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REVIEW

Oncolytic viruses: a step into cancer immunotherapy

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Abstract: Oncolytic virotherapy is currently under investigation in phase I–III clinical trials for approval as a new cancer treatment. Oncolytic viruses (OVs) selectively infect, replicate in, and kill tumor cells. For a long time, the therapeutic efficacy was thought to depend on the direct viral oncolysis (virocentric view). The host immune system was considered as a brake that impaired virus delivery and spread. Attention was paid primarily to approaches enhancing virus tumor selectivity and cytotoxicity and/or that limited antiviral responses. Thinking has changed over the past few years with the discovery that OV therapy was also inducing indirect oncolysis mechanisms. Among them, induction of an antitumor immunity following OV injection appeared to be a key factor for an efficient therapeutic activity (immunocentric view). Indeed, tumor-specific immune cells persist post-therapy and can search and destroy any tumor cells that escape the OVs, and thus immune memory may prevent relapse of the disease. Various strategies, which are summarized in this manuscript, have been developed to enhance the efficacy of OV therapy with a focus on its immunotherapeutic aspects. These include genetic engineering and combination with existing cancer treatments. Several are currently being evaluated in human patients and already display promising efficacy.

Keywords: oncolytic virus, cancer immunotherapy, tumor antigen, cancer vaccine, combination strategies

Cancer and the immune system

In 2006, the World Health Organization reported cancer as the second cause of death in developed countries and the third cause worldwide.[1] Numerous risk factors responsible for cancer development have been characterized, and two-thirds appeared to be associated with lifestyle. In high-income countries, smoking, alcohol use, and obesity represent the main risk factors.[2] Age aside, other factors include an unhealthy diet and lack of exercise, chronic infection (eg, hepatitis B virus [HBV], hepatitis C virus, human papillomavirus [HPV], Helicobacter pylori), or exposure to carcinogens: natural (eg, aflatoxin B1), chemical (eg, benzene, arsenic), radionuclide, or radiation (eg, ultraviolet). Some inherited genetic factors can also predispose to cancer development (eg, mutations in BRCA1 and BRCA2 genes for breast cancer).[3] Although treatments for the disease have significantly improved, conventional therapies still have limited effects against many forms of neoplasm. As a consequence, projections to 2030 are pessimistic, with an increased impact of cancers on global mortality.[4] Thus, reducing cancer-associated mortality will mean behavioral changes together with improvement and expansion of therapeutic strategies.

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Carcinogenesis

Upon exposure to the cancer risk factors mentioned, carcinogenesis takes decades in adults and occurs in three consecutive steps: initiation, promotion, and progression. Tumor initiation results from genetic and/or epigenetic mutations in growth-regulatory genes encoding tumor suppressors (eg, p53, Rb) or proto-oncogenes (eg, Ras, Myc). Tumor promotion consists of expansion of some initiated cells that have acquired a growth advantage over noninitiated cells. During replication, cancer cells accumulate mutations affecting genes involved in various cellular functions such as gene regulation (eg, TP53, RB1, JUN, MYC), DNA repair (eg, BRCA1, ATM), DNA replication (eg, hTERT, CDC6), chromosome segregation (eg, BUB1B), cell cycle checkpoints (eg, TP53, CCND1, CDKN2), viability (eg, TP53, BCL2, PTEN), or intra-/inter-/extracellular signalling components (eg, RAS, APC, AKT, EGFR, HER2, CDH1, CTNNB1).[5–9] Tumor progression refers to the stepwise transformation of a benign tumor to a neoplasm and to malignancy. Hallmarks of tumor malignancy were summarized by Hanahan and Weinberg[10] as follows: (1) sustaining proliferative signalling, (2) evading growth suppressors, (3) enabling replicative immortality, (4) resisting cell death, (5) inducing angiogenesis, and (6) activating invasion and metastasis.

Cancer immunoediting

Intrinsic and extrinsic tumor suppressor mechanisms help prevent or slow down carcinogenesis all through life. Intrinsic tumor suppressor mechanisms consist of DNA repair and the death of mutated cells through apoptosis or senescence. After cellular transformation occurs and intrinsic tumor suppression fails, extrinsic tumor suppressor mechanisms are engaged. Extrinsic tumor suppressor mechanisms refer to the involvement of the immune system in eliminating tumor cells or preventing their outgrowth. In 2001, Schreiber et al observed that the immune system not only protects the host against tumor formation but also shapes tumor immunogenicity.[11] This notion led to revision of the cancer immunosurveillance hypothesis by introducing the concept of cancer immunoediting.[12] They postulate that cancer immunoediting proceeds sequentially through three distinct phases termed elimination, equilibrium, and escape.

The elimination phase would correspond to cancer immunosurveillance in which innate (natural killer cells [NKs], dendritic cells [DCs], macrophages) and adaptive (T cells and NK T cells) immune systems are involved in detecting and destroying developing tumors. Mechanisms involved in tumor recognition by the immune cells are not fully understood. Induction of type I interferons (IFNs) during early steps of tumor development, release of different damage-associated molecular patterns (DAMPs) from dying tumor cells or damaged tissues (eg, HMGB1), and expression of stress ligands (eg, NKG2D ligands MICA/B) on the surface of tumor cells have been described. Additionally, effective cancer immunosurveillance requires the expression of tumor antigens that are able to induce expansion of effector $CD4^+$ and $CD8^+$ T cells.[13,14]

A limited number of tumor cell variants can survive elimination and enter the equilibrium phase. Functionally, these surviving tumor cells appear dormant. Disruption of crosstalk between growth factor and adhesion signalling during the elimination phase is, in part, responsible for this state.[15] During the equilibrium phase, which may last for the lifetime of the host, the adaptive immune system (T cells, interleukin [IL]-12, and IFN-γ), but not innate immunity, prevents tumor outgrowth but also edits cancer immunogenicity. Indeed, by eliminating immunogenic tumor cells, T cells exert a selective pressure on occult tumor cell populations and may favor the emergence of variants able to escape immunity.[13,14]

Tumor cell variants that enter the escape phase evolve different processes to elude or inhibit immune recognition and/or destruction (Figure 1A). First, they are poorly immunogenic and no longer recognized by adaptive immunity due to antigen loss or defects in antigen processing or presentation (eg, decreased major histocompatibility complex [MHC] class I expression). Second, they become resistant to immune cytotoxic effect by overexpression of antiapoptotic molecules (eg, Bcl-2, Bcl-xL), persistent activation of pro-oncogenic transcription factors (eg, STAT3), or expression of surface molecules inducing cytotoxic T lymphocyte (CTL) killing (eg, FasL, PD-L1). Third, they can generate an immunosuppressive microenvironment by secreting cytokines that inhibit effector immune cell functions (eg, transforming growth factor [TGF]-β, IL-10, galectin-1, indoleamine 2,3-dioxygenase) or by recruiting effector cells of immunosuppression like regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). MDSCs can block T-cell function by secreting TGF-β, arginase 1, and nitric oxide synthase. Tregs can inhibit effector T cells by expressing PD-L1 and CTLA-4 on their surface or secreting TGF-β and IL-10. Over time, these selected tumor cell variants acquire the capacity to grow, leading to clinically apparent disease.[13,14]

