Going viral with cancer immunotherapy

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Abstract | Recent clinical data have emphatically shown the capacity of our immune systems to eradicate even advanced cancers. Although oncolytic viruses (OVs) were originally designed to function as tumour-lysing therapeutics, they have now been clinically shown to initiate systemic antitumour immune responses. Cell signalling pathways that are activated and promote the growth of tumour cells also favour the growth and replication of viruses within the cancer. The ability to engineer OVs that express immune-stimulating ‘cargo’, the induction of immunogenic tumour cell death by OVs and the selective targeting of OVs to tumour beds suggests that they are the ideal reagents to enhance antitumour immune responses. Coupling of OV therapy with tumour antigen vaccination, immune checkpoint inhibitors and adoptive cell therapy seems to be ready to converge towards a new generation of multimodal therapeutics to improve outcomes for cancer patients.

Our immune systems have the capacity to evolve in response to any invading pathogen and the ability to detect differences in protein structure at the atomic level. Tumours arise through a combination of genetic and epigenetic changes that favour immortality but at the same time create ‘neo-antigens’ that should render the malignant cell detectable by the immune system and target it for destruction. However, tumours survive and flourish in the hostile environment of a healthy immune system through its manipulation. Tumours dampen their responses to innate immune effectors, limit their display of neo-antigens and paralyze infiltrating immune effector cells. In effect, the tumour creates a niche of innate and adaptive immune suppression where the evolving repertoire of mutant regulatory proteins (the mutanome) within the tumour is hidden, allowing escape from an array of normal cellular homeostatic regulatory systems. Although this region of ‘immune privilege’ protects the tumour from assault by the immune system of its host, it does come at a price. Tumour cells have a more limited ability to respond to viral infections than normal tissues and can hence be targeted and destroyed by engineered viruses that are unable to attack normal tissues. Furthermore, we now understand that the ‘hallmarks of cancer’ that favour tumour cell growth can mostly be superimposed onto the ‘hallmarks of a successful viral infection’. Viruses have evolved sophisticated multifunctional gene products that usurp cellular apoptotic programmes, deregulate cellular energetics and inactivate growth suppressors. The appreciation of the parallels in biology between the requirements for effective propagation of a virus within the mammalian cell and the pathways that drive malignant cell growth has led to the development of the oncolytic virus (OV) field, with initial clinical testing of the concept as early as the 1950s. Current OVs are viruses that are unable to manipulate antiviral programmes in normal host cells but are complemented by malignantly activated or deregulated pathways in tumour cells that favour growth of OVs. This leads to selective infection of tumour cells by OVs, with minimal toxicity to normal tissues, as shown in preclinical models and patients.

OVs cause acute tumour debulking owing to tumour cell infection and lysis (termed oncolysis). Furthermore, some OVs have been shown to trigger acute vascular-disrupting effects that are capable of contributing to acute tumour debulking. In addition, OVs have been shown to induce antitumour immunity. The multiple, complementary mechanisms of action of OVs distinguish these therapeutics from tumour vaccines and immune adjuvants. All three of the above mechanisms of action contribute to efficacy, although this Opinion article focuses on OVs and cancer immunotherapy. We review the ability of OVs to induce antitumour immune responses in a single-agent setting and propose strategies to boost the activity of immunotherapeutic agents through optimizing OV engineering and combination therapy regimens. Furthermore, we discuss the potential of OVs to be an ideal candidate therapy to sensitize patients to other active immunotherapies.

Clinical experience with OVs
Clinical development of OVs that were engineered for cancer specificity was initiated in the 1990s. Recent reviews give detailed summaries of the recent progress in the clinical development of OVs. More than 1,000 patients have now been treated with OVs by intratumoural injection and/or intravenous infusion during Phase I–III clinical trials. The agents that are the most advanced in clinical development include talimogene laherparepvec (TVEC; Amgen; herpes simplex virus (HSV); Phase III trial for melanoma (ClinicalTrials.gov identifier: NCT00769704)), pexastimogene davacene (Pexa-Vec, JX-594; SillaJen Biotherapeutics and Transgene S.A.; an oncolytic vaccinia virus; Phase I fb trial for hepatocellular carcinoma (ClinicalTrials.gov identifier: NCT01387555)) and pelareorep (Reolysin; Oncolytics Biotech; reovirus; Phase III in combination with chemotherapy in head and neck cancer (ClinicalTrials.gov identifier: NCT01166542)). H101 (Oncorine; Shanghai Sunway Biotech), a recombinant adenovirus, is approved for the treatment of head and neck cancer in China.

An acceptable safety and tolerability profile has been shown for OV treatment of patients to date, with acute, transient
Tumour-associated antigens

A summary of the origins of tumour-associated antigens is provided below.

