

Oncolytic Viruses and Their Application to Cancer Immunotherapy

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Abstract

Oncolytic viruses (OV) selectively replicate and kill cancer cells and spread within the tumor, while not harming normal tissue. In addition to this direct oncolytic activity, OVs are also very effective at inducing immune responses to themselves and to the infected tumor cells. OVs encompass a broad diversity of DNA and RNA viruses that are naturally cancer selective or can be genetically engineered. OVs provide a diverse platform for immunotherapy; they act as *in situ* vaccines and can be armed with immunomodulatory transgenes or combined with other immunotherapies. However, the interactions of OVs with the immune system may affect therapeutic outcomes in opposing fashions: negatively by limiting virus replication and/or spread, or positively by inducing antitumor immune responses. Many aspects of the OV–tumor/host interaction are important in delineating the effectiveness of therapy: (i) innate immune responses and the degree of inflammation induced; (ii) types of virus-induced cell death; (iii) inherent tumor physiology, such as infiltrating and resident immune cells, vascularity/hypoxia, lymphatics, and stromal architecture; and (iv) tumor cell phenotype, including alterations in IFN signaling, oncogenic pathways, cell surface immune markers [MHC, costimulatory, and natural killer (NK) receptors], and the expression of immunosuppressive factors. Recent clinical trials with a variety of OVs, especially those expressing granulocyte macrophage colony-stimulating factor (GM-CSF), have demonstrated efficacy and induction of antitumor immune responses in the absence of significant toxicity. Manipulating the balance between antiviral and antitumor responses, often involving overlapping immune pathways, will be critical to the clinical success of OVs. *Cancer Immunol Res*; 2(4); 295–300. ©2014 AACR.

Oncolytic Viruses

Oncolytic virus (OV) therapy is based on selective replication of viruses in cancer cells and their subsequent spread within a tumor without causing damage to normal tissue (1, 2). It represents a unique class of cancer therapeutics with distinct mechanisms of action. The activity of OVs is very much a reflection of the underlying biology of the viruses from which they are derived and the host–virus interactions that have evolved in the battle between pathogenesis and immunity. This provides a diverse set of activities that can be harnessed and manipulated. Typically, OVs fall into two classes: (i) viruses that naturally replicate preferentially in cancer cells and are nonpathogenic in humans often due to elevated sensitivity to innate antiviral signaling or dependence on oncogenic signaling pathways. These include autonomous parvoviruses, myxoma virus (MYXV; poxvirus), Newcastle disease virus (NDV; paramyxovirus), reovirus, and Seneca valley virus (SVV; picor-

navirus); and (ii) viruses that are genetically manipulated for use as vaccine vectors, including measles virus (MV; paramyxovirus), poliovirus (PV; picornavirus), and vaccinia virus (VV; poxvirus), and/or those genetically engineered with mutations/deletions in genes required for replication in normal but not in cancer cells including adenovirus (Ad), herpes simplex virus (HSV), VV, and vesicular stomatitis virus (VSV; rhabdovirus; refs. 1, 3). Genetic engineering has facilitated the rapid expansion of OVs in the past two decades, enabling a broad range of potentially pathogenic viruses to be manipulated for safety and targeting (3). Many of the hallmarks of cancer described by Hanahan and Weinberg (4) provide a permissive environment for OVs; they include sustained proliferation, resisting cell death, evading growth suppressors, genome instability, DNA damage stress, and avoiding immune destruction. In addition, insertion of foreign sequences can endow further selectivity for cancer cells and safety, as well as altering virus tropism through targeting of translation with internal ribosome entry sites (IRES) or microRNAs (PV and VSV), transcription with cell-specific promoter/enhancers (Ad, HSV), or transduction with altered virus receptors (HSV, Ad, MV, and VSV; refs. 1, 3). These strategies are also being used to target replication-deficient viral vectors for gene therapy applications in cancer immunotherapy.