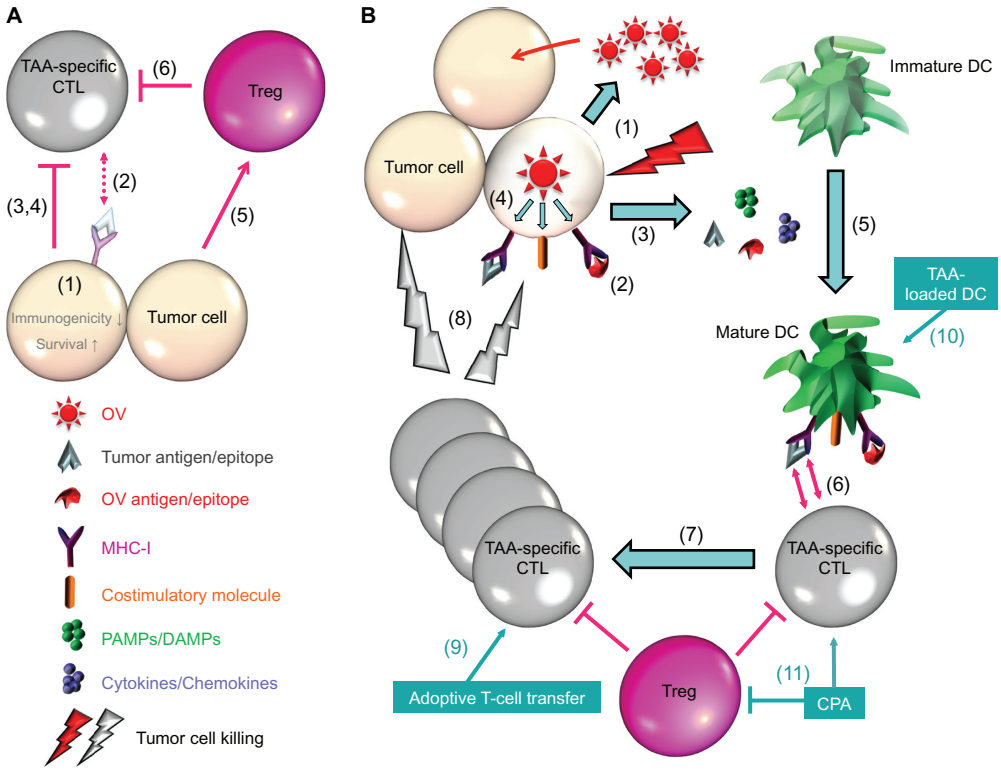

Figure 1 Tumor immunotolerance (**A**). Tumor cells evolve different processes to elude or inhibit immune recognition and/or destruction. (1,2) First, they are poorly immunogenic and no longer recognized by adaptive immunity due to antigen loss or defects in tumor-associated antigen (TAA) processing or presentation. Second, they become resistant to immune cytotoxic effect by overexpression of antiapoptotic molecules (eg, Bcl-2, Bcl-xL) and persistent activation of pro-oncogenic transcription factors (eg, STAT3). Third, they can generate an immunosuppressive microenvironment by (3) expressing surface molecules that induce inactivation/killing of cytotoxic T lymphocytes (CTLs) (eg, FasL, PD-L1), (4) by secreting cytokines that inhibit effector immune cell functions (eg, transforming growth factor [TGF]-β, interleukin [IL]-10, galectin-1, indoleamine 2,3-dioxygenase), or (5) by recruiting effector cells of immunosuppression-like regulatory T cells (Tregs). (6) Tregs can inhibit effector T cells by expressing PD-L1 and CTLA-4 on their surface or secreting TGF-β and IL-10. (**B**) Oncolytic virotherapy as/in cancer immunotherapy. (1) Tumor cell infection by oncolytic viruses (OVs) leads to cell death and release of progeny virions that are able to infect adjacent tumor cells. Additionally, OV infection produces and releases immunostimulatory molecules that contribute to break immunotolerance and to reactivate antitumor immunity. (2, 3) First, OV replication produces viral pathogen-associated molecular patterns (PAMPs) including viral antigens that can be presented onto the surface of infected tumor cells or released in the tumor microenvironment. (3) Second, stress induced by OV infection also releases cytokines and chemokines, damage-associated molecular patterns (DAMPs) (eg, HMGB1, uric acid, heat-shock proteins), and TAA. (4) Insertion of transgenes expressing TAA, cytokines/chemokines, or costimulatory molecules (eg, CD80) into OVs can improve stimulation of antitumor immunity. (5) All these immunostimulatory molecules contribute to dendritic cell (DC) maturation. (6, 7) After taking up released TAAs, mature DCs can present them to cognate T cells, including CTLs, which will undergo proliferation. (8) TAA-specific CTLs can then migrate to the tumor site and kill tumor cells that have not been infected by OVs. DC activation following OV therapy has also been described to stimulate natural killer cell-mediated antitumor activity.[179,191,200] OV-induced antitumor immunity can be enhanced by combination with other cancer treatments such as cell therapies like (9) adoptive transfer of TAA-specific T cells and (10) DC-based vaccines or immunomodulatory drugs like (11) cyclophosphamide (CPA), which can both deplete Tregs and promote T-cell activation.
Abbreviation: MHC-I, major histocompatibility complex class I.

Cancer immunotherapy

Together with surgery, common cancer treatments consist of radiotherapy, chemotherapy, and immunotherapy. Alternatively, hormone therapy and tissue transplantation can be used to treat some neoplasms such as breast cancer (blocking estrogen synthesis or binding to its receptor) and lymphoma (bone marrow transplant).[16,17] Radiotherapy and chemotherapy induce tumor cytotoxicity by breaking DNA (X-rays), blocking DNA replication (eg, cisplatin) or its transcription (eg, histone deacetylase inhibitors [HDACis]), and/or blocking mitosis (eg, paclitaxel). Immunotherapy mainly consists of stimulating the extrinsic tumor suppressor mechanisms by increasing the quality and/or quantity of immune effector cells, increasing tumor immunogenicity, or decreasing cancer-induced immunosuppressive mechanisms. Multiple immunotherapeutic strategies are being explored (for review, see Dougan and Dranoff[18]).

Passive immunotherapy

First, administration of monoclonal antibodies (mAbs) is a potent cancer treatment. Depending on the nature of the target (soluble or membrane-bound), binding of mAbs results in steric inhibition and neutralization, modulation of downstream signalling pathways, complement activation,

and/or induction of the antibody-dependent cellular cytotoxicity. Twelve mAbs are clinically approved for treatment of various cancers. Nine are targeting tumor-associated surface proteins. Most of these targets are markers of hematologic tumors. Rituximab, ibritumomab tiuxetan, tositumomab, and ofatumumab bind to CD20 (associated to non-Hodgkin's lymphoma and chronic lymphocytic leukemia [CLL]), alemtuzumab binds to CD52 (CLL), and gemtuzumab ozogamicin binds to CD33 (acute myeloid leukemia). Trastuzumab, cetuximab, and panitumumab bind to epidermal growth factor receptor (EGFR) family members expressed on solid tumors.[18,19] The last three mAbs approved do not directly target malignant cells. Bevacizumab binds to soluble vascular endothelial growth factor (VEGF), inhibiting tumor angiogenesis, while denosumab binds to RANKL, thus inhibiting osteoclast-mediated bone destruction and preventing skeletal-related events in patients with bone metastases. Finally, ipilimumab binds to CTLA-4 expressed on Tregs and CTL and blocks its inhibitory activity, thereby sustaining immune responses.[18,20,21]

