

# Oncolytic viruses and cytokine-based gene therapies reprogram the tumor microenvironment

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Solid tumors are sustained by profoundly immunosuppressive tumor microenvironments (TMEs) that underlie resistance to immunotherapy. Engineered oncolytic viruses and cytokine-based gene therapies can reprogram the TME, converting ‘cold’ tumors into immune-responsive states and amplifying antitumor immunity. Several agents have achieved regulatory approval, and clinical studies demonstrate that even limited dosing can induce durable changes in immune infiltration and cytokine signaling. Yet consistent and lasting clinical responses remain elusive. Here, we synthesize translational insights from recent trials and highlight emerging strategies to overcome barriers and enhance the therapeutic impact of these TME-modulating biologics.

Modern cancer therapy has been transformed by the advent of immune checkpoint inhibitors (ICIs), which have become a cornerstone of treatment. ICIs, including antibodies targeting programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated protein 4 (CTLA-4), have shifted oncological therapeutics toward harnessing host immunity, yielding durable responses in a subset of patients<sup>1</sup>. Yet many patients derive little or no benefit, and even among initial responders, acquired resistance often limits the durability of therapeutic effects<sup>2</sup>.

The tumor microenvironment (TME) is a critical determinant of this resistance<sup>3,4</sup>. A hallmark of the TME is its profoundly immunosuppressive state, driven by diverse cellular and molecular mechanisms<sup>4</sup>. Difficult-to-treat solid ‘cold’ tumors often exhibit low or no infiltration of immune cells, resulting in so-called immune-deserted or immune-excluded phenotypes that are resistant to ICIs<sup>5</sup>. Even when present, CD8<sup>+</sup> cytotoxic T cells acquire features of exhaustion under chronic stimulation, impairing tumor rejection<sup>6</sup>. Immunosuppressive CD4<sup>+</sup> regulatory T (T<sub>reg</sub>) cells, myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages further reinforce tolerance through the expression of interleukin-10 (IL-10), transforming growth factor- $\beta$  (TGF $\beta$ ), vascular endothelial growth factor (VEGF), and nutrient-depleting enzymes such as arginase and indoleamine 2,3-dioxygenase (IDO)<sup>3,7–12</sup>. Metabolic derangements, including lactate

accumulation and acidification, impair immune cell metabolism and effector function<sup>13</sup>. Immune checkpoints such as PD-1, PD-L1, CTLA-4, T cell immunoglobulin and mucin-domain containing 3 (TIM3), lymphocyte-activation gene 3 (LAG3), and CD161 are frequently upregulated across immune cells<sup>4,14</sup>. Nonimmune stromal elements contribute to this state of the TME: cancer-associated fibroblasts produce extracellular matrix (ECM) proteins and immunosuppressive cytokines, while tumor-associated endothelial cells limit immune infiltration by downregulating adhesion molecules<sup>3,8</sup>. The TME is highly dynamic, with composition and signaling that adapt in response to both tumor-intrinsic changes and external pressures, further driving resistance to immunotherapies<sup>9</sup>.

Reprogramming the TME could be an important strategy for overcoming immunosuppression. Biologic immunotherapies, such as oncolytic viruses (OVs) and cytokine-based gene therapies (GTs), initially conceived as tumor-selective cytotoxic agents, increasingly appear to act primarily as TME reprogrammers. Mounting clinical evidence suggests that their dominant activity lies in converting immunosuppressive TMEs into inflamed, immune-responsive states, activating innate and adaptive immunity and enhancing responsiveness to subsequent therapies<sup>15,16</sup>. For example, histological analyses from trials in glioblastoma and melanoma often reveal limited direct viral infection but profound shifts in immune infiltration and cytokine signaling<sup>15,17–19</sup>, as further discussed below.

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## OVs and cytokine GTs as modulators of TME phenotypes

In this section, we will discuss how OVs and cytokine-based GTs (Table 1) reshape the TME.

### Oncolytic viruses

OV infection destroys malignant cells through iterative cycles of tumor cell entry, intracellular replication and lytic release of viral progeny. This process results in direct cytorreduction and facilitates local viral spread to adjacent cancer cells, thereby amplifying oncolytic activity within the tumor bed. Beyond direct cytorreduction, virus-mediated lysis of tumor cells is inherently immunogenic and triggers immunogenic cell death, leading to the release of pathogen-associated molecular patterns, damage-associated molecular patterns, proinflammatory cytokines and other danger signals<sup>20</sup>. These factors stimulate innate immune pathways<sup>20,21</sup>. Concomitantly, tumor-associated antigens (TAAs) liberated from dying cells are processed and presented, driving the activation and expansion of tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells<sup>20</sup>. It is postulated that this mechanism—termed epitope spreading—may be a way for OV therapy to establish an adaptive immune response and, in some cases, long-term immunological memory<sup>22–27</sup>. Epitope spreading in cancer has been shown to occur in mouse models as well as in some human clinical trials<sup>28</sup>. It is a mechanism needed to counteract tumor immune evasion caused by the emergence of antigen-loss tumor clone variants, and it is invoked as a determinant of ultimate success not only by OVs and GTs but also by several other types of immunotherapies, such as peptide vaccines<sup>29</sup> and chimeric antigen receptor (CAR) T cells<sup>30</sup>.

Clinical evidence supports the occurrence of systemic immunity induced by the initial cytotoxic insult from OV infection. In both pediatric and adult patients with gliomas, intratumoral injection of the oncolytic herpes simplex virus (oHSV) G207 (Table 1) increased the infiltration of CD3<sup>+</sup> and CD8<sup>+</sup> T cells as well as monocytes and macrophages, effectively converting cold tumors into hot ones<sup>17,31</sup>. The adenovirus DNX-2401 similarly enhanced CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration in recurrent glioma<sup>18</sup>. G47Δ, an oHSV recently approved in Japan for glioma, increased CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration without increasing FOXP3<sup>+</sup> cells<sup>32</sup>. In a clinical trial of the modified oHSV rQNestin34.5v.2 (CAN-3110), changes in T cell clonality and intratumoral CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration correlated with overall survival, with these immune effects persisting for several months following a single intratumoral injection<sup>23</sup>. Even ONYX-015, one of the earliest oncolytic adenoviruses, demonstrated durable immune activation, with lymphocyte and plasma cell infiltration detectable 3 months after treatment<sup>33</sup>. In melanoma, talimogene laherparepvec (T-VEC) induced systemic immune responses, resulting in regression of noninjected metastases and notable reductions in immunosuppressive cell populations such as T<sub>reg</sub> cells and MDSCs<sup>19,34,35</sup>.

### Cytokine GTs

Unlike OVs, the primary activity of cytokine-encoding GTs is mediated through transgene-driven immune stimulation rather than viral replication. For example, nadofaragene firadenovec encodes interferon-α2b (IFNα2b), which activates both innate and adaptive immune responses. Notably, the composition of peritumoral immune infiltrates can be influenced by the cytokine that is expressed: granulocyte-macrophage colony-stimulating factor (GM-CSF) expression increases monocyte and macrophage recruitment, whereas IL-2 expression increases local T cells<sup>36</sup>. In gliomas, an IFNβ-encoding adenovirus induced apoptosis in tumor cells<sup>37</sup>, while a regulatable IL-12 vector promoted tumor-infiltrating lymphocyte (TIL) infiltration and IFNγ production<sup>16</sup>. In metastatic melanoma, intratumoral electroporation of IL-12 plasmids activated transcriptional programs associated with natural killer (NK) cell activation, cross-presenting dendritic cells and T helper 1 (T<sub>H</sub>1) responses while simultaneously inducing adaptive resistance pathways exemplified by PD-L1 and TGFβ upregulation<sup>38</sup>.

Clinical responders were characterized by increases in systemic IL-1β, macrophage inflammatory protein 1α (MIP-1α), proliferating effector memory CD8<sup>+</sup> T cells and cytolytic NK cells<sup>38</sup>. Combination approaches further underscore the immunomodulatory potential of GTs. In glioblastoma, delivery of the 'suicide gene' HSV thymidine kinase (HSV-TK) together with IL-2 was characterized by an increase in T<sub>H</sub>1 cytokines in the tumor and plasma of patients with recurrent glioblastoma, with tumor responses seen in approximately half of treated patients<sup>39</sup>. Adenoviral vectors co-encoding HSV-TK and FMS-like tyrosine kinase 3 ligand (FLT3L) transgenes enhanced both dendritic cell recruitment and CD8<sup>+</sup> T cell infiltration, coupling direct tumor cytotoxicity with antigen exposure and immune priming<sup>40</sup>.

Therefore, this clinical evidence supports a mechanism whereby OVs and cytokine GTs function less as direct cytotoxic agents and more as broad reprogrammers of the TME. While the mode by which the initial cytotoxic antitumor insult occurs depends on the specific OV or GT modality, both converge on pathways that promote innate immune activation, epitope spreading and the generation of adaptive antitumor immunity. The magnitude, durability and cellular composition of these responses may vary, but their shared capacity to remodel the TME positions them as a distinct class of multimodal immunotherapies (Fig. 1). Notably, OVs can also be engineered to encode cytokine transgenes, blurring the distinction between these modalities<sup>34,41–44</sup>.