OVs have many features that make them advantageous and distinct from current therapeutic modalities: (i) there is a low probability for the generation of resistance (not seen so far), as

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OVs often target multiple oncogenic pathways and use multiple means for cytotoxicity; (ii) they replicate in a tumor-selective fashion and are relatively nonpathogenic and, in fact, only minimal systemic toxicity has been detected; (iii) virus dose in the tumor increases with time due to *in situ* virus amplification, as opposed to classical drug pharmacokinetics that decrease with time; and (iv) safety features can be built in, such as drug and immune sensitivity. These features should result in a very high therapeutic index. An important issue for OV therapy is delivery. Although systemic intravenous administration is simpler than intratumoral injection and can target multiple tumors, it has drawbacks, including nonimmune human serum, anti-OV antibodies that preexist for human viruses or can be induced by multiple administrations, lack of extravasation into tumors, and sequestration in the liver (1). Cell carriers [i.e., mesenchymal stromal cells, myeloid-derived suppressor cells (MDSC), neural stem cells, T cells, cytokine-induced killer cells, or irradiated tumor cells] can shield virus from neutralization and facilitate virus delivery to the tumor (5). The effectiveness will vary depending upon the cell phenotype, permissiveness to virus infection, tumor-homing ability, and transfer of infectious virus to tumor cells. To block virus neutralization and extend vascular circulation, viruses can also be coated in nanoparticles (i.e., PEGylation; ref. 1).

OV Immunotherapy

Virus infection and pathogenicity have been major drivers in the evolution of the human immune system, and vaccination against viruses is the quintessential exploitation of adaptive immunity. A major goal of OV-mediated immunotherapy is to activate and redirect functional innate and adaptive immune responses toward the tumor. Interactions between innate and adaptive immune cells and signaling factors (i.e., cytokines and chemokines), often involved in virus infections, play a large role in antitumor immunity or lack thereof, as well as successful immunotherapies (Fig. 1). Virus infection induces an inflammatory response leading to adaptive antiviral immunity. Thus, the immune system was seen initially as a negative factor in OV therapy for limiting virus infection/delivery because of preexisting or therapy-induced immunity, virus replication because of innate antiviral responses, and virus spread because of the infiltration of innate immune cells (6). In addition, most early studies were performed in human xenograft tumor models in immunodeficient mice lacking adaptive immune responses because some viruses were species selective or replicated better in human cells, and because there was availability of a broad diversity of human cancer cell lines. With the use of syngeneic tumor models in immunocompetent mice, it became clear that the consequences of the immune system were complex, but that the induction of antitumor immunity was feasible and efficacious (6). In particular, many OVs act as *in situ* vaccines, inducing robust, long lasting, and specific adaptive antitumor responses, often CD8⁺ T cell-mediated (7, 8). Interestingly, adaptive antiviral immunity can enhance antitumor immunity for HSV, but not for VSV (8, 9).

The inflammatory cascade and immunogenic cell death (ICD) induced by OV infection of tumors makes OVs particularly powerful inducers of antitumor immunity (8, 10). Among

the many different types of cell death, some are immunogenic and characterized by the release of danger-associated molecular patterns (DAMP), such as calreticulin, high-mobility group protein B1 (HMGB1), and ATP, along with tumor-associated antigens (TAA; ref. 10). Multiple forms of ICD have been observed after OV (Ad, VV, HSV, MV, and coxsackievirus) infection of cancer cells, and there is a suggestion that ICD occurs in patients after treatment with oncolytic Ad and temozolomide (11). However, much remains to be learned about the mechanisms of OV-mediated cell death and how it can be exploited to enhance immunogenicity. Inflammation, typically chronic, can also promote tumorigenesis and inhibit T-cell antitumor activity (12). Restraining antiviral immune responses and minimizing pathology, while promoting antitumor immune responses, is a complex and poorly understood balancing act that will dictate OV therapy outcomes. In some cases, where minimal OV replication occurs in mouse tumors (i.e., HSV) or no replication is required (i.e., reovirus; ref. 13), antitumor efficacy is principally due to OV-induced immune responses. Understanding, harnessing, modulating, and/or enhancing OV-mediated immune responses for effective antitumor immunity are major areas in current research that intersect with other immunotherapeutic strategies.

