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Design and application of oncolytic viruses for cancer immunotherapy

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The approval of the first oncolytic virus (OV) for the treatment of metastatic melanoma and the recent discovery that the use of oncolytic viruses may enhance cancer immunotherapies targeted against various immune checkpoint proteins have attracted great interest in the field of cancer virotherapy. OVs are designed to target and kill cancer cells leaving normal cell unharmed. OV infection and concomitant cancer cell killing stimulate anti-tumour immunity and modulates tumour microenvironment towards less immunosuppressive phenotype. The intrinsic capacity of OVs to turn immunologically cold tumours into immunologically hot tumours, and to increase immune cell and cytokine infiltration, can be further enhanced by arming OVs with transgenes that increase their immunostimulatory activities and direct immune responses specifically towards cancer cells. These OVs, specifically engineered to be used as cancer immunotherapeutics, can be synergized with other immune modulators or cytotoxic agents to achieve the most potent immunotherapy for cancer.

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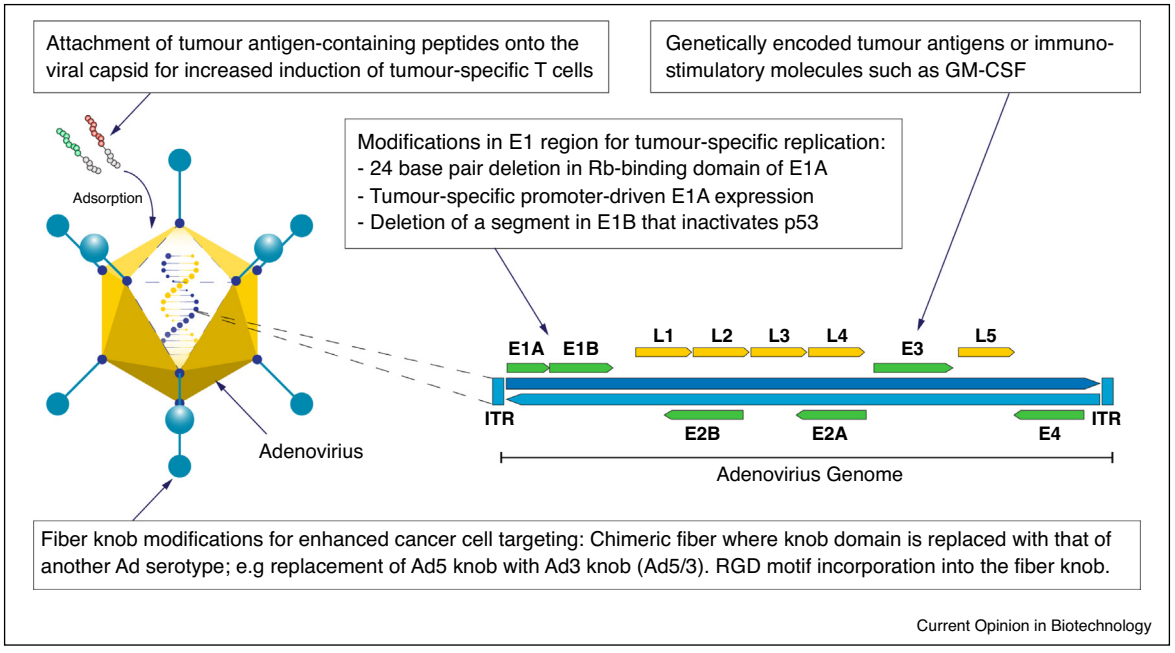
Introduction

Cancer immunotherapy aims to increase the amount and function of tumour-infiltrating immune cells such as dendritic cells (DCs) and tumour-infiltrating lymphocytes (TILs) in order to elicit therapeutic efficacy. This may be achieved via multiple different strategies. For example, DC vaccinations that aim to increase tumour antigen presentation, TIL and chimeric antigen receptor (CAR) T cell therapies that aim to increase cancer killing T cells, and immune checkpoint inhibitor (ICI) therapies that aim to enhance endogenous anti-tumour immune responses [1]. In particular, ICIs such as antibodies targeted against programmed cell death 1 (PD-1) or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) have drastically changed the treatment paradigm for many cancers. However, objective responses to ICI therapies have predominantly been seen in patients with prior anti-tumour immune response (10–30% of patients are responding to ICIs) [2]. OV therapies have been shown to modulate the tumour microenvironment (TME) towards a less immunosuppressive phenotype and to enhance anti-tumour immune responses. Combining ICI therapies with OVs may help patients overcome resistance to ICI therapies. OVs are currently in clinical evaluation in combination with multiple cancer immunotherapeutic platforms. In this review, we discuss the current engineering strategies to enhance OVs and their application as cancer immunotherapeutics (see Figure 1 for schematic representation of the design strategies of a typical OV used in the clinics). In addition, we discuss the most recent synergistic combinations of OVs with other immunotherapeutic platforms.