**The mutanome**
Advances in DNA sequencing have revealed the cellular mutanome — a comprehensive map of all of the somatic mutations in individual tumours. A subset of these somatic mutations occurs in protein-coding sequences and might create new antigenic structures that are recognized by the immune system of the patient. These novel antigens could arise from amino acid substitutions or deletions, protein truncations or fusions of two unique polypeptides.

**Oncofetal proteins**
Some gene products are only expressed during normal embryonic development and not in adult tissues. In cancers, the expression of these developmental genes can be reactivated and produce antigens that the adult immune system recognizes as foreign.

**Viral antigens**
More than 20% of cancers are known to arise as a result of infection with a cancer-causing virus. These oncoviruses drive the growth of the tumour through the expression of one or more oncoproteins. These virally encoded proteins are unique tumour-associated antigens that can be recognized by the immune system.

**Differentiation antigens**
Differentiation antigens are associated with the differentiation of a specific tissue and are overexpressed on tumour cells.

**Post-translational modifications**
An almost universal feature of cancer cells is altered glycosylation patterns that could be recognized by the immune system. For example, a loss of glycosylation can reveal protein peptide antigens that are not visible on normal cells.

Flu-like symptoms being the most common adverse event[s][20–22]. Pexa-Vec was shown to selectively infect tumour cells after intratumoral injection or intravenous infusion in patients[s][23]. Antitumour efficacy has been shown in Phase II and Phase III trials in patients with advanced cancers[s][23–24]. In an abstract presented at the 2013 American Society of Clinical Oncology (ASCO) annual meeting[s][24], a Phase III trial of T-VEC versus granulocyte–macrophage colony stimulating factor (GMCSF) treatment in patients with melanoma was reported to have achieved the primary endpoint of improvement in durable response rate for T-VEC compared with GMCSF treatment. Intratumoral administration of T-VEC in the setting of melanoma has proven to be effective. However, given that the feasibility of systemic dosing of other OV backbones has been shown in patients, it is likely that intravenous OV administration will be more relevant in other disease settings.

OVs have been reproducibly shown to have an acute, transient toxicity profile. Furthermore, the common adverse events associated with OVs are non-overlapping with other anticancer agents; therefore, OV therapy lends itself to combination with current therapeutic modalities. Several trials evaluating the safety and efficacy of OVs in combination with other treatments have been completed or are currently underway. To date, OVs have been well-tolerated in combination with chemotherapy, radiation, low-dose cyclophosphamide and targeted therapy[s][25–28]. With several OV products in late-stage clinical development, OVs are likely to become a new tool in the toolbox of oncolologists.

**Cancer immunotherapy breakthroughs**
After decades of unfulfilled promise, technologies that aim to use the immune system of cancer patients to control their tumours are now achieving unprecedented success. For instance, sipuleucel-t (Provenge; Dendreon), an autologous dendritic cell (DC) vaccine, has shown a survival benefit in patients with castration-resistant prostate cancer and has received US Food and Drug Administration (FDA) approval[s][29,30]. A novel class of antibody therapeutics blocks immune checkpoints at the T cell priming or effector stages. Ipilimumab (Yervoy; Bristol-Myers Squibb), which was initially developed for patients with melanoma, is the first agent in this class to get FDA approval[s][31]. Ipilimumab enhances T cell priming by inhibiting cytotoxic T lymphocyte-associated antigen 4 (CTLA4), a receptor involved in the negative-feedback loop that blocks a co-stimulatory signal from DCs[s][32]. Alternate strategies include agonistic antibodies that are designed to potentiate co-stimulation (for example, OX40 or CD137 agonists). In addition, multiple antibodies that target programmed cell death protein 1 (PD1) and its ligand, PDL1, which block inhibitory signals in the tumour microenvironment during the effector phase of the immune response, are currently in development (reviewed in REF[2]). Alternatively, cell-based therapeutics build on early work extracting tumour-infiltrating lymphocytes (TILs) that are expanded and re-infused into patients[s][33]. Current trials are evaluating T cell receptor (TCR) transgenic cell therapeutics, as well as chimeric antigen receptor (CAR) technology (reviewed in REF[34]). Given this initial validation of cancer immunotherapies in patients, a rapid effort has been initiated to identify combination therapeutics that can prime or sensitize to immunotherapeutics that are currently approved or in advanced-stage development. It is clear that when the immune system is appropriately induced, a durable benefit can be achieved (for example, in approximately 20% of patients with melanoma treated with ipilimumab[s][35]). A concerted effort has been initiated to assess which combination therapy approaches have the potential to markedly increase the percentage of patients achieving durable benefit.