Many viruses express immune evasion genes that enable them to establish infections and spread within their host (14). Mutations in these genes (i.e., HSV *Us11*, VV *E3L*, MYXV *M156R*, Ad *VAI*, and reovirus $\sigma 2/\sigma 3$, inhibitors of PKR; HSV *ICP0*, VV *N2*, NDV *V*, and MV *V*, inhibitors of IRF3; HSV *ICP0*, MYXV *M13L*, MV *V*, PV *3C*, and VSV *M*, inhibitors of NF- κ B; VV *B8R* and MYXV *MT-7*, inhibitors of IFN- γ ; HSV *ICP47* and Ad *E3-19K*, inhibitors of MHC class I presentation; MV gp, inhibitor of T cells; and MYXV *M128L* and MV *H*, inhibitors of CD46) are likely to enhance the induction of immunity and possibly cross-presentation of TAAs. Such mutations should improve the safety of OVs by making them more visible to the immune system, as well as increasing antitumor immune responses. Conversely, they may diminish virus replication and spread. An additional problem not as easily addressed is OV infection of immune cells, especially dendritic cells (DC), that interferes with their function (15, 16).

Innate Immunity

Although adaptive immunity seems to provide and, in fact, represent even the major mode of anticancer action for OVs, it is also evident that an initial host response against an administered OV could destroy it along with the infected cells before the OV has a chance to replicate and induce cytotoxicity of a magnitude that is sufficient to set up an effective vaccination response (17). Location and site of OV administration is an important determinant of the characteristics of these initial host responses against the OV. For instance, intravenous or intra-arterial administration of OVs, such as recombinant HSV1, leads to its rapid recognition and elimination by the circulating complement and antibodies of the humoral defense system (18, 19). This has also been shown for VV (20), NDV (21), MV (22), and Ad (23, 24). Intratumoral administration can also lead to complement- and antibody-mediated destruction of the OV. In addition, intracellular and microenvironmental

