295 patients) for T-VEC *vs* 1·4% (2 of 141 patients) for GM-CSF (unadjusted odds ratio 16·6; 95% CI 4·0–69·2; p<0·0001); ORR 31·5% for T-VEC *vs* 6·4% for GM-CSF; median OS 23·3 months (95% CI 19·5–29·6) *vs* 18·9 months for GM-CSF (95% CI 16·0–23·7) |
| Todo et al (2022)[50] | Teserpaturev | Japan (2021) | Refractory high-grade glioma | Phase 2; median PFS 4·7 months (95% CI 3·3–6·1); 1-year survival 84·2% (16 of 19 patients, 95% CI 60·4–96·6); median OS 20·2 months (95% CI 16·8–23·6) |
| Boorjian et al (2021)[51] | Nadofaragene firadenovec (Adstiladrin) | USA (2022) | BCG-non-responsive NMIBC | Phase 3; CR 53·4% (55 of 103 patients) at 3 months; maintained in 25 (45·5%) of 55 patients at 12 months |

CR=complete response. DRR=durable response rate. NMIBC=non-muscle invasive bladder cancer. ORR=objective response rate. OS=overall survival. PFS=progression-free survival.

***Table 2:*** **T-VEC and beyond—viral agents approved since 2015**

patients is wrought with difficulty. Now, with clear clinical rationale and promising data, these settings are becoming fertile ground to explore the clinical potential of oncolytic viruses alongside their immune biology in patients.

## Oncolytic viruses as monotherapies: regulatory approvals and application in settings beyond melanoma

Only two other viral agents have gained regulatory approval since 2015 (table 2), highlighting the length and complexity of clinical translation. The oncolytic HSV-1 teserpaturev (G47Δ) was approved for the treatment of therapy-resistant high-grade glioma in Japan in 2021.[6] This was the first oncolytic agent to be approved for primary brain cancer, representing a promising emerging area of oncolytic virus application, particularly with neurotropic viruses such as HSV-1. Similar to T-VEC, teserpaturev has deletions of neurovirulence factor ICP34.5 and ICP47 protein with inactivation of *ICP6 (RIR1)* for enhanced selectivity. After encouraging results in a small phase 1/2 trial (three of 13 patients surviving for longer than 46 months),[52] a pivotal, investigator-led, single-arm phase 2 study evaluated the effects of up to 6 doses of stereotactically administered teserpaturev in 19 patients with recurrent or residual glioblastoma. Adverse events included fever, headache, and vomiting, and a 1-year survival rate of 84·2% (95% CI 60·4–96·6) was reported (median overall survival was 20·2 months [range 16·8–23·6]).[50] Longitudinal tissue biopsies showed T-cell infiltration through treatment, with a reduction in FoxP3$^+$ regulatory T cells and no viral persistence.[52] This trial led to conditional approval for the treatment of malignant glioma in Japan, with acknowledgment of the study's limitations, including a small cohort, high frequency of IDH1 mutations (which are associated with better-prognosis) and absence of a control group. This conditional approval was a positive step for oncolytic viruses in the treatment of high-grade glioma, given the paucity of other options, mirroring the clinical scenario in melanoma at the time of the OPTiM trial; however, more definitive clinical testing will be required for wider clinical integration. This study showed the unprecedented feasibility (and tolerability) of repeat stereotactic dosing with longitudinal tissue sampling in high-grade glioma, which had been a major limitation in the field, and has guided further trial development.[53]

Other agents under evaluation in the setting of high-grade glioma include modified HSV-1 CAN-3110. Although the majority of HSV-1 oncolytic virus constructs are attenuated through mutation of both copies of viral neurovirulence factor ICP34.5, CAN-3110 restores one copy under the control of nestin-1, which is highly expressed in glioblastoma cells.[12] In a recent phase 1 study of 41 patients, tumour-selective HSV-1 infection was shown after stereotactic administration, with a significant survival advantage reported in patients with baseline HSV-1 seropositivity (hazard ratio 0·16, 95% CI 0·053–0·47), enhanced T-cell infiltration, and HSV-1 clearance, highlighting a role for robust antiviral immunity.[22] The serotype 5 adenovirus (Ad5) DNX-2401 is also under investigation in adult and paediatric brain tumours, with reports of durable tumour regression in a minority.[54]

Despite considerable promise, research into oncolytic viruses in primary brain cancers faces practical challenges, along with scepticism aligned with other facets of immune neuro-oncology, where early-phase success has not yet translated into meaningful benefit in late-stage trials. One example is the replication-competent retroviral therapy vocimagene amiretrorepvec (Toca 511), which was evaluated at phase 3 in refractory high-grade glioma, yet was not superior than physician's choice,[3] despite encouraging early-phase results.[55] An exploratory subgroup analysis at second recurrence (60 patients) in