A second strategy for cancer immunotherapy consists of administering cytokines (eg, IL-2, IFN-α, granulocyte-macrophage colony-stimulating factor [GM-CSF]), adjuvants (eg, BCG, imiquimod), or immunomodulating agents (eg, thalidomide) to boost patient immune systems. Cytokines are also often used as adjuvants. IL-2 induces the proliferation of responsive T cells. GM-CSF stimulates the production of granulocytes and monocytes and promotes DC recruitment and activation. IFN-α shows pleiotropic effects. It increases tumor immunogenicity by upregulating genes encoding MHC-I and some tumor antigens, has antiangiogenic properties, promotes T- and B-cell activity, stimulates macrophages and DCs, and upregulates Fc receptors.[22–27]

Third, adoptive T-cell transfer strategy involves ex vivo identification and expansion of autologous or allogeneic tumor-specific lymphocytes that are then infused into cancer patients. So far, this approach is the most effective treatment for patients with metastatic melanoma. It can mediate objective cancer regression in approximately 50% of patients with metastatic melanoma refractory to all other treatments. Lymphocytes can be extracted from blood, tumor-draining lymph nodes, malignant effusions, or, if possible, from the tumor (tumor-infiltrating lymphocytes [TILs]) of patients. Their antitumor activity arises either naturally or after genetic engineering (eg, expression of tumor antigen-specific T-cell receptors). Ex vivo expansion (using growth factors such as IL-2) allows administration of 10–100 billion tumor-specific lymphocytes to patients. In vivo injection usually follows a lymphodepleting regimen (eg, body irradiation or cyclophosphamide/fludarabine chemotherapy) and often comes along with growth factors (eg, IL-2) or vaccines to stimulate survival and proliferation of infused lymphocytes.[28,29]

Active immunotherapy (cancer vaccines)

All strategies mentioned previously depend on supplying the immune system with infused short-lived molecules or cells. Because the immune system is not directly engaged to fight the tumor (passive immunity), infusions must be repeated and treatment efficacy may not be optimal. In the last decade, cancer vaccine strategies have expanded. They aim at priming an endogenous antitumor response to generate active immunity. Because some cancers are caused by chronic infections, therapies that clear or prevent infection of the corresponding agent are defined as prophylactic cancer vaccines (eg, HBV and HPV16/18 vaccines against liver and cervical cancers, respectively, or antibiotics versus H. pylori against stomach cancer). Their efficacy relies on priming an immune response specific to foreign antigens expressed by the infectious agent. The development of therapeutic cancer vaccines is much more challenging. Indeed, their efficacy depends on priming a response against an established tumor mediating immune tolerance. Many approaches for therapeutic cancer vaccines have been attempted with limited efficacy so far. Antigen-specific, idiotype-specific, DC-based, and whole tumor cell-based vaccines currently figure among the most promising.

DCs are the most potent professional antigen-presenting cells (APCs). They play a critical role in priming and regulating T- and B-cell responses. For this reason, their enrollment appears to be the key for the success of any vaccine approach. Immature DCs (iDCs) patrol in tissues looking for dying cells or pathogens. They undergo maturation once exposed to inflammatory signals (eg, TNF-α, IL-1β, IL-6), DAMPs (eg, HMGB1, heat-shock proteins [HSPs], nucleic acids), and/or pathogen-associated molecular patterns (PAMPs) (eg, LPS, dsRNA). Mature DCs have improved antigen-presenting abilities and increased expression of T-cell costimulatory molecules (CD80, CD83, CD86, CD40). They also acquire migratory potential by upregulating the chemotactic receptor CCR7, which will bring them to a lymph node or to the spleen. There, mature DCs will encounter and present antigen on MHC-II to cognate $CD4^+$ T cells. Interaction between CD40 on the DCs and CD40L, expressed on the antigen-activated $CD4^+$ T cell, induces the final maturation step of DCs, known as licensing. Licensed DCs upregulate additional cell surface products, such as OX40L and 4-1BBL,

and present antigen on MHC-I to cognate $CD8^+$ T cells. They also secrete IL-12 and stimulate survival and proliferation of antigen-activated $CD8^+$ and $CD4^+$ T cells through the crosslinking of 4-1BBL with 4-1BB expressed on activated $CD8^+$ T cells, and of OX40L with OX40 expressed on activated $CD4^+$ T cells.[30]

Antigen-specific vaccines are based on the delivery of tumor antigens. Antigens are delivered as recombinant proteins[31,32] or immunogenic peptides,[33] often in combination with adjuvant (eg, CpG) or using naked plasmid DNA,[34] bacterial vectors (eg, Salmonella typhimurium),[35] or viral vectors (eg, recombinant poxviruses).[36] In vivo, tumor antigens can then be taken up and presented by APCs and elicit cellular and humoural antitumor responses. Tumor antigens can be shared by both normal and tumor cells and described as tumor-associated antigens (TAAs), or expressed only on tumor cells and defined as tumor-specific antigens (TSAs). These antigens can be aberrantly expressed differentiation or embryonic markers, overexpressed or mutated cellular proteins, or viral proteins (eg, retrovirus, HBV). A list of characterized tumor antigens with their immunogenic epitopes is available at http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm.[37] A wide range of neoplasms displays aberrant expression of differentiation factors that mainly belong to the cancer-testis antigen (CTA) family. Two CTA vaccines, against MAGE-A3 and NY-ESO-1 antigens, are being evaluated clinically.[38] Results from numerous clinical trials in melanoma and nonsmall-cell lung cancer patients are encouraging, revealing their ability to elicit antitumor B- and T-cell responses.[31,36,39–42]

Idiotype-specific vaccines are based on immunization of patients against their own tumor idiotype. This type of vaccination is being improved for treating various cancers and, more particularly, mature B-cell neoplasms.[43–45] Each plasma B cell produces one single kind of antibody. Antibodies produced are either secreted or anchored to the plasma membrane functioning as the B-cell receptor. The variable regions of the heavy and light chains of an immunoglobulin contain a unique set of antigenic determinants (idiotopes) called idiotypes. Idiotypes have the characteristics of tumor antigens, in this case the immunoglobulins expressed at the surface of malignant B cells, and can then be targeted with anti-idiotype antibodies. Idiotype-specific vaccines often include anti-idiotype antibodies together with a carrier (eg, keyhole-limpet hemocyanin) and an adjuvant (eg, GM-CSF) to overcome immune tolerance. Several idiotype-specific vaccines have reached phase III clinical trials for the treatment of follicular lymphomas with limited results so far.[43]