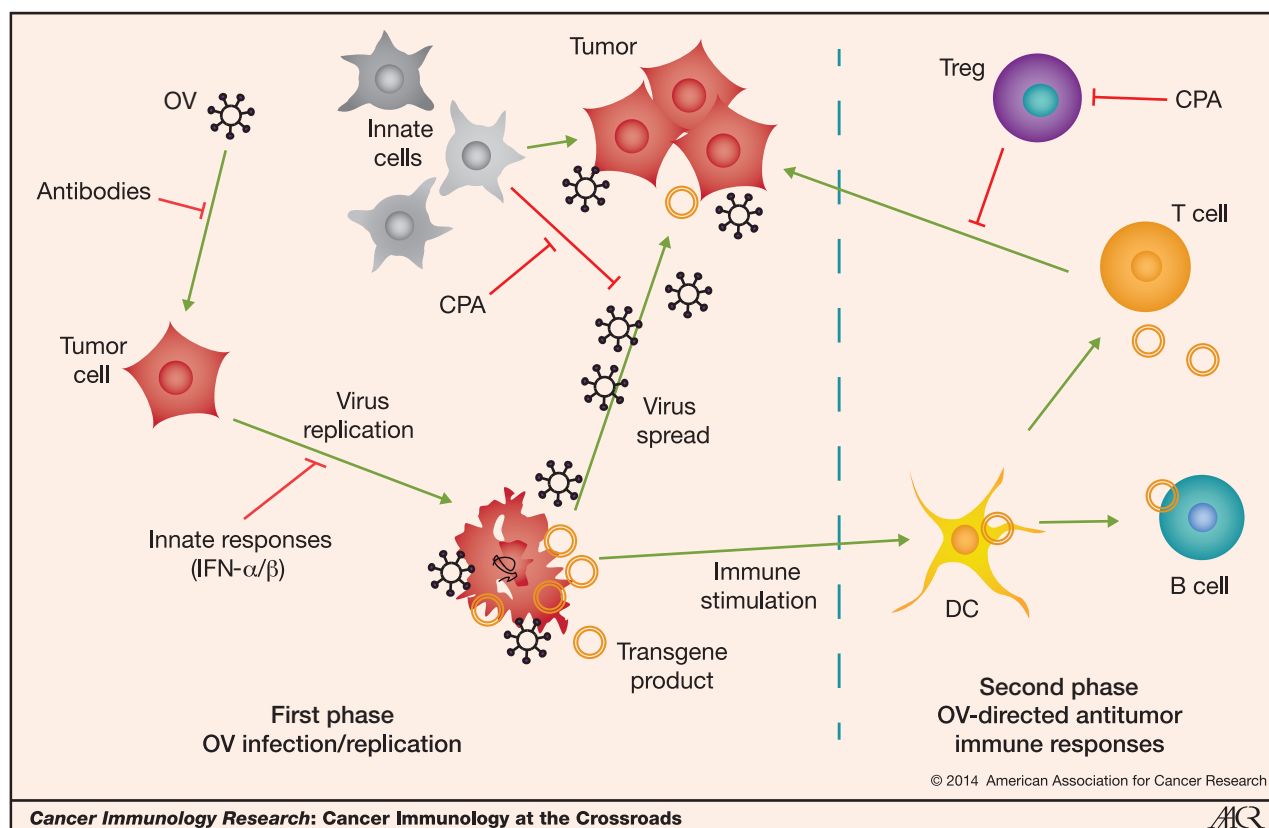


Figure 1. Cartoon of OV-mediated effects in tumor. First phase, OV delivered intratumorally or systemically, infects tumor cells (can be blocked by humoral defense systems; antibodies). After infection, OV replicates (can be blocked by innate responses; i.e., IFN- α/β), kills cells often by ICD, and spreads throughout the tumor (can be blocked by innate immune cells, i.e., NK cells and macrophages), eliciting an inflammatory response. When an armed OV is used, the immunomodulatory transgene is expressed (transgene product). Second phase, ICD and inflammation recruit DCs to the tumor, where they take up TAAAs and induce an adaptive immune response (T and B cells), which targets the tumor (can be blocked by Tregs and MDSCs). Innate cells such as NK cells also have antitumor activities. Antitumor immune responses can be further enhanced by transgene products. CPA, cyclophosphamide.

antiviral defense responses in infected tumor cells can also greatly limit the magnitude of OV replication (25–31). Finally, innate immune cells can rapidly respond to an administered OV, further limiting its survival and that of OV-infected tumor cells (32–35). In all these models, circumvention of such responses using pharmacologic agents, such as histone deacetylase (HDAC) inhibitors or immunomodulating drugs, or genes that block antiviral defense mechanisms, has led to improved OV replication and tumor cytotoxicity (reviewed in ref. 36). When pharmacologic agents are used, the interference of antiviral responses can be applied in a transient fashion usually right before or at the time of OV administration. This should lead to an initial burst of OV replication leading to tumor cell lysis. As the pharmacologic effects against host innate immunity wane, a large debris field of OVs and tumor antigens could be more promptly recognized by the antiviral host response, leading to a secondary long-term vaccination effect responsible for effective tumor immunity (Fig. 1). However, quantification of responses to OV therapy is a sorely needed area of investigation. For instance, the number of OV-replicative rounds, the tumor cell-OV burst size, the number of OV-replicative tumor foci, and the temporal kinetics of innate response suppression that are needed for an efficient lytic and

vaccination effect are still undetermined. In fact, current applications of innate immunity modulation with OV administration remain to be determined in an empirical manner.