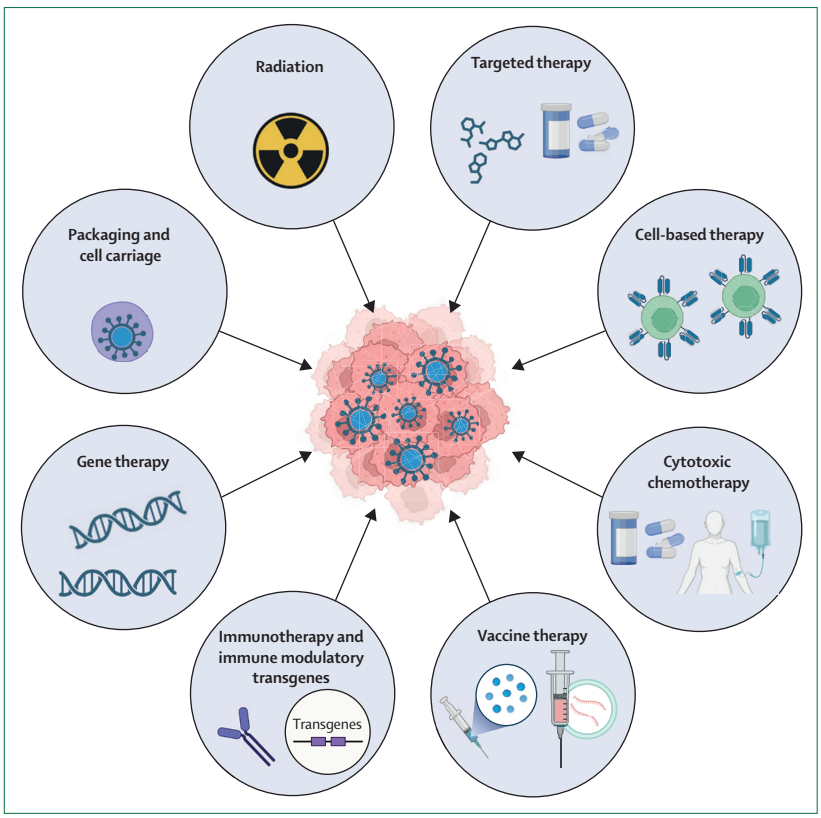


***Figure 3*: Oncolytic viruses in combination with other treatment modalities**
Oncolytic viruses do not have universal therapeutic potency as single agents; however, they are well tolerated, with non-overlapping side-effects, and have diverse effects on the tumour microenvironment, making them an appealing addition to the therapeutic armoury when used in combination with other treatments. Combinatorial strategies currently under investigation include immunotherapy, conventional chemotherapy, cancer vaccines, cell therapy, and targeted therapy. More research is needed, including translational mechanistic readouts from clinical trials of combinations, to evaluate where oncolytic viruses as a tool might be applied most effectively. With broad and ever-complex combinations, questions such as scheduling become of paramount importance, and testing this huge variety of constructs, combinations, and schedules in a clinical setting remains a challenge.

this study was suggestive of tumour phenotypes that might be selectively responsive, including a trend towards a survival benefit in patients treated with Toca 511 with IDH1 mutant tumours and anaplastic astrocytoma.[29] This result emphasises the need for a translational commitment to understanding intertumoural and intratumoural heterogeneity, to guide patient selection and combinatorial strategies. Repeat stereotactic injections also have safety and economic implications, yet the blood–brain barrier presents a unique hurdle for systemic delivery. Some viruses, including reovirus, have been shown to hitchhike across the blood–brain barrier on immune cells,[56] and other strategies, including stem-cell carriers and pressure gradients, have also been used for delivery of oncolytic viruses to the brain. For example, a phase I study evaluating delivery of human mesenchymal stem cells loaded with oncolytic adenovirus DNX-2401 in recurrent high-grade glioma is currently recruiting (NCT03896568).[4] Despite these challenges, oncolytic viruses are an exciting prospect for treatment of high-grade glioma, and scientific discovery with longitudinal sampling will be instrumental in driving clinical progress and personalised approaches.

The second recent approval of a viral agent was granted for replication-defective adenoviral vector nadofaragene firadenovec (Adstiladrin; Ferring Pharmaceuticals, Bridgewater, NJ, USA) in the treatment of BCG-unresponsive non-muscle invasive bladder cancer.[57] 55 (53·4%) of 103 patients with carcinoma in situ in the treatment group had complete response,[51] making it a promising application of a viral monotherapy (here applied as a viral vector rather than oncolytic agent) in this early yet difficult-to-treat disease setting. In the same setting, oncolytic adenovirus cretostimogene grenadorepvec (CG0070) was granted fast-track designation, after the single-arm, BOND-003 study (NCT04452591) showed an encouraging complete response rate (79 [75·2%] patients had complete response) with no dose-limiting adverse events.[58]

Despite encouraging results and scattered approvals, therapy with multiple modalities will likely be needed to overcome tumour heterogeneity and achieve wider benefit. The direction of travel has therefore been towards oncolytic viruses as versatile tools in combination with other agents (figure 3). Clinical trials are summarised in table 3.

## Oncolytic viruses in combination therapies

### Oncolytic viruses in combination with immunotherapies

Oncolytic viruses in combination therapies with immune checkpoint inhibitors are among the most clinically advanced combinations to date, supported by preclinical evidence of synergy.[17] T-VEC has been shown to increase CD8$^+$ T-cell infiltration and immune checkpoint expression in melanoma (factors that have been associated with response to checkpoint inhibitor therapy),[16,73] yet clinical studies have yielded mixed results. In early-phase studies, T-VEC enhanced ORR when used in combination with ipilimumab (ORR 50%)[74] and pembrolizumab (ORR 62%),[16] compared with historical control patients (ORR 35–40%),[16,74,75] with one study reporting immune activation including CD8$^+$ T-cell infiltration and IFNγ signatures in patients with response.[16] A comparative randomised phase 2 study of T-VEC in combination with ipilimumab subsequently showed improved ORR (39% for combination therapy *vs* 18% for ipilimumab alone), with enhanced regression of visceral lesions (52% in patients who had combination therapy *vs* 23% in patients who had ipilimumab alone).[59] The MASTERKEY-265 phase 3 trial, evaluating T-VEC and pembrolizumab in 692 patients with stage IIIb to IVM1c melanoma who had not received immune checkpoint inhibitors, did not show significant survival benefit (ORR was 48·6% for T-VEC plus pembrolizumab and 41·3% for placebo plus pembrolizumab).[1] Researchers hypothesised that the study might have been underpowered, with a high proportion of patients with stage IVM1b or stage IVM1c