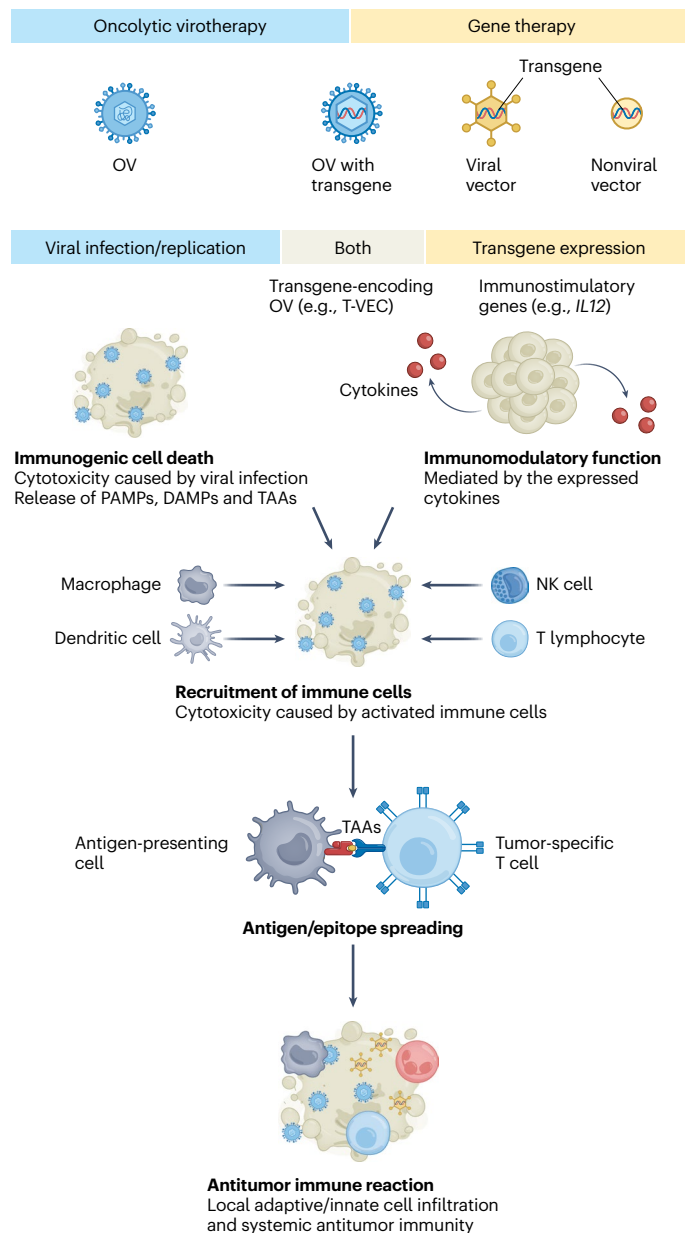
### OVs and cytokine GTs with regulatory approval

Table 2 lists some clinical trials of OVs and cytokine GTs for solid tumors, selected based on first-in-human application, signs of efficacy, regulatory approvals, and/or the generation of informative translational and correlative data. Five OV or cytokine GT modalities have received regulatory approval worldwide (Fig. 2), although they have not been widely implemented in routine clinical practice. Oncorine (H101), an oncolytic adenovirus nearly identical to ONYX-015, was approved in China in 2005 for the treatment of head and neck cancer in combination with chemotherapy, but its use remains limited to that country<sup>45</sup>. Rigvir, an echovirus approved in Latvia for melanoma, was suspended in 2019 following concerns about its efficacy and manufacturing quality<sup>46,47</sup>. Teserpaturev (G47Δ; DELYTACT, Daiichi Sankyo), an oHSV, conferred a median overall survival of 20.2 months following treatment (28.8 months from initial surgery) in patients with supratentorial high-grade glioma treated after chemoradiation, leading to its regulatory approval in Japan<sup>32</sup>. Unfortunately, to date, access to this therapy has been limited to one specialized center in Japan due to the low supply by the pharmaceutical company, likely influenced by the low price set by the Ministry of Health, Labour and Welfare of Japan. However, this issue is expected to be resolved soon (T. Todo, The University of Tokyo, Japan, personal communication).

In the United States, two biologic agents have received approval from the Food and Drug Administration (FDA). T-VEC (IMLYGIC), a genetically modified oHSV encoding GM-CSF, demonstrated a durable response rate of 16.3% in the phase 3 OPTIM trial, with the greatest benefit observed in earlier-stage melanoma. It exhibited an acceptable safety profile, leading to its approval for injectable but unresectable melanoma<sup>34</sup>. Notably, a subset of patients experienced prolonged responses associated with improved survival<sup>48</sup>. Neoadjuvant studies have suggested that T-VEC may reduce recurrence rates relative to surgery alone<sup>49</sup>. Despite these signs of activity, its clinical use has been limited, reflecting a narrow indication, the requirement for intralésional administration, and the difficulty of demonstrating a clear superior and/or additive benefit over established immunotherapies<sup>50</sup>. For example, a particularly anticipated strategy was the combination of T-VEC, intended to enhance intratumoral T cell infiltration, with immune checkpoint blockade<sup>51</sup>. However, the phase 3 MASTERKEY-265 trial did not demonstrate statistically significant improvements in progression-free or overall survival for the combination compared to pembrolizumab alone in patients with advanced melanoma<sup>51</sup>.

**Table 1 | Properties of OV and viral GT platforms**

| Backbone                                    | Agent  | Modification  |
|---|--|---|
| HSV-1                                       | G207 (ref. 78)   | – $\gamma_1$ 34.5 deleted (attenuates viral replication and reduces evasion of the interferon response in infected cells)<br>– ICP6 encoding viral gene inactivated (enables replication selectivity in cells defective in <i>p16</i> tumor suppressor signaling)   |
|   | CAN-3110 (ref. 23)                                       | – $\gamma_1$ 34.5 deleted but one copy of $\gamma_1$ 34.5 expressed under the control of a nestin promoter (enables viral replication and increases evasion of the interferon response in cells that express nestin)<br>– ICP6 encoding viral gene inactivated  |
|   | G47 $\Delta$ (ref. 78)                                   | – $\gamma_1$ 34.5 deleted<br>– ICP6 inactivated<br>– $\alpha$ 47 deleted (improves MHC-I presentation in infected cells)<br>– Promoter region of <i>US11</i> deleted, placing the <i>US11</i> gene under the control of the immediate early promoter of $\alpha$ 47, which improves viral replication in infected cells |
|   | T-VEC <sup>34</sup>                                      | – $\gamma_1$ 34.5 deleted<br>– $\alpha$ 47 gene deleted, leading to increased expression of <i>US11</i> (improves viral replication in infected cells)<br>– Encodes GM-CSF  |
|   | VG161 (ref. 72)  | – $\gamma_1$ 34.5 deleted<br>– Encodes IL-12, IL-15, IL-15R $\alpha$ and a fusion protein capable of blocking PD-1/PD-L1 interactions (TF-Fc)   |
|   | M032 (ref. 44)   | – $\gamma_1$ 34.5 deleted<br>– Encodes IL-12  |
| Adenovirus                                  | DNX-2401 (ref. 18)                                       | – 24-base-pair ( $\Delta$ 24) deletion in the <i>E1A</i> gene (enables replication selectivity in cells with defective tumor suppressor <i>Rb/p16</i> signaling)<br>– Fiber knob modification with an RGD motif to enable use of $\alpha$ v $\beta$ 3 or $\alpha$ v $\beta$ 5 integrins for cell entry                  |
|   | ONYX-015 (refs. 81,162)                                  | – Deletion in the <i>E1B-55K</i> region (reduces viral RNA export kinetics; however, tumor cells can complement this defect, allowing replication)<br>– Partial <i>E3</i> region deletion (enables less immune evasion and more apoptosis in infected cells)  |
|   | HI01 (ref. 45)   | – Almost identical to ONYX-015 but with a larger deletion in the <i>E3</i> gene   |
|   | VCN-01 (ref. 63)   | – Insertion of an integrin-binding motif into the adenovirus 5 fiber<br>– Expresses hyaluronidase to degrade tumor stroma and facilitate drug delivery  |
|   | Ad5/35- $\Delta$ 24 (ref. 142)                           | – 24-base-pair ( $\Delta$ 24) deletion in the <i>E1A</i> gene<br>– Chimeric adenovirus whose Ad5 fiber knob was replaced with Ad35  |
|   | CG0070 (ref. 43)   | – Human <i>E2F-1</i> promoter drives the expression of viral <i>E1A</i> genes (allows tumor-selective replication)<br>– Encodes GM-CSF  |
|   | TILT-123 (ref. 42)                                       | – Oncolytic serotype 5/3 chimeric adenovirus<br>– $\Delta$ 24 deletion in the <i>E1A</i> gene<br>– Addition of an <i>E2F</i> promoter upstream of <i>E1A</i><br>– Encodes IL-2<br>– Encodes TNF   |
|   | Ad-RTS-hIL12 (refs. 16,149)                              | – Replication-defective adenoviral vector<br>– Encodes the human <i>IL12</i> gene under the transcriptional control of the RTS regulatable promoter<br>– The promoter is activated by a small oral ligand (veledimex) that turns on <i>IL12</i> transcription.  |
|   | Ad-TD-nsIL12 (ref. 54)                                   | – Replication-defective adenoviral vector<br>– Encodes nonsecreting IL-12<br>– Deletion in three genes ( <i>E1A CR2</i> , <i>E1B19K</i> and <i>E3gp19K</i> ) (promotes tumor-selective replication based on <i>Rb/p16</i> tumor suppressor signaling; inhibits immune evasion and apoptosis in infected cells)          |
|   | CAN-2409 (ref. 117)                                      | – Replication-defective adenoviral vector expressing the HSV-TK gene (increases inflammation and renders infected cells susceptible to antiherpetic agents)<br>– Given in combination with valacyclovir or acyclovir  |
|   | VB-111 (ref. 140)  | – Nonreplicating adenovirus carrying a proapoptotic human Fas-chimera transgene (Fas and human TNF receptor 1) under the control of a modified murine pre-proendothelin promoter<br>– <i>E1</i> deletion  |
|   | Ad-hCMV-TK+ Ad-hCMV-Flt3L <sup>40</sup>                  | – Two replication-defective adenoviral vectors, one encoding HSV-TK to increase immunogenicity and chemosensitivity to antiherpetic agents and the second one encoding FLT3L to increase dendritic cell activity  |
| Nadofaragene firadenovec-vncg <sup>34</sup> | – Nonreplicating adenovirus<br>– Encodes IFN $\alpha$ 2b |   |
| Measles                                     | MV-CEA <sup>100</sup>                                    | – Naturally occurring measles virus strain that also expresses CEA  |
| Echovirus                                   | Rigvir <sup>47</sup>                                     | – Oncolytic echovirus   |
| Reovirus                                    | Pelareorep <sup>55</sup>                                 | – Naturally occurring nonenveloped human reovirus serotype 3 Dearing strain   |
| Parvovirus                                  | H-1PV <sup>105</sup>                                     | – Oncolytic parvovirus  |
| Vaccinia virus                              | TG6002 (ref. 156)  | – Vaccinia-based OV encoding the FCU1 prodrug-converting enzyme, which locally converts orally administered 5-FU into active 5-FU within tumor tissue<br>– Deletion of thymidine kinase and ribonucleotide reductase genes  |
|   | JX-594 (ref. 41)   | – Inactivation of the vaccinia thymidine kinase gene<br>– Encodes GM-CSF<br>– Encodes $\beta$ -galactosidase  |
| Coxsackievirus                              | V937 (ref. 132)  | – Bioselected, genetically unmodified, oncolytic coxsackievirus   |
| Newcastle disease virus                     | Newcastle disease virus <sup>73</sup>                    | – Oncolytic Newcastle disease virus   |



**Fig. 1 | OVs and cytokine GTs elicit antitumor immune responses.** Infection of tumor cells with OVs induces direct cytotoxicity and immunogenic cell death, resulting in the release of danger-associated molecular patterns and TAAs that initiate an antitumor immune response. Cytokine-based GTs promote the recruitment and activation of innate and adaptive immune cells, including macrophages, NK cells, dendritic cells and T cells, through the localized expression of proinflammatory cytokines. Subsequent immune-mediated tumor cell killing leads to the release of additional tumor antigens, driving epitope spreading and the expansion of effector T cell responses against a broader repertoire of TAAs. Together, these processes support the development of durable antitumor immunity. DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns.