Tumour microenvironment and immune evasion

Cells of the TME consist of a heterogeneous population of neoplastic cells together with a number of different non-transformed cells including mesenchymal cells, for example, cancer stem cells (CSCs), mesenchymal stem cells (MSCs), endothelial cells (ECs), fibroblasts and myofibroblasts, hematopoietic cells, for example, innate and adaptive immune cells such as macrophages, T cells, natural killer (NK) cells, B cells, neutrophils, DCs, and mast cells (MCs) and myeloid-derived suppressor cells (MDSCs). In addition to cells, the TME consists of secreted factors such as cytokines, and extracellular vesicles and proteins of the extracellular matrix (ECM) [3]. Cancer cells, as well as non-transformed cells, for example, cancer-associated fibroblasts (CAFs), adipocytes, T regulatory cells (Tregs), MDSCs and tumour-associated macrophages (TAMs) support immune evasion and tumour growth by producing and releasing cytokines such as interleukin-10 (IL-10), chemokines such as chemokine C-X-C motif ligand 12 (CXCL12), growth factors such as transforming growth factor beta (TGF-β), matrix remodelling factors such as collagen, fibronectin and fibrin and other soluble factors such as adenosine into the TME [3,4]. The immunosuppressive environment is established via multiple mechanisms: TGF-β and IL-10 mediate an anti-inflammatory response by dampening the activity of tumour suppressor

Figure 1



Schematic representation of various strategies for the design of an oncolytic adenovirus. Modifications in the viral E1, E3 and fiber knob regions are commonly used in oncolytic adenoviruses used in clinical trials. For more information on viral modifications, see Ref. Kaufman *et al.* [117]. Ad, adenovirus; Rb, retinoblastoma protein; p53, cellular tumour antigen p53; ITR, inverted terminal repeat.

cells such as cytotoxic T cells (CTLs) and NK cells and enhancing the activity of tumour promoting cells such as Tregs and tumour-associated neutrophils (TANs) [5,6]. In addition, cancer cells have acquired the ability to activate different immunosuppressive immune checkpoint pathways such as CTLA-4/CD80/86 and PD-1/PD-L1 signalling pathways that, in normal cells, are associated with immune homeostasis and prevent an overactivated immune response leading to autoimmune reactions [7]. Despite the hostile and highly immunosuppressive environment of the TME, some tumour suppressor cells may still be activated to combat the growing lesion. Indeed, it has been shown in a variety of cancers that the number of infiltrating lymphocytes positively correlates to patient survival [8–10].

Oncolytic viruses can stimulate anticancer immunity and modulate the TME

OVs induce anticancer immunity by multiple mechanisms: i) Virus-mediated lysis of tumour cells releases tumour-associated antigens and neoantigens (TAAs and TANs) which can be captured and processed by tumour-infiltrating antigen presenting cells (in particular, DCs), ultimately leading to a tumour-specific T cell response against a wide spectrum of the released antigens. ii) OVs can promote several forms of immunogenic cell death (ICD) including necrosis, necroptosis, pyroptosis, autophagic cell death and immunogenic apoptosis, leading to the release of danger-associated molecular patterns (DAMPs) such as surface-exposed calreticulin (ecto-CRT), secreted adenosine triphosphate (ATP) and released high mobility group box 1 protein (HMGB1) [11,12]. In addition to DAMP release, OV-mediated cancer cell lysis is usually associated with the release of various pathogen-associated molecular patterns (PAMPs) including viral components such as nucleic acids (DNA, dsRNA, ssRNA, and 5′-triphosphate RNA), proteins and capsid components [12]. DAMPs and PAMPs are recognised by pattern recognition receptors on innate immune cells such as DCs and NK cells and function as ‘danger’ and ‘eat me’ signals. This signalling attracts more DCs to the TME which in turn leads to increased recruitment and maturation of tumour-specific T cells into the TME [11,12]. iii) Some OVs such as HSV-1 and vaccinia virus can infect and replicate in endothelial cells causing disruption of tumour vessels potentially facilitating immune cell migration into the TME [13,14]. iv) Tumour cell infection by an OV leads to an inflammatory response and localized cytokine production followed by infiltration of innate immune cells and CTLs that help repolarize the TME towards less immunosuppressive phenotype [15].