DC-based vaccines consist of the autologous transfer of DCs loaded ex vivo with tumor antigen(s). DCs are often generated from circulating monocytes through culture in serum-free medium with GM-CSF in combination with IL-4 or IL-15.[46] These iDCs are then loaded with TAA/TSA. Antigen(s) can be provided in many forms: (1) exogenously as peptides, protein, tumor lysate, complexed with antibody, or by fusing DCs with tumor cells (using polyethylene glycol or electrical fields) or (2) endogenously by transfection or transduction (eg, using adenoviral vector) of nucleic acids encoding a TAA/TSA. Loaded iDCs can be infused into a patient's tumor together with adjuvant (eg, TLR7 agonist imiquimod) to stimulate their maturation in situ or, more often, delivered subcutaneously or intravenously.[47,48] These loaded iDCs can also be matured ex vivo prior to infusion. Protocols often involve their incubation with a cocktail of inflammatory cytokines (eg, TNF-α, IL-1β, IFN-α, IFN-γ) together with activators of the TLR signalling (eg, polyI:C/TLR3, LPS/TLR4, imiquimod/TLR7, CpG/TLR9).[30,49] In addition, DCs can be modified ex vivo to express chemokines, cytokines, and costimulatory molecules to provide a more robust and persistent anticancer immunity in vivo (for review, see Boudreau et al[50]). Sipuleucel-T is a DC-based vaccine applied for treating patients with hormone-refractory prostate cancer. The vaccine is prepared from isolated DC precursors matured by incubation with a fusion protein consisting of GM-CSF and the cancer prostate antigen PAP. In April 2010, Sipuleucel-T became the first therapeutic cancer vaccine approved by the Food and Drug Administration, opening a new era for cancer vaccines.[51,52]

Tumor cell-based vaccines are made from autologous or allogeneic tumor cells removed during surgery and manipulated ex vivo. These cells are expanded in culture, then irradiated or lysed before their in vivo infusion, often together with adjuvants. To improve the vaccine potency, tumor cells can also be genetically modified to express cytokines (eg, GM-CSF), growth factors (eg, EGF), human leucocyte antigen, or costimulatory molecules (eg, CD80). Compared with other cancer vaccine approaches, whole tumor cell vaccines are not restricted to a limited number of TAAs/TSAs. Patients' APCs are virtually able to take up the full pattern of tumor antigens (including undiscovered ones) and present it to T cells. As a consequence, the immune response primed is highly specific to the patient's tumor. However, induction of autoimmunity is more likely to occur. Several tumor cell vaccines have already reached phase III clinical trials with promising results (eg, autologous whole cell vaccine OncoVax given with BCG adjuvant for colon cancer).[53]

Immunotherapy and combination strategies

As mentioned, radio-, chemo-, and immunotherapies have limited clinical efficacy for many cancer types. Optimal cancer treatment would imply acting simultaneously on multiple "fronts" such as viability/growth/immunogenicity of tumor cells, quality/quantity of immune effector cells, or factors responsible for tumor immunosuppression, angiogenesis, and evasion. Thus, combining various therapeutic approaches that act separately or cooperatively on these "fronts" must be considered. Combination of passive immunotherapy with radiotherapy (ibritumomab tiuxetan, tositumomab), chemotherapy (gemtuzumab ozogamicin), or biotherapy (denileukin diftitox) as immunoconjugates has been approved for over a decade.[18] Most of these immunoconjugates induce both direct and immune-mediated tumor cytotoxicity. Combining immunotherapy with chemotherapy can appear antagonistic at first sight. Indeed, chemotherapy induces not only tumor cell apoptosis, which has been regarded as nonimmunogenic or even tolerogenic, but also lymphodepletion. However, several studies provide mounting evidence that, depending on the dose and timing of administration, some chemotherapeutic agents such as cyclophosphamide (CPA) can improve antitumor immunity.[54,55] CPA showed pleiotropic effect that covers most of the "fronts": (1) direct tumor cytotoxicity, (2) depletion of immunosuppressive Tregs, (3) activation and proliferation of T and B cells, (4) promote infiltration of tumor-specific lymphocytes inside the tumor, (5) increase number and activation status of myeloid DCs, and (6) promote emergence of tumor-infiltrating DCs secreting more IL-12 and less IL-10.[54,56,57] Combining several immunotherapeutic approaches is also very promising. So far, this mostly implies coadministration of well-characterized adjuvants (eg, IL-2, GM-CSF, BCG) together with tumor-specific mAbs, adoptively transferred cells, or cancer vaccines to stimulate the recruitment and activity of immune effector cells. New adjuvants like TLR9 agonists and α-galactosylceramide are also being tested.[18] Approved mAbs such as ipilimumab and bevacizumab reducing, respectively, immunosuppression and angiogenesis may also become common additives. In the same perspective, new mAbs are currently being evaluated. They block immunosuppressive cytokines (eg, anti-IL-10, IL-13, TGF-β, or VEGF) or immune inhibitory signals in lymphocytes (eg, anti-PD-1) or their ligand (eg, anti-PD-L1), or act as agonist of immunostimulatory receptors (eg, anti-CD40, 4-1BB, OX34).[18] Other immunotherapeutic combinations are being evaluated to improve efficacy of cancer vaccines, such as (1) combining cancer vaccine with mAbs targeting the same TAAs/TSAs,[58,59] (2) sequential administration of cancer vaccines expressing/carrying the same TAAs/TSAs (prime-boost strategy),[60–64] or (3) targeting different TAAs/TSAs simultaneously.[65,66] Finally, combining cancer immunotherapy with oncolytic virotherapy also raises a lot of hope and will be discussed in this review.

Oncolytic viruses

Oncolytic virotherapy consists of administering viruses that selectively infect, replicate in, and kill tumor cells with no or limited impact on normal tissues. Viral oncolytic properties have been reported since the middle of the 19th century, before the actual discovery of viruses. At that time, some patients with hematologic malignancy showed transient remission after naturally occurring infections.[67] In 1949, Moore[68,69] demonstrated selective destruction of murine tumors by the Russian Far East encephalitis virus, opening the field of oncolytic virotherapy. After a peak of interest in the 1950s–60s with the first clinical trials, the field was nearly abandoned. Extended knowledge in virology and molecular biology led to its rebirth 20 years ago with the first human clinical trial using a recombinant oncolytic virus (OV).[67,70] Since then, several viruses showing oncolytic ability have been identified and engaged in preclinical and clinical studies (Table 1). The first OV was approved in China in 2005 for treating nasopharyngeal cancer (in combination with chemotherapy), and several are undergoing phase III clinical trials in the US (Table 1).[71,72]

Tumor selectivity

OVs are human (eg, herpes simplex virus [HSV], adenovirus [Ad], measles virus [MV]) or veterinary (eg, vesicular stomatitis virus [VSV], Newcastle disease virus [NDV], myxomavirus [MYXV]) viruses engineered to have, or naturally having, little pathology in humans. Also, their oncotropism can be inherent or acquired after genetic engineering.

Inherent oncotropism refers to OVs that are naturally able to infect and replicate in tumor cells. First, it implies that tumor cells express the surface receptor(s) required for OV binding/entry. Expression of these surface receptors can be aspecific (eg, CAR for Ad, CD46/CD150 for MV) or specific to malignant phenotype (eg, overexpression of the high-affinity laminin receptor used by the Sindbis virus,[112,113]

Table 1 List of candidates for oncolytic virotherapy identified during the last 20 years