More recently, nadofaragene firadenovec-vncg (rAd-IFN $\alpha$ /Syn3; ADSTILADRIN, Ferring Pharmaceuticals) has received FDA approval. Delivered intravesically, this adenoviral vector encodes human IFN $\alpha$ 2b, which has immunostimulatory, antiangiogenic and antitumor effects<sup>52</sup>. In 2022, it was approved for patients with *Bacillus Calmette–Guérin*-unresponsive non-muscle-invasive bladder cancer<sup>52</sup>. Unlike earlier therapies, it has been met with favorable reception among oncologists, and manufacturer-reported expansion of production capacity suggests anticipated growth in clinical demand<sup>53</sup>.

## Why have OVs and GTs not been widely adopted into clinical practice?

Multiple factors contribute to the limited use of OVs and GTs in routine oncology practice, including (1) immunological changes in the TME that do not consistently translate into durable clinical benefits; (2) absence of clearly defined oncogenic targets; (3) the perception that these agents are limited to single-dose administration; (4) reliance on intralesional rather than systemic intravenous administration; (5) uncertainties regarding potential viral toxicity; and (6) economic and logistical considerations (Table 3).

### Durable TME changes do not consistently result in durable clinical responses

As discussed, OVs and GTs frequently induce initial immune activation within the TME<sup>38,54</sup>, but many patients ultimately fail to achieve durable clinical responses<sup>34</sup>. Clinical trial data suggest that tumors adapt to the initial immune activation induced by OVs and GTs by upregulating compensatory immune mechanisms that attempt to restore an immunosuppressive TME, thereby limiting durable clinical benefits<sup>16,38,54–56</sup>.

For example, intravenous administration of the reovirus pelareo-rep increased circulating proinflammatory cytokines in patients with metastatic pancreatic ductal adenocarcinoma but was also associated with the expansion of T<sub>reg</sub> cells and CTLA-4<sup>+</sup> T cells, without improving progression-free survival compared to chemotherapy alone<sup>55</sup>. Similarly, in recurrent high-grade glioma, treatment with the IL-12-encoding adenovirus Ad-TD-nsIL12 increased CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration, but there was also a predominance of immunosuppressive M2-like over M1-like macrophages in post-treatment samples<sup>54</sup>. In patients treated with the oHSV CAN-3110, the expression of insulin-like growth factor 2 (IGF2) was upregulated following therapy, and subsequent mechanistic studies implicated its role as a mediator of resistance to oHSV therapy<sup>56</sup>.

Adaptive immune resistance has also been reported following cytokine GTs. In a phase 2 trial of intratumoral electroporation of IL-12-encoding plasmids in metastatic melanoma, immune infiltration was enhanced in both injected and distant lesions, but this was accompanied by increased PD-L1 and TGF $\beta$  expression<sup>38</sup>. Likewise, after treatment with a regulatable adenoviral vector encoding IL-12 in glioma, durable intratumoral IFN $\gamma$  expression and upregulation of PD-1 and PD-L1 were observed<sup>16</sup>.

Sustained IFN $\gamma$  signaling may itself drive paradoxical immunosuppression (Fig. 3). Preclinical and translational data suggest that chronic IFN $\gamma$  exposure activates counter-regulatory mechanisms, including epigenetic pathways mediated by noncoding RNAs, leading to checkpoint upregulation<sup>57,58</sup>. Therefore, strategies aimed at prolonging immune activation following OV or GT treatment should take into account therapy-induced secondary immunosuppression. Analysis of molecular data from clinical trials can help in understanding the mechanisms of tumor evasion following therapy. For instance, results from a single-dose injection of oHSV in recurrent glioblastoma suggest that a mechanism of tumor escape is the formation of VEGF-expressing tumor niches characterized by profound hypoxia that exclude cytotoxic T cells<sup>59</sup>. Therefore, anti-VEGF therapies may be a rational sequential combination to overcome tumor immune evasion in this context.

### OVs and GTs as multimodal immunomodulators in the absence of clearly defined oncogenic targets

Durable tumor rejection depends on epitope spreading, which broadens effector T cell responses to TAAs beyond the initial therapeutic target, thereby counteracting antigen escape<sup>60</sup>. OVs and GTs engage multiple signaling pathways rather than a single oncogenic driver, positioning them to elicit broader and more diversified immune responses<sup>15,20,21,35,61</sup>. OVs can also alter tumor vasculature: some OVs are engineered to remodel stromal architecture through enzymatic degradation of ECM components, reducing physical barriers and facilitating therapeutic delivery<sup>62,63</sup>. However, the absence of a single, clearly

**Table 2 | Translational findings from landmark clinical studies of OVs and cytokine GTs in solid tumors**

| Tumor type   | Agent   | Phase/trial   | Delivery method  | Key translational findings   | Key clinical findings   |
|--------------|---|---|--|--|---|
| Melanoma     | T-VEC (talimogene laherparepvec; IMLYGIC; oHSV encoding GM-CSF)     | Oncovex <sup>GM-CSF</sup> phase 2 (ref. 35) (NCT00289016)<br>OPTIM phase 3 (ref. 34) (NCT00769704)<br>MASTERKEY-265 phase 3 (ref. 51) (NCT02263508)   | • Intratumoral   | <ul style="list-style-type: none"> <li>Systemic immune activation: regression of uninjected lesions (abscopal effect)<sup>44,45</sup></li> <li>Induced antigen-specific T cell responses and decreased T<sub>reg</sub> cells and MDSCs in responders<sup>35</sup></li> </ul>   | <ul style="list-style-type: none"> <li>DRR of 16.3% with T-VEC versus 2.1% with GM-CSF<sup>34</sup></li> <li>Median OS of 23.3 months with T-VEC versus 18.9 months with GM-CSF<sup>34</sup></li> <li>Best responses in patients with earlier-stage metastatic disease compared to those in later stages<sup>34</sup></li> <li>Combination with pembrolizumab did not significantly improve PFS or OS compared to placebo plus pembrolizumab but was safe<sup>51</sup></li> </ul> |
|              | RP1 (vulolimogene oderparepvec; oHSV encoding GM-CSF and GALV-GP-R) | IGNYTE phase 1/2 with anti-PD-1 therapy <sup>65</sup> (NCT03767348)   | • Intratumoral   | <ul style="list-style-type: none"> <li>Abscopal effect<sup>65</sup></li> <li>Increased CD8<sup>+</sup> T cells and PD-L1 expression in tumors after treatment<sup>63</sup></li> <li>Upregulation of immune-related genes (inflamed TME) associated with response<sup>63</sup></li> </ul>   | <ul style="list-style-type: none"> <li>ORR was 32.9%, with a median response duration of 33.7 months<sup>63</sup></li> <li>Combination with anti-PD-1 therapy was safe<sup>63</sup></li> </ul>  |
|              | OrienX010 (oHSV encoding GM-CSF)                                    | OrienX010 in phase 1b <sup>64</sup> (NCT04200040)<br>Neoadjuvant OrienX010 with anti-PD-1 therapy in phase 1b <sup>65</sup> (NCT04197882)   | • Intratumoral   | <ul style="list-style-type: none"> <li>Abscopal effect<sup>64</sup></li> <li>Enhanced proinflammatory cytokine secretion upon combination with anti-PD-1 therapy<sup>65</sup></li> <li>Increased tertiary lymphoid structures and TILs in tumor beds of responders<sup>65</sup></li> </ul>   | <ul style="list-style-type: none"> <li>ORR of 28.6% and median OS of 17.4 months at the recommended phase 2 dose<sup>64</sup></li> <li>Neoadjuvant combination with anti-PD-1 therapy is tolerable<sup>65</sup></li> </ul>  |
|              | V937 (coxsackievirus A21)   | CALM phase 2 (ref. 166) (NCT010227551);<br>MITCI phase 1b with anti-CTLA-4 therapy <sup>67</sup> (NCT02307149)<br>CAPRA phase 1b with anti-PD-1 therapy <sup>68</sup> (NCT02565992)   | • Intratumoral   | <ul style="list-style-type: none"> <li>Abscopal effect<sup>166,167</sup></li> <li>Responses upon intratumoral injection despite neutralizing antibodies at baseline<sup>66</sup></li> <li>Responses observed even in patients without an inflamed TME at baseline<sup>68</sup></li> </ul>  | <ul style="list-style-type: none"> <li>PFS rate at 6 months of 38.6% with monotherapy, with DRR of 21.1% (ref. 166)</li> <li>ORR of 30% in combination with anti-CTLA-4 therapy<sup>67</sup></li> <li>ORR of 47% in combination with anti-PD-1 therapy<sup>68</sup></li> </ul>  |
|              | Tavo kinase telseplasmid (tavo; IL-12 GT)                           | IL-12MEL phase 2 (ref. 38) (NCT01502293)  | • Intratumoral (followed by electroportation)  | <ul style="list-style-type: none"> <li>Abscopal effect<sup>38</sup></li> <li>Immune activation with increased expression of inflammatory genes (including IFN<math>\gamma</math>), but adaptive immune resistance (PD-L1 and TIGFB expression) also observed<sup>38</sup></li> </ul>   | <ul style="list-style-type: none"> <li>ORR was 35.7%; median PFS was 3.7 months<sup>38</sup></li> </ul>   |
| Brain tumors | AdCD40L (CD40 ligand GT)  | Phase 1/2a with or without cyclophosphamide <sup>69</sup> (NCT01455259)   | • Intratumoral   | <ul style="list-style-type: none"> <li>Increased systemic T effector to T<sub>reg</sub> cell ratio following therapy<sup>69</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Treatment (up to six repeated administrations) was safe and demonstrated efficacy (median OS of 20.2 months and PFS of 4.7 months), leading to conditional approval for malignant glioma in Japan<sup>32</sup></li> </ul>  |
|              | G47D (teterparepvec; DELTYACT; oHSV)                                | Phase 2 (ref. 32) (UMIN-CTR Clinical Trial Registry UMIN000015995)  | • Intratumoral   | <ul style="list-style-type: none"> <li>Increased infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells after treatment, while FOXP3<sup>+</sup> cells remained low<sup>32</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Treatment (up to six repeated administrations) was safe and demonstrated efficacy (median OS of 20.2 months and PFS of 4.7 months), leading to conditional approval for malignant glioma in Japan<sup>32</sup></li> </ul>  |
|              | G207 (oHSV)   | Phase 1 (ref. 146) (NCT00028158)<br>Phase 1b before and after resection <sup>31</sup> (NCT00028158)<br>Phase 1 combination with radiation <sup>127</sup> (NCT00157703)<br>Phase 1 combination with radiation in pediatric patients <sup>7</sup> (NCT02457845) | • Intratumoral   | <ul style="list-style-type: none"> <li>Evidence of viral replication<sup>31</sup></li> <li>CD3<sup>+</sup>, CD8<sup>+</sup> and HAM56<sup>+</sup> cells observed after treatment<sup>31</sup></li> <li>Increased TILs in pediatric patients and converted tumors toward an immunologically inflamed phenotype<sup>7</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Intratumoral injection was feasible<sup>146</sup></li> <li>Encouraging radiographic response in combination with radiation<sup>127</sup></li> <li>Median OS in pediatric patients was 12.2 months, with some long-term survivors<sup>7</sup></li> </ul>  |
|              | rQNestin34.5v2 (CAN-3110; linoserparepvec; oHSV)                    | Phase 1 (refs. 23,59,137) (NCT03152318)   | • Intratumoral as a single-time-point dose <sup>23,137</sup> or up to six serial doses <sup>59</sup> | <ul style="list-style-type: none"> <li>Longer OS in patients with positive HSV-1 baseline serology<sup>23</sup></li> <li>Changes in T cell clonotypes and intratumoral CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration were correlated with OS<sup>23</sup></li> <li>T cell clonotype expansion and proximity of effector T cells to apoptotic tumor cells correlated with OS and PFS<sup>37</sup></li> <li>Upregulation of immunopeptidome repertoire and other immune-activated metrics<sup>34,37</sup></li> </ul> | <ul style="list-style-type: none"> <li>Median OS of 14.2 months in patients with positive HSV-1 baseline serology and IDH<sup>wt</sup> recurrent glioblastoma<sup>33</sup></li> </ul>   |