Main hurdles limiting the effects of armed OVs for cancer immunotherapy

Although OVs can induce anticancer immunity by multiple mechanisms (as described in the previous section), in most cases, clinical experience with OVs as a monotherapy has

shown modest activity [16••,17•,18–20]. There are a number of potential reasons that may explain this, including the inability to optimally infect cancer cells due to: i) neutralizing antibodies; ii) other antiviral clearance mechanisms; iii) physical barriers that prevents OVs from reaching their entry receptors, or due to viral intrinsic factors such as; iv) engineered cancer selectivity or transgene expression that may reduce viral fitness or v) expression of potent transgene (s) that may result in potent antiviral immune response leading to a premature clearance of the OV [21]. In the following sections, we will discuss current design strategies for optimising OVs for cancer immunotherapy.

Oncolytic viruses as cancer vaccines: strategies to boost tumour-specific T cell responses

Although OVs can mediate the release of TAAs and TANs through virus-mediated lysis of tumour cells and act as an *in-situ* cancer vaccine, it may not, at least in some situations, be enough to induce a potent tumour-specific T cell response [22]. One approach to enhance the priming efficiency of OVs is to genetically encode one or more TAAs into the OV genome to enhance T cell priming and to guide it towards tumour-specific T cell immune responses. Various viral platforms have been engineered to encode TAAs. For example, de Vries *et al.* engineered an oncolytic vaccinia virus to encode for an HER2/neu TAA (VVneu) and used it in combination with another oncolytic vaccinia virus armed with granulocyte macrophage colony stimulating factor (GM-CSF) (VVGMCSF) as an adjuvant to treat an aggressive orthotopic model of HER2/neu-driven mammary carcinoma. Intratumoural injection of the VVneu in combination with VVGMCSF resulted in the generation of systemic neu-specific immunity, a significant reduction in tumour-associated and systemic MDSCs and a significant control of the tumour growth [23]. A very interesting and recently developed OV platform that has been exploited to encode various TAAs is the genetically modified Maraba virus MG1 [24]. In the first study describing the potency of this platform, the authors engineered MG1 to encode human dopachrome tautomerase (DCT), a well-characterized TAA and an enzyme involved in melanogenesis. Interestingly, DCT-encoding MG1 (MG1-hDCT) was not able to prime detectable DCT-specific T cell responses when administered as a sole agent (either in tumour-bearing or in tumour-free mice), but when combined with recombinant adenoviral vector expressing human DCT (Ad-hDCT) used as a priming vector, the MG1-hDCT displayed very potent boosting of DCT-specific T cell responses leading to strong anti-tumour immunity and extended survival of melanoma metastasis-bearing mice [25]. After this proof of concept study for MG1 suitability as a cancer vaccine, various other TAAs have been engineered to be encoded by the MG1 including human papillomavirus (HPV) E6 and E7 antigens for the treatment of HPV-positive tumours [26], human six-transmembrane antigen of the prostate (hSTEAP) for prostate cancer [27], the human placenta-specific 1 (hPLAC1) for hPLAC1-expressing tumours such as breast/mammary tumours [28] and the human melanoma-associated antigen-A3 (MAGE-A3) for melanoma and lung carcinoma [29••]. Currently, there are two phase I/II clinical trials evaluating the Ad:MG1 prime-boost combination as an oncolytic cancer vaccine platform in patients with MAGE-A3-positive solid tumours (NCT02285816) and in patients with previously treated metastatic non-small-cell lung cancer (NCT02879760).

Another strategy to enhance OV-induced tumour-specific T cell responses is the coating of OVs with specifically designed tumour epitope peptides. Our group has tested this approach with oncolytic adenovirus [30••,31], and vaccinia and HSV-1 viruses [32•]. We showed that intratumoural administration of adenoviruses coated with modified tumour epitope peptides (PeptiCRAd) including tumour epitope peptides derived from tyrosinase-related protein 2 (TRP-2), human glycoprotein 100 (gp100), human melanoma-associated antigen A1 (MAGE-A1), transmembrane and TPR repeat-containing protein 2 (TMTC2), WD repeat domain 11 (WDR11), zinc finger RNA-binding protein (Zfr) and a disintegrin and metalloproteinase with thrombospondin motifs 9 (Adamts9) increase tumour-specific T cell responses, enhance tumour growth control and induce systemic anticancer immunity in mouse and humanized mouse cancer models of melanoma and triple negative breast cancer [30••,31]. Similarly, intratumoural administration of HSV-1 and vaccinia viruses coated with modified tumour epitope peptides (PeptiENV) were shown to increase intratumoural as well as systemic peptide-specific T cell responses [32•]. Induction of strong tumour-specific T cell responses through the virus-attached peptides might be advantageous in personalized cancer vaccine settings, since changes in patients' tumour antigen profile can be rapidly adapted to by coating the virus with a new set of tumour-specific peptides without the need to manufacture another good manufacturing practice (GMP)-grade virus.