**The oncolytic virus paradigm revised**
The initial aim of OV technology (during the 1990s) was to create viral machines that could outstrip tumour cell defence mechanisms and lead to massive tumour cell death by ‘viral oncolysis’. We now believe that curative viral oncolysis on its own probably only occurs in the instance when tumours are completely devoid of any virus defence systems and the therapeutic agent can outpace the adaptive antiviral immune response of the patient. The most effective treatment regimens will be those that combine potent viral oncolysis with an effective and long-lasting antitumour immune response. Although OVs on their own can sometimes do this, the conditions that favour this outcome are not clearly understood.

There are numerous OV platforms and mechanisms of selectivity, which have been reviewed in detail[s][16], and although there is an unquestionable need to create more potent and selective agents, we suggest that perhaps the greatest therapeutic strides could be achieved by combining agents such as OVs that cause acute disruption to tumours with novel antitumour immunomodulating agents. We believe that the real value of the OV platform is to initiate exquisitely targeted infection of tumour beds, disrupt the immune tolerance that cancers create and re-engage powerful multicellular immune surveillance mechanisms to eliminate malignancies.
We envisage at least three requirements for an effective ‘viro-immunotherapy’ response: first, targeted replication of OVs in the tumour bed; second, initiation of an immune-stimulating or immune-recruiting inflammatory response; and third, exposure of tumour-associated antigens (TAAs) that can be targeted by the immune system (Box 2). Therefore, identification of enhanced oncolytic viral platforms should seek to maximize tumour-specific replication and lytic ability, while enhancing immunogenic properties.

**Oncolytic viruses: ‘in situ vaccination’**

It is clear from both preclinical and clinical studies that robust and specific infection of tumour beds by OVs is achievable after intravenous infusion or intratumoural injection using various platforms. Emerging evidence shows that the oncolytic efficacy of many viruses is at least partly related to the induction of potent antitumour immune responses. Oncolytic in situ vaccination is presumably initiated during the viral lysis of tumour cells, which releases tumour antigens into the microenvironment that are cross-presented to T cells by endogenous antigen-presenting cells (APCs). This is a crucial step for mounting and sustaining antitumour T cell responses. One example of such virus-mediated therapeutic immune recruitment to tumour beds comes from Phase II and Phase III studies with T-VEC, in which patients with advanced disseminated melanoma showed complete responses in both OV-injected and non-OV-injected tumours. Inflammatory cell infiltration into tumours was also shown with Pexa-Vec treatment, and antitumour antibodies mediating complement-dependent cytotoxicity were induced after Pexa-Vec treatment of patients with liver tumours. A series of elegant studies examining the ability of certain chemotherapeutics to induce antitumour immunity has revealed several chemical signals that are crucial for the induction of immunological cell death (ICD) (Box 3). These studies are striking because of the parallels drawn between chemotherapeutically induced ICD and the induction of cell death in association with damage-associated molecular pattern molecules (DAMPs) following viral infection. As with chemotherapy, it is therefore tempting to speculate that OV-induced DAMPs may underlie the innate and acquired antitumour immune responses that have been seen following OV therapy in preclinical and clinical results to date. Further studies using the tools developed to study chemotherapy-induced ICD will be necessary to give direct evidence to support this concept. Nonetheless, it is reasonable to believe that as our understanding of ICD and DAMP stimulation increases, so too will our opportunities to engineer oncolytic strains or combination therapies to optimally shape antitumour immunity at the tumour bed. Similarly, radiation therapy has been shown to have the potential to induce antitumour immunity, with responses being observed in non-irradiated lesions (termed the abscopal effect). The potential for radiation therapy to prime immunomodulating agents is currently being clinically investigated.

There is still some controversy in the OV field as to the relative contribution of the lytic infection versus antitumour immune response induction to antitumour efficacy. We propose that in the absence of an existing antitumour immune response, slower replicating viruses that may manipulate the immune response are preferred — functioning to prime the immune response. Conversely, rapidly replicating and spreading viruses (which may be cleared rapidly by the adaptive antiviral immune response) might function better in an immune-boostering situation, by reactivating existing antitumour immune cells.

**Box 2 | Requirements for a ‘viro-immunotherapy’ response**

**Selective virus replication**

Oncolytic viruses (OVs) seem to ‘kick-start’ immune reactions against infected and uninfect ed tumour cells. The specificity of replication is essential to focus the immune system on the tumour and prevent off-target virus- or immune-mediated damage.

**Localized inflammatory response**

In response to viral infection, cells within the tumour microenvironment express an array of immune-stimulating cytokines. These cytokines attract various innate and adaptive immune cells into the tumour and activate resident lymphocytes.

**Exposure of tumour antigens**

The destruction of tumour cells by OVs generates cell debris that is ingested by antigen-presenting cells and delivered to the immune system of the patient. Antigens that are unique to the tumour cells trigger cellular or antibody-mediated immune responses.