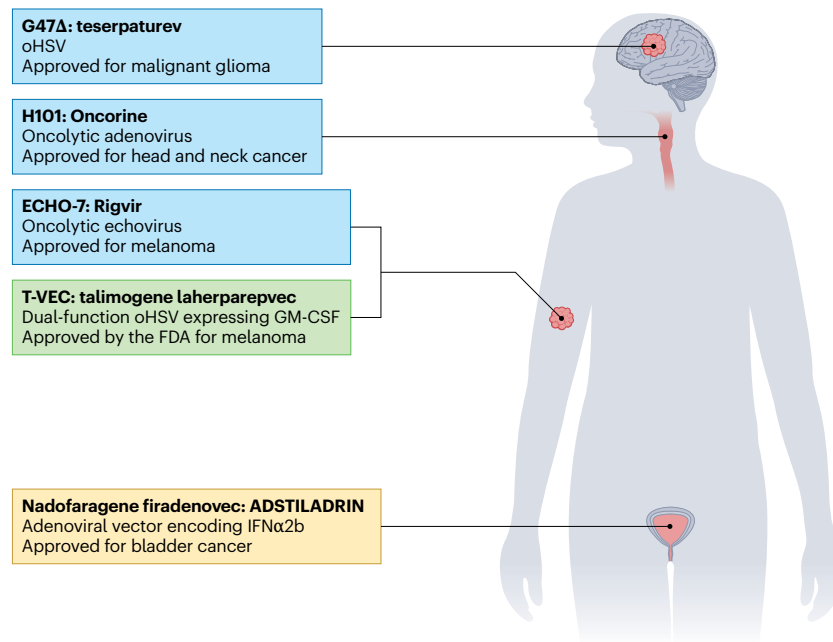
**Table 2 (continued) | Translational findings from landmark clinical studies of OV and cytokine GTs in solid tumors**

| Tumortype | Agent  | Phase/trial  | Delivery method  | Key translational findings   | Key clinical findings  |
|-----------|--|--|--|--|--|
|           | DNX-2401 (tasadenovirev; adenovirus)   | Phase 1 in adult patients with malignant glioma <sup>16</sup> (NCT00805376)<br>Phase 1 DNX-2401 followed by radiotherapy in pediatric patients with DIPG <sup>29</sup> (NCT03178032)<br>CAPTIVE phase 1/2 combination with anti-PD-1 therapy in patients with recurrent glioblastoma <sup>16</sup> (NCT02798406) | • Intratumoral   | <ul style="list-style-type: none"> <li>Evidence of viral replication, spread and oncolysis in tumors<sup>16</sup></li> <li>Infiltration of tumor by CD4<sup>+</sup> and CD8<sup>+</sup> T cells following treatment<sup>16</sup></li> <li>Increased microglia, macrophages and lymphocytes in post-treatment tumor specimens from patients with objective responses<sup>16</sup></li> <li>Patients with tumors showing intermediate immune infiltration (TME<sub>intermediate</sub>) were more likely to experience clinical benefits</li> </ul> | <ul style="list-style-type: none"> <li>Survival of &gt;3 years in 20% of patients after a single injection in recurrent malignant glioma<sup>16</sup></li> <li>Median OS of 17.8 months in the DIPG trial<sup>29</sup></li> <li>ORR of 10.4% and median OS of 12.5 months with some long-term survivors in combination with anti-PD-1 therapy<sup>16</sup></li> </ul>  |
|           | PVSRIPO (lerapopturev; polio-rhinovirus chimera)   | Phase 1 in adult patients <sup>70</sup> (NCT01491893)<br>Phase 1b in pediatric patients <sup>71</sup> (NCT03043391)  | • Intratumoral   |  | <ul style="list-style-type: none"> <li>OS in adult patients plateaued at 21% at 24 months and was maintained at 36 months<sup>70</sup></li> <li>Treatment in adult and pediatric patients was safe<sup>70,71</sup></li> </ul>  |
|           | Ad-RTS-hIL12   | Phase 1 in adult recurrent glioblastoma and malignant glioma <sup>16</sup> (NCT02026271)<br>Phase 1 in combination with nivolumab in glioblastoma <sup>16,9</sup> (NCT04006119)  | <ul style="list-style-type: none"> <li>Perilesional injection of Ad-RTS-hIL12 after resection of tumor;</li> <li>oral activator administered before (to assess its tumor penetrance) and after (to turn on IL12 transcription)<sup>16</sup></li> <li>Same as above but nivolumab administered in a neoadjuvant fashion<sup>16,9</sup></li> </ul> | <ul style="list-style-type: none"> <li>Dose-dependent increase in oral activator levels in tumors with concomitant increase in IL-12 levels in serum<sup>16</sup></li> <li>Durable increases in IFN<math>\gamma</math> expression in injected tumors with infiltration of cytotoxic T cells expressing PD-1 (ref. 16)</li> <li>Addition of nivolumab reduced both activated TILs and PD-1<sup>+</sup> T cells in tumors<sup>16,9</sup></li> </ul>  | <ul style="list-style-type: none"> <li>OS of 12.7 months, with a subgroup that had reduced dexamethasone dosing and the full course of the oral activator showing a median OS of up to 17.8 months. A cytokine-like release syndrome was observed at high doses of the oral activator due to high levels of IL-12 spilling into the blood<sup>16</sup></li> <li>Combination of Ad-RTS-hIL12 and neoadjuvant IL-12 was well tolerated with a median OS of 16.9 months<sup>16,9</sup></li> </ul> |
|           | MV-CEA (oncolytic measles virus expressing CEA)  | Phase 1 in adult recurrent glioblastoma <sup>15</sup> (NCT00390299)  | • Perilesional (group A) or intratumoral (group B) injection   | <ul style="list-style-type: none"> <li>A 22-ISG (interferon-stimulated gene) diagonal linear discriminant analysis classification algorithm inversely correlated with viral replication and TME remodeling<sup>72</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Median OS of 11.6 months and 1 year survival of 45.5%</li> </ul>  |
|           | Ad-hCMV-TK+Ad-hCMV-Fit3L   | Phase 1 in adult patients with newly diagnosed glioblastoma <sup>40</sup> (NCT01811992)  | • Perilesional injection of both adenoviral vectors  | <ul style="list-style-type: none"> <li>Significant increase in CD8<sup>+</sup> and CD3e<sup>+</sup> T cells in tumors when they recurred<sup>40</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Median OS of 21.3 months</li> </ul>   |
|           | Ad-ID-nslIL12 (adenovirus encoding membrane-anchored IL-12)                                    | Phase 1 in pediatric patients with DIPG <sup>50</sup> (NCT05717742; NCT05717699)   | • Intratumoral (Ommaya reservoir used for OV injections) <sup>50</sup>   | <ul style="list-style-type: none"> <li>Increased lymphocytic infiltration following treatment<sup>50</sup></li> </ul>  | <ul style="list-style-type: none"> <li>Treatment was safe, with partial responses and disease stabilization in some patients<sup>50</sup></li> <li>Median OS of 10.3 months after the first virus in primary DIPG and 6.4 months after the first virus in progressive DIPG, comparing favorably to retrospective institutional controls<sup>50</sup></li> </ul>  |
|           | CAN-2409 (aglatimagene besadenovec; adenovirus encoding HSV-TK; combination with valacyclovir) | Phase 2 with standard of care <sup>73</sup> (NCT00589875)<br>Phase 1b with standard of care and anti-PD-1 therapy <sup>17</sup> (NCT03576612)  | • Intratumoral   | <ul style="list-style-type: none"> <li>Systemic immune activation, including changes in circulating cytokines, immune cells and T cell clonotype diversity<sup>17</sup></li> <li>Baseline tumor genomics and immune composition were associated with outcomes, including enrichment of B cells, dendritic cells and memory CD4<sup>+</sup> T cells<sup>17</sup></li> </ul>   | <ul style="list-style-type: none"> <li>Treatment was safe<sup>17,73</sup></li> <li>Median OS of 17.1 months (25 months in patients with GTR) with CAN-2409 plus valacyclovir and standard of care versus 13.5 months (16.9 months in patients with GTR) with standard of care alone<sup>73</sup></li> <li>Median OS of 30.6 months in a subset of patients with newly diagnosed glioblastoma who had MGMT promoter methylation and GTR<sup>17</sup></li> </ul>                                 |