| | Agent | Phase | Disease setting | Key outcomes |
|---|---|---|---|---|
| **Oncolytic viruses as monotherapies** | | | | |
| HSV-1 | | | | |
| Senzer et al (2009)[34] | T-VEC | 2 | Stage IIIc to stage IVM1c melanoma | ORR was 26% (13 of 50 patients); OS was 58% at 1 year; 92% responses maintained for between 7 months and 31 months |
| Andtbacka et al (2015);[20] Andtbacka et al (2019)[36] | T-VEC | 3 | Stage IIIb to IV melanoma | DRR was 19·3% (57 of 295 patients) for T-VEC *vs* 1·4% (two of 141 patients) for subcutaneous GM-CSF (unadjusted odds ratio, 16·6; 95% CI 4·0–69·2; p<0·0001); ORR 31·5% for T-VEC *vs* 6·4% for GM-CSF; median OS was 23·3 months for T-VEC (95% CI 19·5–29·6) *vs* 18·9 months for subcutaneous GM-CSF (95% CI 16·0–23·7); median time to CR was 8·6 months |
| Stahlie et al (2022)[42] | T-VEC | Meta-analysis (eight studies) | Stage III to stage IV melanoma | For stage IIIb to stage IVM1a disease, CR was 41% (141 of 344 patients; 95% CI 25–58) and ORR was 64% (181 of 283 patients; 95% CI 41–82); for stage IVM1b to stage IVM1c disease, CR was 4% (six of 151 patients; 95% CI 2–9) and ORR was 9% (12 of 135 patients; 95% CI 3–27); 0–11% of participants across all studies had severe adverse events (grade 3–4) |
| Dummer et al (2021)[43] | T-VEC | 2 | Neoadjuvant; resectable stage IIIb to stage IVM1a melanoma | pCR was 17·1% for T-VEC (n=76); 2-year RFS was 29·5% for T-VEC *vs* 16·5% for surgery alone (HR 0·75, 80% CI 0·58–0·96); benefit maintained at 3 years; response correlated with increased CD8 density |
| Todo et al (2022)[52] | Teserpaturev | 1/2 | High-grade glioma | n=13 patients; median OS was 7·3 months (95% CI 6·2–15·2); median PFS was 8 days from last G47 administration; 1-year survival from last G47 administration was 38% |
| Ling et al (2023)[22] | CAN-3110 | 1 | Recurrent glioblastoma | Single arm, n=41 patients; median OS was 11·6 months (95% CI 7·8–14·9); enhanced survival in baseline seropositive patients (14·2 months in HSV-1 seropositive patients [95% CI 9·5–15·7] *vs* 7·8 months in HSV-1 seronegative) |
| Adenovirus | | | | |
| Klaassen (2024)[58] | Cretostimogene grenadorepvec | 3 | BCG-unresponsive high-risk non-muscle invasive bladder cancer | CR was 75·2% (75 of 102 patients) at any time in the study |
| Boorjian et al (2021)[51] | Nadofaragene firadenovec | 3 | BCG-unresponsive non-muscle invasive bladder cancer | CR was 53·4% (55 of 103 patients with carcinoma in situ) within 3 months, maintained in 45·5% (25 of 55 patients) patients at 12 months; micturition urgency was the most common grade 3 or grade 4 TRAE (2 of 157 patients) |
| van Putten et al (2022)[54] | Tasadenoturev | 1 | Recurrent glioblastoma | Median PFS was 82 days (range 28–287); median OS was 129 days (range 68 days to >7 years); OS >6 months was 32% (six of 19 patients), one patient was alive at 2·5 years and another at 7·5 years |
| Replication-competent retrovirus | | | | |
| Cloughesy et al (2020)[3] | Vocimagene amiretrorepvec | 2 or 3 | High-grade glioma | No significant improvement on physicians' choice therapy |
| Reovirus | | | | |
| Samson et al (2018)[56] | Pelareorep | 1 | Primary and metastatic brain tumours | Infection of tumour cells after intravenous delivery; upregulation of IFNγ gene expression and PD-1–PDL-1 axis |
| Vaccinia virus | | | | |
| Samson et al (2022)[46] | Pexa-Vec | Biological endpoint | Neoadjuvant; metastatic colorectal cancer or melanoma liver lesions, before resection | 9 patients; pexastimogene devacirepvec found in tumours; 22% (two of nine patients) had tumour necrosis |
| **Oncolytic viruses in combination therapy** | | | | |
| Checkpoint blockade | | | | |
| Ribas et al (2017)[16] | T-VEC plus pembrolizumab | 2b | Advanced melanoma | ORR was 62% (13 of 21 patients); CR was 33% (7 of 21 patients); response was associated with IFNγ gene expression, PD-L1 expression, and CD8 infiltration was elevated after treatment |
| Chesney et al (2023)[1] | T-VEC plus pembrolizumab | 3 | Stage IIIb to stage IVM1c melanoma | No significant improvement in OS or PFS; ORR was 48·6% (168 of 346 patients) for combination therapy *vs* 41·3% (143 of 346 patients) for pembrolizumab alone; grade 3 or higher TRAE in 20·7% patients who had combination therapy *vs* 19·5% patients who had pembrolizumab alone |
| Chesney et al (2018)[59] | T-VEC plus ipilimumab | 2 | Stage IIIb to stage IV melanoma | ORR was 39% (38 of 98 patients) for combination therapy *vs* 18% (18 of 100 patients) for ipilimumab alone; decrease in visceral lesions in 52% patients in the combination group *vs* 23% of patients treated with ipilimumab alone; grade 3 or higher TRAE in 45% patients who had combination therapy *vs* 35% patients who had ipilimumab alone |
| Harrington et al (2020)[60] | T-VEC plus pembrolizumab | 1b | Head and neck squamous cell carcinoma | PR was 13·9% (5 of 36 patients); median PFS was 3 months (95% CI 2·0–5·8); phase 3 was not pursued |
| Robert et al (2024)[61] | T-VEC plus pembrolizumab | 2 | Anti-PD-1 refractory melanoma (primary and acquired resistance in unresectable or metastatic, and progression following adjuvant therapy) | ORR 3·8% (primary resistance; 95% CI 0·1–19·6), 6·7% (acquired resistancel; 95% CI 0·2–32·0), 53·3% (recurrence <6 months after adjuvant anti-PD-1; 95% CI 26·6–78·7), 46·7% (recurrence ≥6 months after adjuvant anti-PD-1; 95% CI 21·3–73·4) |