**Improving OV-elicted immune responses**

**Drug combinations.** Guided by a tremendous increase in our understanding of host–virus interactions during the past decade, researchers continue to develop more potent viruses through engineering or biological selection. As a complement to these strategies, some groups have explored tumour-specific enhancement of OV-mediated infection and killing by pharmacological complementation. Compounds that specifically dampen tumour cell antiviral responses can be used to regulate the extent of virus replication and tumour killing. For instance, histone deacetylase inhibitors have been shown to increase the killing of virus-resistant tumour cells and have the added benefit of promoting tumour-specific immune responses in preclinical models. One interesting compound that warrants further clinical testing in combination with OVs is the receptor tyrosine kinase inhibitor sunitinib. Already approved for the treatment of renal cancer, sunitinib is now known to augment the growth of oncolytic versions of vesicular stomatitis virus (VSV) in mouse models, to have anti-angiogenic properties and to enhance antitumour immunity. A proof-of-principle for the synergistic activity of sunitinib in combination with Pexa-Vec was clinically shown with a durable complete response (>6 years) of a patient with treatment-refractory renal cell cancer receiving Pexa-Vec injections followed by standard sunitinib treatment. Second mitochondria-derived activator of caspases (SMAC) mimetics (agents mimicking an endogenous pro-apoptotic protein that resides in the mitochondria and is released when a cell is triggered to undergo apoptosis) in clinical testing for the treatment of various malignancies have been recently shown in mice to synergize with virally induced tumour necrosis factor-α (TNFα), which diffuses from infected tumour cells, creating a ‘cloud of death’ around infected foci. These compounds alone stimulate antitumour immunity, so it will be interesting to see whether recent preclinical data translates into the clinic.

**Genetic engineering.** Many of the currently used OV vectors have large genomes encoding for immunomodulatory proteins that can blunt immune responses against the virus. Whether these are interfering with the ability of the virus to induce or enhance antitumoural immunity is not clear. Many immunosuppressive viral mediators are known and could be deleted from these vectors, especially in the instance of oncolytic pox and herpes viruses.
Tumour antigens that are released by oncolysis can be taken up by dendritic cells (DCs), which then prime T cells to mediate additional tumour control. Oncolytic vaccines retain the functions of a standard OV but are designed to ensure induction of antitumoural immunity. Systemic delivery leads to loading of DCs (dark red) distal to the tumour and the induction of tumour-specific T cells. The virus also infects the tumour bed to directly destroy tumour cells while reducing local immunosuppression to facilitate T cell killing. Released tumour antigens can be taken up by DCs for additional T cell activation. Therapies for cancer immunotherapy rely on the induction of an antitumoural immune response distal to the tumour (or generated ex vivo). Effector T cells then need to traffic into the tumour bed to attack the target tumour cells. However, the immunosuppressive nature of tumours impedes immune cell infiltration and function. Therapy with OVs involves the delivery of a virus to the tumour bed, where it can infect and kill both tumour cells and tumour vasculature (green cells), leading to immune activation and reduced local immunosuppression.

Figure 1 | Oncolytic virus (OV) infection of the tumour niche and principles of ‘oncolytic vaccines’. a | Most forms of cancer immunotherapy rely on the induction of an antitumoural immune response distal to the tumour (or generated ex vivo). Effector T cells then need to traffic into the tumour bed to attack the target tumour cells. However, the immunosuppressive nature of tumours impedes immune cell infiltration and function. b | Therapy with OVs involves the delivery of a virus to the tumour bed, where it can infect and kill both tumour cells and tumour vasculature (green cells), leading to immune activation and reduced local immunosuppression.

An example of this strategy is T-VEC, in which the viral gene encoding ICP47, which blocks antigen presentation by infected cells, was deleted. A rational approach to deleting this class of viral genes could be used in the hope of enhancing the immunogenicity of OVs. However, testing whether these deletions enhance antitumoural immunity could prove to be challenging, as many of these viral factors show species specificity and might only be functional in their natural host (and, as a result, current vectors used in mice might be more immunogenic in this natural host than in human patients). It may be desirable to enhance immunogenicity without impairing the ability of the virus to replicate. Therefore, OV mutants or backbone genomes with enhanced replication could be selected in screens that identify constructs with enhanced immunogenicity.