Genome	Family	Genus	Strain(s)	Evaluation stage[a]/patented OV (cancer treated)
DNA	Adenoviridae	*Mastadenovirus*	Human adenovirus C serotype 5	Phase I–II/ONYX-015 (HNSCC, oropharyngeal cancers – Clinicaltrials.gov identifier[b]: NCT00006106); Approved in China/H101 Oncorine (nasopharyngeal cancer)[72,73]
			Human adenovirus C serotype 6	Experimental[74]
			Human adenovirus B serotype 3	Experimental[75]
			Human adenovirus B serotype 11	Experimental[76]
	Herpesviridae	*Simplexvirus*	Herpes simplex virus 1	Phase III/OncoVEX^{GM-CSF} (melanoma – Clinicaltrials.gov identifier: NCT00769704)[71,77]
			Herpes simplex virus 2	Preclinical (breast cancer, neuroblastoma)[78,79]
		Varicellovirus	Bovine herpesvirus 1	Experimental[80]
			Suid herpesvirus 1	Preclinical (bladder cancer)[81]
		Rhadinovirus	Bovine herpesvirus 4	Preclinical (glioma)[82]
			Herpesvirus saimiri	Experimental[83]
	Parvoviridae	*Parvovirus*	H-1PV	Phase I–II/ParvOryx (GBM – Clinicaltrials.gov identifier: NCT01301430)
			Minute virus of mice	Experimental[84]
	Poxviridae	*Orthopoxvirus*	Vaccinia virus	Phase II/JX-594 (HCC – Clinicaltrials.gov identifier: NCT00554372)[85]
			Raccoonpox virus	Preclinical (colon carcinoma, glioma)[86]
		Leporipoxvirus	Myxomavirus	Preclinical (glioma)[87]
RNA	Coronaviridae	*Coronavirus*	Feline infectious peritonitis virus	Experimental[88]
			Murine hepatitis virus	Experimental[89]
	Orthomyxoviridae	*Influenzavirus*	Influenza A	Experimental[90]
	Paramyxoviridae	*Avulavirus*	Newcastle disease virus	Phase I–II (metastatic cancers, GBM – Clinicaltrials.gov identifiers: NCT00348842, NCT01174537)
		Morbillivirus	Measles virus	Phase I (ovarian cancer, multiple myeloma, plasma cell neoplasm – Clinicaltrials.gov identifiers: NCT00408590, NCT00450814)[91]
		Respirovirus	Sendai virus	Preclinical (GBM)[92]
		Rubulavirus	Mumps virus	Experimental[93]
	Picornaviridae	*Cardiovirus*	Encephalomyocarditis virus	Experimental[94,95]
		Enterovirus	Coxsackievirus A21	Phase I (melanoma, HNSCC – Clinicaltrials.gov identifiers: NCT00832559, NCT00438009)
			Coxsackievirus A13, A15, A18	Experimental[96]
			Poliovirus	Preclinical (neuroblastoma)[97]
			Echovirus 1	Experimental[98–100]
			Bovine enterovirus	Experimental[101]
		Senecavirus	Seneca valley virus	Phase I/NTX-10 (advanced solid tumors with neuroendocrine features – Clinicaltrials.gov identifier: NCT00314925)
	Reoviridae	*Orbivirus*	Bluetongue virus-10	Experimental[102]
		Orthoreovirus	Reovirus serotype 3	Phase III/Reolysin (HNSCC – Clinicaltrials.gov identifier: NCT01166542)[71]
	Retroviridae	*Gammaretrovirus*	(Moloney) Murine leukemia virus	Experimental[103,104]
		Spumavirus	Foamy virus	Experimental[105]
	Rhabdoviridae	*Vesiculovirus*	Vesicular stomatitis virus	Phase I (HCC)[106]
			Maraba virus, Farmington virus	Preclinical studies ongoing in our group and in Stojdl's group (melanoma, glioma)[107]
			Bahia Grande virus, Carajas virus, Muir Springs virus, Tibrogargan virus	Experimental[107]
	Togaviridae	*Alphavirus*	Semliki forest virus	Preclinical (ovarian cancer)[108,109]
			Sindbis virus	Experimental[110,111]

Notes: [a]Experimental stage = in vitro/in immunodeficient animal; preclinical stage = in immunocompetent animal; clinical stage (phase I, II, or III) = in human; [b]ongoing clinical trials involving OVs are detailed in http://clinicaltrials.gov/; some corresponding identifier numbers are included in the table.
Abbreviations: GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; OV, oncolytic virus.

overexpression of ICAM-1 and DAF used by Coxsackievirus A21).[114] Second, it implies that permissiveness of OV replication depends on factors associated with neoplasia. These factors include defective IFN response (eg, VSV, MYXV),[115,116] aberrant cell cycle control (eg, parvovirus),[117] resistance to apoptosis (eg, NDV),[118] constitutive activation of Ras (eg, reovirus)[119] or Akt (eg, MYXV),[120] or, again, alteration of the extracellular matrix (eg, HSV-1).[121]

In most cases, viral oncotropism is genetically engineered. Genetic modifications aim at improving virus targeting to tumor cells, reducing virulence in normal tissues, and/or increasing dependency of OV genome expression/replication to the malignant phenotype.

Virus retargeting mainly involves fusing the virus attachment protein (eg, Ad fibre knob, MV hemagglutinin/H protein, HSV glycoprotein C) to a single-chain antibody or a peptide known to bind a tumor-associated receptor.[122–128] The receptor targeted can be overexpressed in a wide range of tumors (eg, EGFR, integrin αvβ6, uPAR)[122,126,128] or limited to particular cancers (eg, EGFRvIII mutant/glioma, HER2/breast cancer, PSMA/prostate cancer).[123,125,127,129] Another way to improve OV targeting to tumor cells consists of rendering their binding/entry dependent on proteases secreted in the tumor microenvironment (eg, MMP, uPA).[92,130,131] For example, the F protein of the Sendai virus needs to be cleaved by trypsin to allow fusion between viral and cellular membranes. Replacing the trypsin cleavage site by one of the uPA proteases, secreted in the extracellular matrix of solid tumors, limits virus infection to tumor tissue.[92,130]

Oncotropism can also be generated or improved by mutating viral genes required for virus survival in normal cells but not in malignant cells. For example, deletion/modification of the HSV genes encoding the neurovirulence factor ICP34.5 and the nucleotide reductase ICP6 abolishes its natural neurotropism without affecting its oncotropism.[132,133] Deletion of E1A and E1B genes results in a restriction of Ad replication to cells with defects in Rb and p53-controlled tumor suppressor pathways. This defect is characteristic of well over 50% of tumor cells.[134,135] Deletion of the vaccinia virus (VV) genes encoding the thymidine kinase (TK) and the vaccinia growth factor ensures that virus replication is limited to rapidly dividing cells.[136] Oncotropism can also be ensured or improved by inserting regulatory elements in viral genes. These elements can be promoter sequence from active tumor genes (eg, hTERT promoter),[137–140] 5′UTR sequence recruiting tumor-associated translation factors (eg, eIF4E overexpressed in various cancers),[141] or 3′UTR sequence complementary to cellular miRNA (eg, let-7a miRNA downregulated in many tumors).[142–145]

Tumor killing

Tumor killing by OV can result directly from the viral cycle. Cell death can be the consequence of a lytic viral replication (eg, Ad, HSV). Some viral proteins can also induce apoptosis or necrosis, such as the adenoviral proteins E3 11.6K and E4ORF4 or the F protein of paramyxoviruses (eg, MV, NDV) responsible for syncytia formation.[146,147] Autophagic cell death has also been described following infection of brain cancer-initiating cells with oncolytic Ad.[148] Additionally, OVs can be genetically armed to improve direct oncolysis. In this case, cell death can be induced through transgene expression of viral or cellular proapoptotic proteins (eg, TRAIL, IL-24),[149–153] tumor-suppressors (eg, p53, p16, SOCS3),[154–156] or small hairpin RNA targeting factors involved in cell survival or proliferation (eg, hTERT, survivin, apollon, Ki67).[157–161]