Clinical Outcomes

There have been numerous clinical trials of OVs for cancer. As expected, most have been phase I with a few phase II trials. Currently, a phase III trial is investigating an oncolytic HSV1-expressing granulocyte macrophage colony-stimulating factor (GM-CSF) for melanoma (talimogene laherparepvec, T-Vec) sponsored by Amgen, Inc. Subjects were randomized to intratumoral injections of the agent T-Vec or of GM-CSF alone. Preliminary reports show a 16% durable response rate for the T-vec arm compared with only 2% for the GM-CSF alone arm, and a 26% overall response rate (vs. only 6% for the GM-CSF alone arm). Encouraging trends toward improved overall survival were also shown at an interim analysis (37). Although there is hope that the final analysis will bring results that will lead to the first U.S. Food and Drug Administration (FDA) approval of an OV for tumors, detractors already have pointed out that a recent explosion of several new promising pharmacologic agents against melanoma may impede approval or, if approved, the market utilization of T-Vec.

Two OV therapy phase II trials have been completed recently. An oncolytic VV that expresses GM-CSF (Pexa-Vec, JX-594) has completed a randomized phase II trial in subjects with hepatocellular carcinoma, sponsored by Jennerex, Inc. (38). Low- or high-dose JX-594 was administered intravascularly into liver tumors. Surprisingly, the authors discovered that response rates and intrahepatic disease control were equivalent in both injected and distant noninjected tumors at either dose, suggesting that an immune response might be responsible for the observed anticancer effects. They also report that JX-594 anticancer immunity occurred after JX-594 replication and GM-CSF expression. Although such radiologic tumor responses were independent of dosage, subject survival duration was related to dosage (median survival of 14.1 months compared with 6.7 months on the high and low doses, respectively; HR, 0.39; $P = 0.020$). These results seem highly encouraging, but some of the data related to immunity were equivocal. For instance, although the authors directly injected only some tumors, viral genomes were detected in the peripheral blood within 15 minutes. This implies that even the noninjected tumors were likely exposed to JX-594, rendering more equivocal the conclusion that an immunologic effect was responsible for the observed response in the noninjected tumors (39). In addition, the authors observed humoral immunity in subject sera raised against hepatocellular carcinoma cell lines rather than the actual subject tumors. Although they posited that such humoral immunity, based on antibody-mediated complement activation against tumor antigens, is a critical determinant of the JX-594 effects (40), assays of direct T cell-mediated cytotoxicity against autologous hepatocellular carcinoma cells would enhance the evidence for an immunologic mechanism as a critical determinant for the observed antitumor response.

A phase II trial has been completed for an oncolytic reovirus (Reolysin, sponsored by Oncolytic Biotech) after intravenous administration in subjects with metastatic melanoma (41). Although the authors report no objective responses in the 21 treated patients, they were able to obtain posttreatment biopsies that were evaluable for 13 patients. In 2 patients, there was immunohistochemical suggestion of productive reoviral replication in the tumors. The authors also note that neutralizing antibody titers increased in these subjects. They conclude that the lack of a response precluded further progression of the trial to subsequent phases and reason that this failure was due to the rising neutralizing antibody titers against reovirus. In fact, they state that a trial of Reolysin with cyclophosphamide to reduce serum neutralization of reovirus was needed. In summary, these advanced clinical trials are providing tantalizing pieces of evidence that seem to invoke the critical role of an immunologic mechanism of anticancer action for effective virotherapy. However, there remains a lack of unequivocal demonstration of OV-induced immunologic activity, and the alternative explanation related to direct viral cytotoxicity as a predominant mechanism is still possible. This would be important to establish in the context of clinical trials rather than in preclinical studies. This knowledge would point clinicians toward avenues of even more stimulation of immune responses versus blocking antiviral immunity, as suggested by the Reolysin trial discussed above.