Arming OVs with immunostimulatory cytokines

One of the most used cytokines for arming oncolytic viruses is GM-CSF [15]. GM-CSF is an immunomodulatory cytokine playing a dominant role in the survival, proliferation, differentiation, and function of myeloid lineage cells [33]. Local GM-CSF expression by OVs enhances DC migration and maturation, eventually leading to enhanced priming of T cell responses [34]. The first virus armed with GM-CSF, a modified oncolytic Herpes simplex virus 1 (HSV-1), was shown to significantly enhance the anti-tumour properties of the virus in a preclinical model of murine lymphoma [35]. Recently, the same virus has been tested in a phase III OPTiM trial of 436 patients with unresected stages IIIB-IV

melanoma, where it demonstrated significant improvement in durable response rate, objective response rate and progression-free survival [36,37••]. Considering the results of the OPTiM trial, this virus was approved by the FDA and EMA under the name talimogene laherparepvec (T-VEC) for the treatment of metastatic melanoma patients. Other OVs have also been successfully armed with GM-CSF including various oncolytic vaccinia viruses (e.g. Pexa-Vec currently in multiple clinical trials) [38], measles virus [39], Newcastle disease virus [40], and various adenoviruses (e.g. ONCOS-102 currently in phase I trial in combination with pembrolizumab [NCT03003676]). Another cytokine that has been used for arming OVs is interleukin 12 (IL-12). IL-12 is a pleiotropic cytokine activating both innate and adaptive immunity and acting as a major orchestrator of Th1-type immune response against cancer [41]. Markert *et al.* showed that HSV-1 expressing murine IL-12 (M002) prolonged survival of immunocompetent mice in intracranial models of brain tumours [42]. Currently, a derivative of the M002, M032, an HSV-1 expressing human IL-12 is being evaluated in a Phase I clinical trial (NCT02062827) as a treatment modality for high-grade glioma [43,44]. Interestingly, studies with multiple preclinical cancer models comparing herpes viruses as well as adenoviruses expressing either GM-CSF or IL-12 show markedly enhanced tumour growth inhibition and systemic anti-cancer immune responses with IL-12 expressing viruses as compared to GM-CSF-expressing viruses [45–47]. In addition to IL-12, various other interleukins, including IL-2, IL-15 and IL-18, have also been used to arm OVs and have shown promising immune activating properties in multiple preclinical cancer models including melanoma, hepatoma, colon carcinoma and squamous cell carcinoma [46,48–54].

Arming OVs with chemokines

Chemokines, the largest subfamily of cytokines, are small, secreted proteins that mediate immune cell trafficking and lymphoid tissue development. In response to specific chemokines, different immune cell subsets migrate into the TME and regulate tumour immune responses in a spatiotemporal manner, thus contributing to the immunomodulation of the TME [55]. Li *et al.* armed an oncolytic vaccinia virus with CCL5, a chemokine that attracts leukocytes into the site of inflammation, and showed that CCL5-armed vaccinia virus (vvCCL5) enhanced immune infiltration of mouse colorectal tumours *in vivo* and enhanced therapeutic effects such as tumour suppression and survival [56]. Interestingly, vvCCL5 was also shown to have prolonged persistence specifically within the tumour as compared to the unarmed vaccinia virus. Another chemokine that has been tested recently in the context of OVs is CCL19. This chemokine, that attracts naive or central memory T (TCM) cells and mature DCs to the site of CLL19 production, was used to arm oncolytic vaccinia virus (vvCCL19) [57]. vvCCL19 displayed enhanced tumour growth control and was shown to selectively attract DCs and $CD4^+$ T cells into the TME. In addition to CCL5 and CCL19, other chemokines such as CCL20 and CCL21 have shown to enhance anti-tumour effects when used to arm OVs [58–60].