(Table 3 continues on next page)

| | Agent | Phase | Disease setting | Key outcomes |
|---|---|---|---|---|
| (Continued from previous page) | | | | |
| Wong et al (2025)[62] | RP1 plus nivolumab | 1/2 | PD-1 refractory melanoma | n=140 patients; ORR was 32·9% (95% CI 25·2–41·3); CR was 15·0%; median duration of response 33·7 months; OS at 2 years 63·3% (95% CI 53·6–71·5); |
| Nassiri et al (2023)[28] | Tasadenoturev plus pembrolizumab | 1/2 | Recurrent glioblastoma | n=49 patients; ORR was 10·4% (95% CI 4·2–20·7); OS at 12 months was 52·7%; median OS was 12·5 months (95% CI 40·1–69·2) |
| Sacco et al (2023)[63] | RP2 plus nivolumab | 1 | Refractory uveal melanoma | ORR was 28·6% (4 of 14 patients); favourable safety profile compared to historical studies of dual checkpoint blockaged in a similar cohort |
| Cytokines | | | | |
| Ji et al (2023)[64] | T3011 (expressing IL-12 and anti-PD-1 monoclonal antibody) | 1 | Advanced solid tumours | ORR was 11% (six of 55 patients); no dose-limiting toxic effects; grade 3 or higher TRAE in 2·2% (two of 90 patients) |
| Santos et al (2023)[65] | Igrelimogene litadenorepvec (encoding IL-2 and TNF) | 1 | Advanced solid tumours | Disease control in 22% (two of nine patients), including PR in 11% (one of nine patients) |
| Chemotherapy or radiotherapy | | | | |
| Arnold et al (2023)[66] | Palareorep plus gemcitabine–nab-paclitaxel in combination with atezolizumab | 1/2 | Pancreatic cancer | ORR was 62% (eight of 13 patients); confirmed OR 53% |
| Soliman et al (2023)[44] | T-VEC plus neoadjuvant chemotherapy | 2 | Neoadjuvant, stage II or stage III triple negative breast cancer | pCR rate was 45·9%; 2-year disease-free rate was 89% |
| Clark et al (2025)[67] | Palareorep plus paclitaxel and avelumab | 2 | Hormone receptor positive breast cancer | n=48 (15 patients in paclitaxel group, 16 patients in paclitaxel plus palareorep group, 14 patients in paclitazel plus palareorep plus avelumab group, and three patients in safety run-in group); median PFS was 6·4 months (paclitaxel), 12·1 months (paclitaxel plus palareorep), and 5·8 months (paclitaxel plus palareorep plus avelumab); ORR was 20% (three of 15 patients in paclitaxel group), 31% (five of 16 patients in paclitaxel plus palareorep group), and 14% (two of 14 patients in paclitaxel plus palareorep plus avelumab group) |
| Candel Therapeutics (2024)[45] | CAN-2409 plus valacyclovir plus chemoradiotherapy | 2 | Neoadjuvant borderline resectable pancreatic ductal adenocarcinoma | Survival benefit was 28·8 months in patients who had combination therapy *vs* 12·5 months in patients who received standard of care |
| Monga et al (2021)[68] | T-VEC plus external beam radiation therapy | 2b or 2 | Neoadjuvant, soft tissue sarcoma | No dose-limiting toxic effects; no increase in proposed rate of pathological necrosis |
| Gállego Pérez-Larraya et al (2022)[69] | Tasadenoturev plus radiotherapy | 1 | Diffuse intrinsic pontine glioma; paediatric (patients aged 3–18 years) | Median OS was 17·8 months |
| Toulmonde et al (2022)[70] | Pexastimogene devacirepvec plus cyclophosphamide | 2 | Advanced soft tissue sarcoma | Evidence of systemic immune activation but no patients were progression-free at 6 months; tumour shrinkage on MRI in 75% (nine of 12 patients) |
| Targeted agents | | | | |
| Abou-Alfa et al (2023)[71] | Pexastimogene devacirepvec plus sorafenib | 3 | Advanced hepatocellular carcinoma | Median OS was 12·7 months (95% CI 9·89–14·95) in patients who had combination therapy *vs* 14·0 months (94% CI 11·01–18·00) in patients who had sorafenib alone; early termination of the study due to futility; 53·7% (117 of 218 patients) had serious adverse events with combination therapy *vs* 35·5% (77 of 217 patients) had serious adverse events with sorafenib alone |
| Cell carriage | | | | |
| Fares et al (2021)[72] | Adenovirus Ad5 NSC-CRAd-S-pk7 | 1 | Malignant glioma, after neurosurgical resection; treatment with radiotherapy and temozolomide was initiated within 10–14 days | Delivered by use of neural stem cells; median PFS was 9·05 months; median OS was 18·4 months; PR in 8% (one of 12 patients) |

CR=complete response. DRR=durable response rate. HR=hazard ratio. OR=overall response. ORR=overall response rate. OS=overall survival. pCR=pathological complete response. PFS=progression-free survival. PR=partial response. RFS=relapse-free survival. TRAE=treatment-related adverse events.