Enhancing OV Immunotherapy

Many OVs can accommodate gene insertions and thus can be "armed" with therapeutic transgenes, combining local gene delivery with oncolytic activity (42). Local expression in the tumor obviates toxicity arising from systemic administration of potent immune modulators. GM-CSF, based on its effects in cytokine-transduced cancer cell vaccines (i.e., clinically approved Sipuleucel-T), has been incorporated into a number of OVs [HSV T-Vec, VV JX-594, Ad Ad5/3-D24-GMCSF (43), and CG0070 (44)] that have entered clinical trials (8). GM-CSF-expressing OVs demonstrated only moderate activity in preclinical studies (45, 46), while JX-594 was not compared with a VV lacking GM-CSF (47). Other therapeutic transgenes include interleukin (IL)-2 (NDV, HSV, and parvovirus), IL-12 (Ad and HSV), IL-15 (VSV), IL-18 (HSV), IFN- α/β (Ad, VSV, and VV), soluble CD80 (Ad and HSV), 4-1BB (VV), CD40L (Ad, and no effect with VSV), Flt3L (Ad and HSV), CCL3 (Ad), CCL5 (Ad and VV), and combinations thereof (2). In addition to transgenes that enhance adaptive immune responses, cytokines/chemokines directed at the tumor microenvironment can alter the immune cell balance toward productive therapeutic immunity (Fig. 1). IL-12, a potent antitumor cytokine with antiangiogenic activities, when expressed from oncolytic HSV, reduced neovasculature and tumor regulatory T cells (Treg) and induced T cell-mediated immunity in an immunocompetent cancer stem cell model (48). Expression of a CXCR4 antagonist from oncolytic VV reduced tumor vasculature and accumulation of bone marrow-derived epithelial and myeloid cells and induced antitumor humoral responses (49).

Like many cancer vaccine strategies, OVs expressing TAAs can be used to induce tumor-selective adaptive immune responses. The combination of TAA expression in the tumor and OV-mediated cell killing induces enhanced T-cell migration and activation compared with OV-infected tumor cells expressing the TAA (50). This can be coupled to a prime (replication-deficient Ad or oncolytic Semliki Forest virus expressing a TAA)-boost (oncolytic VSV or VV expressing the same TAA) vaccine strategy, in which the boosted secondary response to the tumor dominates the primary anti-OV response (6, 8). To expand the antigenic repertoire, cDNA libraries from normal tissue (e.g., prostate for prostate tumors) or recurrent tumors have been inserted into VSV, and induced therapeutic immunity (51). Further enhancement was obtained by expressing xenogeneic TAAs (51, 52). The ability of oncolytic VSV expressing TAAs to induce IL-17 in the context of tumor immunity has been exploited to screen tumor cDNA libraries for individual TAAs and optimal TAA combinations, limiting potentially inappropriate responses of whole-cell or cDNA vaccines (53). Developing a similar strategy in a human setting would be a major advance.

A number of immunomodulatory agents have been examined to restrain antiviral immune responses and promote OV replication and spread. Cyclophosphamide can increase OV replication and inhibit tumor growth by suppressing innate immune cell (34) and antibody responses (54), depleting Tregs, and enhancing the antitumor activity of CTLs (Fig. 1; ref. 8). A challenge is to identify immunosuppressive strategies that can blunt acute innate cells from blocking

virus replication and spread, while permitting sufficient inflammation and cross-priming for robust antitumor immunity. Conversely, it will be of interest to combine OV with chemotherapies that induce ICD (e.g., cyclophosphamide, oxaloplatin, or anthracyclines such as doxorubicin and mitoxantrone), increase tumor cell antigenicity (e.g., gemcitabine, cisplatin, or etoposide) or susceptibility to immune cells (e.g., HDAC inhibitors, paclitaxel, or doxorubicin), or suppress MDSCs (e.g., gemcitabine and paclitaxel) and Tregs (e.g., cyclophosphamide or sunitinib; ref. 55) in immunocompetent preclinical models.

In conclusion, the field of virotherapy is becoming mature in its knowledge of effective anticancer mechanisms in animal tumor models with OVs that are also safe in human clinical

trials. It seems that there may soon be a first-in-humans OV approved for use in the United States, which will further stimulate laboratory and clinical endeavors with this therapeutic strategy.

Disclosure of Potential Conflicts of Interest

E.A. Chiocca has an ownership interest (including patents) and is a consultant/advisory board member of DNATRIX. S.D. Rabkin has an ownership interest (including patents) as coinventor on oncolytic HSV patents.

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