Arming OVs with immune-activating ligands

One of the most studied immune-activating ligands is the ligand for cluster of differentiation 40 (CD40), the CD40L. CD40 is a member of the tumour necrosis factor receptor family and is expressed on antigen-presenting cells such as DCs and myeloid cells. CD40L is expressed on activated $CD4^+$ T cells, B cells and NK cells as well as memory $CD8^+$ T cells [61]. Signalling through CD40 on APCs greatly increases their antigen-presentation and co-stimulatory capacity and allows for efficient $CD8^+$ CTL priming [62,63]. OVs and viral vectors armed with CD40L have been extensively tested in clinical [64–67] and preclinical [68–73] settings and have been shown to mediate multiple antitumoural activities including tumour growth control, cancer cell apoptosis, induction of T-cell responses, increase in $T_{effector}/T_{regulatory}$ cell ratios and the upregulation of Th1 cytokines. As an example, Pesonen *et al.* used oncolytic adenovirus armed with CD40L (CGTG-401) to treat multiple patients with advanced solid tumours and reported that five out of six evaluable patients (83%) displayed disease control and, importantly, induction of tumour-specific T cell responses was seen in the majority of patients. Three patients had injected and non-injected lesions that the authors were able to assess separately. In all three patients, the non-injected lesions responded similarly to the injected lesions, suggesting induction of systemic immune responses against the tumour [64]. Another member of the tumour necrosis factor receptor family, the tumour necrosis factor receptor superfamily, member 4 (TNFRSF4, also known as OX40 receptor) and its ligand (OX40L) have gained interest as therapeutic target molecules for cancer immunotherapy. Signalling through OX40 plays an important role in the survival and homeostasis of effector and memory T cells as well as controlling the function and differentiation of $Foxp3^+$ regulatory T cells [74]. In a recent study, OX40L-armed oncolytic adenovirus (Delta-24-RGDOX) was shown to have superior tumour-specific lymphocyte activation and proliferation of $CD8^+$ T cells specific to tumour-associated antigens in addition to increased survival when compared to the unarmed Delta-24-RGD virus in two mouse glioma models [75]. The same virus (Delta-24-RGDOX) was further tested in disseminated subcutaneous and intracranial melanomas and localized treatment with Delta-24-RGDOX in the subcutaneous tumour was able to reject intracranial tumours, suggesting an induction of strong systemic anticancer immunity [76•]. Currently, a phase I trial is going on to evaluate the effects of Delta-24-RGDOX treatment in patients with recurrent glioblastoma (NCT03714334). Combinations of two different co-stimulatory molecules have also been used

to arm OVs, for example, Eriksson *et al.* armed oncolytic adenovirus with CD40L together with another tumour necrosis factor receptor family ligand named 4-1BBL (4-1BB is expressed on activated T cells. Signalling through 4-1BB/4-1BBL stimulates T cell expansion, acquisition of effector function, survival and development of T cell memory). The double-armed virus, named LOAd703, was shown to efficiently reduce established tumours in an *in vivo* murine xenograft model of human pancreatic cancer and to induce strong activation of immune responses based on assessment of LOAd703-infected human monocyte-derived immature DCs [77]. Currently, LOAd703 is undergoing two Phase I/II clinical trials in patients with pancreatic cancer (NCT02705196) and in patients with pancreatic adenocarcinoma, ovarian cancer, biliary carcinoma or colorectal cancer (NCT03225989). Other costimulatory molecules that have successfully been used to arm OVs include B7-1 [78] and GITR [79].

Arming OVs with bispecific T cell engager (BiTE) molecules

BiTE molecules are a novel class of immunotherapeutic agents that can activate T cells independently of MHC expression to lyse target cells. One arm of the BiTE molecule binds CD3epsilon on the T cell receptor, while the other arm binds to a chosen target antigen. Binding of both arms to their corresponding target antigens triggers T cell activation leading to target cell lysis by apoptosis [80•]. The first BiTE-armed OV that has been tested in preclinical models is an oncolytic vaccinia virus armed with a BiTE molecule targeting the tumour cell surface antigen EphA2 (EphA2-TEA-VV) [81]. The authors showed that in a murine xenograft model of human lung cancer, EphA2-TEA-VV had very potent anti-tumour activity when administered in combination with human peripheral blood mononuclear cells (PBMCs). Recently, Freedman *et al.* armed oncolytic adenovirus to express a BiTE molecule that binds to the epithelial cell adhesion molecule (EpCAM) overexpressed on target cancer cells (EnAd-SA-EpCAM) [82]. Remarkably, EnAd-SA-EpCAM could activate endogenous T cells within the immune-suppressive microenvironment of liquid cancer biopsies (malignant peritoneal and pleural exudates) and exhibited killing of endogenous tumour cells without addition of exogenous T cells [82].