**Table 2 (continued) | Translational findings from landmark clinical studies of OV and cytokine GTs in solid tumors**

| Tumor type                       | Agent  | Phase/trial  | Delivery method  | Key translational findings  | Key clinical findings   |
|----------------------------------|--|--|--|---|---|
| Bladder cancer                   | CG0070 (oncolytic adenovirus encoding GM-CSF)  | Phase 1 (ref. 174) (NCT02143804)<br>BOND phase 2 (ref. 175) (NCT02365818)  | • Intravesical   | • Findings consistent with viral replication <sup>74</sup>  | • Overall complete response rate of 47% at 6 months <sup>75</sup>   |
|                                  | Nadofaragene firadenovec-vncg (ADSTILADRIN; rAd-IFN $\alpha$ /Syn3)                          | Phase 3 (ref. 84) (NCT02773849)  | • Intravesical   |   | • Complete response in 53.4% of patients with carcinoma in situ within 3 months, which was maintained in 45.5% of those patients at 12 months <sup>84</sup><br>• Received FDA approval <sup>82</sup>  |
| Pancreatic ductal adenocarcinoma | Pelareorep (REOLYSIN; oncolytic reovirus)  | Phase 2 combination with paclitaxel/ carboplatin <sup>55</sup> (NCT01280058)<br>Phase 2 combination with gemcitabine <sup>18</sup> (NCT00998322)<br>Phase 1b combination with anti-PD-1 treatment and chemotherapy <sup>37</sup> (NCT03723915) | • Intravenous (systemic)   | • Treatment was associated with increased circulating proinflammatory cytokines but also with increased T <sub>reg</sub> cells and CTLA-4 <sup>+</sup> T cells <sup>55</sup><br>• Increased systemic T and NK cell subsets and certain soluble biomarkers were associated with outcomes <sup>55</sup><br>• Reovirus can replicate and induce apoptosis within tumors <sup>18</sup><br>• Intratumoral PD-L1 upregulation was observed following pelareorep treatment <sup>18</sup><br>• High peripheral T cell clonality was associated with the clinical benefit of pelareorep with anti-PD-1 antibody and chemotherapy <sup>37</sup> | • Addition of pelareorep was safe but did not improve PFS compared to paclitaxel/carboplatin alone <sup>55</sup><br>• Median OS of 10.2 months in combination with gemcitabine <sup>18</sup><br>• Combination with anti-PD-1 antibody and chemotherapy was safe <sup>37</sup> |
| Colorectal cancer                | Pelareorep (REOLYSIN; oncolytic reovirus)  | IND.210 phase 2 study of FOLFIRI/ bevacizumab + pelareorep <sup>30</sup> (NCT01622543)   | • Intravenous (systemic)   |   | • Inferior PFS with pelareorep despite significant increases in ORR (53% with pelareorep versus 35% without) <sup>30</sup>  |
|                                  | TG6002 (oncolytic vaccinia virus encoding the FCUI prodrug-converting enzyme)                | Phase 1 (ref. 153) (NCT04194034)   | • Intra-arterial virus (hepatic artery) with oral 5-FC               | • Robust systemic immune activation characterized by proinflammatory cytokine induction, cathectinulin release (immunogenic cell death) and expansion of T cell receptor clones <sup>56</sup>   | • No objective responses were observed, but TG6002 with oral 5-FC was feasible <sup>56</sup>  |
| Hepatocellular carcinoma         | Pexa-vec (pexastimogene devacirpvec; JX-594; oncolytic vaccinia virus encoding GM-CSF)       | Phase 2 dose-finding trial <sup>137</sup> (NCT00554372)<br>PHOCUS phase 3 pexa-vec followed by sorafenib <sup>76</sup> (NCT02562755)   | • Intratumoral   | • Higher doses of pexa-vec correlated with significantly improved OS (median OS of 14.1 months with high-dose pexa-vec versus 6.7 months with lower dosing) <sup>137</sup><br>• Pexa-vec replicated and induced a humoral immune response <sup>37</sup>   | • PHOCUS study was terminated early because of inferior median OS in interim analysis (pexa-vec plus sorafenib: 12.7 months versus sorafenib: 14.0 months) <sup>76</sup>  |
|                                  | VG161 (oHSV encoding IL-12, IL-15, IL-15R $\alpha$ and a PD-1/PD-L1-blocking fusion protein) | Phase 1 in patients with failed second-line treatments <sup>51</sup> (NCT04806464)   | • Intratumoral   | • TME remodeling with increased CD8 <sup>+</sup> T and NK cells, clonal T cell expansion, and abscopal immune effects <sup>51</sup><br>• Renewal of therapeutic responsiveness in tumors that had progressed on prior systemic therapies <sup>51</sup><br>• Certain gene signatures were associated with clinical benefits and OS <sup>51</sup>   | • ORR of 17.65% by mRECIST; median OS of 9.4 months (17.3 months in a subgroup that received prior checkpoint inhibitor therapy for >3 months) <sup>51</sup>  |
| Advanced solid tumors            | TILT-123 (igralimogene tildenorepvec; TNF/IL-2-armed adenovirus)                             | Phase 1 in advanced solid tumors (TUNIMO, TUNINTIL, PROTA) <sup>102</sup> (NCT04695327; NCT04695327; NCT04217473; NCT04217473; NCT05271318)  | • Intravenous  | • Systemic delivery resulted in tumor transduction, with detection of viral DNA/proteins and viral gene expression in post-treatment biopsy specimens <sup>102</sup><br>• Significant intratumoral immunomodulation, including increased immune cell infiltration and upregulation of immune-related transcriptional programs <sup>102</sup><br>• Robust systemic inflammatory and adaptive immune responses following treatment, despite preexisting neutralizing antibodies <sup>102</sup>  | • Patients with detectable TILT-123 in post-treatment tumor samples showed longer median OS compared to those without detectable virus (280 versus 190 days) <sup>102</sup>   |
|                                  | Measles-CEA or measles-NIS   | Phase 1 in ovarian cancer (NCT00408590), mesothelioma (NCT01503177), malignant peripheral nerve sheath tumors (NCT02700230), multiple myeloma (NCT02192715) and several others <sup>100</sup>  | • Intravenous, intraperitoneal or intratumoral (based on the cancer) | • Expression of serum CEA can be used to follow measles persistence. Expression of NIS can be used for imaging and treatment  | • Several examples of responses based on imaging or a reduction in serum levels of cancer markers were reported   |

CEA, carcinoembryonic antigen; DFIG, diffuse intrinsic pontine glioma; DRR, durable response rate; GTR, gross total resection; IDH<sup>wt</sup>, IDH wild-type; MGMT, methylguanine methyltransferase; mRECIST, modified Response Evaluation Criteria in Solid Tumors; NIS, sodium-iodide symporter; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.



**Fig. 2 | Approved OVs and cytokine GTs for the treatment of cancer.** As of today, multiple OVs (blue) and GTs (yellow) have been approved for treating different types of cancers. T-VEC occupies a special position as an OV that encodes GM-CSF, also qualifying it as a GT vector (green). Notably, Rigvir was suspended in Latvia in 2019 due to concerns about its efficacy and manufacturing quality.

defined oncogenic target may reduce clinicians' perceived mechanistic rationale for these approaches, potentially hindering broader adoption. Nevertheless, engineering strategies have been developed to confer a degree of tumor specificity. These include retargeting viral entry through receptors enriched on tumor cells<sup>18</sup>, using tumor-selective promoters to enhance replication<sup>23</sup> or transgene expression<sup>64</sup>, and designing constructs responsive to microRNAs (miRNAs) differentially expressed in malignant and normal tissues<sup>65</sup>. These approaches maintain the multimodal immunomodulatory activity of OVs and GTs while enhancing tumor selectivity.

### Antiviral immunity is a context-dependent determinant of therapeutic efficacy

The impact of antiviral immunity on the efficacy of OVs and viral vector-based GTs has been debated for more than a decade. Early assumptions were that antiviral immunity would limit viral replication and spread, thereby reducing oncolysis and therapeutic efficacy; however, this view has since evolved<sup>66</sup>. A more nuanced understanding now recognizes that antiviral immune responses can, in certain contexts, help overcome tumor-induced immunosuppression and convert immunologically cold tumors into hot ones, thereby facilitating the development of antitumor immunity<sup>66</sup>.

Antiviral immunity can be broadly divided into innate and adaptive components<sup>67</sup>. Innate antiviral immunity includes humoral factors, such as neutralizing antibodies and the complement system, as well as cellular elements<sup>67</sup>. For systemically administered OVs or viral vector-based GTs, neutralizing antibodies and complement activation can significantly reduce therapeutic efficacy<sup>20,68,69</sup>. For example, in patients treated with oncolytic adenoviruses, higher baseline antiviral antibody titers have been associated with shorter survival, although heterogeneity across trials complicates interpretation<sup>68</sup>. These observations initially motivated strategies aimed at dampening antiviral immunity to enhance viral efficacy. Transient immunosuppression with cyclophosphamide has been explored and, in preclinical models, has been shown to reduce complement activation and antiviral cytokine production, improving the infection of glioma cells following intra-arterial delivery of oHSV therapy<sup>70</sup>.

Suppression of antiviral immunity must be carefully balanced against potential negative effects on antitumor immune responses. Prolonged or excessive immunosuppression can impair outcomes in patients receiving immunotherapies<sup>16</sup>. Notably, the influence of preexisting antiviral immunity appears to be highly dependent on the route of administration. When OVs are delivered intratumorally, viral spread through direct cell-to-cell contact may shield virions from neutralizing antibodies<sup>71</sup>. In fact, preclinical studies across multiple tumor models have shown that preimmunized mice exhibit enhanced antitumor efficacy and increased immune infiltration following intratumoral administration of agents such as the oHSV VG161 or Newcastle disease virus<sup>72,73</sup>.

Clinical observations further support this context dependence. In glioblastoma, patients with baseline HSV-1 seropositivity who were treated with intratumorally administered CAN-3110 demonstrated improved survival, potentially reflecting more robust antiviral and antitumor immune responses<sup>23</sup>. In contrast, in pediatric high-grade glioma, patients with preexisting HSV-1 antibodies had poorer survival following intratumoral G207 treatment compared to those who seroconverted after therapy, underscoring that the relationship between antiviral immunity and efficacy is not uniform and may vary by disease context, patient population and viral platform<sup>17</sup>.