The efficacy of oncolytic virotherapy also relies on indirect means of oncolysis. Among the mechanisms involved, stimulation of antitumor immunity plays a critical role. The discovery that OV therapy was acting like immunotherapy deeply redefined the strategies for applying OVs to cancer treatment. This point will be detailed in the next paragraphs. OV therapy can also induce tumor vasculature shutdown, leading to massive tumor necrosis. This phenomenon has been described, both in preclinical and clinical studies, following administration of oncolytic VSV and VV, respectively.[162,163] Additionally, strategies involving genetic engineering have been developed to enhance indirect oncolysis. First, some recombinant OVs have been developed to sensitize tumor cells to chemotherapy. In this case, the OV expresses an enzyme that activates an administered prodrug. For example, the TK of herpesviruses converts ganciclovir into a guanine analog responsible for DNA synthesis inhibition and cell death. TK-expressing oncolytic Ad combined to ganciclovir have already shown efficacy against various types of cancers.[134,164,165] Alternative combinations have been tested, converting prodrugs into other cytotoxic nucleotide analogs (eg, purine nucleoside phosphorylase/fludarabine, cytosine deaminase/5-fluorocytosine) or into alkylating agents (eg, nitroreductase/CB1954).[166–169] Second, some OVs have been developed to sensitize tumor cells to radiotherapy. As examples, VSV, Ad, and MV expressing the sodium symporter NIS allowed tumor-targeted radio iodide uptake in multiple myeloma, prostate cancer, and hepatocellular

carcinoma cells, respectively. This combination significantly improved the efficacy of OV therapy.[170–175]

Oncolytic viruses as/in immunotherapy

The field of viral oncolytics has come to recognize the importance of the host immune response in determining clinical outcomes. Immune responses against viral vectors likely impair viral oncolysis, thereby representing a barrier to clinical success. On the other hand, immune responses against the tumor should aid in tumor destruction and can potentially prevent disease relapse.

Induction of antitumor immune responses following oncolytic virotherapy

As mentioned, the impact of OVs is not restricted to direct tumor cytolysis but also depends on the resultant immune response. Tumor cell infection by OVs should be highly immunogenic due to cell death, production of cytokines and danger signals, and release of tumor antigens (Figure 1B).[176–178] These changes inside the tumor bed may affect the established immunosuppressive microenvironment and initiate antitumor immunity.

As an illustration, melanoma cell lines infected with reovirus secreted the proinflammatory cytokines IL-6, IL-8, and IFN-β together with the chemokines CXCL-10/IP-10, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL11/eotaxin.[179–181] At the same time, some infected cell lines showed a decreased secretion of the immunosuppressive IL-10.[181] Additionally, OV infection may be a source of immunogenic danger signals. First, it produces PAMPs: viral proteins and nucleic acids (dsRNA, unmethylated CpG motif DNA). Then, virus-induced stress and cell death programs may trigger the synthesis and spread of DAMPs in the tumor microenvironment. Immunogenicity of cell death depends on its type: necrosis, apoptosis, or autophagy, each of them differing by the pattern of cytokines and DAMPs produced (for review, see Kepp et al[182] and Tesniere et al[183]). Release of intracellular HMGB1, uric acid, HSP70, or HSP27, related to necrosis, has been reported following infection with oncolytic Ad, VV, MV, or, again, NDV.[177,184–187] To note, even apoptotic cell death can be immunogenic under certain circumstances.[182,183] Finally, like tumor cell vaccines, viral oncolysis may also lead to the release of the whole set of TAAs/TSAs.[188]

Taken together, this cloud of inflammatory molecules facilitates immune cell recruitment and homing to the tumor and promotes their activation (Figure 1B). Among them, APCs, mainly DCs, can take up TAA/TSA from dying tumor cells (by phagocytosis) or released in the microenvironment (by extracellular processing and capture on empty surface MHC or by endocytosis).[189,190] APCs are then able to crosspresent tumor antigens to the adaptive immune system, thereby leading to the induction of tumor-specific T cells. Such immune reaction has been characterized for reoviruses, both in vitro and in vivo, including clinical trials. In this case, tumor cell infection led to DC activation, which, in turn, stimulated the cytolytic ability of NK cells, expansion of T-cell populations, and induction of tumor-specific CTLs.[178–181,191–193] A number of other studies have found that both innate and adaptive immune responses are generated following viral oncolysis mediated by HSV,[79,194–198] adenovirus,[177] parvovirus,[199,200] and VSV.[162,201,202] Antitumor immunity consecutive to viral oncolysis is an important aspect of this therapy, as CTLs will be able to recognize and destroy any remaining tumor cells that are not killed by the virus (Figure 1B). Moreover, preclinical studies showed that such OV-generated antitumor immunity may provide long-term tumor protection, preventing re-engraftment with the same tumor cells.[180,195–198,203]

Immunostimulatory oncolytic viruses

One strategy that has been investigated to increase the immunostimulatory properties of OVs is their combined use with cytokines/chemokines.[204,205] As we have seen, recombinant cytokines have been used in the clinic as cancer immunotherapy. However, toxicities associated with higher doses of systemically administered cytokines are substantial.[206] By incorporating cytokines and chemokines as a transgene into OVs, it is possible to safely increase immune stimulation through their local expression (Figure 1B). Various arms of the immune system can be targeted for stimulation by these transgenes. These could include APCs (DCs, macrophages, neutrophils) and/or lymphocytes (NK, NKT, T and B cells).

In an effort to recruit and activate APCs, a number of groups have added cytokines to their OVs such as GM-CSF[77,163,207–212] or Flt3L,[213,214] or chemokines like CCL3[213] or CCL5.[215,216] Studies involving OV-mediated expression of GM-CSF started in 2001 in the HSV-1 backbone.[217] Since then, GM-CSF has been inserted in many other OVs, including VV,[85,211,218,219] Ad,[212,220,221] NDV,[222] MV,[223] and VSV.[224] Oncolytic Ad (KH901), VV (JX-594), and HSV (OncoVEX) expressing GM-CSF

have, respectively, reached clinical phase I, II, and III for the treatment of various neoplasms.[77,85,207–212] JX-594 is an oncolytic VV deleted for viral TK and expressing human GM-CSF. In a preclinical context, JX-594 demonstrated significant antitumor efficacy, with concomitant induction of tumor-specific CTLs, in two liver tumor models,[218] and this virus has been evaluated in clinical trials following intratumoral[211] or systemic delivery.[85] In a phase I trial, out of 21 patients suffering from different cancers and treated systemically with various doses of JX-594, eight showed progressive disease, one patient had a partial response, and 12 had stable disease, according to Response Evaluation Criteria in Solid Tumors.[85]

In order to stimulate lymphocytes, genes expressing other cytokines such as IL-12,[206,225–228] IL-2,[222,229,230] IL-4,[231,232] IL-18,[225,233,234] IFN-α/β,[172,235–238] IFN-γ,[239] or, again, TNF-α[240] have been inserted into OVs. IL-12 targets NK, NKT, and T cells, inducing proliferation, expression of cytotoxic mediators, and production of cytokines. Among these cytokines figures IFN-γ, thereby inducing Th-1 $CD4^+$ T-cell response thought to yield superior antitumor immunity.[241–243] Apart from its immunomodulatory role, IL-12 has also been shown to suppress tumor-associated angiogenesis, also in an IFN-γ-dependent manner.[244–247] As a consequence, exogenous administration of IL-12 impaired the growth of various tumors in vivo.[248–251] However, its systemic delivery has been associated with substantial toxicity.[252–255] Therefore, amount and tissue diffusion of IL-12 should be restricted. Such restriction has already demonstrated significant impact on tumor growth when IL-12 was expressed from the liver following hydrodynamic injection of DNA.[256] Similarly, introduction of IL-12 transgene into OVs improved efficacy of OV therapy.[206,226–228,257–259]

Alternative strategies have been evaluated to improve OV-induced antitumor immune response. These involved transgenes encoding costimulatory molecules (Figure 1B) such as CD80/B7-1,[206,260] 4-1BBL,[261,262] or CD40L[232,263]. Additionally, fusion proteins like CD80 fused to an Fc fragment of IgG1 (CD80-Ig)[233,234,264] or HSP proteins, which have the ability to chaperone peptides and activates APCs,[265–268] have been added to OVs.