OVs in combination with other immunotherapies

Since the recent approval of ICIs such as ipilimumab (targeted against CTLA-4), pembrolizumab and nivolumab (both targeted against PD-1), there has been an immense amount of interest in using OVs in combination with ICIs. Multiple OVs, both unarmed and armed, have been tested in several preclinical cancer models in combination with various ICIs. These preclinical studies have shown that the combination is highly synergistic [31,83–92]. However, the first clinical indication of synergistic effects on anti-tumour activity by the combination of an OV and an ICI was seen in a phase Ib study using T-VEC in combination with ipilimumab in patients with advanced melanoma. In the T-VEC + ipilimumab combination therapy, the objective response rate was 50%, and 44% of patients had a durable response lasting for 6 months or longer. Importantly, the combination had a tolerable safety profile, and appeared to have greater efficacy than either T-VEC or ipilimumab monotherapy [93••]. These positive results were later confirmed in a follow-up phase II study showing a significant increase in confirmed objective response rate with T-VEC + ipilimumab compared with ipilimumab alone (39% versus 18%, respectively; $p = 0.002$) [94]. Recently, Ribas *et al.* showed, in a phase Ib study using T-VEC combined with pembrolizumab, exceptionally high overall and complete response rates of 62% and 33%, respectively, in patients with advanced melanoma [95]. Also a reported case series of 10 unresectable stage III–IV melanoma patients treated with T-VEC in combination with pembrolizumab, nivolumab or ipilimumab + nivolumab, showed overall response rates for injected lesions of 90% and complete response rates of 60%. Importantly, two patients who had un-injected lesions experienced complete resolution of both the injected and un-injected lesions indicating induction of a systemic anti-tumour immune response [96]. Currently, there are at least 12 different clinical trials evaluating the combination of T-VEC with pembrolizumab, atezolizumab (ICI targeted against PD-L1), nivolumab or ipilimumab in patients with melanoma, lung cancer, breast cancer, colorectal cancer, sarcoma, and hepatocellular carcinoma, carcinoma of the head and neck and malignant pleural effusion [97]. See Table 1 for more information on recent clinical trials of OVs in combination various checkpoint inhibitors.

In addition to combining OVs with checkpoint inhibitors, OVs have recently been tested in combination with DC vaccines. Preclinical studies have shown that OVs can modulate the TME by reducing the immunosuppressive conditions and thus allowing enhanced induction of tumour-specific T cells by DC vaccines leading to greatly enhanced tumour growth control [98,99•,100]. Komorowski *et al.* used oncolytic vaccinia virus armed with CXCR4 antagonist (OVV-CXCR4-A-Fc) in combination with DCs pulsed with whole tumour lysates and showed that TME modulation by OVV-CXCR4-A-Fc had a significant positive impact on the efficacy of the DC vaccine [98]. The OV-enhanced DC cancer vaccine strategy has now entered into early clinical trials; Autologous CD1c (BDCA-1)$^+$ myeloid DCs together with T-VEC will be tested in patients with non-visceral metastases of melanoma (NCT03747744), while a DC vaccine for prostate cancer (DCVAC/PCa) together with ONCOS-102 will be tested in patients with metastatic castration-resistant prostate cancer (NCT03514836). Similar to DC vaccines, OVs have also been shown