Cellular components of innate immunity also have a critical role. NK cells are key mediators of antiviral responses, and because virus-infected tumor cells often downregulate major histocompatibility complex class I (MHC-I) molecules, they become susceptible to NK cell-mediated cytotoxicity<sup>67,74</sup>. This antiviral activity may, in turn, contribute to antitumor effects and influence therapeutic outcomes<sup>67,74</sup>. As infected cells are taken up and processed by antigen-presenting cells, T cells are activated and become a central component of the adaptive antiviral immune response. Although such responses have the potential to limit viral persistence, accumulating evidence suggests that adaptive immunity more often amplifies, rather than undermines, the therapeutic efficacy of OVs<sup>66</sup>. Virus-specific T cells can contribute directly to tumor control by recognizing and eliminating OV- or GT-infected malignant cells that present viral peptides on MHC-I molecules<sup>66</sup>. Consistent with this paradigm, preclinical studies using oncolytic adenovirus and

**Table 3 | Barriers to widespread adoption of approved OV and viral GTs**

| Barrier                         | Explanation   |
|---------------------------------|---|
| Durability of response          | OVs and GTs induce durable biological changes in the TME, but tumors often adapt through compensatory immunosuppressive pathways (for example, PD-L1 and TGF $\beta$ upregulation, MHC-I loss), leading to disease progression. |
| Lack of specificity             | Unlike targeted therapies or ICIs, OVs and GTs do not act on a single oncogenic or immune pathway, which may reduce the perceived mechanistic rationale for their adoption.   |
| Dosing misconceptions           | Concerns over neutralizing antibodies or antiviral responses have led to the misconception that only single-dose administration is feasible; in reality, repeat dosing strategies are increasingly being explored.              |
| Cumbersome delivery             | Many OVs and GTs require intralesional or other locoregional delivery, which is perceived as less practical than systemic intravenous administration and limits the eligible tumor types and patient populations.               |
| Safety concerns                 | Concerns about viral shedding, toxicity and transmission persist despite consistently favorable safety profiles in clinical trials and no observed evidence of secondary transmission.  |
| Economic and logistical factors | Multistep preparation, injection procedures or storage requirements can be costly and logistically challenging.   |

GT approaches that exploit vaccinia virus-specific T cell memory have demonstrated that antiviral T cell immunity can enhance intratumoral immune activation and antitumor efficacy<sup>75,76</sup>. At the same time, overly rapid clearance of infected tumor cells before sufficient intratumoral viral dissemination has occurred may attenuate both direct oncolytic activity and the subsequent antitumor immune response<sup>77</sup>.

How strongly antiviral immunity should be engaged without compromising viral persistence remains an open question, as illustrated by divergent strategies that manipulate antigen presentation. Early engineering efforts sought to enhance OV immunogenicity by deleting viral inhibitors of the transporter associated with antigen processing (TAP), such as the HSV-1  $\alpha$ 47 gene, thereby restoring MHC-I expression on infected tumor cells and promoting CD8<sup>+</sup> T cell recognition<sup>78</sup>. Although this approach was hypothesized to strengthen antitumor immunity, direct comparative studies evaluating how modulation of TAP activity influences overall therapeutic performance are lacking<sup>77</sup>. In contrast, later preclinical work demonstrated that arming OVs with a TAP inhibitor enhanced both local and systemic antitumor immune responses following intratumoral administration, although whether these effects are mediated by reduced antiviral clearance remains unresolved<sup>77</sup>. Other exploratory strategies have leveraged preexisting immune memory by encoding well-characterized recall antigens—against which the host is already immunized—into OVs to amplify a primed immune response within the TME<sup>79,80</sup>.

Importantly, multiple clinical studies indicate that, once antitumor immunity is established, therapeutic effects can persist even after viral clearance<sup>17,23,32,66</sup>. For example, the oHSV G47A is typically eliminated from glioblastoma tissue within approximately 4 weeks; yet, a subset of patients achieved durable local tumor control<sup>32</sup>. Similar findings have been reported with CAN-3110, where patients with the longest survival often exhibited preexisting HSV-1 seropositivity and clearance of detectable virus from tumor tissue within months after treatment<sup>23</sup>. Despite the absence of recoverable virus, these tumors displayed sustained infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, along with transcriptional signatures consistent with ongoing immune activation<sup>23</sup>. In line with this paradigm, post-treatment biopsy specimens

from pediatric patients with high-grade glioma following intratumoral G207 administration showed no detectable HSV-1 at 3–5 months after therapy, with many of these samples obtained from patients with the most favorable survival<sup>17</sup>. Collectively, these observations suggest that, in a subset of patients, clinical benefit from OVs or viral vector-based GTs can outlast active viral replication, underscoring that immune reprogramming—rather than long-term viral persistence—is the dominant mechanism supporting durable responses.

### Finding the optimal route of delivery

The route of administration determines both efficacy and toxicity. Intratumoral injection is the most common method, achieving high local concentrations with limited systemic exposure<sup>34</sup>. Image-guided methods, such as endoscopic ultrasound and intraoperative magnetic resonance imaging, can guide precise delivery<sup>23,81</sup>. Yet barriers remain, including abnormal vasculature and a dense ECM, which restrict distribution. VCN-01, an adenovirus encoding hyaluronidase, was designed to overcome these barriers and has been shown to improve the delivery of other therapeutic agents in early-phase studies<sup>63</sup>. Of note, time-consuming intratumoral injection techniques have been cited as one reason for the slow adoption of T-VEC<sup>50</sup>.

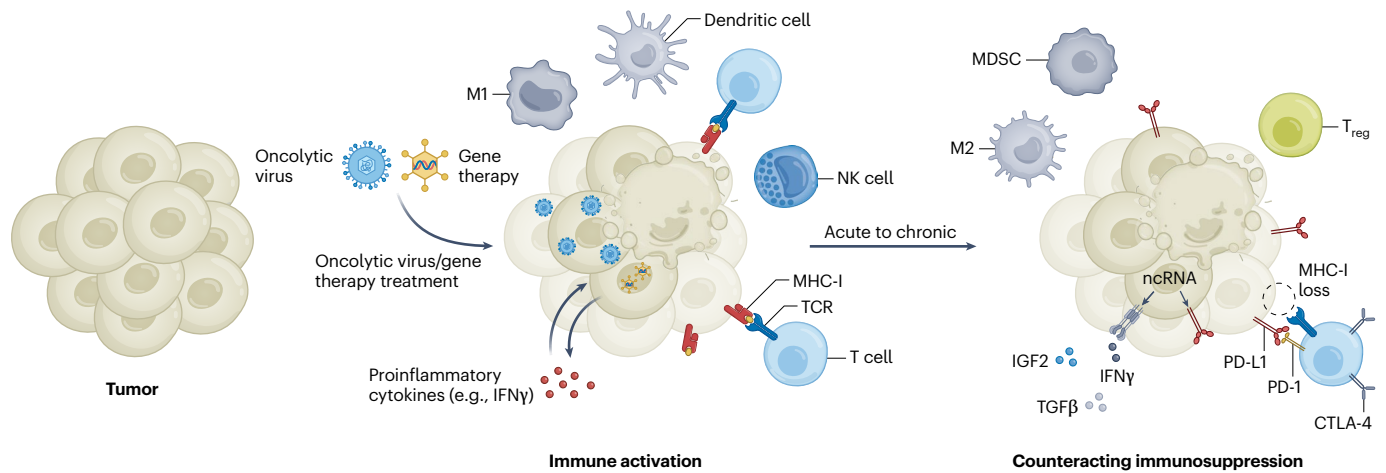
Other locoregional approaches include intravesical delivery in bladder cancer<sup>82–84</sup>, intraperitoneal therapy in ovarian cancer<sup>85–87</sup> and hepatic artery infusion in colorectal cancer<sup>88,89</sup>. Although the preferred route for brain tumors has been intralesional injections, intraventricular delivery has generally been avoided due to neurotoxicity, although pretreatment with poly(I:C) or a low-dose virus reduced toxicity in preclinical studies<sup>90</sup>. In rare cases, unconventional routes such as nasal inhalation have been attempted, including one case report of intravenous and inhalational OV delivery in a child with astrocytoma<sup>91</sup>. Encapsulation of OVs or GT vectors within hydrogels or carrier cells is being developed to protect against immune clearance and to prolong exposure<sup>92–94</sup>. It is important to note that, for locoregional delivery to have a systemic impact, antitumor responses must extend beyond the treated site.

Intravenous administration is often preferred in medical oncology. Trials have demonstrated the feasibility and safety of systemic delivery with VCN-01, parvovirus H-1PV, pelareorep, JX-594, carcinoembryonic antigen-expressing measles virus, and others<sup>41,55,95–100</sup>. Challenges include rapid clearance by antibodies and the complement system, dose escalation requirements, and hepatic sequestration<sup>69,99,101–104</sup>. In brain tumors, the blood–brain barrier limits delivery, but H-1PV has been shown to cross into gliomas and induce T cell responses<sup>105</sup>.

### Safety concerns: perception and clinical reality

OVs and cytokine GTs are generally well tolerated, and dose-limiting toxicities are uncommon. Reported systemic reactions include injection-site inflammation, fever, fatigue, flu-like symptoms, and route-specific effects such as bladder spasm, dysuria and hematuria with intravesical delivery<sup>34,52,84</sup>. These events are typically mild, transient and managed with supportive care<sup>106</sup>. Trials testing combinations of GT vectors or OVs encoding multiple cytokines have also shown acceptable tolerability<sup>40,42</sup>. In some cases, ulceration of injected melanoma lesions has been described<sup>107</sup>. Cytokine release syndrome has been observed only rarely, particularly with systemically administered OVs encoding immunostimulatory cytokines<sup>108</sup> or with GTs encoding IL-12 (ref. 16).

Local organ-specific toxicities vary by delivery method<sup>52,109</sup>. Intracerebral administration can produce peritumoral edema with neurological sequelae, including seizures, cognitive dysfunction or motor deficits<sup>109</sup>. Management includes low-dose corticosteroids ( $\leq 4$  mg per day dexamethasone) or bevacizumab as a steroid-sparing option in severe cases<sup>109</sup>. Other central nervous system-related adverse events, such as hydrocephalus and aseptic meningitis, are infrequent and usually responsive to medical or surgical treatment<sup>109</sup>. For oHSV,



**Fig. 3 | Chronic immune activation can lead to counteracting immunosuppression.** Treatment with OV and GTs initially leads to immune-activating changes in the TME. However, chronic immune activation by cytokines such as IFN $\gamma$  can result in counteracting immunosuppression mediated by several signaling pathways (including noncoding RNAs) with increased

immunosuppressive cytokines and cells, as well as upregulated immune checkpoint molecules. M1, classically activated macrophage; M2, alternatively activated macrophage; MDSC, myeloid-derived suppressor cell; ncRNA, noncoding RNA; TCR, T cell receptor.

antiherpetic agents are effective in mitigating side effects. Although clinical studies have not reported secondary transmission, monitoring for OV shedding in body fluids is often included in study protocols to determine the possibility of transmission to others<sup>106</sup>.