All these approaches contributed to increase efficacy of OV therapy in animal models. Some have also been evaluated in human patients with promising antitumor activity.[77,211,212,265] Importantly, combining OV-mediated expression of both cytokines and costimulatory factors has been shown to further enhance the therapeutic efficacy. In this case, cytokines and costimulatory molecules can be coexpressed from the same OV or expressed from different ones that are coadministered. Various combinations have already been tested with success, such as GM-CSF+CD80,[260,269] IL-12+4-1BBL,[262] IL-12+CD80,[206] IL-18+CD80-Ig,[233] or even IL-12+IL-18+CD80-Ig.[234]

Oncolytic viruses expressing tumor antigens (oncolytic vaccines)

Vaccination against pathogens has been one of the great successes in medicine. The development of therapeutic cancer vaccines is far more challenging due to the fact that successful vaccines will have to target tumor-associated antigens that the host may be tolerized against and which must be tumor-specific to avoid toxicities.

Phase I and II clinical trials have been performed to assess the ability of viral vaccine vectors expressing tumor antigens to induce immune responses in cancer patients. Some examples of TAA that have been evaluated include 5T4, carcinoembryonic antigen, MAGE, and NY-ESO-1.[36,210,270–274] In these trials, some patients were found to develop an antibody and/or cell-mediated immune response against the immunizing antigen. When using viral vectors to raise immune responses versus self-antigens, it is possible that the overwhelming immune response to the viral antigens may have limited the expansion of specific immune responses to the TAA of interest.[275] One method to circumvent this biology is through the use of heterologous prime-boost strategies where the priming and boosting vectors are immunologically distinct. The large majority of studies assessing heterologous vaccination have used a variety of poxviruses, as well as Ad and Semliki Forest virus.[276–281] Using a heterologous boost led in most cases to an expansion of TAA-specific T cells in both murine models and clinical trials. However, the results vary and there is a need to identify pairs of vectors that work well together.

Although classical viral vaccine vectors are nonreplicating, the use of replicating OVs as vaccine vectors has begun to be interrogated and led to the introduction of the concept of oncolytic vaccines (Figure 1B). As with Ad and modified VV Ankara, other OVs can be engineered to express TAA/TSA. Along with expressing their TAA/TSA transgene to induce a specific immune response, these viruses will also infect and debulk the tumor, thus leaving residual tumor for the immune system while releasing other tumor antigens that may allow for antigen spreading. VV, VSV, and NDV engineered to express model TAAs have been used in an attempt to lytically destroy the tumor while inducing a specific immune response.[201,205,282] Importantly,

we have recently shown that OVs expressing TAA can be excellent boosting vectors.[108,188] Combining the benefits of viral oncolysis (tumor debulking and reversal of local immunosuppression) with that of heterologous prime-boost strategies has led to substantially enhanced therapeutic benefit in animal models.[188]

Combining oncolytic viruses with cell (immuno)therapy

Cell therapies are based on the adoptive transfer of immune cells (eg, T cells, DCs), tumor cells, or progenitor cells that have been manipulated ex vivo. Initially, the idea was to use these cells as a carrier or "Trojan horse" for OV to decrease their detection by the immune system (eg, neutralizing antibodies). The objective was to improve OV delivery to the tumor following systemic administration and so to enhance tumor oncolysis.[283–289] Interestingly, some combinations between OV and cell therapies also happen to further enhance antitumor immunity, when compared with each therapy taken separately.[290–295] Cytokine-induced killer (CIK) cells are tumor-trafficking, non-MHC-restricted, cytolytic immune cells targeting NKG2D ligands; ligands present on most tumor cells. These cells not only displayed potent antitumor efficacy but also were able to carry and deliver oncolytic VV to primary tumors and metastases. As a consequence, administration of VV-infected CIK cells mounted very efficient antitumor immunity characterized by an increased number of TILs and a decreased Treg population infiltrating the tumor.[290,292,296] The tumor cell-based vaccine approach has also been combined to OV therapy. After ex vivo infection with OV (eg, parvovirus, NDV), tumor cells were lethally irradiated and injected in vivo. Such a combination demonstrated better efficacy to break immune tolerance and to generate antitumor immunity than the standard tumor cell-based vaccine.[293,295] Extracts of tumor cells infected by OVs (oncolysates) have been used to pulse DCs. These pulsed DCs displayed better immunostimulatory properties than DCs pulsed with uninfected tumor cell lysate.[187] After infection with some OVs (eg, reovirus, VSV), DCs display improved immunostimulatory ability, underwent activation, and acquired the capacity to prime TAA-specific T cells.[180,297] Reovirus virotherapy was shown to enhance the efficacy of DC- or T cell-based anticancer immunotherapies and synergistically enhances the survival of tumor-bearing mice.[180] Finally, we demonstrated that OVs carrying TAA transgenes could also be used to transduce DCs as a vaccine platform.[297] The expression of TAA from VSV in transduced DCs was able to enhance tumor-specific CTL responses and very efficiently activate NK cells. Interestingly, in this model, although CTLs played a role in protection, NK cells appeared to be the main effectors responsible for tumor protection.[297]

Assets, limitations, and improvements of oncolytic virotherapy

For safety reasons, OV repertoire is currently restricted to viruses that are rendered non- or weakly pathogenic for humans. Coupled to a preferential targeting to and/or replication in tumor cells, it means that OV therapy has shown very limited toxicity. If required, OV replication could also be controlled by administering antiviral drugs, neutralizing antibodies, or type I IFNs.[298,299] Symptoms commonly described are transient and include fever, headache, fatigue, flu-like symptoms, pain, and some events of hepatic dysfunction.[209,211,300] Most of them remain tolerable when compared with side effects associated with common cancer treatments, mostly tumor-aspecific. Nausea, diarrhea, hair loss, anemia, infection, and infertility are frequently reported with chemo- and radiotherapies. Side effects following passive immunotherapy (eg, cytokine administration) are considerable and can be severe, such as allergic reaction, diabetes, or heart, liver, and thyroid problems.

Antitumor efficacy of OV therapy has often been limited for various reasons. First, numerous clinical trials performed so far involved intratumoral injection of the oncolytic agent. This route ensures that the OV is delivered at high dose to the tumor. Unfortunately, oncolytic efficacy may rapidly drop as tumor size increases. Indeed, OV infection mainly concerns tumor cells that are proximal to the injection site. Replication competency of OVs should contribute to viral spreading from cell to cell. However, diffusion of injected and progeny virions through the tumor environment may be limited. Genetic engineering helped overcome this issue by introducing transgenes affecting the extracellular matrix (eg, relaxin; for review, see Smith et al[301]). Additionally, for practical evidence, intratumoral delivery would limit OV therapy to surface neoplasms (eg, head and neck cancer, melanoma).