Table 1

Recent clinical trials with OVs in combination with checkpoint inhibitors

OV	Virus type	Transgene	Checkpoint inhibitor	Indication	Clinical phase	Number of participants	Identifier
T-VEC	Herpes simplex virus 1	GM-CSF	Atezolizumab	Early breast cancer	Exploratory study	30	NCT03802604
T-VEC	Herpes simplex virus 1	GM-CSF	Pembrolizumab	Metastatic and/or locally advanced sarcoma	Phase II	60	NCT03069378
T-VEC	Herpes simplex virus 1	GM-CSF	Nivolumab	Malignant pleural effusion	Phase Ib/II	24	NCT03597009
T-VEC	Herpes simplex virus 1	GM-CSF	Pembrolizumab	Melanoma	Phase II	100	NCT04068181
T-VEC	Herpes simplex virus 1	GM-CSF	Atezolizumab	Triple negative breast cancer and colorectal cancer with liver metastases	Phase Ib	36	NCT03256344
T-VEC	Herpes simplex virus 1	GM-CSF	Pembrolizumab	Melanoma	Phase II	47	NCT02965716
T-VEC	Herpes simplex virus 1	GM-CSF	Nivolumab	Sarcoma	Phase II	40	NCT03886311
T-VEC	Herpes simplex virus 1	GM-CSF	Pembrolizumab	Melanoma	Phase II	28	NCT03842943
T-VEC	Herpes simplex virus 1	GM-CSF	Pembrolizumab	Liver tumours	Phase Ib/II	244	NCT02509507
Pexa-Vec; JX-594	Vaccinia virus	GM-CSF	Nivolumab			Hepatocellular carcinoma	Phase I/IIa
30	NCT03071094						
Pexa-Vec; JX-594	Vaccinia virus	GM-CSF	Ipilimumab	Metastatic/ advanced solid tumours	Phase I	66	NCT02977156
Pexa-Vec; JX-594	Vaccinia virus	GM-CSF	Durvalumab and/or Tremelilumab	Colorectal cancer	Phase I/II	35	NCT03206073
DNX-2401	Adenovirus	None	Pembrolizumab	Brain cancers	Phase II	49	NCT02798406
ONCOS-102	Adenovirus	GM-CSF	Pembrolizumab	Melanoma	Phase I	24	NCT03003676
ONCOS-102	Adenovirus	GM-CSF	Durvalumab	Advanced peritoneal malignancies	Phase I/II	78	NCT02963831
AD-E6E7 MG1-E6E7	Adenovirus vector Maraba virus	Mutant HPV E6 and E7 proteins	Atezolizumab	HPV associated cancers	Phase I/Ib	75	NCT03618953
Ad-MAGEA3 MG1-MAGEA3	Adenovirus vector Maraba virus	Melanoma-associated antigen 3	Pembrolizumab	Melanoma or cutaneous squamous cell carcinoma	Phase Ib	40	NCT03773744
Ad-MAGEA3 MG1-MAGEA3	Adenovirus vector Maraba virus	Melanoma-associated antigen 3	Pembrolizumab	Non-small cell lung cancer	Phase I/II	75	NCT02879760

to have synergistic effects with chimeric antigen receptor (CAR) and adoptive T cell therapies [101,102,103•,104,105]. Havunen *et al.* armed oncolytic adenovirus with human interleukin 2 (IL-2) and tumour necrosis factor alpha (TNF-α) (TILT-123) and treated a hamster HapT1 model of pancreatic cancer with TILT-123 in combination with tumour infiltrating leucocytes (TILs) [106]. The authors reported that the combination therapy was able to cure 100% of tumour-bearing hamsters and importantly, when these hamsters were rechallenged with the same HapT1 cell line, they completely rejected the reintroduced tumours, indicating that the curative therapy was also able to induce protective T cell memory response [106]. These compelling preclinical data encouraged the study authors to initiate a human trial studying the utility of TILT-123 in patients with advanced cancer receiving TIL therapy.

Considerations for clinical trials

Increasing evidence from preclinical and clinical studies indicates that OVs are potent immunostimulators and have an impact on treating cancer, but clearly (at least in a majority of cases) have the most effect on therapeutic outcomes when combined with other immunotherapies, such as ICIs. There are a large number of clinical trials on the run with OVs in combination with various ICIs, but there are still considerable discrepancies on the timing of OV therapy in combination with ICI therapy. Which treatment should be given first to patients, or should they be administered simultaneously? Studies are now emerging describing the importance of timing of the ICI therapy with respect to OV therapy (and other cancer vaccine therapies) in achieving the most potent synergy of both therapies [107,108••]. In the phase 1b portion of MASTERKEY-265, pembrolizumab was given five weeks following the initiation of T-VEC therapy, to allow robust anti-cancer immune responses to mount, and viral oncolysis to occur, prior ICI therapy. This resulted in an ORR of 62% and CR of 33% [95]. However, a recent study of a case series of stage III–IVM1b melanoma patients treated with T-VEC in combination with pembrolizumab, nivolumab or nivolumab + ipilimumab presented an even higher ORR of 90% and CR of 60% [96]. Patients in this study started ICI therapy either before T-VEC injections or simultaneously with T-VEC injections. Although the small patient cohort and variability of the treatments have an effect on the analysis of the results, the enhanced response rate may be linked to different sequencing of the two therapies, as well as to different ICIs used. Clearly, the timing of checkpoint inhibitor

Table 2

Characteristics of OVs used in cancer immunotherapy.