### Economic and logistical barriers to widespread implementation

Manufacturing, storage and administration requirements for OVs and GTs remain complex. Viral amplification, purification, batch release testing and long-term storage substantially increase the per-patient costs. Manufacturing capacity is limited to a small number of specialized facilities, constraining supply and geographic accessibility<sup>110,111</sup>. Intratumoral injections and other locoregional delivery approaches impose additional procedural demands, including image guidance, operating room or interventional radiology resources, and close coordination among multidisciplinary teams. These factors increase overall costs, complicate reimbursement pathways and hinder integration into routine oncology practice. Resource constraints at centers and hospitals without specialized capabilities may restrict patient access and slow broader clinical adoption.

### Moving the field forward

The failure of several candidates in late-stage clinical trials highlights that immune reprogramming alone may be insufficient to overcome established tumor immune resistance and that the full therapeutic potential of OVs and GTs is more likely to be realized through appropriate patient selection, optimized dosing and delivery strategies, and rational combination approaches.

### Biomarker-guided patient selection

Responses to OVs and GTs vary widely among patients. Even within tumors classified by similar histology, significant spatial and temporal heterogeneity exists in the TME, genomic signatures and immunophenotypes, both between patients and between primary and metastatic lesions<sup>112,113</sup>. Stratifying patients based on predictive biomarkers may, therefore, improve therapeutic outcomes.

Several clinical studies have begun to identify such predictors. For example, a gene expression-based predictive algorithm linked resistance to measles virus-based OV therapy to the constitutive activation of interferon signaling<sup>114,115</sup>. In glioblastoma, a TME characterized by intermediate immune cell scores and moderate expression of *PDCD1* (the gene that encodes PD-1), but relatively low expression of other

checkpoint proteins, correlated with responses to DNX-2401 combined with pembrolizumab<sup>116</sup>. In a different clinical trial, select tumor mutations and immune features—such as baseline mutated gene pairs (for example, *MED15/HRC*), tumor immune cell composition, and changes in systemic cytokines, immune cells and T cell diversity—were associated with survival following therapy with CAN-2409 delivered before chemoradiation and nivolumab<sup>117</sup>. These findings underscore the potential of biomarker-guided patient selection to match patients with the biologics most likely to benefit them.

### Combination therapy

Cancer monotherapies yield modest efficacy, and the full potential of OVs and GTs may be realized through combinatorial approaches.

1. Chemotherapy. Early studies aimed to enhance tumor lysis by combining OVs with chemotherapy. While feasibility was demonstrated<sup>95,118,119</sup>, several trials failed to show a survival benefit or even reported worse outcomes<sup>120–122</sup>. A meta-analysis of pelareorep plus chemotherapy in 492 patients with advanced solid tumors found no improvement in overall survival, progression-free survival or response rates—but a partial increase in adverse events—compared to chemotherapy alone<sup>123</sup>. In a larger meta-analysis across 12 studies and nearly 1,500 patients, OV therapy in combination with traditional chemotherapy was found to improve objective responses but had no survival advantage compared to traditional treatment alone<sup>124</sup>. Therefore, it is unclear whether there is a benefit to combining OVs or GTs with traditional chemotherapy.
2. Radiotherapy. The synergy between OVs or GTs and radiotherapy is well established. Certain OVs have been shown to sensitize tumor cells to ionizing radiation by impairing DNA repair, while radiation promotes viral replication and antigen release<sup>125–127</sup>. Radiotherapy also induces the release of damage-associated molecular patterns and proinflammatory cytokines and enhances antigen presentation, functioning as an *in situ* vaccine<sup>126</sup>. Clinically, combined regimens have shown feasibility and signs of efficacy in esophageal cancer<sup>125</sup>, rectal cancer<sup>128</sup> and malignant glioma<sup>17,129</sup>.
3. Immunotherapy. OVs and cytokine-based GTs are frequently associated with the upregulation of immune checkpoint molecules, including PD-1, PD-L1 and CTLA-4, providing a strong biological rationale for their combination with ICIs<sup>16,38,55</sup>.

Consistent with this concept, spatially resolved immune profiling in pediatric high-grade glioma following intratumoral oHSV therapy demonstrated robust CD8<sup>+</sup> T cell infiltration accompanied by upregulation of PD-1, PD-L1 and CTLA-4, providing direct human tissue evidence for OV-induced checkpoint engagement in central nervous system tumors<sup>130</sup>. However, early-phase clinical trials exploring OV-ICI combinations have yielded mixed results: some have suggested enhanced efficacy<sup>15,131</sup>, while others have failed to demonstrate clear survival or response benefits<sup>51,132,133</sup>. In advanced melanoma, intratumorally administered T-VEC combined with ICIs has demonstrated a favorable safety profile and encouraging activity in early-phase studies. When T-VEC was combined with CTLA-4 inhibition using ipilimumab, objective response rates reached 50%, with durable responses lasting at least 6 months observed in 44% of patients and 18-month progression-free and overall survival rates of approximately 50% and 67%, respectively—exceeding historical outcomes for either agent alone<sup>131</sup>. Similarly, initial reports of T-VEC combined with the anti-PD-1 antibody pembrolizumab showed objective responses in 62% of patients, including complete responses in 33%, and an association with increased CD8<sup>+</sup> T cell infiltration, IFN $\gamma$ -associated transcriptional programs and PD-L1 expression in responding tumors<sup>15</sup>. However, a large randomized phase 3 trial comparing T-VEC plus pembrolizumab to placebo plus pembrolizumab in advanced melanoma failed to demonstrate improvements in progression-free or overall survival, despite modest increases in objective and durable response rates<sup>51</sup>.

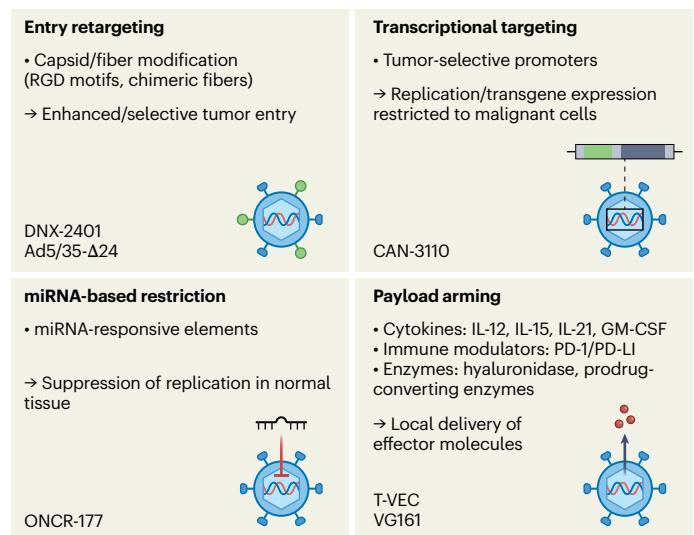
Additional evidence supports context-dependent synergy. In melanoma, patients progressing on IL-12 plasmid therapy often responded to subsequent anti-PD-1 treatment<sup>38</sup>, suggesting a potential benefit from sequential immune modulation. In recurrent glioblastoma, intratumorally administered DNX-2401 combined with pembrolizumab was safe and associated with prolonged survival in a subset of patients, despite modest overall response rates<sup>116</sup>. Notably, this study demonstrated upregulation of multiple immune checkpoints following treatment, suggesting that effective combinations may require blockade of more than one inhibitory pathway<sup>116</sup>.

Not all OV-ICI combinations have translated into meaningful clinical benefits, as exemplified by the phase 3 T-VEC plus pembrolizumab trial discussed above, as well as other negative studies. In the neoadjuvant setting for stage IIIB–D melanoma, the addition of the oncolytic coxsackievirus V937 (gebasaxturev) to pembrolizumab did not improve pathological complete or major response rates compared to pembrolizumab alone and was associated with higher rates of grade 3 or greater toxicity<sup>132</sup>. Similarly, in a phase I study, intravenous delivery of V937 alone or combined with pembrolizumab was well tolerated but yielded objective response rates comparable to historical outcomes with PD-1 blockade alone in non-small cell lung cancer and urothelial carcinoma, despite evidence of viral tumor infection<sup>133</sup>.

Beyond checkpoint inhibition, other immunomodulatory strategies have been explored to condition the immune environment before OV administration. In pediatric patients with recurrent or refractory high-grade brain tumors, administration of the oncolytic reovirus pelareorep following subcutaneous GM-CSF priming was feasible and safe, although all patients progressed within 60 days<sup>134</sup>. Emerging approaches are now combining OVs with adoptive cell therapies, showing early signs of durable responses; however, these strategies remain in the early stages of clinical evaluation<sup>42</sup>.

### Rethinking dose optimization

The optimal dosing of OVs and GTs does not follow the traditional paradigms observed with chemotherapy. Retrospective analyses indicate that lower doses of vaccinia virus were associated with improved tumor responses and prolonged survival among responding patients,



**Fig. 4 | Engineering strategies for next-generation OVs.** Engineering strategies to enhance tumor selectivity and improve safety and immune activation include the following: (1) modification of viral entry into tumor cells; (2) transcriptional targeting using tumor-selective promoters to restrict replication or transgene expression to malignant cells; (3) miRNA-responsive elements to suppress activity in normal tissues; and (4) arming with payloads such as cytokines, costimulatory ligands or matrix-modifying enzymes.

consistent with a hormetic dose–response relationship<sup>135</sup>. In a pediatric glioma trial, some long-term survivors were observed at lower doses of G207 compared to higher doses<sup>17</sup>. Some preclinical data support the superiority of repeated low-dose administration over single high doses<sup>136</sup>. In a trial of CAN-3110 in glioblastoma, dose did not correlate with survival<sup>23</sup>.

By contrast, in some studies, higher doses improved outcomes. In hepatocellular carcinoma, patients receiving higher-dose JX-594 had a median overall survival of 14.1 months compared to 6.7 months with lower dosing<sup>137</sup>. In colorectal cancer, repeated intravenous injections of JX-594 achieved disease stabilization more frequently at higher cumulative doses<sup>41</sup>. A phase 1 study of an IL-12- and suicide gene-encoding adenovirus in pancreatic cancer also demonstrated a dose-dependent survival benefit, with patients receiving the highest dose showing markedly longer survival<sup>138</sup>.

Repeat dosing is gaining traction. In Japan, G47 $\Delta$  was administered intratumorally up to six times in patients with glioblastoma in the study that led to its conditional approval<sup>32</sup>. A trial of CAN-3110 is now evaluating six prespecified injections (NCT03152318)<sup>139</sup>. The timing of combinations also seems to matter: in glioblastoma, VB-111 monotherapy initially followed by VB-111 plus bevacizumab at progression prolonged survival compared to VB-111 alone or an upfront combination<sup>140</sup>.