Infection of tumour cells by OVs can lead to the robust induction of various inflammatory cytokines, thereby producing a highly optimized cocktail of molecules to attract and activate immune effector cells. In some situations, this cocktail is sufficient to trigger a potent antitumour immune response; and so the question becomes, can engineering OVs to express specific cytokines improve on mother nature? One rationale for engineering cytokines into OVs may be that some tumour cells might not detect or respond to OVs and, thus, might not produce an optimal inflammatory cytokine profile. In addition, the goal of OV therapy is to overcome immune suppression, so the skewing of cytokine expression to favour immune cell recruitment and activation is probably desirable. In preclinical models, many different cytokines have been expressed from OV backbones for various reasons, but no clear winner has emerged to date. However, identification of the optimal cytokine to express in the context of OVs will probably require detailed correlational analyses in the context of clinical trials, as mouse models are probably not predictive of the effect in humans.

Various immune effector cells can be targeted for stimulation by OV-expressed cytokines, including APCs (DCs, macrophages and neutrophils) and/or effector lymphocytes (for example, natural killer (NK) cells, NKT cells, T cells and B cells). APCs have a pivotal role in the induction of immune responses, and several groups have sought to recruit and activate these cells using OVs that express cytokines such as GMCSF or FMS-related tyrosine kinase 3 ligand (FLT3L), or chemokines such as CC-chemokine ligand 3 (CCL3) or CCL5. The most widely adopted of these has been GMCSF, which has been inserted into many OVs in efforts to induce differentiation and recruitment of DCs into the tumour bed and draining lymph nodes and thereby facilitate the presentation of tumour antigens that are released during viral oncolysis. In particular, GMCSF cDNA has been grafted into the backbones of OVs that have been clinically tested, and it was shown to have a biological effect.
For example, detectable concentrations of GMCSF in plasma that coincided with peak Pexa-Vec replication were measured within 1 week of Pexa-Vec administration and associated with white blood cell induction in a subset of patients receiving intravenous or intratumoural Pexa-Vec treatment. FLT3L is a potentially interesting immunostimulatory transgene that has undergone some preclinical testing. It is notable that, when expressed from irradiated cells that were then used as a whole-cell tumour vaccine, both GMCSF and FLT3L (G-VAX and Fi3VAX, respectively) were effective if administered distal to existing tumours, but only Fi3VAX was effective when administered into the tumour microenvironment, implying that expression of this cytokine within the tumour milieu from an OV may be more effective than GMCSF.

A great variety of other cytokines have been engineered into a wide range of OVs. These are generally intended to stimulate various lymphocyte populations to aid oncolytic therapy. For example, interleukin-12 (IL-12) targets NK cells, NKT cells and T cells, inducing proliferation, expression of cytotoxic mediators and production of additional cytokines. IL-15 also affects these lymphocyte populations and seems to be able to reverse intratumoural immunosuppression, but its secretion from infected cells and presentation to responding cells presents a challenge.

Various alternative strategies have been evaluated to enhance OV-induced antitumour immune responses. Many of these strategies are directed towards providing co-stimulation to intratumoural T cells by allowing the expression of co-stimulatory molecules on infected tumour cells, thereby generating T cell-activating signals that are usually provided by APCs. Interestingly, the combined expression of cytokines and co-stimulatory factors from OVs has been shown to further enhance their therapeutic efficacy in preclinical models. Various combinations have already been tested with success, but as the matrix of possible combinations is obviously huge, it is not clear how one should go about identifying combinations for clinical advancement.

A new class of immunotherapeutic molecules — the bispecific T cell engagers (BiTEs) — could be interesting ‘cargo’ to load into OVs. These bispecific antibodies tether T cells to surface-exposed tumour antigen targets when delivered to a tumour-bearing recipient. BiTEs have recently been expressed from oncolytic vaccinia virus backbones, and their localized expression mediated T cell bystander killing of uninfected tumour cells. The expression of checkpoint inhibitors from OVs that selectively infect tumour cells is also a very attractive strategy and might reduce systemic toxicities attributed to checkpoint inhibition while relieving intratumoural immunosuppression. Multiple groups in the field are currently developing such approaches.

**Oncolytic vaccines**

Although vaccination against foreign pathogens has proved to be highly successful, the development of therapeutic vaccines against cancer has been less successful to date. It is much more challenging to induce efficacious immune responses against tumour antigens, as these tend to be autologous in nature and therefore the immune system of the patient will be tolerant against them. Thus, generating an antitumour immune response requires the breaking of tolerance and may result in autoimmune toxicities.

Clinical trials using various traditional tumour vaccines that do not use OV vectors have been carried out with a range of tumour antigens. In these trials, some patients developed a cell-mediated immune and/or antibody response against the targeted antigen. However, the use of viral vectors (rather than autoantigens) to induce immune responses may predominantly induce a highly competitive immune response against expressed viral antigens rather than against the expressed tumour antigen transgene. In order to focus the immune response on the tumour antigen target, strategies have been used that involve priming of the immune response with one vector, followed by boosting with another vector expressing the same tumour antigen. These strategies can induce robust immune responses against the target antigen, but there is a need to identify compatible pairs of vectors and to determine which vector platforms are best suited to priming versus boosting.