***Table 3:*** **Examples of viral agents as monotherapy and in combination**

disease (193 of 346 patients), a group that had previously been identified to benefit less from T-VEC monotherapy than patients with stage IIIB to stage IVM1a disease. Concordantly, subgroup analysis revealed a progression-free survival benefit in patients with low LDH and smaller tumours, although this could not be conclusively assessed. Melanoma is more responsive than many other cancers to checkpoint inhibitor therapy in the first-line setting (ORR was 41·3% for pembrolizumab as a single agent in the MASTERKEY-265 study); yet, the checkpoint inhibitor–refractory setting remains an area of clinical need. The phase 1/2 IGNYTE study (NCT03767348) recently treated 140 patients with anti-PD-1-refractory melanoma with RP1 and nivolumab, reporting a 32·9% ORR (21 [15%] of

140 patients had complete response),[62] with plans for phase 3 evaluation. This study reported regression of injected and non-injected (including visceral) lesions with a similar frequency, highlighting systemic immune activation. Notably, 48·6% of patients had stage IVM1b, IVM1c, or IVM1d disease, a subgroup that was previously shown to derive less benefit from T-VEC. T-VEC has also been evaluated in combination with pembrolizumab in the refractory settling, with encouraging results in patients with progression after adjuvant therapy.[61] These results show a potential advantage for oncolytic viruses in immune checkpoint inhibitor-refractory cancers, rather than as a first-line approach.

Outside melanoma, a phase 1/2 trial of T-VEC in combination with pembrolizumab in head and neck squamous cell carcinoma showed similar results to pembrolizumab monotherapy in historical studies, and the combination did not proceed to phase 3.[60] Similarly, a phase 2 study of RP1 in combination with cemiplimab in metastatic cutaneous squamous cell carcinoma did not show significant benefit (NCT04050436).[76] Importantly, both these trials and the MASTERKEY-265 study administered oncolytic viruses and immune checkpoint inhibitors concomitantly, whereas the previous positive phase 1b study of T-VEC in combination with pembrolizumab initiated oncolytic virus therapy 5 weeks before immune checkpoint inhibitor therapy, and the IGNYTE study initiated it 3 weeks before immune checkpoint inhibitor therapy.[16] These are unlikely to be trivial differences. Concomitant immune checkpoint inhibitor treatment could strengthen the rapidly expanding antiviral response at the expense of antitumour T-cell priming, and improved outcomes with sequential dosing have been shown preclinically,[77] although few studies have directly compared sequencing approaches.[78] Longitudinal translational sampling will be key to elucidate the dynamic changes within the tumour microenvironment in response to oncolytic viruses and inform rational scheduling.

The high-grade glioma tumour microenvironment is highly immunosuppressive, and immune checkpoint inhibitors have not delivered meaningful benefit. A phase 1/2 study of intratumoural oncolytic adenovirus DNX-2401 in combination with pembrolizumab in high-grade glioma showed response or stable disease in 56·2% of patients, with a 12-month overall survival of 52·7% (95% CI 40·1–69·2).[28] Responses were shown to exclusively occur in patients with a moderately inflamed tumour microenvironment. A trial of non-oncolytic adenoviral delivery of the potent pro-inflammatory cytokine IL-12 in recurrent glioblastoma also showed some evidence of clinical efficacy, however this was not improved upon by the addition of an immune checkpoint inhibitor despite evidence of upregulation of PD-1–PD-L1 signalling,[79] highlighting the complexity of the tumour ecosystem and perceived immunogenicity.

CAR T-cell therapy has shown substantial promise in haematological cancers, with little effect in solid tumours to date. Several preclinical studies have shown synergy between oncolytic viruses and CAR T-cell therapy,[25] where sequential oncolytic virus delivery has been shown to enhance CAR T-cell persistence and tumour infiltration. However, concurrent oncolytic virotherapy has also been shown to restrict CAR T-cell therapy via oncolytic virus-derived type-I interferon preclinically,[80] emphasising the importance of the timing of combination treatments. One study combining *HER2*-specific CAR-T with oncolytic adenovirus is currently recruiting (NCT03740256), highlighting the practical difficulties of this approach, namely production, regulation, toxic effects, and cost.[81] Tumour-infiltrating lymphocyte therapy has recently been approved for metastatic melanoma,[82] and was shown to be safe in combination with intratumoural and intravenous delivery of oncolytic adenovirus igrelimogene litadenorepvec (TILT-123) in a 2025 phase 1 study in checkpoint-refractory disease (NCT04217473).[83] In a further T-cell centric strategy, bispecific T-cell engagers (BITEs) bypass the T-cell receptor–major histocompatibility complex interaction by engaging T cells (via CD3), and a tumour cell target.[84] Several preclinical studies have shown feasibility and efficacy of oncolytic viruses encoding BITEs,[85] where local delivery presents an appealing prospect to overcome off-target effects of BITEs.