Family	Characteristics	Cancer selectivity	Examples of viruses in clinical development	Transgenes
Herpesviruses: HSV-1	Enveloped viruses with large dsDNA genome. Replicate in the nucleus. Large transgene insertion capacity.	Viral gamma 34.5 gene deletion. Thymidine kinase deletion. Control of gene expression with tumour selective promoters or microRNA targeting.	T-VEC [37••]	GM-CSF
Poxviruses: Vaccinia virus Myxoma virus	Enveloped viruses with large dsDNA genome. Replicate in the cytoplasm. Large transgene insertion capacity.	Viral B18R gene deletion. Thymidine kinase deletion. Ribonucleotide reductase deletion. Viral growth factor deletion.	Pexa-Vec [38]	GM-CSF
Adenoviruses	Non-enveloped viruses with intermediate-sized dsDNA genome. Replicate in the nucleus. Medium transgene insertion capacity.	Partial deletion in viral gene E1A Control of gene expression with tumour selective promoters or microRNA targeting.	ONCOS-102 [22] TILT-123 [106] DNX-2440 [76•] LOAd703 [77]	GM-CSF IL-2 and TNF-α OX40L Trimerized CD40L and 4-1BBL
Paramyxoviruses: Measles virus Newcastle disease virus	Enveloped viruses with small ssRNA genome. Replicate in the nucleus. Medium transgene insertion capacity.	Naturally IFN sensitive.	MV-NIS [111] MV-CEA [111] MEDI5395 [112]	Sodium iodide symporter (NIS) Human carcinoembryonic antigen GM-CSF
Rhabdoviruses: vesicular stomatitis virus Maraba virus	Enveloped viruses with small ssRNA genome. Replicate in the nucleus. Medium transgene insertion capacity.	Naturally IFN sensitive. Partial mutation of M protein increases IFN sensitivity. MicroRNA mediated cancer cell selectivity.	VSV-IFNβ-NIS [113] Maraba MG1-MAGE-A3 [29••]	Interferon beta and NIS TAA MAGE-A3
Reoviruses: mammalian orthoreovirus Type 3 Dearing	Morphologically complex non-enveloped viruses with segmented intermediate-sized dsRNA genome. Replicate in the cytoplasm.	Naturally IFN sensitive.	Reolysin [114]	No transgenes
Picornaviruses: Coxsackievirus A21 Poliovirus	Non-enveloped viruses with small ssRNA genome. Replicate in the cytoplasm. Very small transgene insertion capacity.	Naturally IFN sensitive.	CAVATAK [115] PVS-RIPO [116]	No transgenes

HSV-1, Herpes simplex virus 1; ds, double-stranded; ss, single-stranded; IFN, interferon; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-2, interleukin 2; TNF-α, tumour necrosis factor alpha; TAA, tumour-associated antigen; MAGE-A3, melanoma-associated antigen 3.

therapy administration in combination with OV therapies, as well as choosing the best ICI(s) will require further investigation to derive the best outcome for future patients. The recent unfortunate failure of Pexa-Vec to show a synergistic effect in combination with sorafenib in patients with hepatocellular carcinoma in two clinical trials (NCT01387555 and NCT02562755) highlights the difficulty in translating preclinical success to clinical success [109,110]. Accumulating clinical evidence suggests that OV therapy is more effective when given to earlier-stage or treatment-naïve patients, which may be expected since OV-induced anti-tumour immunity might be more achievable in earlier stage patients than pre-treated late stage patients with, on average, more suppressed immune status [16••,18,110].

Conclusions and perspectives

OVs have markedly increased the efficacy of immune checkpoint inhibitors in recent early/mid stage clinical evaluations, and the OV field is now eagerly waiting for the confirmation from the first phase III clinical trial of OV + ICI combination using T-VEC with pembrolizumab (NCT02263508). In addition to T-VEC, various other OVs have entered into clinical evaluation with ICIs as well as other immunotherapeutic platforms. The diversity of OVs in clinical testing indicates that there is no clear winner, a virus that could be efficiently used in all indications, but rather, the choice of OV may depend on patient intrinsic factors such as size, location and origin of the tumour, as well as virus intrinsic factors such as replication speed, oncolytic activity, immunogenicity, cancer cell tropism and suitability for systemic administration (see Table 2 for main characteristics of OVs used in cancer immunotherapy). Recent advances on improving oncolytic viruses as cancer vaccines as well as the ability of OVs to enhance other cancer vaccine platforms enable OV field to move swiftly towards personalized cancer immunotherapy. OVs in combination with other cancer immunotherapies have the potential to deliver more safe and efficacious treatment modalities for patients in the foreseeable future.

Conflict of interest statement

V.C. is a co-founder and shareholder of VALO therapeutics.

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