Although classical viral vaccine vectors are non-replicating, the use of replicating OVs as vaccine vectors has begun to be investigated and has led to the introduction of the concept of oncolytic vaccines. Classical viral vectored vaccines are only able to engage the immune system, whereas OVs are able to infect and destroy tumour cells while potentially altering the immunosuppressive tumour microenvironment. An emerging concept in oncolytic viral therapeutics is the use of so-called oncolytic vaccines. These oncolytic vaccines retain all of the beneficial oncolytic properties of the parent vector but also express a TAA transgene to induce a specific immune response. These viruses will also infect and debulk the tumour; this leaves any residual tumour for the immune system and leads to the release of other tumour antigens that could allow for antigen spreading and reduce local immunosuppression.

**Box 3 | Immunogenic cell death and viral mimicry**

Several groups have now reported that not only the quantity but also the quality of cell death can have a marked influence on the immunological response to antigens from dying cells. Although there is currently no consensus on which form of cell death is more immunogenic (apoptosis, necrosis, autophagy or necroptosis), several markers and signalling pathways are necessary to skew immune reactions from tolerogenic to immunogenic responses. Immunogenic cell death is driven through the sensing of damage-associated molecular pattern molecules (DAMPs) by dendritic cells (DCs) as they take up antigen from dying cells. The endoplasmic reticulum stress-mediated cell surface marker calreticulin (ecto-CRT), has emerged as a hallmark DAMP of immunological cell death (ICD). Ecto-CRT binds to the low-density lipoprotein receptor-related protein-6 on engulfing DCs to drive cellular activation. Autophagy-dependent ATP that is released from dying cells binds to P2X purinoceptor 7 (P2X7) on DCs, activating the NALP3–ASC inflammasome and driving the secretion of interleukin-1β (IL-1β). This cytokine, together with antigen presentation, is required for the polarization of interferon-γ (IFNγ)-producing CD8+ T cells and the development of the adaptive antitumour immune response. Similarly, the nuclear protein high mobility group protein B1 (HMGB1) is released during necrotic and apoptotic cell death and binds to toll-like receptor 4 (TLR4) on DCs to induce their activation. In addition to DC activation, some DAMPs, such as Fas-ligand (which binds to DC natural killer lectin group receptor 1 (DNKR1); also known as CLEC9A) on DCs, function to regulate the intracellular trafficking of internalized antigens, thereby diverting them from a lysosomal proteolytic fate to a non-degradative recycling endosomal compartment that favours antigen extraction for antigen peptide transporter 1 (MHC class I, also known as TAP)-dependent cross-presentation on major histocompatibility complex (MHC) class I. Thus, oncolytic virus-delivered DAMPs might function not only to increase the amplitude of an antitumour response by driving the activation of DCs but also to target selection by efficiently routing antigens to MHC class I for presentation to T cells.
Newcastle disease virus (NDV) that express model tumour antigens (that is, foreign protein) have been used to achieve direct tumour lysis accompanied by a directed vaccination effect\(^9,27,29\). However, the induction of responses to bona fide tumour (self) antigens by replicating oncolytic vaccines is weak, as the response to the replicating viral vector dominates in this case\(^9,80\). However, these oncolytic vaccines could prove to be very effective boosters of antitumour immunity, as the recall response against the tumour antigen transgene can dominate the response against the OV\(^78,80\). The use of an oncolytic vaccine to boost antitumour immunity combines the benefits of viral oncolysis (that is, tumour destruction and reversal of local immunosuppression) with the immunological enhancement associated with vaccine boosting. Importantly, the use of a replicating OV for this purpose allows for replication of the tumour vaccine and an amplification of dose that can lead to larger antitumour antigen transgene immune responses in tumour-bearing animals than can be achieved in tumour-free hosts\(^78,80\). Furthermore, concurrent destruction of tumour cells with a virus and with T cells could provide an environment that is highly permissive of antigen spreading and therefore encourage the development of responses to diverse tumour antigen targets\(^9,81\).