Oncolytic viruses can also be delivered with cancer vaccines, a strategy that has been shown to enhance induction of tumour-specific T-cell populations.[23] Oncolytic viruses are being investigated as adjuvants for off-the-shelf and personalised anticancer vaccinations. For example, the peptide-coated conditionally replicating adenovirus (PeptiCRAd; Valo Therapeutics, Helsinki, Finland) has been modified for immunogenicity with insertion of CD40-L and OX40L and coated with immunogenic cancer peptides. A phase 1 study (NCT05492682) targeting commonly expressed shared cancer antigens MAGE-A3 and NY-ESO-1 is currently recruiting, and personalised, immunopeptidomics-based approaches using this platform are also in development.[86] Other tactics include prime-boost regimens,[87,88] which deliver different oncolytic viruses sequentially with the same tumour-associated antigens to resist virus-specific immunity and amplify vaccine responses. This prime-boost strategy has been shown to generate antigen-specific T-cell responses in patients,[89] and early-phase trials are underway (NCT04046445 and NCT05846516) evaluating prime-boost regimens. Timing of administration of vaccine and virus, or sequential viruses, as with all combinations, is likely to be crucial, as has been shown in a recent preclinical study which showed that vaccination before oncolytic HSV-1 therapy was associated with enhanced survival.[90]

### Incorporation of immunomodulatory transgenes

Oncolytic virus combination regimens can either be delivered separately or through transgene incorporation into the viral backbone. Transgene incorporation has several theoretical advantages, including self-amplifying tumour-selective delivery, reduced toxicity, and avoidance of multiple infusions or oral dosing. However, these advantages come at a cost of unpredictable pharmacokinetics, reliance on viral infectivity and replication, and a reduced ability to modify schedules with patient-centred flexibility.[91] Although no clinical studies have directly compared the two approaches, in 2023, results were reported from a phase 1 study of RP2, a HSV-1 modified to express an anti-CTLA-4 antibody, alone or in combination with nivolumab in treatment-resistant uveal melanoma.[63] Six (35%) of 17 patients had grade 3 adverse events, with no grade 4 or 5 treatment-related adverse events, in comparison to 30 (57·7%) of 52 patients in a trial evaluating systemic dual immune checkpoint inhibitors in the same setting,[92] suggesting modified oncolytic viruses might circumvent elements of systemic immune checkpoint inhibitor toxic effects. Outside of checkpoints, systemic administration of cytokines and other immune modulators is often precluded by pharmacokinetics or toxic effects.[93] For example, systemic delivery of IL-12 is associated with prohibitive toxic effects,[94] yet a phase 1/2 study of an HSV-1 modified with IL-12 and anti-PD-1 in advanced solid tumours showed grade 3 or higher treatment-related adverse events leading to treatment discontinuation in two (2·2%) of 90 patients.[64]

An outstanding question is whether simultaneous administration of immunostimulatory agents within the tumour microenvironment is optimal for therapy. One emerging approach is to attempt to uncouple viral infection and therapeutic gene expression.[13] This approach involves the use of on–off switches, which can further refine efficacy and enhance safety and toxicity profiles. Such switches include protein stabilisation domains,[95] inducible promoters,[96] and RNA switches.[97] Whether this control can be reflected in improved clinical outcomes with more complex immunomodulatory combinations remains to be evaluated.

### Oncolytic viruses in combination with conventional therapies

Conventional cytotoxic chemotherapies, such as targeted agents and radiotherapy, still dominate the clinical landscape in many settings. Preconditioning chemotherapy with low-dose cyclophosphamide has been shown to enhance oncolytic virus delivery to tumours through modulation of the immune environment including inhibition of regulatory T cells,[98] and several chemotherapeutic agents have been shown to potentiate the cytopathic effects of oncolytic viruses in vitro and in vivo.[99,100] DNA damage and tumour cell destruction might enhance neoantigen availability for priming of oncolytic virus-induced T-cell infiltrates, whereas epigenetic modifiers such as histone deacetylase inhibitors can enhance epitope spreading,[101] restrict antiviral interferon responses,[102] and enhance viral replication.

Oncolytic virus chemotherapy combinations have shown promise in tumour sites where immune checkpoint inhibitors have had little effect to date. For example, in pancreatic cancer, interim analysis showed an ORR of 69% in a small cohort of 13 patients treated with first-line reovirus (pelareorep), atezolizumab, gemcitabine, and nab-paclitaxel, with no additional safety concerns.[103] An overall survival benefit (median 28·8 months in the treatment group *vs* 12·5 months in the control group) was also reported in a small phase 2 study (n=13) of neoadjuvant intravenous adenovirus CAN-2409 (Candel Therapeutics, Worcester, MA, USA) in combination with valaciclovir (a prodrug) and chemoradiotherapy in borderline-resectable pancreatic cancer (NCT02446093).[45] Despite the fact that CAN-2409 is replication defective, it was associated with a survival benefit, highlighting that the importance of viral replication to the efficacy of viral therapies remains unclear.

Also in the gastrointestinal cancer setting, modest efficacy was shown with pelareorep and atezolizumab in combination with chemotherapy in microsatellite stable colorectal cancer, with best response of stable disease in five (33%) of 15 patients, hypothesised to be due to pre-existing immunocompromise from substantial chemotherapy before the trial.[104] In hepatocellular cancer, the large phase 3 PHOCUS study of intratumoural oncolytic virus pexastimogene devacirepvec and tyrosine-kinase inhibitor sorafenib showed worse outcomes than sorafenib alone at interim analysis leading to early termination. Median overall survival was 12·7 months (95% CI 9·89–14·95) in the pexastimogene devacirepvec plus sorafenib group, compared with 14·0 months (95% CI 11·01–18·00) in the sorafenib group at interim analysis, with serious adverse events in 117 (53·7%) of 234 patients in the combination group and 77 (35·5%) of 225 patients in the sorafenib group.[71] These results were partly attributed to antagonistic effects of cell-cycle inhibition on pexastimogene devacirepvec replication, highlighting conflicting mechanisms between oncolytic viruses and many conventional anticancer agents.