Intravenous OV delivery raises a lot of hope for treating the variety of neoplasms: hematologic or solid, primary, or metastatic forms. Unfortunately, through this route, it is likely that some viruses were trapped by off-target tissues (eg, liver, spleen) or failed to escape the vascular compartment. But the main obstacle for systemic delivery remains the components

of the immune system (eg, neutralizing antibodies, complement activation). The nature of the OV administered represents a critical choice.[302] Viruses such as VV or MV evolved to traffic in the bloodstream while Ad or HSV are more rapidly neutralized in the plasma.[303–306] Additionally, natural human viruses may encounter pre-existing immunity in some patients.[286,298,307] Various strategies have been developed to limit clearing of circulating OVs, including injection of virions displaying surface proteins unrecognized by neutralizing Ig,[75,308,309] cell carriers,[283,284,286–289] modulation of tumor vasculature,[204] or transient immunosuppression.[310–313] Systemic administration of OVs allows widespread infection of tumors. However, as is mentioned previously, the dose of virus that effectively reaches the target is lowered. To compensate, OV must be injected at high doses.[85] Furthermore, rapidly primed antiviral immunity limits OV replication to a few days. Similarly, anticancer drugs and immunotherapeutic molecules also display short lifespan in the body, but repeated injections circumvent this limitation. Such approaches have been applied to OV therapy but are likely thwarted due to OV-specific immune responses.

Luckily, the efficacy of OV therapy is not limited to direct oncolysis. For some OVs, like VSV or VV, a limited number of infection sites can initiate tumor vasculature shutdown and lead to necrosis of the whole tumor mass.[162] As we have seen, infection of tumor cells by OVs also contributes to breaking tumor immunotolerance and reactivating tumor suppression mechanisms. Antitumor immunity consecutive to viral oncolysis might actually be the key point determining the overall therapeutic efficacy. Not only does it allow for destruction of non OV-infected cells but also it may raise tumor-specific memory populations, preventing relapse of the disease.[79,196–198]

Interestingly, OV therapy appears to be particularly suitable for combination with other cancer treatments. Direct oncolysis can be enhanced by associating OVs with radiotherapy and/or chemotherapy (eg, HDACi, cisplatin, paclitaxel, rapamycin).[184,209,291,314] Indirect oncolysis can be enhanced by combining OVs with antiangiogenic molecules administered either exogenously (eg, cRGD peptide, trichostatin A, bevacizumab)[315–317] or as transgene (eg, endostatin, angiostatin, anti-VEGF signalling antibodies),[317–323] or with mAbs targeting tumor-associated surface proteins (eg, cetuximab).[288] Additionally, a multitude of strategies have been developed to improve OV-induced antitumor immunity. These included combination of OVs with tumor cell- and DC-based therapies[50,293–295,297] or with immunomodulatory molecules (eg, cytokines, Treg-depleting mAbs, drugs like CPA).[78,204,205,267,324] Inserting transgenes expressing immunostimulatory factors (eg, cytokines/chemokines, costimulatory molecules) into OVs also displayed efficient enhancement of the antitumor activity in preclinical and clinical settings.[163,206,211,215,260] Once again, the nature of the viral vector appeared to be of importance. For example, expressing CD40L from oncolytic HSV did improve antitumor activity, and no benefit was observed when inserted into VSV.[232,263] Also, when combined with chemotherapy, particular attention must be paid to the dose and timing of administration of both viruses and drugs. Indeed, the transient lymphodepletion must not affect OV-induced antitumor immunity to avoid a deleterious impact on the therapeutic efficacy.[325,326]

With the approval of the DC-based vaccine Sipuleucel-T, therapeutic cancer vaccines officially joined the arsenal of cancer therapies.[51,52] Among them, oncolytic vaccines represent a promising way for establishing potent tumor-specific response. Unlike antigen-, anti-idiotype-, or DC-based vaccines, viral oncolysis may present the full pattern of TAA to APCs. This approach is similar to tumor cell-based vaccines. However, OV therapy also produces viral TLR agonists that act as catalyzers for the activation of APCs, which are key players for priming specific antitumor response. From there, overexpression of particular TAAs, selected for their ability to expand tumor-specific CTLs, should improve therapeutic efficacy.[201,205,282] For this purpose, effort must be maintained to identify highly immunogenic TAA/TSA. Moreover, we have recently shown that including oncolytic vaccines in a heterologous prime-boost can further enhance tumor-specific T-cell response but also reduce anti-OV cellular responses.[188]

Oncolytic viral therapy lends itself well to computer simulation, as there are at least two populations (virus and tumor) that interact, and this interaction can be mathematically modelled. A number of models have been published over the years.[327–339] These models have predicted that replication rate of the virus, tumor size,[335] cytotoxicity of the virus,[334] and distribution of the virus[337] are all important. They have also highlighted the potential for oscillating population sizes following infection[327,330] and how this may be important for the ultimate outcome of virotherapy. Others have attempted to make predictions about how the number of viral doses impacts on outcome.[328] Few have attempted to include the complicating influence of antiviral immunity where it has been shown to negatively impact outcome.[339]

The induction of strong innate immune responses has been predicted to aid therapy, however.[335] Our group recently published a model that attempted to incorporate antitumoral and antiviral immunity, and this predicted that both viral oncolysis and antitumoral immunity were required for tumor clearance with extended longevity of viral replication, leading to further enhancement of treatment.[340] Overall, most of these models provide predictions that are intuitive to those in the field. However it is likely that they will become more useful as they become ever more sophisticated. The danger lies in basing the math on inaccurate assumptions (we may not know enough) and in generating predictions that are not easily tested to validate the models.

Finally, in addition to the points mentioned, OV therapy is attractive for its relatively cheap and low time-consuming procedures, from ex vivo virus production and purification to in vivo injection(s) and follow-up. Additionally, positive interactions (additive or synergic) between OV therapy and other cancer treatments should reduce side effects, because smaller dosages of radiation or therapeutic agents could be administered.

Conclusion and place in therapy

OVs are starting to show promise in the clinic. These advances have required the use of larger doses of less attenuated viruses to begin to achieve robust infection and destruction of tumors. The most promising clinical candidates show evidence of induced antitumoral immunity, and this is most likely the path to success for these agents. We believe that oncolytic viral therapy occurs in two phases: an initial phase where the virus mediates direct oncolysis of tumor cells, leading to a second phase where an induced immune response continues to mediate tumor destruction and control after the viral vector has been cleared. To date, there have been limited opportunities to compare and contrast viruses being tested or to test their combination with thoughtful attempts to modify the immune system to benefit and enhance therapy. As the field matures and the first viruses become approved therapeutics, we will be able to contemplate these possibilities. Ultimately, most cancer therapies are applied in combination, and it is reasonable to predict that OVs will be as well. However, they are very different from those therapies currently in use, and the ways and means to combine these agents with other therapies may require novel clinical trial designs and considerable attention paid to the many facets of their therapeutic effects.

Disclosure

The authors report no conflicts of interest in relation to this article.

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