### Table 1 | Immuno-stimulatory transgenes encoded by oncolytic viruses

<table>
<thead>
<tr>
<th>Transgenes</th>
<th>Vectors</th>
<th>Targets</th>
</tr>
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<tbody>
<tr>
<td>GMCSF</td>
<td>HSV-1, Vacinia virus, Adenovirus, NDV, Measles virus, VSV</td>
<td>Stimulates production of granulocytes and monocytes, promoting differentiation of monocytes into DCs for antigen presentation</td>
</tr>
<tr>
<td>FLT3L</td>
<td>Adenovirus, VSV</td>
<td>Both conventional and plasmacytoid DCs, as well as NK cells</td>
</tr>
<tr>
<td>CCL3</td>
<td>Adenovirus</td>
<td>Attracts polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>CCL5</td>
<td>Adenovirus</td>
<td>T cells (recruitment)</td>
</tr>
<tr>
<td>IL2</td>
<td>HSV-1, NDV</td>
<td>T cells (activation)</td>
</tr>
<tr>
<td>IL4</td>
<td>Adenovirus, HSV-1, VSV</td>
<td>T cells and B cells (replication and T&lt;sub&gt;1&lt;/sub&gt; skewing)</td>
</tr>
<tr>
<td>IL12</td>
<td>Adenovirus, HSV-1, VSV</td>
<td>T cells and NK cells (activation)</td>
</tr>
<tr>
<td>IL15</td>
<td>HSV-1, VSV, Influenza A virus</td>
<td>T cells and NK cells (activation)</td>
</tr>
<tr>
<td>IL18</td>
<td>Adenovirus</td>
<td>T cells and NK cells (activation)</td>
</tr>
<tr>
<td>IFNA1 or IFNB1</td>
<td>Vaccinia virus, Measles virus, Adenovirus, VSV</td>
<td>APCs and T cells (enhanced T cell immunity)</td>
</tr>
<tr>
<td>IFNG</td>
<td>Adenovirus</td>
<td>NK cells, T cells and macrophages (activation)</td>
</tr>
<tr>
<td>CD80</td>
<td>Adenovirus, HSV-1</td>
<td>T cells (co-stimulation)</td>
</tr>
<tr>
<td>CD40L</td>
<td>VSV, HSV-1</td>
<td>T cells (co-stimulation)</td>
</tr>
<tr>
<td>Genes encoding heat shock proteins</td>
<td>Adenovirus</td>
<td>APCs (delivery of peptides and activation)</td>
</tr>
<tr>
<td>IL12 and 4-1BBL</td>
<td>Adenovirus</td>
<td>Combined effects</td>
</tr>
<tr>
<td>IL18 and CD80 (soluble)</td>
<td>HSV-1</td>
<td>Combined effects</td>
</tr>
<tr>
<td>IL12 and CD80</td>
<td>Adenovirus</td>
<td>Combined effects</td>
</tr>
<tr>
<td>GMCSF and CD80</td>
<td>Adenovirus</td>
<td>Combined effects</td>
</tr>
<tr>
<td>IL12, IL18 and CD80 (soluble)</td>
<td>Adenovirus</td>
<td>Combined effects</td>
</tr>
</tbody>
</table>

4-1BBL, 4-1BB ligand; APC, antigen-presenting cell; CCL, CC-chemokine ligand; CD40L, CD40 ligand; CD80, T lymphocyte activation antigen CD80; DC, dendritic cell; FLT3L, FMS-related tyrosine kinase 3 ligand; GMCSF, granulocyte–macrophage colony stimulating factor; HSV-1, herpes simplex virus-1; IFN, interferon; IL, interleukin; NDV, Newcastle disease virus; NK, natural killer; T<sub>1</sub>, T helper 2; VSV, vesicular stomatitis virus.

### Adding immune checkpoint inhibitors

The extent to which immune cells are excluded from or paralyzed within the tumour bed varies among individual patients and disease types. Patients with increased levels of TILs often have a better prognosis and response to therapy than those whose tumours bar lymphocyte entry\(^9\). Exciting emerging clinical data from the use of immune checkpoint inhibitors that can be effective even in patients with advanced disease are consistent with the concept that reversal of immunosuppression and elimination of the tumour with potent immune responses can be achieved\(^1\). The combination of immune checkpoint inhibitors with OVs seems to be a natural marriage (FIG. 2). It is known that some tumours constitutively express PD-L1 as a mechanism to suppress T cell activity and others ‘adaptively respond’ to immune cell infiltration by upregulation of PD-L1 on their surface\(^2\). In the case of OV therapy, it is desirable to override immune checkpoint inhibitor networks and thereby create a pro-inflammatory environment within the cancer. This was demonstrated in preclinical models by combining NDV with CTLA4 blockade\(^3\). OV infection triggered lymphocytic infiltration (including tumour-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells) into both injected and non-injected tumours, which rendered tumours susceptible to CTLA4 blockade. Clearly, in this strategy, the exquisite replicative targeting that limits OV growth within the tumour is crucial to limit unwanted ‘off-target’ immune responses. Ipilimumab is in early clinical testing with T-VEC (ClinicalTrials.gov identifier: NCT01740297), but it is still not known whether this combination will be safe and effective in patients with cancer. In this case, T-VEC is provided as an intratumoural agent, and its ability to selectively infect tumours might help to target the activity of ipilimumab to the tumour. Clearly, in the setting in which
OVs are combined with immunomodulatory therapies, the two agents will need to be carefully dose-escalated and optimal sequencing will need to be established to avoid cumulative toxicity. Early clinical data from the T-VEC–ipilimumab trial that were reported at the 2014 American Society of Clinical Oncology Annual Meeting suggest that this strategy is feasible\(^8^3\), and no substantial toxicities were reported in preclinical studies\(^8^2\).