Although immunotherapy has had little success in hormone receptor positive breast cancer, intravenous palareorep prolonged survival in combination with paclitaxel in heavily pretreated patients (progression-free survival was 12·1 months [95% CI 6·5–24·8] in the treatment group *vs* 6·4 months [2·0 to not reached] in the control group),[67] notably with no additional benefit from avelumab (progression-free survival 5·8 months [95% CI 3·5–9·4]). Contrary to the majority of oncolytic virus studies, dose-limiting reovirus toxic effects, including influenza-like infusion reactions,[105] were noted

in one-third of patients, which might highlight a drawback of more intensive intravenous dose scheduling, particularly in combination with chemotherapy. Also in breast cancer, a phase 2 trial of T-VEC plus neoadjuvant chemotherapy in patients with stage II or stage III triple negative breast cancer showed a pathological complete response (pCR) rate of 45·9%,[44] which compares favourably to a meta-analysis of neoadjuvant chemotherapy alone (pCR 31%);[2] however, these results predate the use of immune checkpoint inhibitor in this setting. This study correlated immune activation (including change in $CD8^+$ T-cell density) with response.

Radiotherapy is known to be immunomodulatory, leading to enhanced immunogenic cell death, antigenicity, and adjuvanticity, with evidence of synergy with immuno-oncology modalities in mice and patients.[106] The inflammatory effects of oncolytic viruses might also potentiate radiotherapy-induced abscopal effects, defined as a response in distant (untreated) tumours, which have been shown to be dependent on $CD8^+$ T-cell infiltration and dendritic cell crosspriming,[107] and alternative strategies include oncolytic viruses modified with radiosensitisers.[108] Radiotherapy in combination with T-VEC was shown to enhance immunogenic cell death and T-cell infiltration in preclinical models, with evidence of further benefit with addition of an immune checkpoint inhibitor.[109] Several ongoing studies are evaluating oncolytic virus and radiotherapy combinations, however published results remain scarce. In a phase 2 study of 17 patients with head and neck squamous cell carcinoma treated with T-VEC and chemoradiotherapy, 14 (82·3%) of 17 patients were shown to have clinical response with locoregional control at 29 months,[110] and in one of the few trials in paediatric patients, tasadenoturev (DNX-2401) followed up by radiotherapy was shown to be safe with evidence of immunostimulatory changes within glioma tumours.[69] In contrast to other studies in the neoadjuvant setting, no benefit was shown with neoadjuvant T-VEC in combination with radiotherapy over radiotherapy alone for soft tissue sarcoma in a phase 1b/2 trial involving 29 patients.[68]

The breadth of combinations continues to show the versatility of oncolytic viruses. A continued commitment to understanding the synergistic (and contradictory) mechanisms in preclinical models, ex-vivo human systems, and patients, including the effect of dosing and scheduling, will be imperative to guide future engineering and combinatorial approaches.

## Ongoing challenges to clinical integration

### Preclinical hurdles

Unravelling the mechanisms and contradictions underlying the complex contribution, positive or negative, of oncolytic viruses to therapy remains difficult. There is a risk of overengineering with concurrent delivery of conflicting immunomodulatory agents, and a paucity of representative models for detailed study of therapeutic immunodynamics. Engineering also presents a risk of overattenuation, with extensive modifications restricting viral replication and efficacy. For example, neurovirulence factor ICP34.5 is almost universally deleted within emerging HSV-1 constructs, yet it is implicated in robust replication in infected cells, and strategies to restore viral replicative capacity through engineered selectivity are in development (NCT03152318 and NCT05477849).[22,111] As engineering capabilities continue to grow, revisiting the age-old principles of virology and basic immunology will be crucial to the rational design of more effective oncolytic virus constructs.

### Mode of delivery and other pharmacological considerations

Most oncolytic viruses in development are delivered by intratumoural injection. This presents several challenges, including treatment of tumours that are difficult to reach, unpredictable pharmacokinetics, practical and regulatory challenges, and a scarcity of approved intratumoural comparators for clinical trials. Strategies to enhance oncolytic virus systemic delivery represent a major area of importance, along with pharmacodynamic studies to interrogate both intratumoural and intravenous delivery of replicating agents to inform dosing and monitoring. To facilitate systemic administration, an oncolytic virus must overcome several hurdles. These include dilution and viral neutralisation in the blood by neutralising antibodies and complement, sequestration by phagocytic cells in the liver and spleen, and barriers to extravasation and tumour infection posed by tumour vasculature and the extracellular matrix.[8] A clinical study evaluating pexastimogene devacirepvec showed that virus could only be detected within tumours at doses of at least $10^9$ plaque forming units (PFU), 1000 times higher than the commonly administered intratumoural dose ($10^6$ PFU), with implications for viral production and toxic effects in patients. Some oncolytic viruses have been shown to associate with immune cells, with capacity to hitchhike to tumours (ie, enabling viruses to be trafficked to tumours, including across the blood–brain barrier), evading systemic neutralisation.[56,112] Cell-associated oncolytic virus delivery, including with CAR T cells and stem cells, has also shown encouraging results.[72] Other strategies include modifying neutralising antibody binding sites to restrict recognition (NCT05222932).[113] Again, the role of robust, antiviral immunity remains incompletely understood, and in some contexts neutralising antibodies might be paradoxically beneficial.[114,115] For example, neutralising antibody complexes have been proposed to facilitate reovirus transport to tissues, including the brain.[116]