Together, these studies suggest that the optimal schedule for OVs and GTs may require balancing repeated lower dosing with context-dependent use of higher intensities, as well as careful sequencing with other therapies.

### Next-generation therapeutics

Early OVs relied largely on the natural tropism and cytolytic activity of wild-type or attenuated viruses<sup>141</sup>. Modern OVs are molecularly engineered to enhance tumor specificity, improve replication, and overcome barriers such as immune evasion and restricted penetration. Two main strategies have been pursued to enhance specificity and replication: (1) facilitating viral entry into tumor cells and (2) restricting replication or transgene expression to malignant cells through tumor-selective promoters or by exploiting dysregulated signaling pathways. Figure 4 provides an overview of these and other engineering strategies used in next-generation therapeutics.

Examples of entry retargeting include DNX-2401, an adenovirus engineered with an arginine–glycine–aspartic acid (RGD) motif in the fiber knob to bind  $\alpha\beta 3$  and  $\alpha\beta 5$  integrins<sup>18</sup>, and Ad5/35- $\Delta 24$ , a chimeric adenovirus whose Ad5 fiber knob was replaced with Ad35 to enable interaction with CD46 (ref. 142). Both integrins and CD46 are frequently enriched on tumor cells<sup>143,144</sup>. Selective replication has been exemplified by G207, in which both  $\gamma_1 34.5$  neurovirulence genes were deleted, preventing the virus from counteracting interferon-mediated protein kinase R (PKR) signaling in normal cells<sup>17,31,127,145,146</sup>. Nevertheless, such modifications might also impair the OV replicative potential in tumor cells<sup>147</sup>. Therefore, next-generation derivatives such as CAN-3110 reintroduce replication capacity under tumor-specific promoters, allowing selective expression of  $\gamma_1 34.5$  in malignant and stem-like tissue<sup>147,148</sup>.

OVs are increasingly being armed with therapeutic payloads, thereby functioning as dual OV–GT platforms. These payloads most commonly include proinflammatory cytokines, with intratumoral expression intended to enhance local immune activation while limiting systemic exposure and toxicity. Clinical examples include T-VEC, JX-594 and CG0070, which encode GM-CSF to promote dendritic cell recruitment and immune priming<sup>34,41,43</sup>. Other engineered OVs encode alternative cytokines; for example, TILT-123 and M032 encode IL-2 plus tumor necrosis factor (TNF) and IL-12, respectively<sup>42,44</sup>.

TILT-123 (igrelimogene litadenorepvec) is an adenoviral vector designed to enhance cytotoxic T cell infiltration through capsid retargeting and tumor-selective control of viral replication, enabling intravenous administration<sup>102</sup>. In patients with advanced solid tumors, intravenously administered TILT-123 was well tolerated, and tumor biopsy specimens obtained 7 days after treatment demonstrated increased immune cell infiltration when viral presence was detected<sup>102</sup>. Notably, patients with detectable TILT-123 in post-treatment tumor samples had a longer median overall survival compared to those without detectable virus (280 versus 190 days)<sup>102</sup>.

Given the risk of systemic toxicity associated with escalating doses of cytokine-armed vectors, an adenoviral vector was engineered with a regulatable IL-12 promoter that is activated by an oral agent (Ad-RTS-hIL12)<sup>16</sup>. Alone<sup>16</sup> or in combination with immune checkpoint inhibition<sup>149</sup>, regulation of IL-12 production in tumors could be observed as a function of the oral activator ligand administered to patients, with durable expression of IFN $\gamma$  and T cell infiltrates in treated tumors. Ad-TD-nslIL12 was engineered to express a nonsecreted, membrane-bound form of IL-12, thereby promoting sustained local immune activation while limiting systemic exposure<sup>150</sup>. In two phase I trials involving pediatric patients with newly diagnosed or progressive isocitrate dehydrogenase (IDH) wild-type diffuse intrinsic pontine glioma, intratumoral administration of Ad-TD-nslIL12 was well tolerated and associated with disease stabilization or partial responses<sup>150</sup>. Treated cohorts also exhibited increased post-treatment lymphocyte counts, and their median overall survival from diagnosis exceeded that of institutional historical controls<sup>150</sup>. Another example is VG161, an engineered oHSV expressing multiple immunostimulatory transgenes, including IL-12, IL-15/IL-15 receptor subunit  $\alpha$  (IL-15R $\alpha$ ) and a PD-1/PD-L1-blocking fusion protein<sup>151</sup>. In a multicenter phase I study of treatment-refractory hepatocellular carcinoma, VG161 demonstrated an acceptable safety profile and encouraging biological activity, including remodeling of the TME and renewed therapeutic responsiveness in tumors that had progressed on prior systemic therapies<sup>151</sup>. Transcriptional profiling identified gene signatures associated with clinical benefit and overall survival<sup>151</sup>.

Beyond the examples discussed above, a broader spectrum of cytokines has been explored to tailor immune activation to the dominant immunological constraints of specific tumor types. FLT3L has been used to recruit dendritic cell populations, thereby enhancing antigen presentation and promoting T cell priming—an approach that is particularly relevant in immunologically cold tumors<sup>40</sup>. In contrast,

cytokines that primarily augment lymphocyte effector function, including IL-15 and IL-21, enhance the infiltration, expansion and activity of NK cells and cytotoxic T cells<sup>151,152</sup>. IL-15 is particularly attractive as an OV-encoded payload because it preferentially expands and sustains cytotoxic CD8<sup>+</sup> T cells and NK cells through physiological transpresentation using IL-15R $\alpha$  (ref. 153). Preclinical studies demonstrate that IL-15/IL-15R $\alpha$ -armed OVs promote robust CD8<sup>+</sup> T cell-mediated antitumor immunity, with one vaccinia-based virus encoding IL-15 and IL-15R $\alpha$  showing potent antitumor activity and synergy with PD-1 blockade in murine cancer models<sup>154</sup>. Another important immune mediator, IL-24, has been incorporated into OVs for its ability to induce tumor-selective apoptosis in addition to its immunomodulatory effects<sup>155</sup>.

Beyond immunomodulatory cytokines, OVs have also been engineered to deliver functional enzymes that enable the localized activation of conventional anticancer agents. TG6002, a vaccinia-based OV encoding the FCU1 prodrug-converting enzyme, locally converts orally administered 5-fluorocytosine (5-FC) into active 5-fluorouracil (5-FU) within tumor tissue<sup>156</sup>. In a multicenter phase I study evaluating intrahepatic arterial delivery of TG6002 combined with oral 5-FC in patients with liver-dominant metastatic colorectal cancer, successful tumor delivery and viral replication were demonstrated, along with robust systemic immune activation characterized by proinflammatory cytokine induction and expansion of T cell receptor clones<sup>156</sup>. Although objective clinical responses were not observed in this early cohort, these findings support the feasibility and immunological activity of this combinatorial prodrug–virus strategy<sup>156</sup>.

Looking ahead, emerging preclinical and early clinical data may enable the development of a more rational framework for cytokine selection based on the dominant immune deficit within the TME (for example, impaired antigen presentation versus effector cell dysfunction) and the desired balance between local and systemic immune activation. Future OV designs will likely prioritize cytokines that synergize with immune checkpoint blockade or adoptive cell therapies.

### Beyond tumor shrinkage: immune-centric response criteria

Traditional oncology endpoints, such as radiographic tumor shrinkage, may not fully reflect the therapeutic impact of OVs and GTs. Pseudoprogression, an apparent tumor enlargement caused by immune infiltration and inflammation, is frequently observed with immunotherapies and can potentially lead to inappropriate treatment decisions if misinterpreted<sup>157,158</sup>. For this reason, immune-related response criteria are increasingly applied to OV and GT trials. For example, in the ongoing CAN-3110 repeat-administration trial for recurrent glioblastoma (NCT03152318), tumor biopsy, cerebrospinal fluid and blood samples are collected at each prespecified injection (days 0, 15, 30, 60, 90 and 120). Samples undergo multimodal analysis<sup>159,160</sup>. A report illustrating these serial analyses in the first two patients enrolled revealed therapeutic effects including longitudinal and spatial reshaping of the recurrent glioblastoma microenvironment, expansion of new tissue-resident T effector memory clonotypes against CAN-3110 epitopes and other undetermined antigens, and expression of human leukocyte antigen (HLA)-presented immunopeptides, such as cancer testis antigens<sup>139</sup>. Moreover, serial integrated multimodal analyses provided evidence of therapeutic responses to CAN-3110, despite traditional magnetic resonance imaging indicating progression. These results demonstrate the value of longitudinal tissue sampling in understanding recurrent glioblastoma evolution during investigational therapy<sup>139,161</sup>.

### Conclusion

OVs and cytokine GTs are multimodal immunotherapies that act primarily by reprogramming the TME. Clinical and translational evidence demonstrates their capacity to convert immunologically cold tumors into inflamed, hot states through immunogenic cell death, epitope spreading and durable T cell activation. Widespread adoption has been hindered by the incomplete durability of clinical responses, delivery

constraints and logistical barriers. Progress will depend on improving our molecular and immunological analyses of tumors in clinical trials. With continued advances in vector engineering and integrated immune profiling, OV and GTs have the potential to become integral components of precision immuno-oncology.

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## Author contributions

J.D.B., L.S. and E.A.C. contributed to the literature review, visualization of concepts through figures and tables, and writing and revising the Review.

## Competing interests

E.A.C. is an advisor to Bionaut Laboratories, Seneca Therapeutics and Relgnite Therapeutics. He has equity options in Bionaut Laboratories, Seneca Therapeutics, Ternalys Therapeutics and Relgnite Therapeutics. He is a cofounder and a member of the board of directors of Ternalys Therapeutics. Patents (US 10,806,761 B2: oncolytic HSV-1 vector and methods of use; US 6,897,057: cell-specific and/or tumor-specific promoter retargeting of herpes  $\gamma$ 34.5 gene expression; US 7,214,515: viral delivery system for infectious transfer of large genomic DNA inserts) related to oHSV and CAN-3110 are held by Brigham and Women's Hospital, with E.A.C. named as a co-inventor. These patents have been licensed to Candel Therapeutics. Present and future milestone license fees, as well as future royalty fees, are distributed to Brigham and Women's Hospital from Candel. J.D.B. has an equity position in Treovir, Inc., and is the chief medical officer of UpFront Diagnostics and Centile Bioscience. J.D.B. is also on the QV Bioelectronics and NeuroX1 boards of scientific advisors, as well as the Synaptive Medical board of directors. He also has a patent (US patent no. US20240374663A1) titled 'Methods and formulations related to the intrathecal delivery of oncolytic viruses'. L.S. declares no competing interests.

## Additional information

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