**Adoptive cell therapy**

The development of adoptive cell therapy (ACT) as an effective strategy for the treatment of some cancers offers exciting opportunities for combination with emerging OV platforms. One of the challenges in getting long-lasting responses with ACT is enhancing trafficking to and survival of donor T cells in the tumour bed. OVs could provide a means to both recruit and activate engineered or selected T cells into the tumour. The use of virus antigen-specific T cells for tumour targeting was shown several years ago in proof-of-principle experiments that used T cells from Epstein–Barr virus (EBV)-infected cancer patients\(^9^1\). EBV-reactive cytotoxic T lymphocytes (CTLs) were engineered to express a CAR against GD2 (a neuroblastoma tumour antigen). These re-targeted CTLs were continually stimulated through their natural anti-EBV receptors and thereby survived and remained active but, moreover, were able to induce antitumour activity through the engagement of their GD2-directed CARs. Using OVs or oncolytic vaccines that are specifically designed to complement ACT products gives an opportunity to substantially improve therapy. For instance, an OV could be engineered to express cytokines that mediate T cell recruitment and survival [Table 1] and could then be used in tandem with ex vivo-expanded TILs to drive CTL activity within the tumour. A variation of the EBV strategy is to modify OV-reactive T cells with a tumour-specific CAR. In principle, these CAR T cells with dual specificity can be sequentially activated by infusion of the OV.

**The future of oncolytic immunotherapies**

The positive Phase III data using the oncolytic immunostimulatory virus T-VEC have been a tremendous boost to the OV field. At the same time, these results challenge some of the original tenets of OV therapy, showing that systemic effects can be achieved following loco-regional administration and that viral oncolysis may be necessary but perhaps not sufficient to provide long-term therapeutic benefit. Thus, for some OV platforms, such as HSV, adenovirus, reovirus and measles virus, pre-existing antiviral immunity, which has long been thought to be an issue if a virus needed to be delivered intravenously, may not be a problem if loco-regional therapy is sufficient to trigger systemic immune responses. Nonetheless, successful intravenous delivery of an OV will potentially give access to all sites of metastasis, thereby creating productive immune stimulation in every tumour bed. This could be important in generating anticancer responses to the full range of TAAs, especially considering the known heterogeneity of cancer cells. Furthermore, infection of all tumour sites may be crucial in ‘de-cloaking’ tumours and allowing or facilitating the infiltration of newly generated immune cells. It seems reasonable to expect that systemic tumour debulking by oncolysis will have a substantial therapeutic benefit, but perhaps the optimal treatment regimen would include serial oncolytic viral boosting of immune responses through intratumoural injections.

The ability of OVs to locally stimulate inflammation, to function as gene delivery vehicles and to lead to direct tumour lysis positions them well as therapeutic partners in rational combination strategies. The events that are associated with the natural interplay of viruses with our immune systems provide multiple opportunities to combine OV therapy with immune checkpoint inhibitors and/or ACT.

### Figure 2: Combining oncolytic viruses (OVs) with new immunotherapeutics

**a** At baseline, T cells within a tumour can be kept in an inactive state through the programmed cell death protein 1 (PD1) receptor binding to programmed cell death 1 ligand 1 (PDL1) expressed on tumour cells. Inhibitory cytokines also prevent immune clearance of tumours. **b** Engineered OVs form factories inside the tumour, triggering expression of pro-inflammatory cytokines and/or immune checkpoint inhibitors. This localized production leads to a focused immune stimulation and recruitment of immune cells. **c** Intravenously administered antibodies, such as those directed against PDL1, can be used to reverse T cell anergy and potentiate OV-induced immune responses. Alternatively, the pro-inflammatory microenvironment in OV-infected tumours can promote recruitment of chimeric antigen receptor (CAR)-expressing T cells.

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The text is a scientific article discussing the efficacy of targeted oncolytic poxvirus in cancer therapy. It highlights the importance of oncolytic viruses in immunotherapy and the potential of using recombinant poxviruses to induce antitumor immune responses. The article mentions studies on various types of viruses, including Newcastle disease virus, canarypox virus, and recombinant avipox virus, and their applications in different cancer models. The text also discusses the role of immune checkpoint inhibitors and the potential of combination therapies.

The article concludes with a call for further research to understand the mechanisms of antitumor immunity and to develop more effective targeted therapies for cancer treatment.