### Prediction of response

Many immunotherapies (including oncolytic viruses) elicit good response in a proportion of patients, suggesting that better predictive criteria would yield more impressive clinical results. Although tumour mutational burden, mismatch repair deficiency, and PD-L1 expression have all shown some use as biomarkers for immune checkpoint inhibitor, no predictive biomarkers of oncolytic virus efficacy or toxicity have been defined to date. A higher tumour mutational burden might enhance the antigen repertoire and facilitate more effective priming upon oncolytic virus-induced immunogenic cell death; however, large-scale immunogenomics or functional studies within oncolytic virus clinical trials are yet to evaluate this hypothesis. JAK mutation-associated interferon signalling defects have been shown to confer acquired resistance to anti-PD-1 therapy but sensitivity to oncolytic viruses in preclinical studies,[117] whereas other potential biomarkers include expression of viral entry receptors. For example, the HSV-1 receptor nectin-1 has been associated with response to T-VEC in preclinical models and melanoma patients,[118] although extensive validation remains scarce. Evaluation of the tumour cell intrinsic properties that facilitate effective viral replication, and the factors predicting immunological response, will be crucial to improving biological understanding. With the limitations in biomarker discovery in preclinical models, continued interrogation of data from clinical trials, with close collaboration between industry, academia, and the clinic, will be essential to refine patient selection (panel 2).

***Panel 2*: Optimising patient selection: Tumour subtype and biomarkers—patient selection**

Predicting which patients will have dramatic and durable responses to oncolytic virus therapy remains a challenge. However, subgroup analysis of clinical trials is beginning to elucidate select populations who might benefit. This warrants further trials based on individualised tumour phenotype rather than broad cancer type, with deep, translational readouts to aid biomarker discovery and patient selection. For example, patients with head and neck melanoma were seen to have greater benefit with talimogene laherparepvec therapy than the overall study cohort,[20,38] and patients with IDH1 mutant high-grade glioma had a higher rate of response to vocimagene amiretrorepvec (Toca 511) than patients with IDH1 wild type high-grade glioma.[29] Interferon signalling defects have also been associated with resistance to checkpoint therapy, but susceptibility to oncolytic viruses such as herpes simplex virus-1 and vesicular stomatitis virus in preclinical models.[117] More research is needed to understand mechanisms of response and resistance to begin to better stratify patient selection.

## Conclusions and future directions

Despite considerable challenges over the last two decades, oncolytic viruses continue to offer considerable promise to the field of oncology. Established and emerging data from the use of T-VEC, along with other virus platforms and encoded transgenes, are providing new insights into patient response, and the number of strategies under development is growing exponentially. Oncolytic viruses continue to be safe and well tolerated, and there is increasing evidence to support their application in particular settings, including in earlier-stage disease.

Methods for seamlessly integrating basket-style trial design into the evaluation of novel agents has been proposed in other settings and could help shorten oncolytic virus clinical timelines. However, this should proceed alongside a preclinical and translational commitment to deeper understanding of the biology of viral therapies in patients. Thus, future careful integration of artificial intelligence, machine learning, and biomarker modelling will be helpful in the analysis of multiomic, longitudinal translational data from clinical trials, to enable construction of cross-tumour biomarker classifiers which might lead towards adaptive personalisation of virotherapy-based treatment schedules.

Integration of preclinical human systems such as organoids and tissue slices will aid iterative, human-relevant mechanistic analysis, which should be integrated with basic virology and translational immunology. Such methods have already been shown in the assessment of oncolytic virus infectivity, and are emerging as powerful tools for therapeutic discovery, particularly of immunotherapeutic strategies that are reliant on the influence of the tumour microenvironment.[119] Bolstering these aspects of oncolytic virus research to match the dramatic advances in viral engineering will further inform rational design and prioritisation amongst the plethora of novel constructs and combinations, and enable biology-based trials, which should include study of practical considerations such as scheduling and delivery.

As with so many novel strategies, clinical integration of oncolytic viruses has been a tale of ups and downs: the initial approval of T-VEC, the negative outcome of T-VEC in combination with anti-PD-1 at phase 3 despite the promise of T-VEC in combination with anti-CTLA-4 and anti-PD-1 in early-phase studies, and, most recently, encouraging early data for the use of oncolytic viruses in immune checkpoint inhibitor-refractory melanoma, signalling a potentially large group of patients who might benefit from virotherapy, after immune checkpoint inhibitor therapy was not effective. The goal of finding the right virus, in the right setting, in the right combination now feels to be within reach. The major challenges are now to continue to critically evaluate emerging preclinical and clinical data, maximise opportunity for translational analysis, and

streamline clinical application towards wider benefit for patients.

#### Contributors

EA, EAC, GU, AM, and RV: conceptualisation and literature review and acquisition. RV: funding acquisition. EA: figure preparation. AM and RV: project administration. EA, AM, and RV: manuscript writing and review. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Declaration of interests

EAC reports grants from the National Institutes of Health and Alliance for Cancer Gene Therapy; a license with Candel TX; consulting fees from Calidi; patents planned, issued, or pending with Brigham and Women's Hospital; leadership or fiduciary role in other board, society, committee, or advocacy group, paid or unpaid for American Association of Neurological Surgery, the Society for Neurological Surgery, and Ternalys Therapeutics; and stock or stock options in Bionaut, Seneca, and Ternalys. RV reports consulting fees from Oncolytics Biotech, Greenfire Bio, and Adze Biotechnology and participation on a data safety monitoring board or advisory board for Oncolytics Biotech and Greenfire. AM reports participation on a data safety monitoring board or advisory board for Turnstone Biologics and Transgene Trials. EA reports grants or contracts from the Institute of Cancer Research, London (CRUK–ICR PhD fellowship and EACR fellowship) and receipt of materials (RP1) from Replimune. GU reports grants or contracts from DFG, German Cancer Aid, and Wilhelm Sander Foundation and consulting fees from Boehringer Ingelheim and Amgen.

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